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Overnight identification of imipenem-resistant *Acinetobacter baumannii* carriage in hospitalized patients

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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 carriers 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 Acinetobacter2 (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 carbapenemase genotypes were tested on CA2 supplemented with a carbapenem (CA2S). Then the 2044 nasal and rectal swabs were plated on CA2S and Drigalski-ceftazidime (DC), our routine medium for identification of multidrug-resistant *Ab*. Suspect colonies were identified by MT-MS (Brüker Microflex).

Results: All collection IR-*Ab* isolates grew as typical *Ab* colonies on CA2S after 18 h. The lowest detection limit ranged between 1 and 5 CFU. Thirteen (1.2%) patients were found to carry IR-*Ab*, (nose only, n = 5, rectum only, n = 1, both, n = 7). All 13 were detected with CA2S while 6 (46%) were detected with DC. On CA2S, 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 = 158), *P. aeruginosa* (n = 28), *Escherichia coli* (n = 4), IS-*Ab* (n = 11), and *Hafnia alvei* (n = 4).

Conclusions: Coupled with MT-MS, CA2S allowed overnight detection of IR-*Ab* with a reduced workload as compared to our routine method. The sensitivity of CA2S 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 (CA2S), 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. CA2S 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 carbapenem-resistance genotypes of worldwide origin (**TABLE 1**) were streaked onto CA2S 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 CA2S.

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 CA2S.

Criteria for presumptive identification of IR-*Ab* colonies. (i) On CA2S, well-separated, ≥ 2 mm, raspberry-red colonies (**FIG. 1B**); (ii) on Dri-CAZ, greeny-yellow, ≥ 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 (Brüker Microflex, Wissembourg, France).

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

Results

1. Growth of IR-*Ab* stock isolates on CA2S.

All IR-*Ab* isolates grew as ≥ 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 CA2S (13 IR-*Ab* isolates)

Specificity and positive predictive value (TABLE 2):

- on CAZ-DRI, 203 false positive colonies were identified as *Enterobacter cloacae* (n = 88), *Enterobacter aerogenes* (n = 70), *Pseudomonas aeruginosa* (n = 13), *Escherichia coli* (n = 4), imipenem-susceptible *A. baumannii* (n = 11), and *Hafnia alvei* (n = 4).
- on CA2S, 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* colonies (**FIG. 2**).
- Positive predictive value was significantly higher with CA2S 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 CA2S. 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**).

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

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

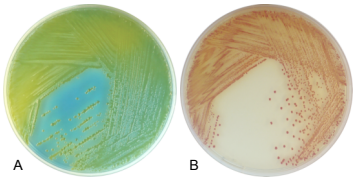


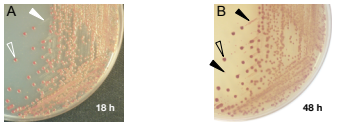
FIG. 1. Colonies plated out from a rectal swab specimen containing imipenem-resistant *Acinetobacter baumannii* and ESBL-producing *Enterobacter cloacae*. (A), Drigalski-ceftazidime (DRI-CAZ) agar. (B), CHROMagar Acinetobacter 2-S (CA2S). Both strains grew on DRI-CAZ as greeny-yellow colonies, while only *A. baumannii* grew on CA2S 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%
	CA2S	13	2*	2,029	0	100%	87%*	99.9%	100%
≥ 48	DRI-CAZ	6	203	203	7	46%	2.9%	90%	99,7%
	CA2S	13	36**	1,995	0	100%	26.5%**	96,6%	100%

* False positives after 18-24 h: imipenem-susceptible *A. baumannii* (n = 2)

** False positives after ≥ 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.



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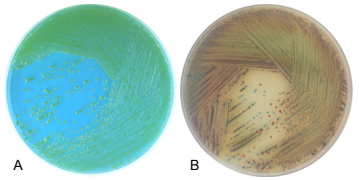

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Summary

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

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References

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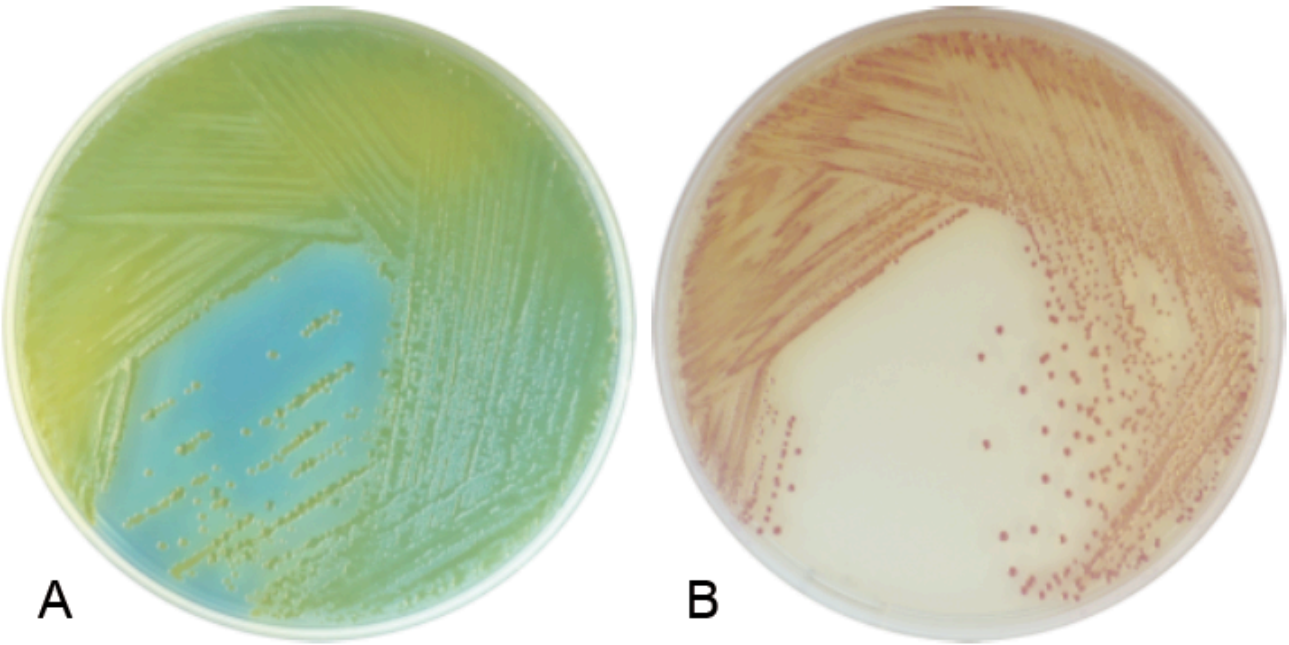

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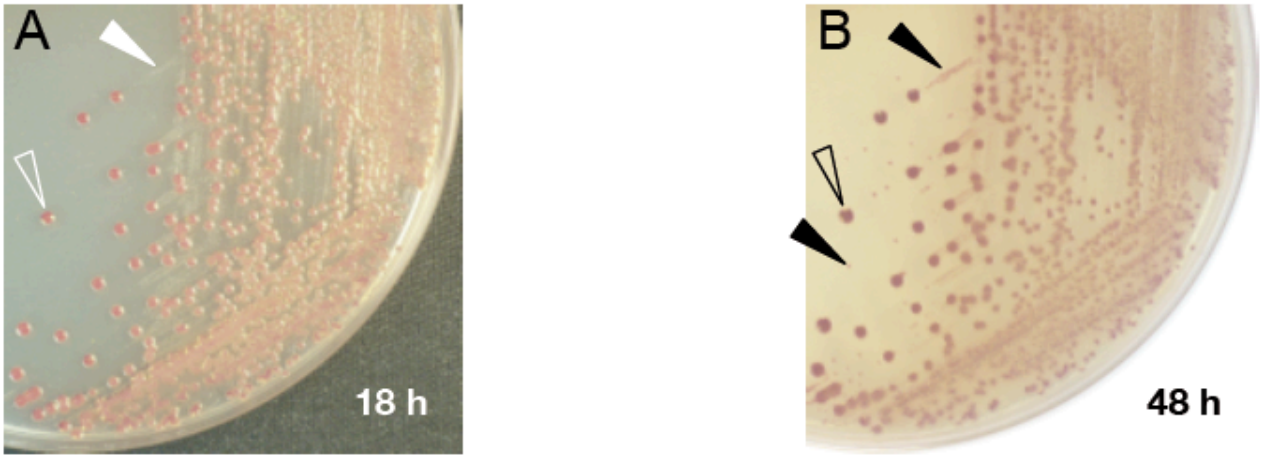

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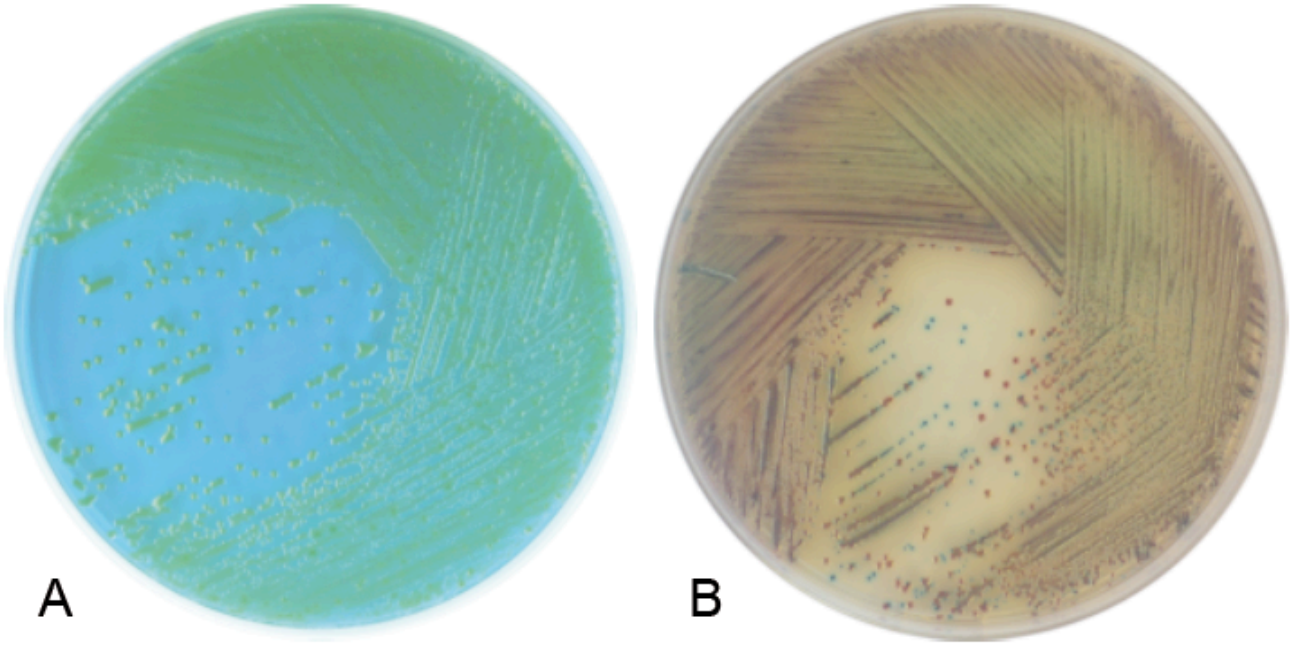

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