P1504

Evaluation of Broth Enrichment for the Detection of Vancomycin Resistant Enterococci on Two Chromogenic Media

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Abstract

Background: Rapid and accurate screening for vancomycin resistant enterococci (VRE) is essential in a hospital setting. We recently evaluated the performance of Brilliance VRE Agar (BVA) and Colorex VRE Agar (CVA) to identify VRE isolates in broth-enriched (BE) rectal swabs and found high sensitivity but also high numbers of false positives (FP). We sought to determine whether eliminating BE step would improve specificity of BVA and CVA without significantly affecting the sensitivity of detection of VRE.
Methods: Evaluation of BVA and CVA were performed at different time periods, following the same protocol. For BE method, rectal swabs were inoculated into BHI enrichment broth and incubated overnight at 35-37°C, followed by streaking 10 µL onto BVA or CVA. For the direct method, swabs were inoculated onto BVA or CVA, and streaked for isolation. BVA plates were read at 24 h and CVA plates were read at 24 h and 48 h. In the BE study, 2160 and 935 samples were evaluated on BVA and CVA, respectively. Comparison of direct versus BE was performed on 101 and 208 samples for BVA and CVA, respectively. Suspicious VRE colonies were confirmed by Gram stain, biochemical identification, and antibiotic susceptibility testing. Enterococcus faecium or E. faecalis with a minimum inhibitory concentration of ≥ 8 µg/mL for vancomycin were considered VRE.
Results: In the BE study for BVA, 689 (32%) samples required further work-up; 199 (9%) were VREs and 490 (23%) were FP. For BE on CVA, further work-up was performed on 135 (14%) with 69 (7%) confirmed as VRE and 66 (7%) FP. The specificity for BVA and CVA were 75% and 92%, respectively. In the direct inoculation versus BE study for BVA, sensitivity decreased from 100% to 83% and specificity increased from 80% to 91%. For CVA, sensitivity was the same (85%) but specificity increased from 86% to 92%. The FP rate decreased by 47% (8/17) for BVA and 54% (15/28) for CVA when BE was eliminated. Direct inoculation of CVA detected 9/11 VRE at 24 h and 13/15 FP at 48 h.
Conclusion: Although BVA and CVA both showed exceptional sensitivity using BE method, the number of FP were significant. When direct inoculation was employed, FP decreased significantly for both agars with a slight decrease in sensitivity for BVA only. There appears to be no benefit in employing a BE step for BVA and CVA when screening for VRE in our hospital.

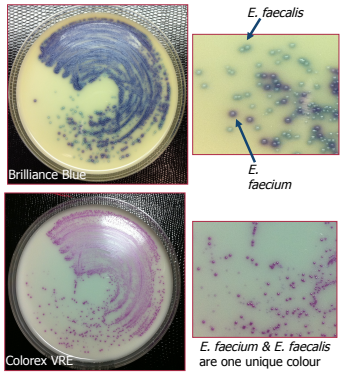
Introduction

- 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.
- In our institution BHI broth enrichment (BE) had previously been determined to increase sensitivity when screening for VRE using a chromogenic agar with minimum inhibitory concentration (MIC) of 8 µg/ml.
- Due to the prevalence of *Enterococcus faecium* and *E. faecalis* strains with low vancomycin MICs in our patient population, we sought out to evaluate a VRE chromogenic agar plate that contained a lower MIC of vancomycin.

Goals of this study:

- To determine the sensitivity and specificity of two chromogenic agars when BE step is incorporated.
- To determine whether a BE step is required, prior to inoculation of a chromogenic media when a chromogenic agar with a lower MIC of vancomycin is utilized.
- To evaluate two new chromogenic agars and select the superior one that meet our requirements for accurate identification with minimal levels of extraneous workload caused by false positive results.

Figure 1. Broth Enrichment with Brilliance VRE and Colorex VRE agar



Methods

Data:

- **Medias included in study (Figure 1):**
  - Brilliance VRE Agar (BVA) (Oxoid, Ottawa,Ont.)
  - Colorex VRE Agar (CVA) (Alere, Ottawa,Ont.)
- **Broth Enrichment study:**
  - 2160 (BVA) and 935 (CVA) rectal/stool swabs were collected from patients admitted to Kingston General Hospital for VRE colonization.
  - 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 BVA and CVA with isoplater streaking for isolation.
  - Incubation times: 35-37ºC: 24 h for BVA and 24 and 48h for CVA as per manufacturer's protocol.
- **Broth Enrichment vs. Direct Plating study:**
  - 101 (BVA) and 208 (CVA) rectal/stool swabs were plated directly onto chromogenic media. Swabs were also inoculated into BHI enrichment broth, incubated overnight and 10µL of broth was plated onto the corresponding media for comparison.
  - BVA were examined for any pink, purple pink or dark blue colonies consistent with VRE *E. faecium* or light blue colonies consistent with *E. faecalis.*
  - CVA were examined for any pink to mauve colonies consistent with VRE *E. faecium* or *E. faecalis.*
- Suspicious VRE colonies were confirmed by subculture, Gram stain, catalase and 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 MIC of ≥ 8µg/mL were considered VRE positive

Results

- **Broth Enrichment Study (Figure 2):**
  - Of the 2160 samples tested on BVA with BE, 689 (32%) required further workup. 199/2160 (9%) were true positive (TP) and 490/2160 (23%) were false positive (FP) (Table 1)
  - BVA with BE had a sensitivity of 100%,specificity of 75%,PPV was 29% and NPV 100%.
  - Of the 935 samples tested on CVA with BE , 135/935 (14%) required further workup. 69/935 (7%) were VRE TP. 66/935 (7%) were FP.
  - CVA with BE had a sensitivity of 100% ,specificity of 92%, PPV of 51% and NPV of 100%.
- **Broth Enrichment vs. Direct Study:**
  - TP and FP results were recorded for BVA plates after 24h incubation and for Colorex agar after 24h and 48h incubation.
  - **BVA results (Table 1) (Figure 3):**
    - 101 samples were tested
    - 18 were TP and 17 were FP with a total of 66 true negative (TN) and 0 false negatives (FN) detected
    - Direct inoculation of samples yielded 15 TP, 8 FP, 78 TN and 0 FN
    - Sensitivity for BE and direct inoculation were 100% and 83%, respectively
    - Specificity for BE and direct inoculation were 80% and 91%, respectively
    - The FP rate decreased by 47% (8/17) when BE was eliminated
  - **CVA Results (Table 1) (Figure 4):**
    - 208 samples were tested
    - Of the 208 samples tested using BE there were 11 TP, 28 FP, 2FN and 167 TN
    - The same samples tested again with direct inoculation showed 11 TP, 15 FP, 2 FN and 180 TN
    - Sensitivity were identical for both methods (85 %) but specificity increased to 92% with direct inoculation.
    - FP rate decreased significantly by 54% (15/28) after elimination of BE.

Table 1. TP and FP VRE results broth vs. direct

	BVA (N = 101)		CVA (N = 208)			
	Direct	BE	Direct		BE	
Incubation time	24 h	24 h	24 h	48 h	24 h	48 h
TP	15	18	9	2	9	2
FP	8	17	2	13	16	12
TN	78	66	195	180	183	167
FN	0	0	0	2	0	2

Figure 2. Broth Enrichment TP and FP percentage rates for BVA and CVA

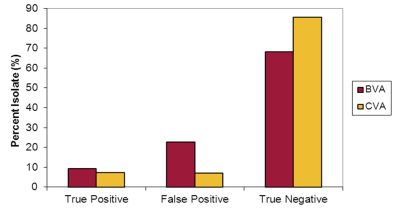


Figure 3. Direct vs. BE results for BVA agar

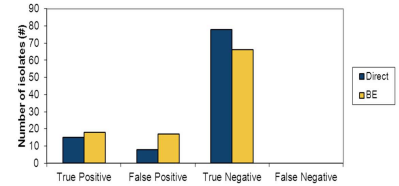
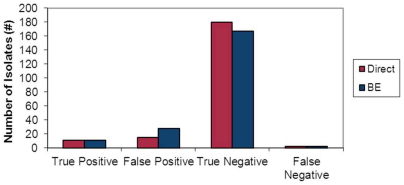


Figure 4. Direct vs. BE results for CVA agar



Conclusions

- Brilliance VRE Agar and Colorex VRE Agar showed exceptional sensitivity but decreased specificity when broth enrichment was included
- Both plates showed a significant increase in specificity after the elimination of BE. There was no change in sensitivity for Colorex VRE Agar and a slight decrease in sensitivity for Brilliance VRE Agar
- There appears to be no benefit in employing a broth enrichment step for Brilliance VRE Agar and Colorex VRE Agar when screening for VRE in our hospital
- When taking into account the overall performances of the agar plates to accurately identify VRE isolates in broth enriched rectal swabs and stool samples, Colorex VRE Agar appears to be the most highly effective screening agar.
- Implementation into the laboratory of Colorex VRE Agar met our criteria for reduced workload with accurate identification