Evaluation of CHROMagar™ Compared with Enterococcosel™ Broth for the Isolation of Vancomycin-Resistant Enterococci

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Introduction

Vancomycin-resistant enterococcus (VRE) is of increasing concern in Australian hospitals. Acquisition most commonly occurs through contact with a contaminated environment or via direct or indirect contact with an infected or colonised patient. The consequences of VRE infection are serious and the cost of controlling an established outbreak of cross-infection can be substantial. It is important for laboratories to provide rapid and accurate screening results so that appropriate infection control measures can be implemented.

This study presents a comparison between a chromogenic agar CHROMagar™ VRE (VR952) and Enterococcosel™ enrichment broth (BBL 212207) with 6 mg/L vancomycin for screening of VRE.

Method

Patient samples and hospital environmental swabs were collected and directly inoculated onto CHROMagar™ followed by inoculation into Enterococcosel™ broth. Plates and broths were both incubated aerobically overnight (18-24 hours) at 35ºC.

CHROMagar™ was examined for characteristic colony colour as illustrated in figure 1: VRE *Enterococcus faecalis* and *Enterococcus faecium* appear as pink to mauve colonies as stated in the manufacturer's insert.[2] Negative cultures were re-incubated overnight (36-48 hours) and re-examined for colony colour. Positive cultures were confirmed by a polymerase chain reaction (PCR) method.

Figure 1 – CHROMagar™ agar plates: VRE *E. faecium* and *E. faecalis* pink to mauve colonies.

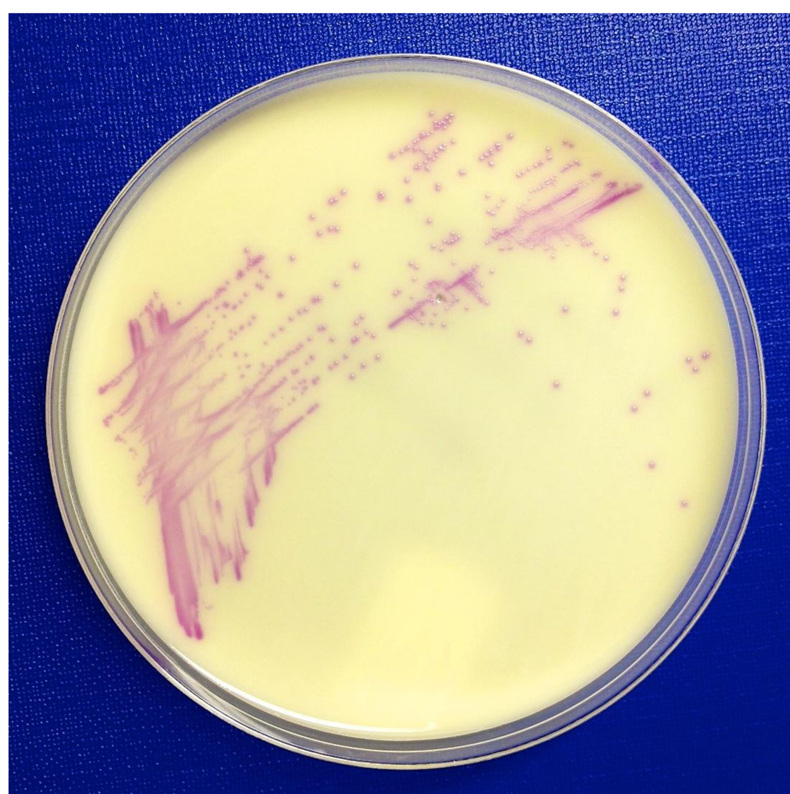
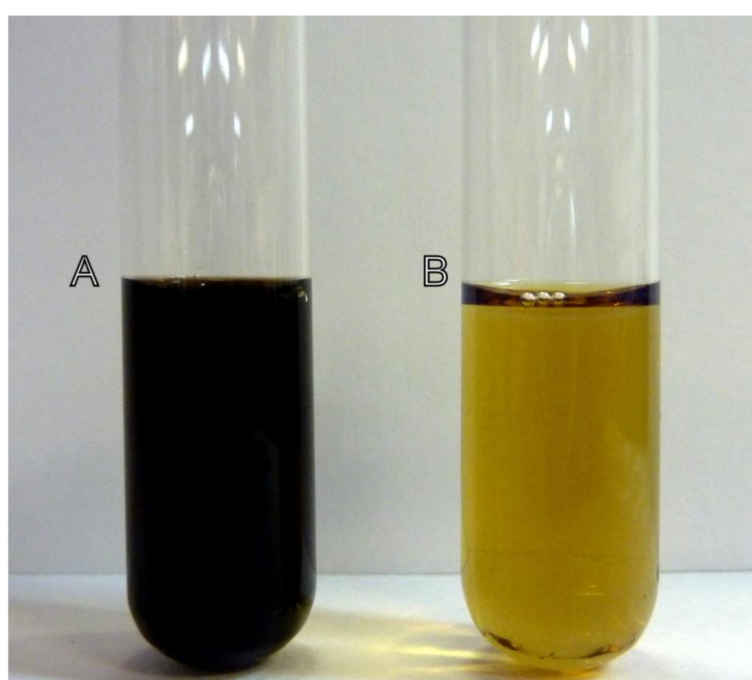


Figure 2 – Enterococcosel™ broth: (A) Black broth, presumptive for VRE. (B) Clear broth, negative for VRE.



Enterococcosel™ broth, as illustrated in figure 2, was examined for a black colour change presumptive of VRE. Clear broths were re-incubated overnight (36-48 hours) and re-examined for colour change. Black broths were sub-cultured onto colistin-nalidixic acid (CNA) horse blood agar plates and incubated with 5% carbon dioxide overnight at 35ºC. Gram stain and motility were performed on colonies resembling enterococci. The identity of non-motile Gram positive cocci was confirmed by PCR. The mean inhibitory concentration (MIC) for vancomycin was determined by Etest® (bioMérieux) for the CHROMagar™ isolates.

Multiplex PCR was performed. These included primers for species-specific *ddl* genes[1] and primers for *vanA* and *vanB* genes. This process is automated by the Easy-Plex ™ system.

Results

Over a six month period during 2011, 290 specimens were submitted for VRE screening (Table 1). These comprised 121 patient specimens (97 rectal swabs, 22 faecal specimens and 2 wound swabs) and 169 hospital environmental swabs.

Table 1 – Number of each specimen type included in comparison of CHROMagar™ and Enterococcosel™ broth.

Specimen Type	*n*
Patient Specimens	121
Hospital Environmental Swab	169
Total	**290**

Table 2 illustrates the results of Enterococcosel™ broth and CHROMagar™ followed by PCR for 121 patient specimens. Using CHROMagar™, all 20 VRE positive patient specimens were detected within 24 hours incubation. With Enterococcosel™ broth, 12 specimens were positive within 48 hours and an additional 7 were positive within 72 hours. Enterococcosel™ broth results were delayed by 24 hours because of the need to subculture onto CNA agar before PCR could be performed.

Table 2 – Comparison of Enterococcosel™ broth and CHROMagar™ at 24 and 48 hours incubation for patient specimens (*n* = 121).

Medium	Enterococcosel™ Broth			CHROMagar™		
Incubation	Black Broth		Clear Broth	Growth of pink to mauve colonies		No Growth
	VRE Positive n (%)	VRE Negative n (%)	VRE Negative n (%)	VRE Positive n (%)	VRE Negative n (%)	
Up to 24 hours	12 (9.9)	20 (16.5)	89 (73.6)	20 (16.5)	9 (7.4)	92 (76.0)
Up to 48 hours	7 (5.8)	17 (14.0)	65 (53.7)	0 (0.0)	4 (3.3)	88 (72.7)

Using Enterococcosel™ broth, there were 37 false positive patient samples, in contrast to CHROMagar™ which yielded 13 false positive cultures.

Table 3 illustrates the results of 169 hospital environmental swabs. As expected, numbers positive for VRE were very low, with three swabs positive on both mediums.

Table 3 – Comparison of Enterococcosel™ broth and CHROMagar™ at 24 and 48 hours incubation for hospital environmental swabs (*n* = 169).

Medium	Enterococcosel™ Broth			CHROMagar™		
Incubation	Black Broth		Clear Broth	Growth of pink to mauve colonies		No Growth
	VRE Positive n (%)	VRE negative n (%)	VRE negative n (%)	VRE Positive n (%)	VRE Negative n (%)	
Up to 24 hours	0 (0.0)	0 (0.0)	169 (100.0)	2 (1.2)	0 (0.0)	167 (98.8)
Up to 48 hours	3 (1.8)	0 (0.0)	166 (98.2)	1 (0.6)	0 (0.0)	166 (98.2)

21 isolates were *E.faecium vanB*, 2 were *E.faecalis vanB* and 1 *E.faecium vanA*. Vancomycin MIC's were performed on CHROMagar™ isolates using Etest® (bioMérieux). MIC's ranged from 12 to >256 mg/L.

Conclusions

A completed result could be issued with the CHROMagar™ agar 24 hours earlier than with Enterococcosel™ broth, as illustrated in table 4. All positive patient specimens could be reported at 24 hours.

Table 4 – Comparison of time to issue a report for Enterococcosel™ broth and CHROMagar™, all specimens combined.

Time Reported (hours)	Enterococcosel™ Broth		CHROMagar™ VRE	
	VRE Positive n (%)	VRE Negative n (%)	VRE Positive n (%)	VRE Negative n (%)
24	0 (0.0)	0 (0.0)	22 (7.6)	9 (3.1)
48	12 (4.1)	251 (86.6)	1 (0.3)	258 (89.0)
72	10 (3.4)	17 (5.9)	0 (0.0)	0 (0.0)

VRE reports were issued at 24 hours for 95.7% of the positive samples found on CHROMagar™, with the remainder of positive reports being issued at 48 hours. In contrast, with Enterococcosel™ broth, initial positive VRE reports were issued at 48 hours for 54.5% and for the remainder only at 72 hours.

Using CHROMagar™ there was a reduction in the number of false positive samples that required PCR confirmation. This number could be further reduced by screening isolates with simple tests such as Gram stain and motility.

These results suggest that the use of CHROMagar™ VRE may improve the speed of VRE detection without loss of sensitivity, thereby improving the implementation of infection control measures.

References

1. Bell, J., J.C., Paton, and J. Turnidge. 1998. Emergence of vancomycin-resistant enterococci in Australia: phenotypic and genotypic characteristics of isolates. Journal of Clinical Microbiology. 36:2187-90.

2. CHROMagar™ Paris, France. 2010. CHROMagar™ VRE Reference VR952.

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