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

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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 facilities. 1,12,13 The survival of multi-resistant A. baumannii is recommended in some clinical settings where patients are at higher risk. 1

Methods

CHROMagar Acinetobacter™ plates and multi-drug resistant (MDR) CHROMagar Acinetobacter™ plates (containing additional supplement CRP02) were prepared and dehydrated base media (AC3038) and supplement (AC3052B) according to manufacturer’s instructions. A collection of 105 Acinetobacter isolates, including multi-resistant A. baumannii group strains as well as Acinetobacter spp. 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 trypticase soy broth (BBL 1194164) and incubated at 35°C to a density of approximately 0.5 McFarland. This suspension was diluted 1:100 in 0.45% saline (Cardinal 300775) 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 CM769), MacConkey agar containing 32mg/L gentamicin (MAC-G, CM75 & PF-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, the number of colonies was performed from blood agar using Vitrek 2 AST-N24卡 (413399, BioMerieux). Identification of any isolate that failed to grow on CHROMagar Acinetobacter™ was confirmed by BioMerieux Vitrek 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 inoculated 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™ plates that were suggestive of Gram negative organisms were identified by BioMerieux Vitrek 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-susceptible to 3 or more antibiotics) by CLSI criteria: carbapenems (MIC ≥ 2mg/L), colistin MIC ≥ 2mg/L, and cephalosporins MIC ≥ 8mg/L. 10 isolates grew on MDR CaA and not MAC-G, and 15 isolates grew on MDR CaA but not MAC-G. Growth of all isolates with meropenem MIC ≥ 2mg/L was obtained on MDR CaA. Growth of 31 of 32 isolates with gentamicin MIC ≥ 4mg/L was obtained on MAC-Gan 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 Vitrek 2 as Serratia marcescens malthophila (2), Chryseobacterium indologenes (2), Pseudomonas 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.malthophilia.

In all, 105 Acinetobacter isolates were grown on different types of media including horse blood 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, carbapenem, aminoglycosides and fluoroquinolones.

Discussion

Multi-resistant strains of A. baumannii group have been associated with outbreaks in healthcare facilities. NSW Health has recently introduced mandatory reporting of multi-resistant A. baumannii infections from intensive care units and in NSW public hospitals surveillance for MRAB is recommended for some high-risk patient groups. 1 Previous resistance to gentamicin was 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 sources. 1,12 However, the earlier red CHROMagar Acinetobacter™ did not demonstrate selectivity for carbapenem-resistant A. baumannii. 1 On closer examination, the other organisms that grew on their CHROMagar Acinetobacter™ agar were easily differentially identified from Acinetobacter spp. as they did not have typical red colour or colony appearance. Ajo 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. 1 More recently Wardan & 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. 1

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. 1 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 50%). Our results also showed that a 24-hour incubation period was sufficient.

While no isolates of A. baumannii were recovered from rectal swabs during this study, the prevalence of Acinetobacter spp. from enteric screening specimens at our healthcare facilities is very low (2% during 2009). 3 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 confirming their 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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Table 1 – Number of Acinetobacter spp. isolates showing growth on different types of media including horse blood 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, carbapenem, aminoglycosides and fluoroquinolones.

Table 2 – Number of Acinetobacter spp. isolates showing particular gentamicin and meropenem minimal inhibitory concentrations (MIC) as tested by BioMerieux Vitrek 2 AST-N24.

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Table 2 – Number of Acinetobacter spp. isolates showing particular gentamicin and meropenem minimal inhibitory concentrations (MIC) as tested by BioMerieux Vitrek 2 AST-N24.

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