Evaluation of BrilliAnt VRE agar for the detection of vancomycin-resistant enterococci in rectal swab specimens

Vancomycin-resistant enterococci (VRE) infections in hospitalized patients cause significant morbidity and mortality, and current recommendations for hospital infection control include VRE faecal surveillance cultures (Stampfer et al., 2007). However, the optimal methods for these cultures have not been defined (Delmas et al., 2007). This study compared the performance of novel chromogenic medium BrilliAnt VRE agar (Oxoid; Thermo Fisher Scientific) with that of traditional culture to screen rectal swab specimens for VRE. BrilliAnt VRE agar is a selective and differential chromogenic agar for the detection of vancomycin-resistant Enterococcus faecium and vancomycin-resistant Enterococcus faecalis. BrilliAnt VRE agar contains two chromogens that are targeted by specific enzymes: phosphatase and z-galactosidase. The presence of phosphatase enzymes in both E. faecium and E. faecalis results in light blue colonies. However, E. faecium also produces z-galactosidase, resulting in a mix of blue and pink chromophores within the bacterium, producing indigo to purple colonies.

VRE surveillance cultures were obtained from patients at Akdeniz University Hospital, Turkey, between August and September 2010. Rectal swab specimens were inoculated onto bile-aesculin-azide agar with 6 μg vancomycin ml⁻¹ (BEAV agar) and BrilliAnt VRE agar. Plates were incubated at 35 °C and were read at 24 and 48 h. Presumptive VRE colonies on each medium were subcultured onto sheep blood agar. After overnight incubation, colonies were identified by colour morphology, Gram stain and biochemical tests. Species identification and antimicrobial susceptibility testing were performed using the BD Phoenix System (BD Diagnostic Systems). The MICs of vancomycin and teicoplanin were also determined by the Etest method. Carriage of the vanA or vanB gene was confirmed using the Cepheid Xpert vanA/vanB RT-PCR assay. Identification and antimicrobial susceptibility test results were used to calculate the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of each medium evaluated at 24 and 48 h.

Over an 8-week period 183 rectal swab samples from 87 patients were screened for VRE. Twenty samples from nine patients were positive for VRE; all were vanA E. faecium, as demonstrated by the BD Phoenix System, MICs (≥32 μg ml⁻¹ for both vancomycin and teicoplanin) and Cepheid Xpert RT-PCR results.

The sensitivities, specificities, PPVs and NPVs of BrilliAnt VRE agar and BEAV agar are shown in Table 1. There were 21 and 32 false positives on BrilliAnt VRE agar, and 46 and 64 false positives on BEAV agar at 24 and 48 h, respectively. Vancomycin-susceptible E. faecium, E. faecalis, E. casseliflavus/E. gallinarum, E. durans and Gram-negative bacilli grew on both media, whereas E. raffinosus, E. durans, Lactobacillus, Leuconostoc and Pediococcus spp. were recovered only on BEAV agar. Coagulase-negative staphylococci and yeast organisms demonstrated white colonies on BrilliAnt VRE agar which make their identification easy.

BrilliAnt VRE agar exhibited sensitivity and specificity exceeding those of BEAV agar at 24 h. When the incubation time was extended to 48 h, both agars showed increased sensitivity and reduced specificity, but the specificity remained higher for BrilliAnt VRE agar. Of the four studies comparable to ours, three (Willey et al., 2011; Scopes & Henry, 2010; Scopes & Silvius, 2010) reported specificity rates at 24 h for BrilliAnt VRE agar that ranged from 88.8 to 99.8 %, higher than that (87.1 %) obtained in our work, whereas in the study by Miller et al. (2011) the reported specificity at 26 h was 71 %, lower than that obtained in the present study. The specificity of BrilliAnt VRE agar at 48 h correlates with that (79.8 %)

Table 1. Performance of BrilliAnt VRE agar and BEAV agar for detection of VRE in 183 rectal swabs including 20 positive for vanA E. faecium

<table>
<thead>
<tr>
<th>Agar medium</th>
<th>Incubation period (h)</th>
<th>No. of:</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TP</td>
<td>FP</td>
<td>TN</td>
<td>FN</td>
<td></td>
</tr>
<tr>
<td>BrilliAnt VRE</td>
<td>24</td>
<td>19</td>
<td>21</td>
<td>142</td>
<td>1</td>
<td>95.0</td>
</tr>
<tr>
<td>48</td>
<td></td>
<td>20</td>
<td>32</td>
<td>131</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>BEAV</td>
<td>24</td>
<td>13</td>
<td>46</td>
<td>117</td>
<td>7</td>
<td>65.0</td>
</tr>
<tr>
<td>48</td>
<td></td>
<td>20</td>
<td>64</td>
<td>99</td>
<td>0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

TP, true positive; FP, false positive; TN, true negative; FN, false negative; PPV, positive predictive value; NPV, negative predictive value.
reported by Willey et al. (2011), whereas it was lower than the one (86.2 %) reported by Scopes & Henry (2010). Although this study has some limitations, including the paucity of samples, the lack of an enrichment step and the exclusive use of vanA strains, it shows that VRE isolation at 24 h is consistently higher on Brilliance VRE agar than on BEAV agar, confirming the former as a reliable medium for the screening and presumptive identification of vancomycin-resistant E. faecium in rectal swab specimens.

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