

Overnight identification of imipenem-resistant *Acinetobacter baumannii* carriage in hospitalized patientsFrédéric WALLET,¹ Nicolas FORTINEAU,² Thierry NAAS,² Patrice NORDMANN,² René COURCOL,¹ and Olivier GAILLOT¹¹ Bacteriology & Hygiene Laboratory, Lille University Hospital, LILLE and ² Microbiology Laboratory, Bicêtre University Hospital, Le Kremlin-Bicêtre, FRANCEOlivier GAILLOT, PhD
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Abstract

Background: Imipenem resistance in *Acinetobacter baumannii* (IR-Ab) is a major concern in health care facilities. As colonized patients are a significant reservoir for its transmission, rapid detection of carriage is crucial in order to limit the spread of IR-Ab. We evaluated the capacity of a new culture medium coupled with MALDI-TOF mass spectrometry (MT-MS) to detect nasal and rectal IR-Ab carriage in 1022 consecutive hospitalized patients.

Methods: CHROMagar Acinetobacter 2 (CA2) is a chromogenic medium for presumptive identification of Ab as raspberry-pink colonies after 18 h incubation. Firstly, sixty-two IR-Ab isolates from 17 countries encompassing 8 carbaapenemase genotypes were tested on CA2 supplemented with a carbapenem (CAZ). Then the 2044 nasal and rectal swabs were plated on CAZS and Drigalski-ceftazidime (DRI), our routine medium for identification of multidrug-resistant Ab. Suspect colonies were identified by MT-MS (Brickear Microflex).

Results: All collected IR-Ab isolates grew as typical Ab colonies on CAZS after 18 h. The lowest detection limit ranged between 1 and 5 CFU. Therein (1/24) patients were found to carry IR-Ab. Inse only, n = 6, rectum only, n = 1, both, n = 7. All 13 were detected with CAZS while 6 (46%) were detected with CAZ. On CAZS, 36 false-positive red or pink colonies were identified as *Stenotrophomonas maltophilia* (n = 19), *Pseudomonas aeruginosa* (n = 13), *Morganella morganii* (n = 2) and imipenem-susceptible Ab (IS-Ab, n = 2, with imipenem MICs of 0.75 and 1.5 µg/mL). On DC, 203 Ab-like greenish colonies were identified as *Enterobacter* spp. (n = 156), *P. aeruginosa* (n = 26), *Escherichia coli* (n = 4), IS-Ab (n = 1), and *Hafnia alvei* (n = 4).

Conclusions: Coupled with MT-MS, CAZS allowed overnight detection of IR-Ab with a reduced workload as compared to our routine method. The sensitivity of CAZS qualifies this medium for primary plating when searching for IR-Ab carriage.

Background

Over the last decade, imipenem-resistant *A. baumannii* (IR-Ab) isolates have become endemic in health care facilities of many countries, and therapeutic options are drastically limited for infected patients. Screening patients for early detection of IR-Ab is essential in order to control cross-contamination. Surveillance cultures are usually performed by plating rectal or nasal swabs on selective media suited for the detection of multidrug-resistant *Enterobacteriaceae*, such as MacConkey or Drigalski agar supplemented with 3rd generation cephalosporins or fluoroquinolones. Recently, attempts have been made to design IR-Ab specific media, including CHROMagar Acinetobacter (CA), a chromogenic medium allowing blue color-based preliminary identification of *A. baumannii* (Gordon & Wareham, 2009). However, CA appeared to lack sufficient specificity and sensitivity (Akers et al., 2010). Here, we evaluated the performance of CHROMagar Acinetobacter 2-S (CAZS), another chromogenic medium, on IR-Ab stock isolates of worldwide origin and for the detection of IR-Ab in over 2000 clinical specimens. Instant and cost effective identification of all suspect colonies was achieved by MALDI-TOF spectrometry.

Materials & Methods

Culture media. CAZS was provided for evaluation as prepared 20 mL-plates by CHROMagar Microbiology (Paris, France). Drigalski agar (Oxoid, France) supplemented with 6 mg/L ceftazidime was poured as 20 mL-plates (DRI-CAZ). All plates were incubated in air at 37°C and examined after 18, 24 and 48 h of incubation.

Stock isolates. Sixty-four stock IR-Ab strains representing 8 carbaapenem-resistance genotypes of worldwide origin (TABLE 1) were streaked onto CAZS plates. Colony color and size were evaluated. Suspensions prepared from fresh colonies were adjusted and diluted in order to inoculate approximately 100 CFU of each strain on tryptic-soy agar and CAZS.

Clinical samples. Consecutive rectal and nasal swabs from 1,022 hospitalized patients were tested for IR-Ab by direct streaking on DRI-CAZ, our routine regimen for detection of multi-resistant Gram-negative bacteria, and then onto CAZS.

Criteria for presumptive identification of IR-Ab colonies. (i) On CAZS, well-separated, 1–2 mm, raspberry-red colonies (FIG. 1B); (ii) on DRI-CAZ, greenish-yellow, 1–2 mm, convex, glistening colonies (FIG. 1A), without further testing.

Identification of suspect colonies. Colonies presumptively identified as *A. baumannii* were directly identified by MALDI-TOF mass spectrometry (Brickear Microflex, Wissembourg, France).

Imipenem resistance was assessed by Elast (bioMérieux, France) on Mueller-hinton agar.

Results

1. Growth of IR-Ab stock isolates on CAZS.

All IR-Ab isolates grew as 1–2 mm, raspberry-red colonies after 18 h, including OXA-58-producing strain with a MIC of 2 µg/mL. The lowest detection limit ranged between 1 and 5 CFU (reference, TS agar).

2. Recovery of IR-Ab from the 2,044 screening samples.

A total of 13 IR-Ab isolates were detected (1.2% of the patients), 12 from nasal swabs and 6 from rectal swabs; 7 isolates were recovered from both rectal and nasal swabs, 5 from nasal swab only, 1 from rectal swab only.

Sensitivity:

- 46% on CAZ-DRI (6 IR-Ab isolates)

- 100% on CAZS (13 IR-Ab isolates)

Specificity and positive predictive value (TABLE 2):

- on CAZ-DRI, 203 false positive colonies were identified as

Enterobacter cloacae (n = 8), *Enterobacter aerogenes* (n = 70), *Pseudomonas aeruginosa* (n = 13),

Escherichia coli (n = 4), imipenem-susceptible *A. baumannii* (n = 11), and *Hafnia alvei* (n = 4).

- on CAZS, 36 false positive (red) colonies were identified as

Stenotrophomonas maltophilia (n = 19), *P. aeruginosa* (n = 13), *Morganella morganii* (n = 2)

and imipenem-susceptible Ab (IS-Ab, n = 2, with imipenem MICs of 0.75 and 1.5 µg/mL, respectively).

However, except for the 2 IS-Ab isolates, those red colonies did not exceed 1 mm after 24 h incubation and with a little experience, they could not be mistaken with *A. baumannii* (FIG. 2).

- Positive predictive value was significantly higher with CAZS than DRI-CAZ (87% and 2.9%, respectively).

Imipenem MICs of the 13 IR-Ab isolates: 3 µg/mL (n = 1), 4 µg/mL (n = 3), 8 µg/mL (n = 4), 16 µg/mL (n = 1), 32 µg/mL (n = 4)

3. Detection of IR-Ab in specimens containing carbapenem-resistant isolates of other species

- Simultaneous growth of *S. maltophilia* (FIG. 2) or imipenem-resistant *E. aerogenes* (FIG. 3B) did not hamper easy recovery of IR-Ab on CAZS. MALDI-TOF MS allowed definitive identification in less than a day after the screening sample was taken.

- CAZ-DRI did not allow easy differentiation of colony types, and subculture was usually required for identification (FIG. 1A/3A).

Carbaapenemase	Geographic origin (no. of isolates)	Imipenem MIC range (µg/mL)
GES-11	Belgium (1), Turkey (1)	8 - 64
GES-12	France (1)	32
GES-14	France (2)	1 - 64
IMP-4	Japan (1), South Korea (1)	2 - 4
OXA-23	Algeria (2), Australia (1), Bahrain (1), Brazil (1), Egypt (1), France (37), Libya (2), Saudi Arabia (1), Thailand (1)	16 - 1 64
OXA-24	Spain (1), Portugal (1)	1 - 64
OXA-40	France (1), Netherlands (1)	1 - 64
OXA-58	France (5), Sweden (1)	2 - 1 64

TABLE 1. Origin and carbapenem resistance characteristics of the 64 *Acinetobacter baumannii* stock isolates used in this study.

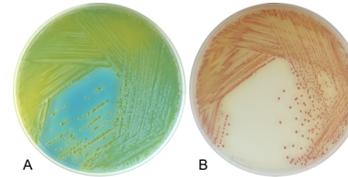


FIG. 1. Colonies plated out from a rectal swab specimen containing imipenem-resistant *Acinetobacter baumannii* and imipenem-resistant, lactose-negative *Enterobacter aerogenes*. (A), Drigalski-ceftazidime (DRI-CAZ) agar; (B), CHROMagar Acinetobacter 2-S (CAZS). Both strains grew on DRI-CAZ as greenish-yellow colonies, while only *A. baumannii* grew on CAZS as characteristic raspberry-red colonies. Plates were incubated for 18 h in air at 37°C.

Incubation Time (h)	Medium	No. of isolates				Sensitivity	PPV	Specificity	PNV
		True positive	False positive	True negative	False negative				
18-24	DRI-CAZ	6	203	1,815	7	46%	2.9%	90%	99.7%
	CAZS	13	2*	2,029	0	100%	87%*	99.9%	100%
1 48	DRI-CAZ	6	203	203	7	46%	2.9%	90%	99.7%
	CAZS	13	20**	1,995	0	100%	86.5%**	98.0%	100%

* False positives after 18-24 h: imipenem-susceptible *A. baumannii* (n = 3)

** False positives after 1 48 h: *S. maltophilia* (n = 19), multidrug-resistant *P. aeruginosa* (n = 13), *M. morganii* (n = 2), and imipenem-susceptible *A. baumannii* (n = 2).

TABLE 2. Sensitivity, specificity, predictive positive and negative values for both media when searching for imipenem-resistant *Acinetobacter baumannii* in 2,044 rectal and nasal swabs of hospitalized patients.

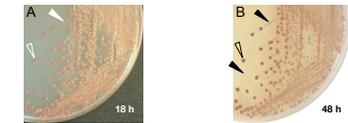


FIG. 2. Mixed growth of carbapenem-resistant *A. baumannii* and *Stenotrophomonas maltophilia* on CHROMagar Acinetobacter 2-S after 18 h (A) and 48 h (B). *S. maltophilia* (black arrowheads) colonies are only barely visible on a black background in the first 24 h. Although appearing as red colonies after 48 h, they are smaller than *A. baumannii* colonies (empty arrowheads).

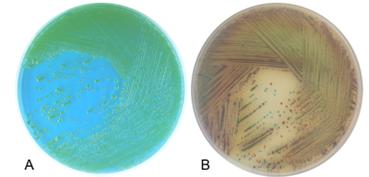


FIG. 3. Colonies plated out from a rectal swab containing imipenem-resistant *Acinetobacter baumannii* and imipenem-resistant, lactose-negative *Enterobacter aerogenes*. (A), Drigalski-ceftazidime (DRI-CAZ) agar; (B), CHROMagar Acinetobacter 2-S (CAZS), incubated for 18 h in air at 37°C. On CAZS, *A. baumannii* (red colonies) can be readily differentiated from *E. aerogenes* (blue colonies), contrarily to colonies on DRI-CAZ.

Summary

CHROMagar Acinetobacter2-S is a new chromogenic medium which

- inhibits the growth of Gram positive bacteria, yeasts, and non-MDR *Enterobacteriaceae*
- inhibits the growth of carbapenem-susceptible *Acinetobacter* species including *A. baumannii*
- partially inhibits the growth of multidrug-resistant *P. aeruginosa* and *S. maltophilia*
- is specific for carbapenem-resistant *A. baumannii*: colonies are ! 2 mm after 18 h incubation

Allowed to grow major epidemic carbapenemase-producing genotypes of *A. baumannii* as typical colonies

Allowed the recovery of a significantly higher number of IR-Ab isolates than our routine screening method

Prolonged incubation is not recommended as it increases the risk of growing false positive colonies

When coupled with MALDI-TOF MS, allows unambiguous identification of IR-Ab carriage in less than 24 h

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