Evaluation of Three Commercial Chromogenic Media and BEAA + van 6μg/mL for the Detection of Vancomycin-Resistant Enterococcus (VRE)

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Introduction

Due to increased use of vancomycin as an agricultural growth promoter and treatment of bacteria such as MRSA, Vancomycin Resistant Enterococci (VRE) have emerged as nosocomial pathogens. In Ontario, the majority of patients acquire VRE from stays in acute care hospitals. In order to maintain further biocides and detection of VRE from nursing homes and determine if the community is equally impacted. The emergence of VRE relies on new and reliable methods of detection. One of the more recent is the advent of various chromogenic VRE media. These media incorporate specific chromophores bearing substrates such as 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside and 5-Bromo-4-chloroindolyl-β-D-galactopyranoside that are inhibited by F, E, and B lactam. The ultimate goal is to detect, with ease, the VRE species which harbour the acquired genes Van A, B, and E. Enterococci are the primary screening sites for VRE, the media tests an economical challenge. Many inhibitors (such as cefoxitin, vancomycin, erythromycin, ampicillin and nalidixic acid) are required to suppress the growth of normal flora, including enterococci vancomycin-resistant Enterococci (E. faecium and E. faecalis). These relatively new chromogenic agar and bile esculin agar Agar Agar (BEAA) and chloramphenicol and Be LLC (CCL) were evaluated for their performance in the detection of VRE.

The chromogenic media tested included ChromoVite, a brand name of CHROMagar VRE from Inverness (COL), ChromID from bioMérieux (CHID) and Ecaffeplus from Oxoid (EC). These media were tested and compared in three phases:

Phase 1: 100 retail screening samples, plated by the direct method (no enrichment step). The samples were pre-selected for previous growth of black colonies of VRE and non-VRE on BEA.

Phase 2: 30 known Van A and B VRE isolates with varying MIC levels from a single patient at the institutions of 10 different institutions.

Phase 3: 101 clinically resistant organisms (in-vitro and 2 small colony variants of VRE.

This was a multicenter study conducted at GDM (Ottawa and C) and GDM (Toronto) performing identical studies.

Materials and Methods

Phase 1: A total of 100 retail swabs, previously deemed suspicious for VRE, were inoculated into 3.5 ml of 0.9% saline and vortexed 50 ml. An aliquot of each was cultured on CCL, CHID, BIA and blood agar's as described. Once the plates were incubated in Lab O, the same saline tubes were transported to Lab T for inoculation.

The chromogenic plates were all read at 24, 36 and 48 hrs. COL was examined for growth of black colonies, which target colonies. CHID for visible purple (E, lactam) and blue-green (F, lactam) target colonies. BIA was examined for indigo/purple (F, lactam) and light blue (E, lactam) target colonies. BIA plates were read only at 48 hrs for black colonies.

All plates were qualitatively assessed for breakthrough growth, colony size and case of detection of suspected VRE.

Phase 2: 30 known PDR identified Van A and B VRE strains of varying MICs were tested in a similar manner as Phase 1, except

Materials and Methods

10% and 10% CFTI and dilutions prepared, which were plated and applied to each plate, using a standard 1 mL loop. Additionally, blood plates (BA) were set up as a control.

Phase 3: 10 known challenging non-VRE isolates, such as Listeria monocytogenes sp., Lactobacillus sp., E. coli, and Staphylococcus sp., were inoculated onto the four types of test plates using a 1 mL loop from a 0.5 McFarland suspension. Additionally, 2 known small colony variants of VRE were included in this phase.

A BA plate was also inoculated, to ensure a growth for the primary set-up was again the same as the first 2 phases.

Phenotypic changes were evaluated, especially the production of erythromycin, chloramphenicol, and vancomycin (E, CL and V). Results

Results

Phase 1: Target colonies appeared as per manufacturer’s descriptions at 48 hrs. However, on an ABI 24 hours of incubation, the colony radii of the suspected colonies appeared to be more diverse than expected. This phenomenon may have occurred because of varying strains of each chromogen. The analysis resulted in proper categorization at 36 hrs. All positive colonies appeared at 36 hrs for 10% and 10% CFTI and COL. Breakthroughs increased as the survey progressed for BEA. Positive colonies (red) were slow-growing colonies demonstrated by the “familiar red” data. Data was averaged between the 2 laboratories.

Phase 2: This phase was a particular challenge for the media. Growth of 10% Van A and B MICs was suppressed to complete inhibition with some samples. Lab T observed that some of these slabs of growth only at 72 hrs or more. The BA growth control plates grew occasional growth at a dilution of 10% and growth 10% for all isolates.

Phase 3: All chromogenic media yielded 2 colonies each of Lactobacillus sp., Pedobacter sp., Lactobacillus sp., Enterococcus gallinarum and E. faecium. BEA allowed Pedobacter and E. gallinarum to grow as black colonies. Lactobacilli grew as a flat, COL, BIA, and BIA supported the growth of the small colony variants of VRE, previously observed in Ontario. CHID did not.

Discussion

• Ease of colony recognition: COL = BEA = CFTI = 24 hrs.
• Color size: BEA = CFTI = BIA = COL = 36 hrs.
• Overall sensitivity: BIA = COL = CHID = BEA = CFTI = 36 and 48 hrs.
• Breakthrough growth: BEA = CFTI = CHID = COL = BIA = 36 hrs.
• Lower vancomycin MIC require longer incubation.
• Phenotypic confirmation testing (PCR) group of presumptive VRE was required prior to reporting.
• The lot of COl, tested in this study had a 12% revertant rate.
• Black pigmentation of colonies were seen in BEA causing difficulty in visualizing distinct positive colonies.
• All plates were bipolar-resistance.
• CHI and BRI differentiated E. faecium and E. faecalis.
• Both laboratories ordered similar results.
• Direct standard testing of colonies from plates were approved by manufacturers.
• PCR positive colony differentiation was difficult with some manufacturers/case variants when not desired.
• CHI was approved for direct PCR card technology.

Conclusions

The cost of chromogenic VRE plates is greater than that of BEA. However, with BEA, VRE detection requires no supplemental identification and susceptibility tests because of possible specificity. This translates to additional technological time, which, by our estimates, was 25% more using the BEA method. Therefore, considering an increase in recovery of VRE and the potential cost savings due to 25% less time and 10 fold reduction in materials, it may be worthwhile for a laboratory to consider using chromogenic VRE plates.

References


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