Evaluation of CHROMagar Acinetobacter™ for detection of multi-resistant *Acinetobacter baumannii* in clinical specimens

Peter G Huntington[1] & Dean A Gosling[1]

[1] Department of Microbiology & Infectious Diseases, Pacific Laboratory Medicine Services, Royal North Shore Hospital, St Leonards, NSW.

Objectives

CHROMagar Acinetobacter™ is a chromogenic medium for the selective culture of *Acinetobacter* spp.. It has recently been reformulated to produce colonies with a distinct red colour. *Acinetobacter baumannii* is associated with a variety of opportunistic infections and survives well in both moist and dry environments. Multi-resistant *A.baumannii* (MRAB) strains have been reported as a cause of outbreaks in healthcare settings.[1] Surveillance for presence of multi-resistant *A.baumannii* is recommended in some clinical settings where patients are at higher-risk.[2]

Methods

CHROMagar Acinetobacter™ plates and multi-drug-resistant (MDR) CHROMagar Acinetobacter™ plates (containing additional supplement CR102) were prepared from dehydrated base media (AC092B) and supplement (AC092S) according to manufacturer's instructions.[3] A collection of 105 *Acinetobacter* isolates, including multi-resistant *A.baumannii* group strains as well as *Acinetobacter lwoffii* strains, were used to evaluate CHROMagar Acinetobacter™. Isolates were sourced during the period 2008 to 2011 from a range of clinical specimens including 27 from wound swabs, 21 from respiratory specimens, 13 from blood cultures and 3 from cerebrospinal fluid. *A.baumannii* ATCC 19606 was included as a control strain.

Isolates were recovered from storage at -80°C and cultured onto horse blood agar overnight, then inoculated into trypicase soy broth (BBL 1194164) and incubated at 35 C to a density of approximately 0.5 McFarland. This suspension was diluted 1:200 in 0.45% saline (Cardinal 3D0775) and sub-cultured using a disposable 1 µL loop onto a quarter plate each of horse blood agar (HBA, Oxoid CM331), MacConkey agar (MAC, Oxoid CM7b), MacConkey Agar containing 32mg/L gentamicin (MAC-Gen, CM7b & Pfizer 11376), CHROMagar Acinetobacter™, and MDR CHROMagar Acinetobacter™ (MDR-CaA). Plates were incubated in air at 37°C for 18-24 hours. CHROMagar Acinetobacter™ plates were examined for bright red colonies suggestive of *Acinetobacter* spp. and growth recorded as semi-quantitative results. Plates were re-incubated for a further 24 hours and re-examined. For each isolate antimicrobial susceptibility testing was performed from blood agar using Vitek 2 AST-N246 card (413395, BioMerieux). Identification of any isolate that failed to grow on CHROMagar Acinetobacter™ was confirmed by BioMerieux Vitek 2 Gram negative identification card (21341, BioMerieux).

In addition, 100 consecutive de-identified rectal swabs collected for surveillance of resistant organisms such as VRE and MRAB were cultured directly onto plates of MacConkey agar, MacConkey-gentamicin agar and MDR CHROMagar Acinetobacter™ following routine processing. Plates were incubated in air at 37°C for 18-24 hours and examined as above, then re-incubated for a further 24 hours. Colonies obtained on CHROMagar Acinetobacter™ that were suspicious of Gram negative organisms were identified by BioMerieux Vitek 2 Gram negative identification card.

Results

All isolates from the collection of *Acinetobacter* strains showed growth on HBA, MAC, and CaA agar plates, with a characteristic red colour as illustrated in Figure 1, except four strains which did not grow on CaA agar that were subsequently re-identified as *E.coli* (2), *S.marcescens* (1) and *Pseudomonas* spp. (1). These strains were excluded from data analysis.

Although one additional isolate was recovered on MAC-Gen agar following re-incubation, no additional isolates were recovered on MDR-CaA agar after a further 24-hour incubation.

Antimicrobial susceptibility testing showed 44% of strains could be classified as multi-resistant (defined as non-susceptibility to 3 or more antimicrobial classes by CLSI criteria: ceftazidime MIC ≥8 mg/L and/or cefepime MIC ≥8 mg/L; meropenem MIC ≥4 mg/L; ciprofloxacin MIC ≥1 mg/L; gentamicin MIC ≥4 mg/L and/or tobramycin MIC ≥4 mg/L and/or amikacin MIC ≥16 mg/L; ticarcillin-clavulanate MIC ≥16 mg/L and/or piperacillin-tazobactam MIC ≥16 mg/L). 38% of isolates were non-susceptible to four or more antimicrobial classes, while 21% were non-susceptible to five antimicrobial classes. 35% of strains remained susceptible to these 5 classes. Antimicrobial minimal inhibitory concentrations of the 101 *Acinetobacter* spp. isolates for gentamicin and meropenem by Vitek 2 are summarised in Table 2.

Figure 1 – CHROMagar Acinetobacter™ agar plate showing typical red colonies of *Acinetobacter* spp. .

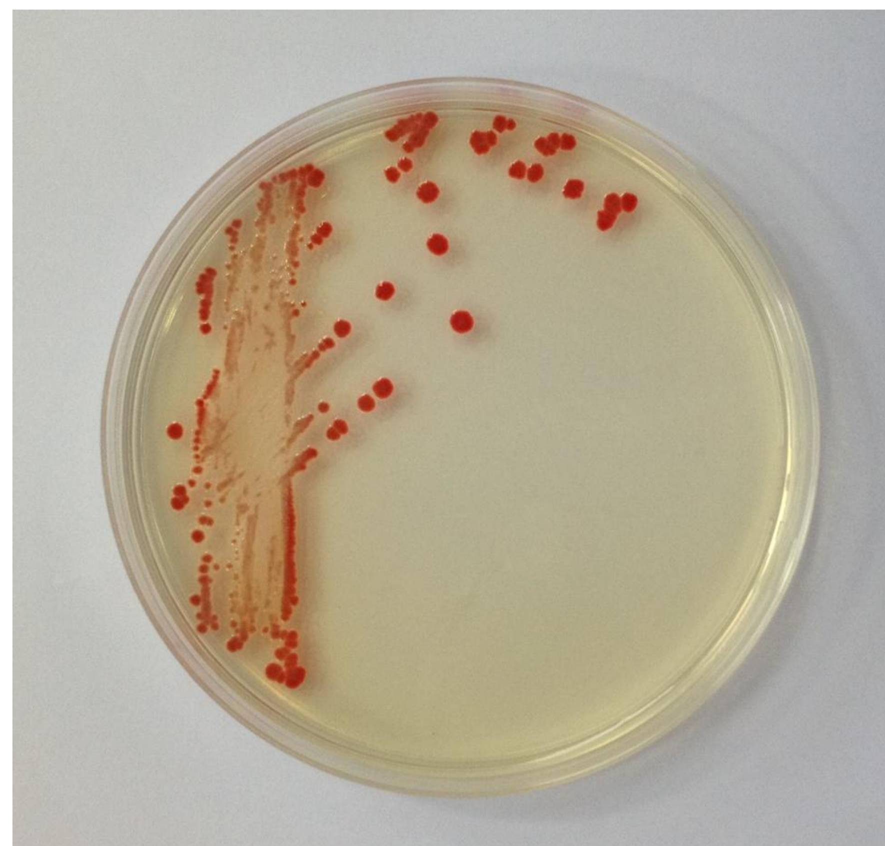

Table 1 – Number of *Acinetobacter* isolates showing growth on different types of media including horse blood agar, MacConkey agar, MacConkey-gentamicin agar, CHROMagar Acinetobacter™ agar and MDR CHROMagar Acinetobacter™ agar containing multi-drug-resistant supplement, after 48 hours incubation, grouped by number of non-susceptible antimicrobial classes including beta-lactam-inhibitor combinations, cephalosporins, carbapenems, aminoglycosides and fluoroquinolones.

Sub-Group of Isolates		Growth of Isolates by Agar Type, n (%)				
		HBA n (%)	MAC n (%)	MAC-Gen n (%)	CaA n (%)	MDR-CaA n (%)
Number of Non-susceptible Antimicrobial Classes	0	36 (100)	36 (100)	0 (0)	36 (100)	0 (0)
	1	11 (100)	11 (100)	0 (0)	11 (100)	0 (0)
	2	10 (100)	10 (100)	1 (10.0)	10 (100)	0 (0)
	3	6 (100)	6 (100)	2 (33.3)	6 (100)	2 (33.3)
	4	17 (100)	17 (100)	7 (41.2)	17 (100)	10 (58.8)
	5	21 (100)	21 (100)	21 (100)	21 (100)	21 (100)
Meropenem MIC ≥ 4 mg/L		33 (100)	33 (100)	21 (63.6)	33 (100)	33 (100)
Gentamicin MIC ≥ 4 mg/L		32 (100)	32 (100)	31 (97.0)	32 (100)	21 (65.6)
All Isolates		101 (100)	101 (100)	31 (30.7)	101 (100)	33 (32.7)

Table 2 – Number of *Acinetobacter* spp. isolates showing particular gentamicin and meropenem minimal inhibitory concentrations (MIC) as tested by BioMerieux Vitek 2 AST-N246.

Number of Isolates		Gentamicin MIC (mg/L)					Sub-total
		≤1	2	4	8	≥16	
Meropenem MIC (mg/L)	≤0.25	50			1	1	*52*
	0.5	5			1	7	*13*
	1	2					*2*
	2					1	*1*
	4						*0*
	8					1	*1*
	≥16	11	1	1	2	17	*32*
Sub-total		*68*	*1*	*1*	*4*	*27*	*101*

22 isolates grew on both MAC-Gen and MDR-CaA agar, while 54 did not grow on either agar. 10 isolates grew on MAC-Gen agar but not MDR-CaA, and 15 isolates grew on MDR-CaA but not MAC-Gen agar. Growth of all isolates with meropenem MIC ≥ 4 mg/L was obtained on MDR-CaA agar. Growth of 31 of 32 isolates with gentamicin MIC ≥ 4 mg/L was obtained on MAC-Gen agar.

Culture of 100 rectal swabs onto CaA and MDR-CaA agar recovered no isolates of *Acinetobacter* spp., but there was minimal growth of other organisms. Growth was present on 11 MDR-CaA plates following 48 hours incubation, including 5 with colonies suggestive of Gram positive organisms or yeast.

The remaining red colonies were identified by Vitek 2 as *Stenotrophomonas maltophilia* (2), *Chryseobacterium indologenes* (2), *Pseuomonas putida* (1) and *Elizabethkingia meningoseptica* (1). MAC-Gen agar showed growth on 36 plates including 5 with Gram negative rods that were not typical of *Acinetobacter spp.*, including two identified as *S.maltophilia*.

Discussion

Multi-resistant strains of *A.baumannii* group have been associated with outbreaks in healthcare facilties. NSW Health has recently introduced mandatory reporting of meropenem-resistant *A.baumannii* group infections from intensive care units[4] and in NSW public hospitals surveillance for MRAB is recommended for some high-risk patient groups.[2] Previously resistance to gentamicin had been used as an indicator for multi-resistance in *Acinetobacter* spp. at our laboratory, however attention has now changed to carbapenems as important last-line agents.

A previous formulation of CHROMagar Acinetobacter™, which produced colonies with an aqua-blue colour, demonstrated high sensitivity and specificity for detection of MRAB from enteric specimens.[5] However Akers *et al.* claimed the new red CHROMagar Acinetobacter™ did not demonstrate selectivity for carbapenem-resistant *A.baumannii.*[6] On closer examination, the other organisms that grew on their CHROMagar Acinetobacter™ agar were easily differentiated from *Acinetobacter* spp. as they did not have typical red colour or colony appearance. Ajao *et* al. evaluated red CHROMagar Acinetobacter™ agar and found it was selective for *A.baumannii* but still allowed growth of certain other Gram negative bacteria.[7] More recently Wareham & Gordon evaluated red CHROMagar Acinetobacter™ agar in a small study and found that it could indeed be used for selective growth of *A.baumannii* and differentiation from other carbapenem-resistant Gram negative bacteria.[8]

Similarly, our results showed that 100% of *Acinetobacter* spp. from a range of clinical specimens were able to grow successfully on CHROMagar Acinetobacter™, and 100% of carbapenem non-susceptible *Acinetobacter* spp. isolates were able to grow on CHROMagar Acinetobacter™ agar containing MDR supplement. This study showed more multi-resistant strains (resistant to ≥3 classes) were detected by a carbapenem-based screening method compared to an aminoglycoside-based method (MDR-CaA 75.0% *v* MAC-Gen 68.2%). Our results also showed that a 24-hour incubation period was sufficient.

While no isolates of *Acinetobacter* spp. were recovered from rectal swabs during this study, the prevalence of *Acinetobacter* spp. from enteric screening specimens at our healthcare facilties is very low (~0.3% during 2002-2011). As been reported by others previously, CHROMagar Acinetobacter™ showed minimal growth of other organisms, but some growth of *Pseudomonas* spp. and *Stenotrophomonas* spp. as small dark red colonies. It would be possible to exclude *Acinetobacter* spp. by further simple tests such as oxidase, or confirmation by routine identification and susceptibility testing of any red colonies.

Conclusions

CHROMagar Acinetobacter™ is an effective and easy-to-use selective medium that may simplify culture and detection of carbapenem-resistant *A.baumannii* group from clinical specimens.

References

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Pathology North

PaLMS
Pacific Laboratory Medicine Services

Royal North Shore Hospital, Sydney

Corresponding author:
Mr Peter Huntington
phuntington@nsccahs.health.nsw.gov.au
+61 2 9926 8470