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

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Abstract

Objective: To evaluate the performance of Brilliance VRE Agar, Colorex VRE Agar and VRE Select Agar for detecting vancomycin-resistant enterococci (VRE) from faecal samples and rectal swabs collected from patients undergoing VRE-screening for VRE colonization at a tertiary care hospital.
Methods: 95 swabs/stool specimens (22 positives and 73 negatives) were collected from patients admitted to Kingston General Hospital to screen for VRE colonization. Swabs or stools were inoculated into BHI enrichment broth and incubated overnight at 35-37ºC, followed by streaking 10 µLof broth onto Brilliance VRE Agar, Colorex VRE Agar and VRE Select Agar. All plates were incubated at 35-37ºC for 22 h, 24 h 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: 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 (96% 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 (18/21) 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 broth-enriched rectal swabs and stool samples, the Colorex VRE Agar appears to be the most highly effective screening agar.

Introduction:

- Antimicrobial resistance is an increasing challenge worldwide and evidence have shown that the rate of transmission of antibiotic-resistant organisms, 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 continues to be part of the mandated workload of most clinical laboratories.
- As methodology has evolved, culture with chromogenic media has become a preferred method for screening for VRE colonization in most hospital laboratories.
- 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 vancomcyin MIC chromogenic agar plate to a lower vancomycin MIC plate.

Goals of this study:

- To evaluate the performance characteristic 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.

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 colonization
- Medias included in study(Figure 1):
  - Brilliance VRE Agar (Oxoid, Ottawa,Ont.)
  - Colorex VRE Agar (Alere, Ottawa,Ont.)
  - VRE Select Agar (Bio-Rad, Mississauga,Ont.)
- Swabs or stools were inoculated into BHI enrichment broth and incubated overnight at 35-37ºC in ambient air,followed by streaking 10µL of broth onto Brilliance VRE Agar, Colorex VRE Agar and VRE Select Agar (Table 1)
- Incubation times at 35-37ºC: 22 h, 24 h and 26 h.
- Suspicious VRE colonies were confirmed by subculture, Gram stain , catalase, Biochemical identification on Vitek 2 (Biomerieux)
- Susceptibility testing was performed by vancomycin Etest where any *E. faecium* or *E. faecalis* that were confirmed to have minimum inhibitory concentration (MIC) of ≥ 8µg/mL were considered VRE positive

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

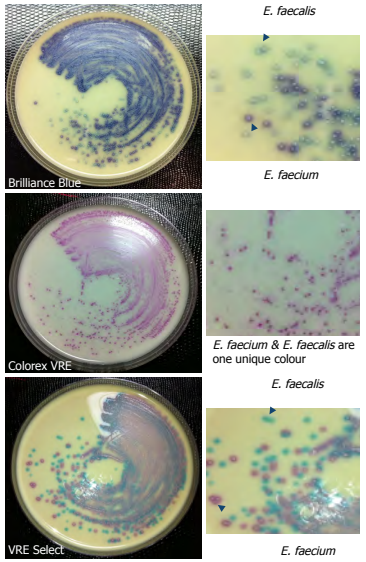

Table 1. VRE media selected for testing showing QC strains and manufacturers recommended incubation.

QC Strain	Brilliance VRE	VRE Select	Colorex VRE
VRE *E. faecalis* (ATCC 51299)	Light blue	Blue	Pink to violet
VRE *E. faecium* (ATCC 51559)	Indigo purple	Pink	Pink to violet
Other Enterococcus spp. not VRE	Inhibited	Inhibited	Light blue, colourless, inhibited

Results

- There were 22/95 positive VRE and 73/95 negatives included in the study. A variable number of positives and negative VRE samples were detected using the three chromogenic media
- The Brilliance VRE plate had 14 false positives at 22 h, 5 at 24h and 2 at 26 h with a total of 21 false positives (Table 2)
- The Colorex VRE had 4 false positives at 22h, 2 at 24h and 3 at 26h with a total of 7 false positives (Table 2)
- The VRE Select had 2 false positives at 22h, 2 at 24h 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 Colorex 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 48h, 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 75% and the Brilliance VRE had a PPV of 51.2%. The Brilliance VRE had a NPV of 100%, followed by Colorex VRE with 98.5% 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 2. VRE results at specific time intervals

	Brilliance VRE			Colorex VRE			VRE Select		
Incubation Time	22h	24h	26h	22h	24h	26h	22h	24h	26h
True Positives	20	20	22	17	18	21	9	13	15
False Positives	14	19	21	4	6	7	2	4	5

Table 3. Overall Positivity rate per chromogenic agar

	BRILLIANCE VRE	COLOREX VRE	VRE SELECT
True Positives	22	21	15
True Negatives	52	66	67
False Positives	21	7	6
False Negatives	0	1	7
Total	95	95	95

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

	BRILLIANCE VRE	COLOREX VRE	VRE SELECT
Sensitivity	100%	95.5%	68.2%
Specificity	71.2%	90.4%	91.8%
Concordance	77.9%	91.6%	86.3%
PPV	51.2%	75%	75%
NPV	100%	98.5%	90.5%

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 broth 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

Limitations

- Colorex VRE Agar and VRE Select agars were not incubated for 48h and 28 h, respectively, as recommended by the Manufacturer's package inserts

Acknowledgements

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