Published in "Diagnostic Microbiology and Infectious Disease
83(3): 234–236, 2015"
which should be cited to refer to this work.

CHROMagar *Acinetobacter* medium for detection of carbapenemase-producing *Acinetobacter* spp. strains from spiked stools

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The recently modified CHROMagar *Acinetobacter* medium was evaluated for detection of carbapenemase-producing *Acinetobacter baumannii* from spiked stools. A total of 45 *Acinetobacter* spp. isolates were tested. The CHROMagar *Acinetobacter* medium had a high sensitivity of 86.5% and a specificity of 75%. This medium is likely to be most useful for controlling outbreaks and in endemic situations.

Acinetobacter spp. are an important source of healthcare-associated infections. The spread of carbapenemase-producing *Acinetobacter* spp. is increasingly reported worldwide associated with multidrug resistance (Bonnin et al., 2013a; Poirel and Nordmann, 2006; Wareham et al., 2008). Therefore, targeted surveillance of high-risk patients based on screening of carriers is essential to control the spread of carbapenem-resistant *Acinetobacter* spp. (CRAB). Resistance to carbapenems in *Acinetobacter* spp. may result from decreased permeability of the outer membrane, modification of penicillin-binding proteins, production of carbapenemases, and mostly from combined resistance mechanisms (Bonnin et al., 2013a; Poirel and Nordmann, 2006; Fernández-Cuenca et al., 2003). Carbapenemases in *Acinetobacter* spp. belong to Ambler class A (KPC and some GES variants) (Bonnin et al., 2013b; Robledo et al., 2010), class B (VIM, IMP, SIM, NDM) (Bonnin et al., 2012; Poirel and Nordmann, 2006), and mostly class D (oxacillinases) (Bonnin et al., 2013a). Five main groups of oxacillinases with carbapenemase activity have been identified in *A. baumannii*, i.e., the intrinsic chromosomal OXA-51-like and the acquired chromosomally and plasmid-encoded OXA-23-like, OXA-40-like, OXA-58-like, and OXA-143-like enzymes (Bonnin et al., 2013a; Higgins et al., 2009; Poirel and Nordmann, 2006). When the $bla_{\text{OXA-51-like}}$ genes are expressed at a basal level, they do not confer carbapenem resistance. However, the insertion of the insertion sequence IS*Aba1* upstream of the $bla_{\text{OXA-51-like}}$ gene may lead to overexpression of this oxacillinase gene leading to carbapenem resistance (Turton et al., 2006). Selective media developed for detection of carbapenem-resistant Enterobacteriaceae are inappropriate for detection of CRAB, since *Acinetobacter* spp. are intrinsically resistant to several carbapenem molecules that are contained in those selective media. CHROMagar *Acinetobacter* (CHROMagar, Paris, France) is a selective medium designed for rapid identification of CRAB inhibiting the growth of yeast, carbapenem-susceptible Gram negatives and Gram positives, and coloring the colonies of *Acinetobacter* spp. in red since it contains a chromogenic molecule (Ajao et al., 2011; Gordon and Wareham, 2009). A new formula of this medium has been recently developed (Song et al., 2013). As compared to the previous version of the CHROMagar *Acinetobacter* medium, it contains an antimicrobial selective supplement (CR102; CHROMagar) aimed to select for CRAB.

While previous studies on previous formulas of this medium included strains for which the molecular mechanisms of resistance was not determined, our aim was to evaluate the performance of this CHROMagar *Acinetobacter* medium using a set of precisely molecular-defined carbapenemase producers. Spiked stools were used to mimic the in vivo colonization of stools.

Forty-five *Acinetobacter* spp. isolates (mostly *A. baumannii*) were tested, including 37 acquired carbapenemase producers as follows: Ambler class A (GES-11-, GES-14-type) producers (n = 2), Ambler class B (IMP, VIM, SIM, NDM-type) producers (n = 7), Ambler class D (OXA-23-type, OXA-40-type, OXA-58-type) producers (n = 28), and 8

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strains that do not produce acquired carbapenemases (n = 8), the last being either carbapenem resistant or carbapenem susceptible. OXA-23 producers are by far the most widespread carbapenemase producers in *A. baumannii* (Bonnin et al., 2013a). MIC values of imipenem and meropenem (*A. baumannii* is naturally resistant to ertapenem) were determined by E-test and interpreted according to the CLSI guidelines updated in 2014. MIC breakpoints for imipenem and meropenem against *A. baumannii* were susceptibility for MICs ≤2 μg/mL, intermediate resistance 4 μg/mL, and resistance for MICs ≥8 μg/mL, as updated in CLSI (2014). The CHROMagar *Acinetobacter* medium was prepared as recommended by the manufacturer from dehydrated powder and liquid supplement added in the form of antimicrobial selective supplement (CR102). Bacterial suspensions of the strains with an optical density of 0.5 McFarland (inoculum of ~5×10^7 CFU/mL) were serially diluted in water. Ten fold dilutions were made. To quantify the viable bacteria in each dilution, trypticase soy agar was inoculated concomitantly with 100 μL of suspension and incubated overnight at 37 °C; the number of viable colonies was counted the following day. Spiked fecal samples were made by adding 100 μL of each dilution to 900 μL of fecal suspension that was obtained by suspending 4 g of freshly pooled feces from four healthy volunteers in 40 mL of distilled water, as previously described (Naas et al., 2011). A fecal suspension without the addition of a bacterial strain was used as negative control. The lowest detection limit of the carbapenemase producers was determined by plating 100 μL of each dilution on CHROMagar *Acinetobacter* medium (CHROMagar). Viable bacteria were counted after 24 h of culture at 37 °C. The sensitivity and specificity were determined using a cutoff value set at ≥1×10^3 CFU/mL, as previously described (Nordmann

Table 1
Sensitivity of detection of the CHROMagar *Acinetobacter* medium from spiked fecal samples.

	β-Lactamase content	IPM MIC (μg/mL)	MEM MIC (μg/mL)	Lowest detection limit (CFU/mL)[a]
Carbapenemase Ambler class A				
A. baumannii KOW	GES-11	6	8	1×10^1
A. baumannii RB	GES-14	32	32	1×10^1
Carbapenemase Ambler class B				
A. baumannii IMP	IMP-1	4	6	1×10^1
A. baumannii IMP4	IMP-4	24	16	1×10^2
Acinetobacter genomospecies 16	VIM-4	>32	>32	1×10^2
A. baumannii SIM	SIM-1	>32	>32	1×10^1
A. baumannii SLO	NDM-1	>32	>32	1×10^1
A. baumannii ALG	NDM-1	>32	>32	1×10^1
A. baumannii EGY	NDM-2	>32	>32	1×10^1
Carbapenemase Ambler class D				
A. baumannii 23-B2	OXA-23	>32	>32	1×10^1
A. baumannii 23-C2	OXA-23	>32	>32	1×10^1
A. baumannii 23-D2	OXA-23	>32	>32	1×10^1
A. baumannii 23-E2	OXA-23	>32	>32	1×10^1
A. baumannii 23-F2	OXA-23 + PER-1	>32	>32	1×10^1
A. baumannii 23-G2	OXA-23	>32	>32	1×10^1
A. baumannii 23-G4	OXA-23	>32	>32	1×10^2
A. baumannii 26-C2	OXA-26	>32	>32	1×10^1
A. baumannii 40-A1	OXA-40	>32	>32	1×10^1
A. baumannii 40-A2	OXA-40	>32	>32	2×10^1
A. baumannii 40-A3	OXA-40	>32	>32	2×10^1
A. baumannii 40-A4	OXA-40	>32	>32	1×10^1
A. baumannii 40-A5	OXA-40	>32	>32	1×10^1
A. baumannii 40-D7	OXA-40	>32	>32	1×10^1
A. baumannii 40-D8	OXA-40	>32	>32	1×10^1
A. baumannii 40-D9	OXA-40	>32	>32	1×10^3
A. baumannii 72-D5	OXA-72	>32	>32	1×10^1
A. baumannii 58-A2	OXA-58	>32	>32	2×10^2
A. baumannii 58-A4	OXA-58	>32	24	2×10^2
A. baumannii 58-A7	OXA-58 + PER-2	>32	12	1×10^1
A. baumannii 58-B1	OXA-58	24	32	1×10^1
A. baumannii 58-B2	OXA-58	>32	32	2×10^2
A. baumannii SWE	OXA-58	>32	24	5×10^3
A. baumannii BAR	OXA-58	4	1	2×10^6
A. baumannii ITA	OXA-58	>32	16	6×10^2
A. baumannii GRE	OXA-58	>32	>32	1×10^3
A. haemolyticus 58-A10	OXA-58	>32	8	$>1 \times 10^6$
Multiple carbapenemases				
A. baumannii LIB	GES-11 + OXA-23	>32	>32	1×10^1
No acquired carbapenemase				
A. baumannii CB3	None	1	0.25	$>1 \times 10^6$
A. baumannii CB4	None	0.25	0.25	$>1 \times 10^6$
A. baumannii CB6	None	0.25	0.12	$>1 \times 10^6$
A. baumannii CA9	RTG-4	0.38	0.25	$>1 \times 10^6$
A. baumannii CA1	GES-12 + OXA-51 + IS*Aba1*	32	32	1×10^2
A. baumannii CA3	OXA-51 + IS*Aba1*	3	3	1×10^2
A. baumannii CA6	SHV-5	6	8	4×10^4
A. baumannii CA7	PER-1	1.5	0.75	$>1 \times 10^6$

Abbreviations: IPM = imipenem; MEM = meropenem.
Underlined CFU counts are considered as negative results (cutoff values set at ≥1×10^3 CFU/mL).
[a] One milliliter of stools contains 100 mg of stool.

et al., 2012), i.e., a limit detection value of 1×10^3 CFU/mL or higher was considered as lack of detection (Table 1). This value may correspond to a low-level carriage of multidrug-resistant bacteria in stools.

Carbapenemase-producing *A. baumannii* were well detected from spiked stools except some OXA-58 producers (Table 1). Lack of detection was noticeable for 2 OXA-58 producers with high-level resistance to carbapenems. The sensitivity of detection of carbapenemase-producing *A. baumannii* using the CHROMagar *Acinetobacter* medium (86.5%) was lower than the sensitivity of detection of carbapenem-resistant *A. baumannii* (91.7%), similarly to what was previously reported for detection of multidrug-resistant *A. baumannii* in an outbreak situation in intensive care unit in 2009 in the UK (sensitivity of 91.7%) (Gordon and Wareham, 2009). This difference may be due to a lower limit of detection that has been set here at 10^3 CFU/mL. Further studies should clinically validate this cutoff value with carbapenem-resistant *A. baumannii* isolates. Sensitivity would have been 94.6% when just considering growth or no growth, as done in this British study (Gordon and Wareham, 2009).

We identified specificity of the CHROMagar *Acinetobacter* medium of 75% that was lower than that previously reported by Gordon and Wareham (2009) (89.7%). Our specificity result was lower since we included in "non-acquired carbapenemase-producers" 2 strains of *A. baumannii* with insertion of IS*Aba1* upstream of the naturally occurring bla_{OXA-51} gene. This resulted in the overexpression of this oxacillinase gene and thus decreased susceptibility to carbapenems, as previously reported (Brown et al., 2005; Turton et al., 2006). Moran-Gilad et al. (2014) showed that CHROMagar *Acinetobacter* medium had a sensitivity of 100% for detection of isolates with MICs of imipenem >32 μg/mL and a specificity of 100% for isolates with MICs of imipenem <1 μg/mL (Moran-Gilad et al., 2014). Our study shows that this rule is not applicable in all cases (Table 1). Neither imipenem nor meropenem MIC could be strictly correlated with the detection limit of the CHROMagar *Acinetobacter* medium, as exemplified by the following strains, *A. baumannii* KOW, IMP, and CA6, which produce GES-11, IMP-1 and SHV-5, respectively. Although these strains showed similar MICs, the carbapenemase producers were specifically detected on the CHROMagar *Acinetobacter* medium (Table 1).

Noticeably, this medium showed a good specificity, since no other bacteria from stools were detected on the CHROMagar *Acinetobacter* medium. This result correlated with that of previous studies on previous formulations of this medium. Wareham and Gordon (2011) showed that the use of the KPC supplement enabled recovery of carbapenem-resistant *A. baumannii*, distinguishable from carbapenem-resistant Enterobacteriaceae by the color of the colonies, and Barsoumian et al. (2013) showed that the CR102 supplement prevented the growth of other bacterial species even if carbapenem resistant (Barsoumian et al., 2013).

Overall, the studied screening medium has a good efficiency for detection of carriers of CRAB and is likely to be most useful during outbreaks or when CRAB is endemic. At least, based on this study performed with spiked stools, the CHROMagar *Acinetobacter* medium can detect not only Ambler class A (GES-type carbapenemase), Ambler class B, but also class Ambler D producers (in particular the most widespread OXA-23) (Mugnier et al., 2010). The CHROMagar *Acinetobacter* medium is well adapted for direct inoculation of patient samples (rectal swabs, stools, skin, and nasal samples) in any clinical settings, as recently shown by Song et al. (2013). Use of this screening medium based on detection of carbapenem resistance is of special interest since most of the carbapenem-resistant *A. baumannii* isolates express an acquired carbapenemase (here 36 out of 37 strains) and the carbapenem resistance trait is associated to multidrug resistance and vice versa.

Funding

This work was supported by a grant from the INSERM (U914) and from the Ministère de l'Education Nationale et de la Recherche, Université Paris XI, Paris, France.

Transparency declarations

None to declare.

References

Ajao AO, Robinson G, Lee MS, Ranke TD, Venezia RA, Harris AD, et al. Comparison of culture media for detection of *Acinetobacter baumannii* in surveillance cultures of critically-ill patients. Eur J Clin Microbiol Infect Dis 2011;30:1425–30.

Barsoumian A, Calvano T, Markelz AE, Cassiy R, Murray CK, Beckius ML, et al. Variations of CHROMagar *Acinetobacter* to detect imipenem-resistant *Acinetobacter baumannii-calcoaceticus* complex. Scand J Infect Dis 2013;45:446–52.

Bonnin RA, Poirel L, Naas T, Pirs M, Seme K, Schrenzel J, et al. Dissemination of New Delhi metallo-β-lactamase-1-producing *Acinetobacter baumannii* in Europe. Clin Microbiol Infect 2012;18:E362–5.

Bonnin RA, Nordmann P, Nordmann P. Screening and deciphering antibiotic resistance in *Acinetobacter baumannii*: a state of the art. Exp Rev Anti Infect Ther 2013a;11:571–83.

Bonnin RA, Rotimi VO, Al Hubail M, Gasiorowski E, Al Sweih N, Nordmann P, et al. Wide dissemination of GES-type carbapenemases in *Acinetobacter baumannii* isolates in Kuwait. Antimicrob Agents Chemother 2013b;57:183–8.

Brown S, Young HK, Amyes SGB. Characterisation of OXA-51, a novel class D carbapenemase found in genetically unrelated clinical strains of *Acinetobacter baumannii* from Argentina. Clin Microbiol Infect 2005;11:15–23.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: 24th informational supplement (M100-S24). Wayne, PA: CLSI; 2014.

Fernández-Cuenca F, Martínez-Martínez L, Conejo MC, Ayala JA, Perea EJ, Pascual A. Relationship between beta-lactamase production, outer membrane protein and penicillin-binding protein profiles on the activity of carbapenems against clinical isolates of *Acinetobacter baumannii*. J Antimicrob Chemother 2003;51:565–74.

Gordon NC, Wareham DW. Evaluation of CHROMagar *Acinetobacter* for detection of enteric carriage of multidrug-resistant *Acinetobacter baumannii* in samples from critically ill patients. J Clin Microbiol 2009;47:2249–51.

Higgins PG, Poirel L, Lehmann M, Nordmann P, Seifert H. OXA-143, a novel carbapenem-hydrolyzing class D β-lactamase in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2009;53:5035–8.

Moran-Gilad J, Adler A, Schwartz D, Navon-Venezia S, Carmeli Y. Laboratory evaluation of different agar media for isolation of carbapenem-resistant *Acinetobacter* spp. Eur J Clin Microbiol Infect Dis 2014;33:1909–13.

Mugnier PD, Poirel L, Naas T, Nordmann P. Worldwide dissemination of the bla_{OXA-23} carbapenemase gene of *Acinetobacter baumannii*. Emerg Infect Dis 2010;16:35–40.

Naas T, Ergani A, Carrër A, Nordmann P. Real-time PCR for detection of NDM-1 carbapenemase genes from spiked stool samples. Antimicrob Agents Chemother 2011;55:4038–43.

Nordmann P, Girlich D, Poirel L. Detection of carbapenemase producers in *Enterobacteriaceae* by use of a novel screening medium. J Clin Microbiol 2012;50:2761–6.

Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. Clin Microbiol Infect 2006;12:826–36.

Robledo IE, Aquino EE, Santé MI, Santana JL, Otero DM, León CF, et al. Detection of KPC in *Acinetobacter* spp. in Puerto Rico. Antimicrob Agents Chemother 2010;54:1354–7.

Song W, Lee J, Lim TK, Park MJ, Kim HS, Kim JS. Modified CHROMagar *Acinetobacter* medium for direct detection of multi-drug resistant *Acinetobacter* strains in nasal rectal swab samples. Ann Lab Med 2013;33:193–5.

Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, et al. The role of IS*Aba1* in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. FEMS Microbiol Lett 2006;258:72–7.

Wareham DW, Gordon NC. Modifications to CHROMagar *Acinetobacter* for improved selective growth of multi-drug resistant *Acinetobacter baumannii*. J Clin Pathol 2011;64:164–7.

Wareham DW, Bean DC, Khanna P, Hennessy EM, Krahe D, Ely A, et al. Bloodstream infection due to *Acinetobacter* spp: epidemiology, risk factors and impact of multidrug resistance. Eur J Clin Microbiol Infect Dis 2008;27:607–12.