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

P. Kornherr, K. Biers, D. Dowhaniuk, L. Lewis, M. Psihramis and J. Kapala

Department of Microbiology, Gamma-Dynacare Medical Laboratories, Ottawa and Toronto Ontario Canada

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 minimize further transmission, detection of VRE from nursing homes and the community is equally important. The containment of VRE relies on rapid and reliable methods of detection. One of the more recent is the advent of various chromogenic VRE media. These media incorporate specific chromophore bearing substrates such as ß-D-galactopyranoside and ß-D-glucopyranoside that are metabolized by *E. faecalis* and *E. faecium*. The ultimate goal is to detect, with ease, the 2 VRE species which harbour the acquired genes Van A, B, D, E and G. As rectum/stool are the primary screening sites for VRE, the media face an additional challenge. Many inhibitors (such as cefoxitin, vancomycin, erythromycin, amphotericin B and nalidixic acid) are required to suppress the wealth of normal flora, including intrinsically vancomycin resistant Streptococci, Enterococci and Enterobacteriaceae.

Three relatively new chromogenic agars and Bile Esculin Azide Agar+6µg/mL vancomycin (BEA) were evaluated for their performance in the recovery of VRE.

The chromogenic media tested included Colorex™, a brand name of CHROMagar™ VRE from Inverness (COL), chromID™ from bioMerieux (CHR) and Brilliance™ from Oxoid (BRI).

These media were tested and compared in three phases:

Phase 1: 100 rectal 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 4->256 µg/ml at a concentration of 10^2 and 10^3 CFU/mL.

Phase 3: 10 intrinsically vancomycin resistant organisms (non-VRE) and 2 small colony variants of VRE.

This was a multi-center evaluation at GDML Ottawa (O) and GDML Toronto (T) performing identical studies.

Materials and Methods

Phase 1: A total of 100 rectal swabs, previously deemed suspicious for VRE, were inoculated into 0.5 ml of 0.9 % saline and vortexed. 50 µL was dispensed onto each of COL, CHR, BRI, and BEA and streaked for isolation. Once the plates were inoculated 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 pink to mauve target colonies, CHR for violet/purple (*E. faecium*) and blue/green (*E. faecalis*) target colonies, BRI was examined for indigo/purple (*E. faecium*) and light blue (*E. faecalis*) target colonies. BEA plates were read only at 48 hrs for black colonies.

All plates were qualitatively assessed for breakthrough growth, colony size and ease of recognition of suspect VRE.

Phase 2: 30 known PCR identified Van A and B VRE strains of varying MICs were tested in a similar manner as Phase 1, except 10^2 and 10^3 CFU/mL dilutions were prepared and applied to each plate via a standard 1 µL loop. Additionally, blood plates (BA) were set-up as a growth control.

Phase 3: 10 known challenging non-VRE isolates : *Pediococcus* spp., *Leuconostoc* spp., *Lactobacillus* spp., *E. gallinarum* and *E. casseliflavus* were inoculated onto the four types of test plates using a 1 µL loop from a 0.5 MacFarland suspension. Additionally 2 known small colony variants of VRE were included in this phase. A BA plate was also inoculated. Pre-analytical set-up was again the same as the first 2 phases.

Phenotypic identification was via catalase, PYR, gram stain, MGP broth, BHI+van 6 , and E-test as necessary. Phase 1 positive VREs were determined by expanded gold standard (any one or combination of positively detected plates with appropriate work-up). Known strains of VRE were kindly submitted by the Ottawa Hospital (Phase 2 study) and Mt. Sinai Hospital -Toronto (Phase 3 study).

All 4 technologists performing these tests in both locations were well experienced.

All tests were performed using manufacturer's guidelines.

Results

Phase 1: Target colonies appeared as per manufacturer's descriptions at 48 hrs. However, on BRI after 24 hours of incubation, the colour shades of the suspect colonies appeared to be more diverse than expected. This phenomenon may have occurred because of varying amounts of each chromophore. These eventually resolved to proper colouration at 36 hrs. All positive colonies appeared by 36 hrs for BRI and 48 hrs for CHR and COL. Breakthrough flora increased as the survey progressed for BRI. Perceived (not true) positive colonies decreased as the study progressed due to the "familiarity factor". Data was averaged between the 2 laboratories.

Phase 2: This phase was a particular challenge for the media. Growth of 10^2 Van A or B with low MICs was suppressed to complete inhibition with some media. Lab T observed that some of these showed signs of growth only at 72 hrs or more.

The BA growth control plates grew occasional growth at a dilution of 10^2 and a light growth at 10^3 for all strains.

Phase 3: All chromogenic agars inhibited 2 strains each of *Leuconostoc* spp., *Pediococcus* spp., *Lactobacillus* spp., *Enterococcus gallinarum*, and *E. casseliflavus*. BEA allowed *Pediococcus* and *E. gallinarum* to grow as black colonies. *Lactobacillus* grew as a haze.

COL, BRI, and BEA supported the growth of the small colony variant VRE previously observed in Ontario. CHR did not.

	24 hr	36 hr	48 hr
BRI	55.7%	92.0%	92.0%
COL	85.7%	85.7%	92.8%
CHR	56.6%	78.2%	76.2%
BEA			80.9%

Table 1 Phase 1 Averaged % Sensitivity

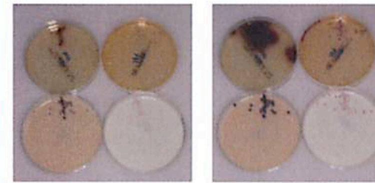
	24 hr	36 hr	48 hr
BRI	91.2%	91.2%	89.1%
COL	93.0%	93.0%	87.4%
CHR	84.6%	84.6%	83.3%
BEA			64.8%

Table 2 Phase 1 Averaged % Specificity

Results

	BRI	COL	COL & BRI	CHR & BRI& COL	CHR & COL
LAB O	1	1	2	3	0
LAB T	0	3	2	0	1

Table 3 Phase 1 exclusive recoveries



Sample 4-0039. Growth of E. faecium Van A >256 µg/mL at 24 hr (left) and 48 hr (right). Clockwise from top left: BEA, CHR, COL and BRI.

Sample 4-0007. Growth of E. faecium VRE only seen on BRI (large purple colony). Breakthrough flora on other plates (turquoise/black) at 24 hr (left) and 48 hr (right). Clockwise from top left: BEA, CHR, COL and BRI.

	24 hr	36 hr	48 hr
BRI	73%	87%	87%
COL	50%	53%	60%
CHR	33%	36%	50%
BEA			36%

Table 3 Phase 2 Averaged summary of % Sensitivity at 10^2 CFU/mL

	24 hr	36 hr	48 hr
BRI	80%	93%	93%
COL	53%	67%	73%
CHR	36%	36%	55%
BEA			68%

Table 4 Phase 2 Averaged summary of % Sensitivity at 10^3 CFU/mL

Ontario VRE small colony variants E. faecium F1240725 (left) and E. faecalis H9132552 (right) at 24 hrs. Strains received courtesy of Barbara Willey (Mt. Sinai, Toronto).

Discussion

- Ease of colony recognition:
  - COL>BRI>CHR>at 24 hrs.
  - COL=BRI>CHR>BEA at 36 and 48 hrs.
- Colony size:
  - BRI>COL=BEA=CHR
- Overall sensitivity:
  - BRI>COL>BEA>CHR
- Breakthrough growth:
  - BEA>CHR>COL=BRI
- Lower vancomycin MIC require longer incubation
- Minimal confirmatory testing (ex. PYR, gram) of presumptive VRE was required prior to reporting.
- The lot of COL tested in this study had a 12% mould contamination rate.
- Black pigment diffuses and spreads in BEA causing difficulty in visualizing distinct positive colonies.
- Not all plates were Isoplater-friendly
- CHR and BRI differentiated *E. faecium* and *E. faecalis*.
- Both laboratories reported similar data.
- Direct standard testing of colonies from plates was approved by manufacturers.
- PYR positive colour differentiation was difficult with some manufacturer/chromogenic plate combinations when used directly.
- CHR was approved for direct Vitek card inoculation

Conclusions

The cost of chromogenic VRE plates is greater than that of BEA. However with BEA, VRE detection requires numerous subcultures, supplementary identification and susceptibility tests because of poorer specificity. This translates to additional technologist time which, by our estimates, was 25% more using the BEA method. When considering an increase in recovery of VRE and the potential cost savings due to 25% less time and a 10 fold reduction in materials, it may be worthwhile for a laboratory to consider using chromogenic VRE plates.

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