A new Screening Medium for Detection of Carbapenem-Resistant Enterobacteriaceae

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Overview
Purpose: Thermofisher™ 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 beta-lactamases. However, the rapid emergence and dissemination of Enterobacteriaceae that are resistant to carbapenems poses a considerable threat to clinical patient care and public health. 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, for the detection of CRE.

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

Brilliance CRE Agar was able to detect more CRE than BBL CHROMagar KPC Agar or 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 88.9%, 89.9%, 84.8% and 83% at 16 hr, 18 hr, 24 hr and 48 hr, respectively. Exclusivity of BBL CHROMagar KPC Agar was 90.0%, 89.9% and 83.9% at 16 hr, 18 hr, 24 hr, 24 hr and 48 hr respectively. Exclusivity of MacConkey Agar with 1µg/ml imipenem was 83.3%, 62.8%, 73.7% and 62.6% at 16 hr, 18 hr, 24 hr and 48 hr, respectively.

Methods
Forty-three CRE (according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines) 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 well 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 10²–10⁵ 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.

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

<table>
<thead>
<tr>
<th>Incubation time (hr.)</th>
<th>Brilliance CRE Agar</th>
<th>BBL CHROMagar KPC Agar</th>
<th>MacConkey Agar + 1µg/ml imipenem</th>
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</thead>
<tbody>
<tr>
<td>16</td>
<td>97.7% (95% CI = 93.3-100)</td>
<td>86.4% (95% CI = 78.9-98.8)</td>
<td>76.6% (95% CI = 61.4-91.3)</td>
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<tr>
<td>18</td>
<td>97.7% (95% CI = 93.3-100)</td>
<td>89.8% (95% CI = 78.6-99.7)</td>
<td>76.6% (95% CI = 61.4-91.3)</td>
</tr>
<tr>
<td>24</td>
<td>97.7% (95% CI = 93.3-100)</td>
<td>88.4% (95% CI = 78.9-98.8)</td>
<td>79.1% (95% CI = 66.9-91.3)</td>
</tr>
<tr>
<td>48</td>
<td>97.7% (95% CI = 93.3-100)</td>
<td>88.4% (95% CI = 78.9-98.8)</td>
<td>79.1% (95% CI = 66.9-91.3)</td>
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</tbody>
</table>

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.
4. European Committee on Antimicrobial Susceptibility Testing (EUCAST) Clinical breakpoints v 1.3 (2011/01/05)

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