EVALUATION OF BRILLIANCE CRE AGAR FOR THE DETECTION OF CARBAPENEM-RESISTANT GRAM-NEGATIVE BACTERIA

Bracco S.,1 Pini B.,1 Migliavacca R.,1 Brigante G.,1 Nucleo E.,2 Pagani L.,2 Luzzaro F.1
1Laboratory of Microbiology, A. Manzoni Hospital, Lecco, and 2SMEC Department, Section of Microbiology, University of Pavia (Italy)

Background
Infections caused by carbapenem-resistant Enterobacteria are an emerging problem worldwide and a serious danger, especially for patients who require long-term hospitalisation (Rice et al., 2009). Since the use of carbapenems has become predominant as a second or third-line drug against multidrug-resistant Gram-negative bacteria, resistance to this class of molecules poses a serious problem in the management of nosocomial infections.

Resistance to carbapenems in Enterobacteriaceae can be mediated by different mechanisms: i) production of extended-spectrum beta-lactamases (ESBLs) associated with decreased membrane permeability (Jaboulay et al., 2004); ii) enzymes that are able to hydrolyse carbapenems such as metallo-beta-lactamases (MBLs) and the recently emerged class A carbapenemases (Queenan et al., 2007). The most common enzymes in the last group are Kibalicola carbapenemases (KPC), which have been reported mostly in Kibalicola pneumoniae and are frequently found on mobile genetic elements with the consequent potential to spread widely (Nordmann et al., 2009). Also, Acinetobacter baumannii and Pseudomonas aeruginosa, common agents of nosocomial infections, often show an MDR phenotype characterised by multiple drug resistance, including carbapenems.

Due to the rapid spread of these Gram-negative resistant bacteria and the scarcity of treatment options, it is essential to understand the mechanism of resistance in order to set up an adequate therapy and to start with appropriate infection control policies (Stuart et al., 2010). Various selective agar media have been developed for preliminary screening allowing differential carbapenem-resistant enterobacterial species to be recognised early (Adler et al., 2011).

Methods
A total of 70 clinical isolates were studied. Of these, 30 were well-characterised carbapenem-resistant enterobacterial strains (Table 1), including Klebsiella pneumoniae producing KPC-type (n=10) or VIM-type (n=3) enzymes, VIM-positive Enterobacter cloacae (n=3) and Escherichia coli (n=1), and isolates characterised by perin loss associated with ESBL production (K pneumoniae, n=3), or AmpC hyperproduction (E. coli, n=2; Serratia marcescens, n=2; E. cloacae, n=1). Ten additional carbapenemase-producing non-fermentative isolates (Pseudomonas aeruginosa, n=7; and Acinetobacter baumannii, n=3) were also included in the study as well as 30 carbapenem-susceptible Gram-negative isolates.

All isolates, shared at 80°C, were inoculated on Columbia Agar with Sheep Blood (Oxoid) with a 10 µg carbapenem disc. After 18-24 h of incubation at 36°C, the colonies were inoculated on MacConkey (Oxoid) with a 0.1 µg carbapenem disc and plates were incubated aerobically at 36°C for 18-24 h. For each isolate MICs (Table 1) for ertapenem (ERT), imipenem (IMP), meropenem (MEM) were determined with Etest (Biolog®; Marcy l’Etoile, France) and results were interpreted according to the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2011).

Table 1. Level of detection on Brilliance™ CRE Agar, sensitivity, and resistance mechanism for the 30 CRE

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>LEVEL OF DETECTION (CFU/ml)</th>
<th>MIC (mg/L)</th>
<th>RESISTANCE MECHANISM</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae</td>
<td>1.5 x 10^1</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>E. coli</td>
<td>1.5 x 10^1</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>1.5 x 10^1</td>
<td>&gt; 32</td>
<td>&gt; 32</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>1.5 x 10^1</td>
<td>&gt; 32</td>
<td>&gt; 32</td>
</tr>
</tbody>
</table>

Figure 1: K. pneumoniae producing KPC

Figure 2: E. coli producing VIM-1

Figure 3: P. aeruginosa producing VIM-1

Figure 4: A. baumannii producing OXA-23

Results
Data about number, size and colour of colonies were collected for all isolates after an overnight incubation. The Brilliance CRE Agar was consistently able to sustain the growth of carbapenem-resistant isolates, showing a detection limit of 1.5 x 10^8 CFU/ml followed by further seven 10-fold serial dilutions (from 1.5 x 10^8 CFU/ml to 1.5 x 10^2 CFU/ml). Finally, 100 µl of each carbapenem-susceptible isolate were inoculated at the fixed dilution of 1.5 x 10^2 CFU/ml.

After 18-24 h of incubation at 36°C the plates were evaluated in order to verify number, size of colonies, and to determine the level of detection (Table 1). All media were provided by Thermo Fisher Scientific.

Conclusions
Our data demonstrates that the new Brilliance CRE Agar allows the growth of carbapenem-resistant isolates with low detection limits thus representing a useful screening medium for carbapenem-resistant enterobacteria. In our experience, carbapenem-resistant P. aeruginosa and A. baumannii were clearly distinguishable from enterobacterial strains based on difference in size and colour.

References