Evaluation of Three Chromogenic Media for Detection of Vancomycin-Resistant Enterococci in a Tertiary-Care Hospital

M.L. Miller², D.E. Zoutman¹,2, L.L. Tomalty¹,2, D. Armstrong², J. Dien Bard¹,2
¹Queen’s University School of Medicine, Department of Pathology and Molecular Medicine, Kingston, ON, Canada, ²Kingston General Hospital, Kingston, ON, Canada

Abstract

Objective: To evaluate the performance of Brilliance VRE Agar, Colorex VRE Agar and VRE Select Agar for detecting vancomycin-resistant enterococci (VRE) from fecal samples and rectal swabs collected from patients undergoing VRE-screening for VRE colonisation at a tertiary care hospital.

Methods: 95 stool and/or stool specimens (22 positives and 73 negatives) were collected from patients admitted to Kingston General Hospital to screen for VRE colonisation. Swabs or stools were incubated in BHI enrichment broth and incubated overnight at 35-37°C, followed by streaking 10 µL broth onto Brilliance VRE Agar, Colorex VRE Agar and VRE Select Agar. All plates were incubated at 35-37°C for 22, 24 and 26 h. VRE colonies were confirmed by Gram stain, biochemical identification, and antibiotic susceptibility testing. Any Enterococcus species that were confirmed to have minimum inhibitory concentration (MIC) of ≥ 8 µg/mL for vancomycin were considered VRE positive.

Results: Of the three chromogenic agars, the Brilliance VRE Agar was able to identify all 22 positives (100% sensitivity), followed by Colorex VRE Agar with 21 positives (95% sensitivity). The one false negative VRE grew only 1 colony on the Brilliance VRE Agar at 26 h. The VRE Select Agar had the lowest sensitivity with 15 true positives (68%) identified. All three agars required 26 h incubation to recover all VRE positive isolates. Although the Brilliance VRE Agar had 100% sensitivity, the specificity was 71%, with 21 false positives identified. The majority of the false positives (16/21) were detected at 24 h. The Colorex VRE Agar and VRE Select Agar had very similar specificities of 90% and 92%, respectively.

Conclusion: Brilliance VRE Agar and Colorex VRE Agar showed exceptionally better sensitivity than VRE Select Agar. In contrast, Colorex VRE Agar and VRE Select Agar had much higher specificity than Brilliance VRE Agar. When taking into account the overall performances of the agar plates to accurately identify VRE isolates in both enriched rectal swabs and stool samples, the Colorex VRE Agar appears to be the most highly effective screening agar.

Goals of this study:

- To evaluate the performance characteristics of three different chromogenic media that contain low vancomycin concentrations to detect the low level resistance strains of VRE that are present at our tertiary care institution.

- To select a chromogenic media that meet our requirements for accurate identification with minimal levels of extraneous workload caused by false positive results.

Introduction:

- Antibiotic resistance is an increasing challenge worldwide and evidence has shown that the role of vancomycin-resistant enterococci strains, such as Vancomycin-Resistant Enterococci (VRE), are directly related to the prevention and control in the hospital settings.

- Our institution cultures for VRE on all admissions as well as weekly prevalence surveys of admitted patients. VRE screens continue to be part of the mandated workflow of most clinical laboratories.

- As methodology has evolved, culture with chromogenic media has become a preferred method for screening for VRE colonisation in most hospital settings.

- Due to the prevalence of Enterococcus faecium and E. faecalis strains with low vancomycin MICs in our patient population, we decided to switch from a higher vancomycin MIC chromogenic agar plate to a lower vancomycin MIC plate.

- To test a chromogenic media that meet our requirements for accurate identification with minimal levels of extraneous workload caused by false positive results.

Methods

Data

95 rectal swabs/stool specimens (22 positives and 73 negatives) were collected from patients admitted to Kingston General Hospital to screen for VRE colonisation.

Media included in study (Figure 1):

- Brilliance VRE Agar (Oxoid, Ontario, Ont.)
- Colorex VRE Agar (Alere, Ottawa, Ont.)
- VRE Select Agar (Bio-Rad, Mississauga, Ont.)

Swabs or stools were incubated in BHI enrichment broth and incubated overnight at 35-37°C in ambient air, followed by streaking 10 µL broth onto Brilliance VRE Agar, Colorex VRE Agar and VRE Select Agar. All plates were incubated at 35-37°C for 22, 24 and 26 h. Suspicious VRE colonies were confirmed by Gram stain, biochemical identification, and antibiotic susceptibility testing. Any Enterococcus species that were confirmed to have minimum inhibitory concentration (MIC) of ≥ 8 µg/mL for vancomycin were considered VRE positive.

Results

- There were 22/95 positive VRE and 73/95 negatives included in the study. A variable number of positives and negatives VRE samples were detected using the three chromogenic media.

- The Brilliance VRE plate had 14 false positives at 22 h, 5 at 24 h and 2 at 26 h with a total of 21 false positives (Table 2).

- The Colorex VRE had 4 false positives at 22 h, 2 at 24 h and 3 at 26 h with a total of 7 false positives (Table 2).

- The VRE Select had 2 false positives at 22 h, 2 at 24 h and 1 at 26 h with a total of 5 false positives (Table 2).

- After 26h incubation Brilliance VRE had the highest sensitivity of 100% (22/22), followed by Colorex VRE and VRE Select with sensitivities of 95.5% (21/22) and 68.2% (15/22), respectively.

- The Colonies VRE plated had one false negative result but there was only one colony of VRE that grew on the VRE Brilliance plate. In addition, the Colorex VRE plate was not incubated for a full 48 h, as per product insert, so it could be postulated that the positive may have grown after prolonged incubation.

- The specificity for the Brilliance VRE was lowest at 71.2%, whereas the Colorex VRE and VRE Select had specificities of 90.4% and 91.8% (Table 4).

- The VRE Select and Colorex VRE had identical PPV at 70% and the Brilliance VRE had a PPV of 51.2%. The Brilliance VRE had an NPV of 100% followed by Colorex VRE with 98.3% and VRE Select with 90.5% (Table 4).

- Concordance results were as follows Colorex VRE with 91.6%, VRE Select with 86.3% and Brilliance VRE with 77.9% (Table 4).

Table 1. VRE media selected for testing showing QC strains and manufacturers recommended incubation

<table>
<thead>
<tr>
<th>QC Strain</th>
<th>Brilliance VRE</th>
<th>Colorex VRE</th>
<th>VRE SELECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>VRE E. faecalis (ATCC 51599)</td>
<td>Light blue</td>
<td>Blue</td>
<td>Pink to violet</td>
</tr>
<tr>
<td>VRE E. faecium (ATCC 51299)</td>
<td>Indigo purple</td>
<td>Pink</td>
<td>Light blue, colourless, inhibited</td>
</tr>
</tbody>
</table>

Table 2. VRE results at specific time intervals

<table>
<thead>
<tr>
<th>Brilliance VRE</th>
<th>Colorex VRE</th>
<th>VRE Select</th>
</tr>
</thead>
<tbody>
<tr>
<td>22h</td>
<td>24h</td>
<td>26h</td>
</tr>
<tr>
<td>True Positives</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>True Negatives</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>False Positives</td>
<td>14</td>
<td>19</td>
</tr>
</tbody>
</table>

Table 3. Overall Positivity rate per chromogenic agar

<table>
<thead>
<tr>
<th>CHROMOGENIC MEDIA</th>
<th>TRUE POSITIVES</th>
<th>TRUE NEGATIVES</th>
<th>FALSE POSITIVES</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRILLIANCE VRE</td>
<td>22</td>
<td>73</td>
<td>21</td>
<td>96</td>
</tr>
<tr>
<td>COLOREX VRE</td>
<td>22</td>
<td>73</td>
<td>21</td>
<td>96</td>
</tr>
<tr>
<td>VRE SELECT</td>
<td>22</td>
<td>73</td>
<td>21</td>
<td>96</td>
</tr>
</tbody>
</table>

Table 4. Sensitivity, specificty, concordance, PPV, NPV

<table>
<thead>
<tr>
<th>CHROMOGENIC MEDIA</th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
<th>CONCORDANCE</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRILLIANCE VRE</td>
<td>100%</td>
<td>95.5%</td>
<td>77.9%</td>
<td>51.2%</td>
<td>75%</td>
</tr>
<tr>
<td>COLOREX VRE</td>
<td>95.5%</td>
<td>90.4%</td>
<td>77.9%</td>
<td>51.2%</td>
<td>75%</td>
</tr>
<tr>
<td>VRE SELECT</td>
<td>98.3%</td>
<td>91.6%</td>
<td>77.9%</td>
<td>51.2%</td>
<td>75%</td>
</tr>
</tbody>
</table>

Conclusions

- Brilliance VRE and Colorex VRE Agar showed exceptionally better sensitivity than VRE Select Agar.

- In contrast, Colorex VRE Agar and VRE Select Agar had much higher specificity than Brilliance VRE Agar.

- 26 h incubation was required for the three agars to detect a few VRE isolates. This is expected of the Colorex VRE and VRE Select agars since they do require further incubation. However, according to the manufacturer’s protocol, the Brilliance VRE need only be incubated up to 24 h, which would have resulted in 2 false negatives.

- When taking into account the overall performances of the agar plates to accurately identify VRE isolates in both enriched rectal swabs and stool samples, the Colorex VRE Agar appears to be the most highly effective screening agar.

- Implementation into the laboratory of Colorex VRE media met our criteria for reduced workload with accurate identification.

Acknowledgments

- We thank Alere, Oxoid and Bio-Rad for supplying the plates for the evaluation.

Other Enterococcus spp. not VRE

Table 2. Other Enterococcus spp. not VRE:

- Brilliance VRE: Pink, inhibited
- Colorex VRE: Light blue, colourless, inhibited
- VRE Select: Blue, inhibited

Figure 1. Chromogenic Medias Growing Vancomycin Resistant E. faecium and E. faecalis

- E. faecalis
- E. faecium
- E. faecium and E. faecalis are one unique colour

Figure 2. Evaluation of three chromogenic media for detection of Vancomycin Resistant Enterococci in a Tertiary Care Hospital

- Brilliance VRE: Orange
- Colorex VRE: Red
- VRE Select: Pink