Prospective Evaluation of Brilliance VRE® and VRE*Select*® Chromogenic Agars for Detection of Vancomycin-Resistant Enterococci from Surveillance Specimens

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Abstract

Objectives: In an effort to improve turn-around-time (TAT) to VRE isolation without loss of sensitivity, this study compared two newly available chromogenic agars to commonly used non-chromogenic agars.

Methods: From Jul-Sep 2010, 3000 specimens from 18 Toronto hospitals were screened prospectively for VRE using 4 agars: Brilliance VRE (Oxoid), VRE*Select* (Bio-Rad), Bile Esculin Azide Vancomycin (Oxoid), and *mEnterococcus* with 6mg/L vancomycin (Difco, prep in-house). To avoid planting bias, agars were arranged in rotating order, inoculated equally, streaked, incubated simultaneously at 37C, and read independently every 24h for 48h (Brilliance VRE, VRESelect, and Bile Esculin Azide Vancomycin) and for 72h (*mEnterococcus* Vancomycin). While non-chromogenic colonies were ignored, *E. faecium* (Brilliance VRE: dark-blue/purple, VRE*Select*: pink) and *E. faecalis* (Brilliance VRE: Demin-blue, VRE*Select*: aquamarine) were identified (ID) directly from agars when possible. Gram-positive cocci from *mEnterococcus* Vancomycin and black colonies from Bile Esculin Azide Vancomycin were subbed to blood agar. Standard rapid presumptive ID was by PYR, arabinose, MGP, ampicillin disc, and CLSI BHI-vancomycin screen agar, while VRE confirmation and genotyping was by PCR.

Results: Overall, 141 VRE were isolated from 140 specimens [137/2926 (4.7%) rectal swabs, 3/74 (4.1%) stools]. These included 126 *vanA E. faecium* (EFE), 11 *vanB* EFE and 4 *vanB E. faecalis* (EFC). Of these, mEnterococcus Vancomycin detected 140, Brilliance VRE 137, VRESelect 135 and Bile Esculin Azide Vancomycin 124; all 15 vanB were detected by each agar. Other isolates requiring >=1 test to rule out VRE included: 527 on Brilliance VRE [334 VS-EFC, 92 coagulase-negative staphylococci (CNS), 87 VS-EFE, 8 yeast, 6 *E. gallinarum* (EGAL)]; 646 on *mEnterococcus* Vancomycin [387 GPB/PYR-, 103 EGAL, 90 VS-EFE, 37 GNB, 16 VS-EFC, 12 yeast, 1 CNS]; 718 on Bile Esculin Azide Vancomycin [325 GPB/PYR-, 192 EGAL, 138 *E. casseliflavus*, 35 VS-EFC, 23 CNS, 4 VS-EFE, 1 yeast]; 804 on VRE*Select* [437 CNS, 169 VS-EFC, 161 GPB/PYR-, 19 EGAL, 12 VS-EFE, 6 yeast]. The daily % sensitivities, % specificities and 95% confidence intervals for each medium are summarized in the table below. No medium was highly specific or capable of detecting all VRE within 24h. However, two tailed P values for 24h sensitivities found a significant improvement in TAT to VRE detection could be gained by implementing the use of either chromogenic agar studied compared the commonly used non-chromogenic media [Brilliance VRE versus *mEnterococcus* Vancomycin/Bile Esculin Azide Vancomycin (0.0026/0.0010); VRE*Select* versus *mEnterococcus* Vancomycin/Bile Esculin Azide Vancomycin (0.0288/0.0132)].

Performance statistics	*mEnterococcus* Vancomycin	Brilliance VRE	VRE*Select*	Bile Esculin Azide Vancomycin
Sensitivity at 24h	80.1% (72.8-85.9)	92.9% (87.3-96.3)	90.1% (83.9-94.1)	78.7% (71.2-84.7)
Sensitivity BY 48h	96.5% (91.8-98.7)	97.2% (92.7-99.1)	95.8% (90.8-98.2)	87.9% (81.5-92.4)
Sensitivity BY 72h	99.3% (95.7->99.9)	ND	ND	ND
Specificity at 24h	92.4% (91.5-93.4)	88.8% (87.6-89.9)	79.4% (77.9-80.9)	84.9% (83.6-86.2)
Specificity BY 48h	83.5% (82.2-84.8)	79.8% (78.3-81.4)	72.0% (70.4-73.7)	70.9% (69.2-72.7)
Specificity BY 72h	78.3% (76.8-79.8)	ND	ND	ND

Conclusions: This evaluation found Brilliance VRE or VRESelect would significantly reduce TAT to VRE detection but highlighted that both required 48h incubation to avoid missing vanA VRE.

Introduction

Nosocomial dissemination of enterococci with acquired vancomycin resistance (VRE) results in an significant drain on healthcare finances. Prevention and control of transmission is multi-factorial but an important aspect contributing to its success is the rapid identification of new VRE cases. PCR amplification of VRE encoding resistance genes can deliver a specimen turn-around-time (TAT) of a few hours, but due to the high associated costs and the high numbers of screening specimens received by large multi-centre laboratories for VRE screening, this technology is generally reserved for use in high risk cases or outbreak situations. Therefore, since conventional selective culture is the only other option, it is crucial for laboratories to accurately assess using well-powered studies, the efficacy of newly developed selective agars as they become available.

To this end, two chromogenic agars, the Brilliance VRE (Oxoid) and VRE*Select* (Bio-Rad), were put to a prospective challenge in parallel to commonly used VRE agars using consecutive rectal swabs and stool specimens received by the clinical laboratory for VRE screening.

Objectives

The main purpose of this prospective study was to identify the most rapid, reliable and cost-effective selective agar for isolating VRE from surveillance specimens.

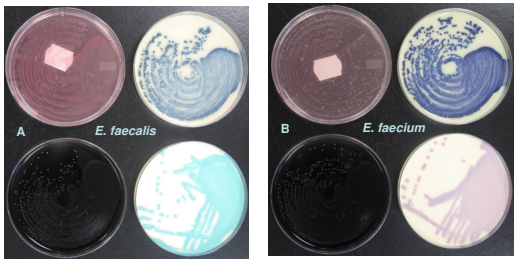

Figure 1A and B: Selective agars (clockwise from top left), Difco *mEnterococcus* with 6mg/L vancomycin, Oxoid VRE Brilliance, Bio-Rad VRE*Select*, and BBL Bile Esculin Azide agar with 6mg/L vancomycin, showing the various typical morphologies for VR-*Enterococcus faecalis* (A) and *VR-Enterococcus faecium* (B), respectively.

Materials and Methods

Between 17 July and 30 September 2010, 3000 prospective specimens were processed to 4 VRE agars: Brilliance VRE (Oxoid), VRE*Select* (Bio-Rad) Bile Esculin Azide with 6mg/L vancomycin (BBL, prep Oxoid), and m*Enterococcus* also with 6mg/L vancomycin (Difco, prep MSH). The chromogenic plates were read by research technologists. The m*Enterococcus* and Bile Esculin Azide plates were read by MSH and SHSC technologists, respectively, as part of the MSH and SHSC standard clinical laboratory protocols.

As summarized in Table 1, study specimens included 2926 rectal swabs received for VRE screening and 74 stools received for *C. difficile* toxin testing on which VRE screening is automatically done. To compensate for the typically clonal nature of circulating VRE, swabs from VRE-positive patients in widely located healthcare facilities situated throughout the Greater Toronto Area and its neighboring regions were also enrolled into the study.

Table 1: Summary: patient, specimen, isolate demographics	No. tested	No. VRE+
Overall specimens	3000	140
Rectal swabs	2926	137
Stools	74 (2.5%)	3
Distinct patients providing specimens	2618	123
Facilities submitting specimens	18	16
A	876	7
B	746	1
C	663	11
D	157	0
E	130	1
F	96	8
G	96	3
H	59	58
I	44	3
J	42	1
K	22	0
L	21	21
M	15	4
N	11	10
O	11	9
P	9	1
Q	1	1
R	1	1

To avoid planting bias, plates of the different media types were pre-arranged in rotating order. The sets of 4 plates were simultaneously inoculated, streaked and incubated at 37ºC, and were read independently every 24h for 48h. Only the *mEnterococcus* was re-incubated for a final read at 72h as per MSH routine VRE protocol.

Non-chromogenic colonies were documented but ignored; colonies resembling *E. faecium* (Figure 1A: Brilliance VRE: dark-blue/purple, VRE*Select*: pink) and *E. faecalis* (Figure 1B: Brilliance VRE: Demin-blue, VRE*Select*: aquamarine) were identified directly from the primary agar when possible.

Gram-positive cocci from *mEnterococcus* and black colonies from Bile Esculin Azide were subbed to blood agar. Presumptive identification was by PYR, arabinose, MGP, ampicillin disc, and CLSI BHI-vancomycin screen agar; VRE were confirmed and genotyped by PCR.

Results from chromogenic agars were entered daily into an Access database; *mEnterococcus* and Bile Esculin Azide data were merged into the database once extracted from the MSH and SHSC laboratory information databases. After cleaning, discrepancy resolution and analyses, 95% confidence intervals and P values were calculated using GraphPad QuickCalcs online software.

Results

Overall, 141 VRE were isolated from 140 specimens: 137 (4.7%) were from the 2926 rectal swabs, while 3 (4.1%) VRE were identified from among the 74 stools. These isolates included 126 *vanA E. faecium*, 11 *vanB E. faecium* and 4 *vanB E. faecalis* (Table 2).

Of the 141 isolates, 140 were detected using *mEnterococcus* vancomycin agar, 137 using Brilliance VRE agar, 135 using the VRE*Select* and 124 were detected using Bile Esculin Azide Vancomycin agar.

Only one rectal swab grew a mix of *vanA E. faecium* and *vanB E. faecalis*. Of note, it was only from the chromogenic agars that this mixture was detected: on the non-chromogenic agars, the smaller and slower growing *E. faecium* were hidden between and under the larger *E. faecalis*, whereas the colonies had distinct chromogenic reactions on Brilliance VRE and VRE*Select* that made the mix obvious (e.g. see Brilliance VRE Figure 2).

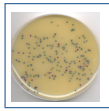

Figure 2: Brilliance VRE with a mixed culture of VR-*E. faecalis* (Denim-blue) and VR-*E. faecium* (purple)

Interestingly, the resistant strains not detected by the various media were invariably of the *vanA* genotype and *E. faecium*, while all 15 *vanB* were detected by each agar as were all VRE *E. faecalis*.

Table 2: Detection of VRE by selective medium [No. (%)]	Total no.	*mEnterococcus* Vancomycin	Brilliance VRE	VRE*Select*	Bile Esculin Azide Vancomycin
VRE+ specimens detected	140	140 (100)	136 (97.1)	134 (95.7)	124 (88.6)
VRE isolates detected	141	140 (99.3)	137 (97.2)	135 (95.7)	124 (87.9)
vanA E. faecium	126	125 (99.2)	122 (96.8)	120 (95.2)	109 (86.5)
vanB E. faecium	11	11 (100)	11 (100)	11 (100)	11 (100)
vanB E. faecalis	4	4 (100)	4 (100)	4 (100)	4 (100)
Detection of *vanA E. faecium* in *vanB E. faecalis* mix	1	0 (0)	1 (100)	1 (100)	0 (0)
vanA E. faecium missed	126	1 (0.7)	4 (2.8)	6 (4.3)	17 (12.1)
vanB VRE missed	15	0 (0)	0 (0)	0 (0)	0 (0)

To save a day, direct identification of query VRE was performed, when possible, from the primary chromogenic agars (Table 3). Since neither routine MSH nor SHSC laboratory protocols includes direct phenotypic work-up from primary agars, this was not attempted from these media during the study. Both chromogenic media performed equally, and direct work-up from these primary agars was successful ~two thirds of the time, enabling results from these screens to be reported one day earlier than otherwise would have occurred.

Table 3: Direct VRE work-up from selective medium (No.%)	Total no.	mEnterococcus Vancomycin	Brilliance VRE	VRE*Select*	Bile Esculin Azide Vancomycin
VRE all	141	ND	94 (66.7)	96 (68.1)	ND
vanA E. faecium	126	ND	82 (65.1)	90 (71.4)	ND
vanB E. faecium	11	ND	9 (81.8)	4 (36.4)	ND
vanB E. faecalis	4	ND	3 (75)	2 (50)	ND

Results

Breakthrough isolates fitting each medium's identification criteria (i.e. appropriate colour on chromogenic agars; black on Bile Esculin Azide; any growth on *mEnterococcus*) that resulted in the requirement of at least one test to rule out VRE are listed below, and summarized by day in Table 4.

Chromogenic agars

- Brilliance VRE (527)
  - 334 vancomycin-susceptible *E. faecalis*
  - 92 coagulase-negative staphylococci
  - 87 vancomycin-susceptible *E. faecium*
  - 8 yeast
  - 6 *E. gallinarum*
- VRE*Select* (804)
  - 437 coagulase-negative staphylococci
  - 169 vancomycin-susceptible *E. faecalis*
  - 161 GPB/PYR-
  - 19 *E. gallinarum*
  - 12 vancomycin-susceptible *E. faecium*
  - 6 yeast

Non-chromogenic agars

- *mEnterococcus* Vancomycin (646)
  - 387 GPB/PYR-
  - 103 *E. gallinarum*
  - 90 vancomycin-susceptible *E. faecium*
  - 37 Gram negative bacilli
  - 16 vancomycin-susceptible *E. faecalis*
  - 12 yeast
  - 1 coagulase-negative staphylococci
- Bile Esculin Azide Vancomycin (718)
  - 325 GPB/PYR-
  - 192 *E. gallinarum*
  - 138 *E. casseliflavus*
  - 35 vancomycin-susceptible *E. faecalis*
  - 23 coagulase-negative staphylococci
  - 4 vancomycin-susceptible *E. faecium*
  - 1 yeast

Table 4: Non-VRE isolates requiring work No. (% spec)	*mEnterococcus* Vancomycin	Brilliance VRE	VRE*Select*	Bile Esculin Azide Vancomycin
By 24h (cumulative)	219 (7.3)	322 (10.7)	591 (19.7)	436 (14.5)
At 48h	290 (9.7)	205 (6.9)	213 (7.1)	162 (9.4)
By 48h (cumulative)	509 (17)	527 (17.6)	804 (26.8)	718 (23.9)
At 72h	237 (4.5)	ND	ND	ND
By 72h (cumulative)	646 (21.5)	ND	ND	ND

The sensitivities, specificities and 95% confidence intervals for each medium, reported by incubation duration are summarized in Table 5 below.

None of the media were highly specific. The *mEnterococcus* incubated for 72h was the most sensitive medium followed by Brilliance VRE then VRE*Select* then Bile Esculin Azide. No medium was capable of detecting all VRE within 24h but two tailed P values for 24h sensitivities found a significant improvement in TAT to VRE detection could be gained by implementing either chromogenic agar compared to non-chromogenic media [Brilliance VRE vs. *mEnterococcus*/Bile Esculin Azide (p=0.003 vs. 0.001); VRE*Select* vs. *mEnterococcus*/Bile Esculin Azide (p=0.03 vs. 0.01)].

Table 5: Performance statistics	*mEnterococcus* Vancomycin	Brilliance VRE	VRE*Select*	Bile Esculin Azide Vancomycin
Sensitivity at 24h	80.1% (72.8-85.9)	92.9% (87.3-96.3)	90.1% (83.9-94.1)	78.7% (71.2-84.7)
Sensitivity BY 48h	96.5% (91.8-98.7)	97.2% (92.7-99.1)	95.8% (90.8-98.2)	87.9% (81.5-92.4)
Sensitivity BY 72h	99.3% (95.7->99.9)	ND	ND	ND
Specificity at 24h	92.4% (91.5-93.4)	88.8% (87.6-89.9)	79.4% (77.9-80.9)	84.9% (83.6-86.2)
Specificity BY 48h	83.5% (82.2-84.8)	79.8% (78.3-81.4)	72.0% (70.4-73.7)	70.9% (69.2-72.7)
Specificity BY 72h	78.3% (76.8-79.8)	ND	ND	ND
Positive Predictive Value at 24h	34.0% (29.1-39.3)	28.9% (24.9-33.3)	17.7% (15.1-20.7)	20.3% (17.1-23.9)
Positive Predictive Value at 48h	21.1% (18.1-24.2)	20.6% (17.7-23.9)	14.4% (12.3-16.8)	15.1% (12.8-17.7)
Positive Predictive Value at 72h	17.8% (15.3-20.7)	ND	ND	ND

Conclusions

Compared to conventional agar, use of either chromogenic agar significantly improved TAT to VRE detection with minimal loss of sensitivity.

It is crucial to note that no medium in this statistically well-powered study, was able to detect all VRE in 24h. And furthermore, nor were either of the two chromogenic agars able to detect all VRE at 48h. However, the 95% confidence intervals for Brilliance VRE (92.7-99.1) and *VRESelect* (90.8-98.2) both indicate that the majority of the time, with a 48h incubation, either formulation should be able to detect >90% of VRE isolates. Therefore, it is in the best interests of patient safety, infection prevention and control, and by default, what makes the most fiscal sense, would be to incubate chromogenic plates for a full 48h before releasing negative results.