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NOTES

Evaluation of CHROMagar *Acinetobacter* for Detection of Enteric Carriage of Multidrug-Resistant *Acinetobacter baumannii* in Samples from Critically Ill Patients[▽]

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CHROMagar *Acinetobacter* was used to screen stool and perineal swabs for enteric carriage of multidrug-resistant *Acinetobacter baumannii* in samples from critically ill patients. Results were compared with a molecular assay resulting in sensitivity and specificity of culture compared to PCR of 91.7% and 89.6%, respectively.

Acinetobacter baumannii has emerged as an important nosocomial pathogen, impacting considerably on patient care (6, 9). In many parts of the world, strains resistant to almost all available antimicrobial classes (multidrug-resistant *Acinetobacter baumannii* [MDRAB]) have been reported and implicated in numerous hospital outbreaks, usually in intensive care units (2). Despite the implementation of infection control measures involving isolation of patients, rigorous hand hygiene, and aggressive environmental decontamination, many centers have continued to have ongoing problems, with rapid reemergence of the organism unless high standards of cleaning are maintained (4, 10).

Although environmental cleaning is of paramount importance, it is likely that patients themselves also represent a significant reservoir of the organism. For many gram-negative bacteria, colonization of the digestive tract is an important prerequisite for the development of nosocomial infections such as ventilator-associated pneumonia (3, 7). Rapid identification of individuals with enteric carriage of MDRAB could therefore be an important component in strategies aimed at limiting the transmission and dissemination of the organism.

CHROMagar *Acinetobacter* (CHROMagar, Paris, France) is a recently developed selective agar for the rapid identification of MDRAB. It contains agents which inhibit the growth of most gram-positive organisms as well as carbapenem-susceptible gram-negative bacilli. It incorporates substrates enabling color-based preliminary identification of colonies recovered within 18 to 24 h of inoculation. In this study, we evaluated the ability of CHROMagar *Acinetobacter* to detect the carriage of MDRAB in enteric samples from critically ill patients in a unit experiencing problems with MDRAB, compared with a molecular method.

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CHROMagar *Acinetobacter* was prepared from dehydrated powder according to the manufacturer's instructions. Each batch of media was quality controlled using a representative isolate of the MDRAB OXA-23 clone 1, obtained from the Health Protection Agency, Colindale, United Kingdom, previously characterized as resistant to carbapenems (2). Stool samples and/or perineal swabs collected from intensive care unit patients as part of routine screening for *Clostridium difficile*-

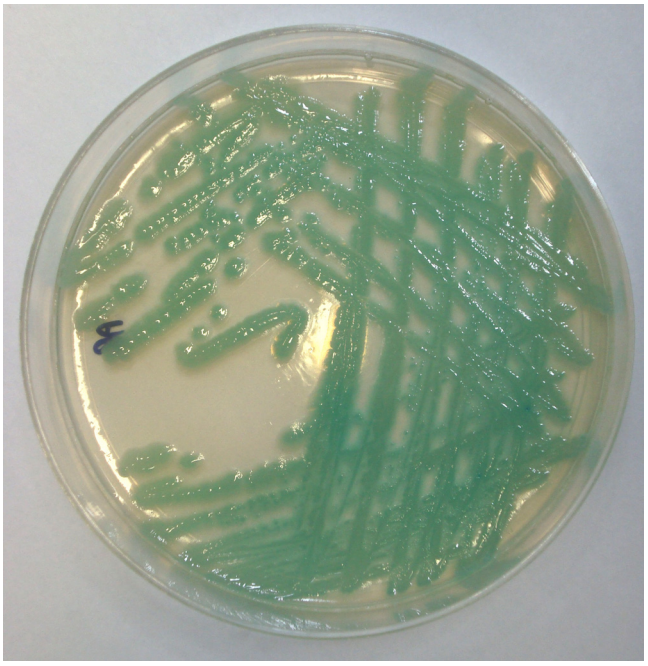

FIG. 1. Appearance of MDRAB on a CHROMagar *Acinetobacter* plate.

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TABLE 1. Comparison of culture and molecular tests for the identification of MDRAB

Culture result	PCR result	No. of specimens examined	No. of specimens known to be colonized
Negative	Negative	60	20
Positive	Positive	33	28
Positive	Negative	7	6
Negative	Positive	3	1
Total		103	55

associated diarrhea or methicillin-resistant *Staphylococcus aureus* carriage were used to inoculate CHROMagar *Acinetobacter* plates and then 3-ml peptone water broths. Plates were incubated in air at 37°C and examined after 24 h for aqua blue colonies indicative of MDRAB (Fig. 1). The peptone broths were also incubated for 24 h and used to generate templates for an MDRAB-specific molecular assay. One hundred microliters of turbid broth was heated at 96°C for 5 min and then centrifuged at 13,000 rpm to pellet lysed bacterial cells. Two microliters of supernatant was used in a multiplex PCR for the *A. baumannii* species-specific OXA-51-like gene and the OXA-23, -24, and -58-like genes, which mediate carbapenem resistance, using the primers and conditions described by Woodford et al. (11). Isolates presumed to be *A. baumannii* based on their growth and appearance on CHROMagar *Acinetobacter* were confirmed as such by Gram staining, oxidase testing, and multiplex PCR assay. The clonal lineage of each isolate was also determined using another multiplex PCR assay, based on sequence variations in the *csuE*, *ompA*, and $bla_{\text{OXA-51}}$ alleles. This enables strains to be assigned to clonal complexes with a reliability equivalent to that obtained by pulsed-field gel electrophoresis (8).

Sixty-six stool samples and 37 perineal swabs obtained from a total of 70 patients were ultimately included in the analysis. Thirty-three of these were positive by both culture and PCR on peptone broths (Table 1). Seven were culture positive but PCR negative, while three were found to be culture negative yet positive by PCR. The gene profiles detected in the PCR assay were identical in every case: OXA-51$^+$, OXA-23$^+$, OXA-24$^-$, and OXA-58$^-$.

Biochemical and molecular testing of colonies identified as MDRAB by CHROMagar *Acinetobacter* confirmed that all of the organisms were *A. baumannii* strains resistant to β-lactams (including carbapenems), quinolones, and aminoglycosides. Multiplex PCR typing placed them as members of European clone II. The only other organisms recovered from samples inoculated onto CHROMagar *Acinetobacter* were *Enterococcus* spp., which were easily distinguishable by their dark blue color and small colonial morphology.

Of the 40 patients with positive MDRAB cultures, 33 were known to be colonized at other sites. In seven patients, MDRAB was isolated from stool alone and not from any other clinical specimens. The sensitivity and specificity of culture compared with the PCR assay were calculated as 91.7% and 89.6%, respectively.

For the seven samples found to be culture positive by CHROMagar *Acinetobacter*, but negative in PCRs performed on peptone broths, follow-up testing of the colonies confirmed that these were all MDRAB. In six of these, the patients had already had MDRAB isolated from other clinical specimens. As the broths were inoculated after the CHROMagar plates, it is possible that either insufficient material remained on the swabs, or in the case of the stool samples, there may have been significant inhibition of the PCR.

Our findings support the use of CHROMagar *Acinetobacter* as a means of identifying enteric carriage of MDRAB, but are limited by the relatively small numbers and the involvement of a single MDRAB clone. Also, as the stool samples were those sent for *C. difficile* testing, only semiformed or liquid stools were obtained. The use of the media with formed stools would therefore require further evaluation. A comparison between stool and perineal swab cultures would also help to determine whether perineal swabs can be used as a proxy marker of gut carriage. These are easier to process and are already routinely obtained to screen for other nosocomial pathogens in many clinical settings.

The study also highlights a role for the gut as a significant reservoir for MRAB in addition to environmental contamination. It raises the question of whether selective decontamination of the digestive tract using nonabsorbable antibiotics such as polymyxin B, to which MDRAB strains remain susceptible, could be a suitable method of control. The approach involving selective decontamination of the digestive tract has been shown to be effective in reducing the carriage and incidence of nosocomial infections caused by gram-negative bacteria but has not been widely adopted (1, 5). The availability of a selective culture media such as CHROMagar *Acinetobacter*, enabling those who may benefit from such a regimen to be rapidly identified, is clearly an important step toward addressing this issue.

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