

Culture media for detection of *Acinetobacter baumannii* selective media for detection of *A. baumannii*

Abstract

Detection of *Acinetobacter baumannii* is of crucial importance to the prevention of the development of nosocomial infections. CHROMagar Acinetobacter[®] is a new selective medium recently developed for the rapid identification of *A. baumannii*. This medium incorporates enzymatic substrates that enable colour-based preliminary identification of bacterial colonies recovered within 18 to 24 h of inoculation. Sheep blood agar (SBA) supplemented with ertapenem contains few modifications and has not yet been defined in the literature. The aim of this study was to evaluate CHROMagar Acinetobacter[®] for detection of *A. baumannii* and compare its performance to that of SBA supplemented with ertapenem.

A total of 73 Gram-negative bacteria were evaluated. *A. baumannii*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and carbapenemase-positive *Klebsiella pneumoniae* were grown on CHROMagar Acinetobacter[®] and supplemented SBA media. Susceptible clinical isolates and standard strains of *Enterobacteriaceae* were inhibited by both CHROMagar Acinetobacter[®] and supplemented SBA. *C. albicans* isolates, inoculated with mixed samples, did not grow on CHROMagar Acinetobacter[®] after 24 or 48 h, but did grow on supplemented SBA after 24 h and exhibited distinct colony morphology. *A. baumannii*, the most clinically relevant of the bacteria tested, have a particular propensity for nosocomial transmission, due in part to their sustained survival on environmental surfaces as well as their multidrug resistance. The prevalence of infection with *A. baumannii* has increased significantly during the last decade. Due to well-designed solid selective culture media are required for detection of *Acinetobacter spp.* We advise the use of supplemented SBA and CHROMagar Acinetobacter[®] medium for rapid detection of nosocomial infection in the absence of a confirmatory procedure. Investigation into the metabolism of *A. baumannii* will facilitate the development of chromogenic media.

Keywords: *Acinetobacter baumannii*, CHROMagar, selective medium

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Abbreviations: MDRAB, multi drug-resistant *A. baumannii*; SBA, sheep blood agar; PCR, *Pseudomonas aeruginosa* carbapenem-susceptible and resistant; KPC, *Klebsiella Pneumoniae* carbapenemase; CFU, colony forming units, CHR, CHROM agar Acinetobacter, CHR⁺, CHROM agar acinetobacter supplemented with 400mg/L KPC; CHR⁺⁺, CHROM agar acinetobacter supplemented with 800mg/L KPC; SBA⁺, sheep blood agar supplemented with 2mg/L ertapenem

Introduction

Members of the genus *Acinetobacter* have become the focus of much research due to their pathogenic potential. Several species persist in hospital environments and cause severe life-threatening infections in immunocompromised patients.¹ Particularly, *Acinetobacter baumannii* is recognised as an increasingly important opportunistic pathogen among such species.² *A. baumannii* have the ability to develop resistance to new antibiotics extremely rapidly. Many clinical isolates of *A. baumannii* are now resistant to all conventional antimicrobial agents including carbapenems.³ Today, in many parts of the world strains resistant to almost all available antimicrobial classes (multidrug-resistant *A. baumannii* or MDRAB) have been reported. These organisms have been implicated in a diverse range of infections and are particularly problematic in intensive care units.⁴ Moreover, detection of *A. baumannii* carriers and the establishment of isolation

measures are of crucial importance to preventing the development of nosocomial infections due to *A. baumannii*.

Several selective and differential media are available for the isolation of *Acinetobacter spp.* One such medium incorporates bile salts and bromocresol purple. Another contains bile salts, ampicillin, cefsulodin, and vancomycin. In the Leeds medium formulation, ampicillin was removed and the concentrations of the other antibiotics were adjusted to optimise performance.⁵ CHROMagar Acinetobacter[®] (CHROMagar, Paris, France) was recently developed as a selective agar for the rapid identification of *A. baumannii*. It incorporates enzymatic substrates, enabling colour-based preliminary identification of colonies recovered within 18 to 24h of inoculation. This medium also contains agents that inhibit the growth of most Gram-positive and negative organisms. Furthermore, CHROMagar Acinetobacter[®] agar supplemented with KPC suppresses carbapenem-susceptible Gram-negative bacilli³. Sheep blood agar (SBA) can be modified and supplemented with common antibiotics for strains that are resistant to for example, ertapenem. Additionally, supplemented SBA has not been used for detection of *A. baumannii* in the literature.

The aim of this study was to evaluate CHROMagar Acinetobacter[®] for detection of *A. baumannii* in comparison to SBA supplemented with ertapenem.

Materials and methods

We conducted this study from March to June 2012 at Sakarya University, School of Medicine Hospital, Sakarya, Turkey. In total, 73 Gram-negative bacteria were included in the study. The isolates consisted of 20 *A. baumannii* (detection of OXA51, OXA23 and OXA58 genes was done by in-house polymerase chain reaction (PCR) 20 *Pseudomonas aeruginosa* (carbapenem-susceptible and -resistant), 15 *Klebsiella pneumoniae* (KPC carbapenemase-positive and negative), 10 *Escherichia coli* and five *Stenotrophomonas maltophilia*. The strains were single (one per patient) clinical isolates recovered from patients who were hospitalised in various clinics. The isolates were identified using conventional techniques (colony morphology, oxidase, and other biochemical tests) as well as the Vitek 2 system using N91 and N90 cassettes (bioMérieux, France).

Two different media were evaluated for their ability to distinguish *A. baumannii* from other bacteria. Twenty mixed cultures of four carbapenem-susceptible isolates (*A. baumannii*, *P. aeruginosa*, *K. pneumoniae*, and *E. coli*) and *Candida albicans* were suspended in 0.9% sterile saline. A 1-µm sterile loop dipper was used to inoculate CHROMagar Acinetobacter® and supplemented SBA plates at a density of 10⁶ colony forming units (CFU). Plates were scored by two independent observers blinded to the inoculum composition for the presence of *A. baumannii*. Colonies thought to be *A. baumannii* were confirmed using the Vitek 2 system. Colony counts were conducted to verify inoculum densities.

CHROMagar Acinetobacter® and SBA (Oxoid, England) were prepared from dehydrated powder according to the manufacturer's instructions. CHROMagar Acinetobacter® was prepared without and

with supplementation with KPC (CHROMagar, Paris, France) at concentrations of 400 and 800 mg/L. KPC was added according to the manufacturer's instructions in the CHROMagar Acinetobacter® at 50°C. SBA was supplemented with 2 mg/L ertapenem (Merck & Co., USA) at 50°C. Inoculated media were incubated at 37°C in aerobic conditions and evaluated after 18-24h. These media were examined for presumptive colonies in accordance with the manufacturer's recommendations. SBA medium cultures were evaluated with conventional methods, such as colony morphology, oxides, and haemolysis. Each medium batch was quality controlled using standard strains (*P. aeruginosa* ATCC 27853, *E. coli* ATCC 602357 and *K. pneumoniae* ATCC 700603) obtained from Oxoid. Medical ethical approval of this study was deemed unnecessary.

Results

A. baumannii, *P. aeruginosa*, *S. maltophilia* and KPC-positive *K. pneumoniae* were grown on CHROMagar Acinetobacter® and CHROMagar supplemented with KPC for 24 h. KPC-positive *K. pneumoniae* were distinguishable by their colony morphology and blue colour. *A. baumannii*, *P. aeruginosa* and *S. maltophilia* colonies all appeared weakly red after the first 24h. However, other Gram-negative bacteria were easily distinguishable by their dark blue colour or lack of colour, and small colony morphology after 48h. Susceptible clinical isolates and standard *Enterobacteriaceae* strains were inhibited by both CHROMagar Acinetobacter® and supplemented SBA. *C. albicans* was inhibited by CHROMagar Acinetobacter®, but formed typical colonies on supplemented SBA. Growth results using CHROMagar Acinetobacter® supplemented with KPC are summarised in the Table 1 and Figure 1.

Table 1 Comparison of CHROMagar Acinetobacter with SBA medium for selection of *A. baumannii* and other multidrug-resistant isolates

Isolate	n	24 h			48 h			CHROMagar Colony Colour		
		CHR	CHR ⁺	CHR ⁺⁺	SBA ⁺	CHR	CHR ⁺		CHR ⁺⁺	SBA ⁺
¹ <i>A. baumannii</i>	10	10	6	4*	10	10	7	6*	10	Reddish
² OXA23+A. bau	5	5	5	5	5	5	5	5	5	Reddish
² OXA58+A. bau	5	5	5	5	5	5	5	5	5	Reddish
¹ <i>P. aeruginosa</i>	10	10	5	3*	10	10	8	6*	10	Reddish
² <i>P. aeruginosa</i>	10	10	10	10	10	10	10	10	10	Reddish
<i>S. maltophilia</i>	5	5	4*	-	5*	5	5*	2*	5*	Reddish
¹ <i>K. pneumoniae</i>	5	3*	-	-	-	5*	-	-	-	Blue
² <i>K. pneumoniae</i>	10	10	10	10	10	10	10	10	10	Blue
¹ <i>E. coli</i>	10	2*	-	-	-	3*	-	-	-	Blue
¹ <i>C. albicans</i>	1	-	-	-	1	-	-	-	1	-
<i>P. aer</i> ATCC27853	1	1	-	-	1	1	-	-	1	Reddish
<i>K. pne</i> ATCC700603	1	-	-	-	-	-	-	-	-	Blue
<i>E. coli</i> ATCC602357	1	-	-	-	-	-	-	-	-	Blue

*Small colonies and/or low colony count,

¹Susceptible clinical isolate,

²Carbapenem-resistant clinical isolate; MIC, >32 µL/mL.

CHR, CHROM Agar Acinetobacter; CHR⁺, CHROM Agar Acinetobacter Supplemented With 400 mg/L KPC; CHR⁺⁺, CHROM Agar Acinetobacter Supplemented With 800 mg/L KPC; SBA⁺, Sheep Blood Agar Supplemented With 2 mg/L ertapenem

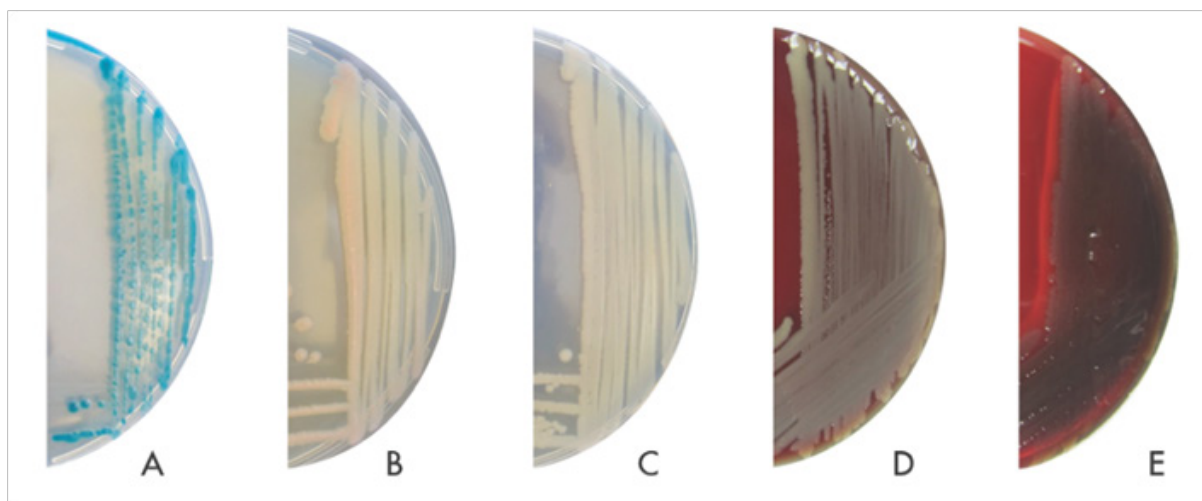


Figure 1 Cultured bacteria view in SBA and CHROMagar *Acinetobacter*.

A, *Klebsiella pneumoniae* in CHROMagar *Acinetobacter*; B, *Acinetobacter baumannii* in CHROMagar *Acinetobacter*; C, *Pseudomonas aeruginosa* in CHROMagar *Acinetobacter*; D, *Acinetobacter baumannii* in SBA; E, *Pseudomonas aeruginosa* in SBA

A. baumannii, *P. aeruginosa*, *S. maltophilia*, KPC-positive *K. pneumoniae* and *C. albicans* were grown on supplemented SBA for 24 h. Other Gram-negative bacteria did not grow on supplemented SBA after 24 or 48 h. *A. baumannii* grown on supplemented SBA were recognisable by their smooth colony morphology, were non-haemolytic and were colourless after 24h. *P. aeruginosa* isolates grown on supplemented SBA were distinct in their colony morphology, colour and demonstration of haemolysis after 24h. *S. maltophilia* were not recognisable by their appearance, as they formed small colonies in the first 24 h. Moreover, KPC-positive *K. pneumoniae* isolates were non-haemolytic, colourless and had mucoid colonies after growth on supplemented SBA for 24h.

A. baumannii, *P. aeruginosa*, *S. maltophilia* and KPC-positive *K. pneumoniae* isolates, which were inoculated as mixed samples, were grown on CHROMagar *Acinetobacter*[®] for 24 h. *P. aeruginosa* and *S. maltophilia* colonies were the same colour as those of *A. baumannii*, and the *S. maltophilia* colonies were small. *C. albicans* isolates, inoculated with mixed samples, did not grow on CHROMagar *Acinetobacter*[®] after 24 or 48 h, but did grow on supplemented SBA after 24 h and exhibited distinct colony morphology.

Discussion

A. baumannii the most clinically relevant of the bacteria tested, have a particular propensity for nosocomial transmission, due in part to their sustained survival on environmental surfaces as well as their multidrug resistance. The prevalence of infection with *A. baumannii* has increased significantly during the last decade. Moreover, *A. baumannii* have developed one of the most impressive patterns of antibiotic resistance ever observed. Therefore, rapid detection of *A. baumannii* in clinical samples could improve the targeted implementation of infection control measures and potentially aid the selection of empirical therapies for infected patients.^{3,5}

To culture *A. baumannii* isolated from clinical samples and to screen for carriers among intensive care unit patients, use of a selective enrichment medium is recommended. In recent years, a few previous reports about CHROMagar *Acinetobacter*[®] have been published with

regard to multidrug resistant *A. baumannii* (MDRAB). Gordon et al.,⁶ reported that the availability of selective culture media, such as CHROMagar *Acinetobacter*[®], enabled those who may benefit from such technology to be identified rapidly. Their findings supported the use of CHROMagar *Acinetobacter*[®] as a (limited) means of identifying the presence of enteric MDRAB. Using a well-characterised panel of carbapenem-susceptible and -resistant *A. baumannii* isolates grown in CHROMagar *Acinetobacter*[®], selectivity for carbapenem-resistant *A. baumannii* was not demonstrated.⁷ In addition, some isolates of *Staphylococcus aureus*, *P. aeruginosa* and *Enterococcus cloacae* formed colonies of the same colour as *A. baumannii*, suggesting the possibility of misdiagnosis. Panagea et al.,⁸ made a similar observation and stated that CHROMagar KPC medium was a useful screening medium both for KPC and VIM carbapenemase-producing *Enterobacteriaceae* in stool samples.

Our data suggest that neither supplemented nor non-supplemented CHROMagar *Acinetobacter*[®] media differed from supplemented SBA in terms of selection of *A. baumannii*. Definitive identification of bacteria grown in either selective medium may require additional testing. *A. baumannii* and *P. aeruginosa* could be differentiated by performing an oxidase test. Supplemented SBA had greater discriminatory power than CHROMagar *Acinetobacter*[®] for *P. aeruginosa*; however, CHROMagar *Acinetobacter*[®] proved to be a reliable rapid-isolation medium for non-fermentative bacteria. Growth of most other Gram-negative bacteria and yeasts was inhibited. CHROMagar *Acinetobacter*[®] cannot be recommended as the sole medium for isolation and screening of *A. baumannii*. Further development of selective media is needed to enable identification of *A. baumannii* or MDRAB. Selective culture media are increasingly necessary due to the exponential increase in the incidence of *A. baumannii* infection. Use of media selective for *A. baumannii* will enable rapid identification.

Conclusion

In conclusion, detection of resistance is crucial for infection control policies and the availability of cost-effective reagents will result in more rapid identification of infected patients compared to

other conventional techniques, permitting immediate implementation of infection control measures to prevent further dissemination. Such measures direct therapy away from inappropriate antibiotics. Well-designed solid, selective culture media thus remain necessary. We advise the use of supplemented SBA and CHROMagar *Acinetobacter*[®] medium for *A. baumannii* for rapid detection of nosocomial infections in the absence of confirmatory procedures. Investigation into the metabolism of *A. baumannii* will facilitate the development of selective agars.

Acknowledgments

This study is an experimental study. In this study in-vitro samples were investigated retrospectively. Samples are not taken from patient.

Conflicts of interest

Authors declare that there is no conflict of interest.

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