A new Screening Medium for Detection of Carbapenem-Resistant Enterobacteriaceae

Withey, S., Scopes, E. Thermo Fisher Scientific, Wade Road, Basingstoke, Hants, RG24 8PW, UK

Overview

Purpose: Thermo Scientific™ *Brilliance*™ CRE Agar (Thermo Fisher Scientific), a new screening medium for detection of carbapenem-resistant Enterobacteriaceae (CRE) was evaluated alongside BBL™ CHROMagar™ KPC Agar (BD Diagnostic Systems) and MacConkey Agar (Thermo Fisher Scientific) with 1µg/ml imipenem.

.

Methods: CRE, carbapenem-resistant non-fermenting organisms and carbapenem-sensitive organisms were inoculated onto *Brilliance* CRE Agar, BBL CHROMagar KPC Agar and MacConkey Agar with 1µg/ml imipenem. Plates were incubated at 36±1°C for up to 48 hr.

Results: *Brilliance* CRE Agar was able to detect more CRE than BBL CHROMagar KPC Agar or MacConkey Agar with 1µg/ml imipenem. *Brilliance* CRE Agar is an effective tool for detecting CRE and other carbapenem-resistant organisms in 16-24 hr., allowing immediate implementation of infection control measures to avoid spread of carbapenem resistance.

Introduction

Carbapenems (imipenem, meropenem, ertapenem and doripenem) are invaluable for the treatment of infections due to multiresistant Gram-negative bacteria, including producers of extended-spectrum b-lactamases[1]. However, the rapid emergence and dissemination of Enterobacteriaceae that are resistant to carbapenems poses a considerable threat to clinical patient care and public health[2]. Early detection of carbapenem-resistant Enterobacteriaceae (CRE) will allow faster implementation of appropriate strategies to limit the spread of these pathogens.

Brilliance CRE Agar is a screening medium designed to detect CRE (figure 1). *Brilliance* CRE Agar was evaluated, alongside BBL CHROMagar KPC Agar and MacConkey Agar (Thermo Fisher Scientific) with 1µg/ml imipenem[3], for the detection of CRE.

FIGURE 1. Carbapenem-resistant NDM-1 *Klebsiella* (blue), *E. coli* (light pink) and *Acinetobacter* (cream) on *Brilliance*™ CRE Agar

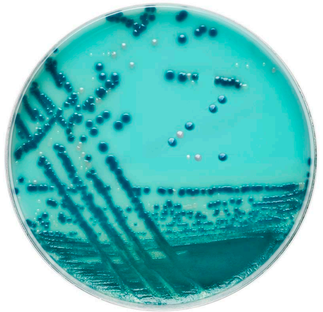

TABLE 1. Inclusivity of three agars for the detection of CRE

Incubation time (hr.)	*Brilliance* CRE Agar	BBL CHROMagar KPC Agar	MacConkey Agar + 1µg/ml imipenem
16	97.7% (95% CI = 93.2-100%)	88.4% (95% CI = 78.8-98%)	74.4% (95% CI = 61.4-87.4%)
18	97.7% (95% CI = 93.2-100%)	88.4% (95% CI = 78.8-98%)	74.4% (95% CI = 61.4-87.4%)
24	97.7% (95% CI = 93.2-100%)	88.4% (95% CI = 78.8-98%)	79.1% (95% CI = 66.9-91.3%)
48	97.7% (95% CI = 93.2-100%)	88.4% (95% CI = 78.8-98%)	79.1% (95% CI = 66.9-91.3%)

Methods

Forty three CRE (according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines[4]) from geographically varied locations, including UK, USA, Scandinavia and Greece, were tested. A further seven non-fermenting organisms showing carbapenem resistance (*Acinetobacter* spp. and *Pseudomonas* spp.) and 99 carbapenem-sensitive organisms (including Enterobacteriaceae, *Pseudomonas* spp., *Acinetobacter* spp., *Staphylococcus* spp. and enterococci spp.) were also tested.

All organisms were cultured onto Columbia Agar with Horse Blood (Thermo Fisher Scientific). A 10µg ertapenem antimicrobial susceptibility testing disc (Thermo Fisher Scientific) was placed in the primary bed of carbapenem-resistant organism inoculum and plates were incubated aerobically overnight at 36±1°C. A 0.5McFarland suspension of each organism was prepared in sterile saline. Carbapenem-resistant organisms were further diluted, 1:100, to give a final inoculum level of 10^2–10^5 cfu/ml. Ten microlitres was streaked onto *Brilliance* CRE Agar, BBL CHROMagar KPC Agar and MacConkey Agar with 1µg/ml imipenem.

All plates were incubated aerobically at 36±1°C and read after 16 hr., 18 hr., 24 hr. and 48 hr. *Brilliance* CRE Agar and BBL CHROMagar KPC Agar were interpreted according to manufacturers' instructions. Growth on MacConkey Agar with 1µg/ml imipenem was interpreted according to typical colonial morphology.

Results

Brilliance CRE Agar was able to detect more carbapenem-resistant Enterobacteriaceae than either BBL CHROMagar KPC Agar or MacConkey Agar with 1µg/ml imipenem, with an inclusivity of 97.7% after 16 hr. incubation compared to 88.4% and 74.4% respectively (see table 1). All non-fermenting carbapenem-resistant organisms tested also showed growth of cream or naturally pigmented colonies on all three agars.

All carbapenem-resistant *E. coli* tested failed to grow on BBL CHROMagar KPC Agar and MacConkey Agar with 1µg/ml imipenem, even after 48 hr. incubation. Growth of carbapenem resistant *E. coli* was observed on *Brilliance* CRE Agar after 16 hr. incubation.

All three agars showed comparable exclusivity: no growth of carbapenem sensitive organisms. Exclusivity of *Brilliance* CRE Agar was 86.9%, 87.9%, 84.8% and 83.8% at 16 hr., 18 hr., 24 hr. and 48 hr., respectively. Exclusivity of BBL CHROMagar KPC Agar was 90.0%, 89.9%, 88.9% and 83.8% at 16 hr., 18 hr., 24 hr. and 48 hr. respectively. Exclusivity of MacConkey Agar with 1 µg/ml imipenem was 83.3%, 82.8%, 73.7% and 62.6% at 16 hr., 18 hr., 24 hr. and 48 hr., respectively.

Conclusion

Brilliance CRE Agar is an effective tool for detecting CRE and other carbapenem-resistant organisms in 16-24 hr., allowing immediate implementation of infection control measures to avoid spread of carbapenem resistance.

References

1. Advice on carbapenemase producers: recognition, infection control and treatment. Department of Health Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infection (ARHAI) and Health Protection Agency. 28 January 2011.
2. Cohen Stuart, J., Leverstein-Van Hall, M. A., on behalf of members of the Dutch Working Party on the Detection of Highly Resistant Microorganisms. (2010). Guideline for phenotypic screening and confirmation of carbapenemases in Enterobacteriaceae. International Journal of Antimicrobial Agents 36, 205–210.
3. Schechner, V., Straus-Robinson, K., Schwartz, D., Pfeffer, I., Tarabeia, J., Moskovich, R., Chmelnitsky, I., Schwaber, M.J., Carmeli, Y., and Navon-Venezia, S. (2009). Evaluation of PCR-Based Testing for Surveillance of KPC-Producing Carbapenem-Resistant Members of the Enterobacteriaceae Family. Journal of Clinical Microbiology. 47 (10) p. 3261-3265
4. European Committee on Antimicrobial Susceptibility Testing (EUCAST) Clinical breakpoints v 1.3 (2011/01/05). http://www.eucast.org/clinical_breakpoints/