REVUE

CHROMagar mSuperCARBA performance in carbapenem-resistant *Enterobacteriaceae* isolates characterized at molecular level and routine surveillance rectal swab specimens

Article sous presse: Épreuves corrigées

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Abstract

Performance of the CHROMagar mSuperCARBA media was assessed in both well-characterized carbapenem-resistant *Enterobacteriaceae* (n = 52) and routine surveillance rectal swab specimens (n = 211). Limit of detection ranged between 10^{1} and 10^{2} CFU/mL except for OXA-48 producers with low-carbapenem MICs (10^{6} CFU/mL). High sensitivity (100%) and specificity (100%) were obtained with rectal swabs.

Highlights

- CHROMagar mSuperCARBA is an accurate media for carbapenem-resistant Enterobacteriaceae screening.
- Limit of detection ranged between 10^{1} and 10^{2} CFU/mL, except for OXA-48 producers with low-carbapenem MICs.
- The media displayed high levels of sensitivity and specificity with rectal swab specimens.

The global spread of carbapenem-resistant *Enterobacteriaceae* (CRE) is a public health issue (Cantón et al., 2012). Rapid methods for the detection of CRE isolates are necessary since this information has an important value from a clinical and infection control perspective (

Munoz-Price et al., 2013; Tzouvelekis et al., 2012).

Different screening methods for detection of CRE have been recently developed. Some of them are based on colorimetric detection in both chromogenic media and enzymatic hydrolysis of the β-lactam ring (Nordmann et al., 2012; , 2012 Vrioni et al. , 2012) while others rely on molecular detection of resistance, as loop-mediated isothermal amplification (LAMP) or microarray techniques (Braun et al., 2014; García-Fernández et al., 2015).

The CHROMagar mSuperCARBA (CHROMagar, France) is a commercial chromogenic media for the detection of CRE from direct clinical specimens. The aim of this study was to evaluate its performance to screen CRE in the Clinical Microbiology laboratory of a University Hospital in Spain with a carbapenemase-endemic situation. The study was performed to: (i) determine the limit of detection (LOD) of CRE, (ii) assess sensitivity using three different inoculum sizes (dilution levels) of well-characterized clinical CRE-strains and (iii) assess sensitivity using routine surveillance rectal swabs.

The LOD was assessed using 7 clinical strains with characterized carbapenemase-resistance mechanisms consisting of: KPC-3 (*Escherichia coli* , *n* = 1, *Klebsiella pneumoniae* , *n* = 1), VIM-1 (*Enterobacter cloacae* complex, *n* = 1; *E. coli, n* = 1), NDM-1 (*K. pneumoniae, n* = 1), OXA-48 (*K. pneumoniae* , *n* = 1) and OXA-48 + CTX-M-15 (*K. pneumoniae* , *n* = 1) producers. Table 1 (t0005) . Each strain was suspended in normal saline (NaCl 0.9%) to a density equivalent to 0.5 McFarland (*ca.* 2x10 ^{8} CFU/mL), followed by serial 10-fold dilutions. An aliquot of 100 μL from each dilution (10 $^{-2}$ to 10 $^{-8}$) was plated onto the chromogenic medium, and on MacConkey agar plates (Oxoid, Wesel, Germany) in order to perform viable colony counts. Viable bacteria were counted after incubation at 35 ± 2 °C during 24 h. Each experiment was performed in triplicate. The LOD was the lowest dilution of inoculated inoculum where bacterial colonies could be counted and was expressed by the mean value of 3 counts (Vrioni et al. , 2012). A collection of 45 characterized CRE was tested to assess sensitivity using a high (10 ^{8} CFU/mL), medium (10 ^{4} CFU/mL) and low (10 ^{2} CFU/mL) inoculum. Strains were selected depending on the bacterial species, carbapenemase-gene content and genetic lineages. All CRE strains were recovered at Ramón y Cajal University Hospital and were identified using both MicroScan system (Beckman, West Sacramento, *CA*) and MALDI-TOF MS (Bruker-Daltonics, Germany). MICs (imipenem, ertapenem and meropenem) were confirmed using MIC gradient strips (MIC-Test Strip, Liofilchem, Italy). The MHT, the Carba NP test (bioMérieux, La Balme-les-Grottes, France) and the assessment of the inhibition profile using the ROSCO KPC/Metallo-β-lactamase and OXA-48 Confirm Kit (ROSCO Diagnostica, Taastrup, Denmark) were used to confirm carbapenemase production and its putative molecular class (CLSI, 2015; Nordmann et al., 2012). *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC BAA-1705 (KPC-2 producer) were used as negative and positive control, respectively.

Table 1

LOD for the 7 clinical strains with characterized carbapenemase-resistance mechanisms.

Microorganism (Reference)	MIC (μg/mL) [a,b] (tf0005)			Carbapenemase(+ESBL)	LOD (CFU/mL) [c] (tf0015)
	IMP	ETP	MEM		
E. coli (Ruiz-Garbajosa et al., 2013)	1	0.38	0.38	KPC-3	1×10^{1}
K. pneumoniae (Ruiz-Garbajosa et al., 2013)	0.5	1.5	1	KPC-3	1.5×10^{1}
Enterobacter cloacae complex (Gijón et al., 2012)	1	1	0.75	VIM-1	2×10^{1}
E. coli (Gijón et al., 2012)	0.5	1	0.75	VIM-1	3×10^{2}
K. pneumoniae (Nordmann et al., 2012)	>32	>32	>32	NDM-1	3.5×10^{1}
K. pneumoniae (Gijón et al., 2014)	>32	>32	>32	OXA-48	1.5×10^{1}
K. pneumoniae (Gijón et al., 2014)	0.25	0.8	0.12	OXA-48 (+ CTX-M-15)	1×10^{6}

a IMP = imipenem, ETP = ertapenem, MEM = meropenem.

b MICs performed by MicroScan system and by gradiente strips.

c LOD: mean value of 3 counts.

Characterization of the carbapenemase genes was performed by conventional PCR assays and sequencing as previously published (Gijón et al., 2012; Gijón et al., 2014; Ruiz-Garbajosa et al., 2013). The strains were KPC- ($n = 19$), VIM- ($n = 14$), and OXA-48- ($n = 12$) producers, respectively. Table 2 (t0010) . An aliquot of 100 μL of the 3 inoculum sizes was individually plated onto the chromogenic media and inspected after 24 h at 35 ± 2 °C. Moreover, rectal swab specimens ($n = 211$) were collected from different patients in medical wards with an endemic epidemiological situation with CRE. In order to compare its performance, the swabs were directly plated onto the new chromogenic medium and the chromogenic medium routinely used in our laboratory and considered as the reference one [ChromID ESBL, CARBA and OXA-48 media (BioMérieux, Marcy-l'Étoile, France)]. The analysis was made after 24 h of incubation at 35 ± 2 °C.

Table 2

Sensitivity of the 45 clinical CRE strains with a high, medium and low bacterial inoculum grouped according to

different carbapenemases.

Carbapenemase type (*n*)	Microorganism (n)	MIC (µg/mL) [a],[b]			Inoculum (CFU/mL)	Positive growth/Total number
		IMP	ERT	MEM		
Class A						
KPC-2 (5)	*Enterobacter cloacae* complex (2)	0.75–1	0.75–1	1	10^8	2/2
					10^4	2/2
					10^2	2/2
	E. coli (1)	6	8	2	10^8	1/1
					10^4	1/1
					10^2	1/1
	K. pneumoniae (2)	2	6–8	12	10^8	2/2
					10^4	2/2
					10^2	2/2
KPC-3 (14)	*E. coli* (1)	1	0.25	0.38	10^8	1/1
					10^4	1/1
					10^2	1/1
	K. pneumoniae (13)	0.5–3	0.19–8	0.38–12	10^8	13/13
					10^4	13/13
					10^2	13/13
Class B						
VIM-1 (14)	*Citrobacter freundii* complex (1)	2	1	1,5	10^8	1/1
					10^4	1/1
					10^2	1/1

	Enterobacter cloacae complex (5)	1–8	0.38–4	0.75–6	10^8	5/5
					10^4	5/5
					10^2	5/5
	E. coli (1)	0.5	1	0.75	10^8	1/1
					10^4	1/1
					10^2	1/1
	K. oxytoca (2)	0.38–2	0.094–0.5	0.25–1	10^8	2/2
					10^4	1/2
					10^2	1/2
	K. pneumoniae (3)	1–6	0.75–1	0.75–3	10^8	3/3
					10^4	3/3
					10^2	3/3
	Serratia marcescens (2)	1.5–2	0.19–0.25	0.38	10^8	2/2
					10^4	0/2 [c] (tf0030)
					10^2	0/2 [c] (tf0030)
Class D						
OXA-48 (12)	*Citrobacter freundii* complex (2)	0,5	0.38	0.25–0,38	10^8	2/2
					10^4	2/2
					10^2	1/2
	C. koseri (1)	0.38	0.25	0.125	10^8	1/1
					10^4	0/1
					10^2	0/1
	Enterobacter cloacae complex (1)	0.75	1.5	0.25	10^8	1/1
					10^4	1/1

					10^{2}	0/1
	E. coli (2)	0.19–0,38	0.25–1,5	0.125–1,5	10^{8}	2/2
					10^{4}	1/2
					10^{2}	1/2
	K. oxytoca (1)	0.5	0.75	0.25	10^{8}	1/1
					10^{4}	0/1
					10^{2}	0/1
	K. pneumoniae (4)	0.25–>32	0.75–>32	0.125–>32	10^{8}	4/4
					10^{4}	3/4
					10^{2}	2/4
	R. ornithinolytica (1)	1	0.75	0.38	10^{8}	1/1
					10^{4}	1/1
					10^{2}	0/1

a IMP = imipenem; MEM = meropenem; ERT = ertapenem.

b MICs performed by MicroScan system and by gradiente strips.

c Low detection levels in these *S. marcescens* strains could be due to low MIC values.

Carbapenem MICs and the LOD of the 7 clinical strains are shown in Table 1 (t0005) . The LOD ranging between 10^{1} and 10^{2} CFU/mL was obtained in 6/7 strains. The exception was the OXA-48 + CTX-M-15-producing *Klebsiella pneumoniae* strain that exhibited low carbapenem-MICs and LOD repeatedly ranging from 10^{6} to 10^{7} CFU/mL. MICs were determined before performing LOD experiments in all strains.

CRE strains tested to assess sensitivity (LOD) with a high, medium and low bacterial inoculum grouped according to different carbapenemases and their carbapenem MICs are detailed in Table 2 (t0010) . The highest sensitivity was shown for KPC producers and the lowest for OXA-48, especially when tested with low inoculum. Sensitivity by carbapenemase type and inoculum was as follows: i) KPC [10^{8} (100%); 10^{4} (100%); 10^{2} (100%)], ii) VIM [10^{8} (100%); 10^{4} (78.6%); 10

^{2} (78.6%)], and iii) OXA-48 [10 ^{8} (100%); 10 ^{4} (66.7%);10 ^{2} (30.6%)]. A slight variability between organisms with similar carbapenem MICs was observed, and could be explained, at least partially, due to the already described heterogeneous expression of carbapenemases (Tato et al., 2013). Additionally, low carbapenems' MICs can influence the sensitivity to detect carbapenemase-producers.

During the period of study (March–July 2015), 211 rectal swabs specimens were analyzed. Of these, 150 were from patients without CRE colonization in their rectal swab. No growth was obtained in either CHROMagar mSuperCARBA or in the comparator media (100% specificity). Seven out of these 150 patients were colonized with ESBL-producing *Enterobacteriaceae* isolates but with the new chromogenic medium they were inhibited. The remaining 61 swabs were from CRE-colonized patients (Those who had CRE colonization in their rectal swab) (2 KPC, 3 VIM, 2 NDM, and 54 OXA-48). Imipenem and ertapenem MICs of these isolates, performed by MicroScan, ranged from ≤1 to >8 μg/mL for imipenem and ≤0.5 to >4 μg/mL for ertapenem, respectively, and was uniformly in the lower limit for OXA-48 producers. A positive growth was obtained for all of them (100% sensitivity). Interestingly, in 8/61 CRE-colonized patients, simultaneous colonization with different carbapenemase-producing isolates was also detected due to a different color of the colonies grown onto the media. Interestingly, the sensitivity of the medium was higher when testing rectal swab' samples than that when using direct colonies. Despite lower detection in OXA-48 producers, we did not obtain false negative results with rectal swabs; even with those containing isolates displaying low MIC values. This result could be explained due to the presence of a high bacterial load in the rectal swabs (Lerner et al., 2013).

One limitation of our study could be the lack of testing AmpC hyperproducer, alone or in combination with ESBL isolates, to fully discard putative false positives. In summary, chromogenic media as the new one assayed in the present study constitute an accurate, easy to perform method for CRE screening that could be afforded by almost all laboratories, leaving alternatives based on PCR or microarray hybridization for specific clinical or epidemiological situations where a rapid intervention is required.

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Conflict of Interests

The authors have no conflict of interests.

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Références

Braun et al., 2014. Braun S.D., Monecke S., Thürmer A., Ruppelt A., Makarewicz O., Pletz M., et al: Rapid identification of carbapenemase genes in gram-negative bacteria with an oligonucleotide microarray-based assay. PLoS One 2014; 9: pp. e102232
Cross Ref (http://dx.doi.org/10.1371/journal.pone.0102232)

Cantón et al., 2012. Cantón R., Akóva M., Carmeli Y., Giske C.G., Glupczynski Y., Gniadkowski M., et al: Rapid evolution and spread of carbapenemases among . Clin Microbiol Infect 2012; 18: pp. 413-431
Cross Ref (http://dx.doi.org/10.1111/j.1469-0691.2012.03821.x)

Clinical and Laboratory Standards Institute, 2015. Clinical and Laboratory Standards Institute : M100-S25. Performance standards for antimicrobial susceptibility testing: 25th informational supplement. Wayne, PA: CLSI, 2015.

García-Fernández et al., 2015. García-Fernández S., Morosini M.I., Marco F., Gijón D., Vergara A., Vila J., et al: Evaluation of the eazyplex® SuperBug CRE system for rapid detection of carbapenemases and ESBLs in clinical . J Antimicrob Chemother 2015; 70:

Gijón et al., 2014. Gijón D., Morosini M.I., García-Fernández S., Coque T.M., Ruiz-Garbajosa P., and Cantón R.: Multiclonal spread of OXA-48 producers including CG258 (ST11) . In (eds): Abstracts of the twenty-fourth European congress of clinical microbiology and infectious diseases. Basel, Switzerland: European Society of Clinical Microbiology and Infectious Diseases, 2014.

Gijón et al., 2012. Gijón D., Curiao T., Baquero F., Coque T.M., and Cantón R.: Fecal carriage of carbapenemase-producing Enterobacteriaceae: a hidden reservoir in hospitalized and non-hospitalized patients. J Clin Microbiol 2012; 50: pp. 1558-1563
Cross Ref (http://dx.doi.org/10.1128/JCM.00020-12)

Lerner et al., 2013. Lerner A., Romano J., Chmelnitsky I., Navon-Venezia S., Edgar R.,

and Carmeli Y.: Rectal swabs are suitable for quantifying the carriage load of KPC-producing carbapenem-resistant Enterobacteriaceae. Antimicrob Agents Chemother 2013; 57: pp. 1474-1479
Cross Ref (http://dx.doi.org/10.1128/AAC.01275-12)

Munoz-Price et al., 2013. Munoz-Price L.S., Poirel L., Bonomo R.A., Schwaber M.J., Daikos G.L., Cormican M., et al: Clinical epidemiology of the global expansion of . Lancet Infect Dis 2013; 13: pp. 785-796
Cross Ref (http://dx.doi.org/10.1016/S1473-3099(13)70190-7)

Nordmann et al., 2012. Nordmann P., Poirel L., and Dortet L.: Rapid detection of carbapenemase-producing . Emerg Infect Dis 2012; 18: pp. 1503-1507
Cross Ref (http://dx.doi.org/10.3201/eid1809.120355)

Ruiz-Garbajosa et al., 2013. Ruiz-Garbajosa P., Curiao T., Tato M., Gijón D., Pintado V., Valverde A., et al: Multiclonal dispersal of KPC genes following the emergence of non-ST258 KPC-producing . J Antimicrob Chemother 2013; 68: pp. 2487-2492
Cross Ref (http://dx.doi.org/10.1093/jac/dkt237)

Tato et al., 2013. Tato M., Morosini M., García L., Albertí S., Coque M.T., and Cantón R.: Carbapenem heteroresistance in VIM-1-producing . J Clin Microbiol 2013; 48: pp. 4089-4093
Cross Ref (http://dx.doi.org/10.1128/JCM.01130-10)

Tzouvelekis et al., 2012. Tzouvelekis L.S., Markogiannakis A., Psichogiou M., Tassios P.T., and Daikos G.L.: Carbapenemases in . Clin Microbiol Rev 2012; 25: pp. 682-707
Cross Ref (http://dx.doi.org/10.1128/CMR.05035-11)

Vrioni et al., 2012. Vrioni G., Daniil I., Voulgari E., Ranellou K., Koumaki V., Ghirardi S., et al: Comparative evaluation of a prototype chromogenic medium (ChromID CARBA) for detecting carbapenemase-producing . J Clin Microbiol 2012; 50: pp. 1841-1846
Cross Ref (http://dx.doi.org/10.1128/JCM.06848-11)