

Comparison of Three Chromogenic Media for Recovery of Vancomycin-Resistant Enterococci from Rectal Swab Samples

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Background: Three chromogenic media using direct inoculation were compared with enriched enterococcosel broth for vancomycin-resistant *Enterococcus faecium* and/or *Enterococcus faecalis* (VRE) surveillance.

Methods: A total of 174 rectal swabs were included for VRE surveillance. The specimens were transferred in enterococcosel broth (EB). An aliquot of the broth was inoculated onto Brilliance VRE, chromID VRE, and VRESelect media and incubated for up to 48 h. We examined each media and EB after 24 h and 48 h of incubation. When appropriately colored colonies were observed, identification was confirmed using the VITEK-2 system and/or VITEK MS. Vancomycin susceptibility was confirmed by disk diffusion test. The presence of resistance genes was confirmed using Anyplex VanR Real-time Detection (Seegene, Korea).

Results: Of the 174 rectal swab specimens, 73 VRE were isolated. For enterococcosel broth, Brilliance VRE, chromID VRE, and VRESelect, the sensitivity at 24 h

was 79.2%, 83.3%, 79.2%, and 79.2%, respectively. The sensitivity at 48 h was 91.7%, 93.1%, 91.4%, and 90.3%, respectively. The specificity at 24 h was 85.3%, 97.1%, 98.0%, and 98.0%, while that at 48 h was 79.4%, 85.3%, 95.2%, and 95.1%, respectively. The specificity of chromogenic media at 24 h and 48 h was significantly higher than that of EB. Furthermore, the specificity at 48 h was significantly higher for chromID VRE and VRESelect than Brilliance VRE, although color distinction was easier with VRESelect.

Conclusion: Based on our results, use of chromID VRE or VRESelect is more reliable and convenient for screening of VRE. In addition, five *vanA*-positive *Enterococcus gallinarum*, *Enterococcus avium* and *Enterococcus durans* were isolated, and two of them (one *E. avium* and one *E. durans*) were detected only on VRESelect. (Ann Clin Microbiol 2015;18:82-87)

Key Words: BioMérieux, Bio-Rad, Oxoid, VRE

INTRODUCTION

Vancomycin-resistant enterococci (VRE) are important causes of nosocomial infections and increasing problem within health care institutions. The rapid and reliable identification of VRE is essential for patient management and infection control measures. The CDC recommends that institutions with moderate to high rates of VRE to perform active surveillance using stool or rectal swab specimens [1]. Bile esculin azide agar with vancomycin (BEAV) has been used as a traditional screening method in many clinical laboratories for detecting VRE in rectal swabs or stool specimens, however confirmation of VRE using this medium requires a minimum of 72 h [2]. Several formulations of

chromogenic media have been developed to shorten this turn-around time and they are supposed to be used with or without enrichment step. Although many investigators reported that various chromogenic VRE agars appeared promising for use in VRE stool screening [3-8], only a few studies compared them with the method including the enrichment step [6].

In addition, there are few studies which compared more than two chromogenic media including Brilliance VRE (Oxoid, Ottawa, Canada), a recently FDA-cleared medium. Therefore, in this study, we evaluated the performance of the three FDA-cleared VRE media Brilliance VRE (Oxoid, Ottawa, Canada), chromID VRE (bioMérieux, Marcy l'Étoile, France) and VRESelect (Bio-Rad, Marnes-la-Coquette, France) using direct inoculation

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to the enterococcosel broth (EB) with enrichment step.

MATERIALS AND METHODS

Stool specimens for VRE surveillance were included for this study between Sep. to Oct. 2014. A total of 174 rectal swab specimens were transferred to the microbiology laboratory in enterococcosel broth (EB) containing 8 μ g/ml of vancomycin (Asan, Seoul, Korea).

A 100 μ L aliquot of the broth was inoculated onto Brilliance VRE (Oxoid, Ottawa, Canada), chromID VRE (bioMérieux, Marcy l'Étoile, France) and VRESelect (Bio-Rad, Marnes-la-Coquette, France) and incubated up to 48 h. Although the recommended incubation time for VRESelect is 24 to 28 h, we examined all the three media at 24 h and 48 h to investigate the effect of extended incubation time.

Colonies on Brilliance VRE and chromID VRE were screened for purple vancomycin resistant *E. faecium* (VREfm) or blue to green vancomycin resistant *E. faecalis* (VREfs). For VRESelect, the pink VREfm or blue VREfs colonies were screened. At the time of examination, growths of appropriately colored colonies from chromogenic media were presumptively regarded as VRE except for the additional catalase testing for *E. faecalis*-like colonies on VRESelect according to the manufacturer's recommendation.

After inoculation of aliquot of the EB, the remaining EB was also incubated at 35°C in ambient air and examined at 24 h and 48 h. EB with black color development was subcultured onto 5% sheep blood agar containing vancomycin disk (30 μ g) and incubated for an additional 18-24 h. The colonies suspected to be *Enterococcus* species on blood agar plates or appropriately colored colonies on chromogenic media were identified using L-pyrroindonyl β -naphthylamide enzyme (PYR), leucine aminopeptidase (LAP), arabinose, methyl- α -D-glucopyranoside, and pigment production for differentiation of *E. faecium*, *E. faecalis*, *E. gallinarum* and *E. casseliflavus*.

Vancomycin resistance was confirmed by disk diffusion test and the presence of resistance gene was determined by Anyplex VanR Real-time Detection (Seegene, Seoul, Korea) which detects *vanA* and *vanB* genes. In addition, breakthrough colonies not showing typical morphology for *E. faecium* and *E. faecalis* were subject to Gram stain and if they were bacteria (not yeast), it was identified to the species level by VITEK-2 system (bioMérieux, Marcy l'Étoile, France) and/or VITEK MS (bioMérieux, Marcy l'Étoile, France).

The true-positive was defined as the presence of confirmed vancomycin resistant *E. faecium* and *E. faecalis* growing on either chromogenic medium or EB at 48 h because the chromogenic media were FDA-cleared for vancomycin resistant *E. faecium* and *E. faecalis*. Growth of appropriately colored colonies was regarded as false positive when the colonies were susceptible to vancomycin or species which were not either *E. faecium* or *E. faecalis*.

The performance of four methods was compared by using the McNemar test. $P < 0.05$ was considered statistically significant.

RESULTS

Of the 174 specimens, 73 vancomycin resistant *E. faecium* and *E. faecalis* were isolated from 71 specimens and among them 60 (82.2%) were detected at 24 h of incubation. All the VRE isolates harbored *vanA* gene.

The concordance rate between EB and all three chromogenic media was 96.6% (168/174: growths of VRE 61, no growth of VRE 107) at 24 h of incubation but it decreased to 93.7% (163/174) at 48 h due to sporadic growths of VRE on EB or chromogenic media.

At 24 h of incubation, the sensitivity and specificity of EB, Brilliance VRE, chromID VRE and VRESelect was 79.2% and 85.3%, 81.9% and 97.1%, 78.9% and 97.1%, 77.8% and 98.0%, respectively. After 48 h incubation, the sensitivity and specificity of EB, Brilliance VRE, chromID VRE and VRESelect was 91.7% and 79.4%, 91.7% and 85.3%, 90.0% and 95.2%, 88.9% and 95.1%, respectively. The difference in sensitivity between all three chromogenic media and EB were not significant either at 24 h or 48 h (Table 1). The false positive cases at 24 h incubation were 15, 3, 3, 2 cases in EB, Brilliance VRE, chromID VRE and VRESelect, respectively. All the false positive cases were vancomycin susceptible *Enterococcus* species except one *Klebsiella pneumoniae* isolated from chromID VRE which resembled *E. faecium*. The specificity at 24 h was significantly higher in three chromogenic media than EB. After 48 h of incubation, false positives were increased in all of the media but especially in EB and Brilliance VRE (21 and 15 cases, respectively). The specificities of chromID VRE and VRESelect were significantly higher than those of Brilliance VRE and EB at 48 h.

Although chromogenic media is selective for VRE, various gram positive or negative bacteria and yeasts were isolated from chromogenic media. The number of breakthrough colonies was highest with chromID VRE. The number of Gram-positive coc-

Table 1. Analysis of three chromogenic media and EB for identification of VRE at 24 h/48 h of incubation

Performance statistics	% Performance (95% CI)			
	EB	Brilliance VRE	ChromID VRE	VRESelect
Sensitivity at 24 h	79.2 (67.7-87.5)	81.9 (70.7-89.7)	78.9 (67.3-87.3)	77.8 (66.2-86.4)
Sensitivity at 48 h	91.7 (82.1-96.6)	91.7 (82.1-96.6)	90.0 (79.9-95.5)	88.9 (78.7-94.7)
Specificity at 24 h	85.3 (76.6-91.3)	97.1 (91.0-99.2)	97.1 (91.1-99.2)	98.0 (92.4-99.7)
Specificity at 48 h	79.4 (70.0-86.5)	85.3 (76.6-91.3)	95.2 (88.6-98.2)	95.1 (88.4-98.2)
PPV at 24 h	79.2 (67.7-87.5)	95.2 (85.6-98.7)	94.9 (84.9-98.7)	96.6 (87.0-99.4)
PPV at 48 h	75.9 (65.3-84.1)	81.5 (71.0-88.9)	92.6 (83.0-97.3)	92.8 (83.2-97.3)
NPV at 24 h	85.3 (76.6-91.3)	88.4 (80.6-93.4)	87.0 (79.1-92.3)	86.2 (78.3-91.7)
NPV at 48 h	93.1 (85.0-97.2)	93.5 (85.9-97.4)	93.4 (86.4-97.1)	92.4 (85.1-96.4)

Abbreviations: CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Table 2. Breakthrough isolates from the three chromogenic media other than *Enterococcus* species (No. of isolates)

Microorganism	Brilliance VRE	ChromID VRE	VRESelect
GPC	<i>Staphylococcus epidermidis</i> (1)	<i>Staphylococcus epidermidis</i> (4)	<i>Staphylococcus epidermidis</i> (3) <i>Staphylococcus hominis</i> (1) <i>Staphylococcus haemolyticus</i> (1)
GPB	<i>Lactobacillus casei</i> (1)	<i>Lactobacillus casei</i> (1)	<i>Lactobacillus casei</i> (1)
GNC	<i>Enterobacter cloacae</i> (1)	<i>Klebsiella pneumoniae</i> (5)	<i>Klebsiella pneumoniae</i> (9)
GNC	<i>Morganella morganii</i> (1)	<i>Acinetobacter baumannii</i> (1)	<i>Acinetobacter baumannii</i> (1)
		<i>Citrobacter freundii</i> (1)	<i>Citrobacter freundii</i> (1)
		<i>Proteus mirabilis</i> (1)	<i>Enterobacter cloacae</i> (1)
		<i>Morganella morganii</i> (4)	
Yeast	Yeast (11)	Yeast (10)	

Abbreviations: GPC, gram positive cocci; GPB, gram positive bacilli; GNC, gram negative cocci; GNB, gram negative bacilli.

Table 3. No. of Vancomycin-resistant *Enterococcus* species other than *E. faecium*/*E. faecalis* on the chromogenic media

<i>Enterococcus</i> species	Brilliance VRE	ChromID VRE	VRESelect
<i>E. gallinarum</i> (2)	2	2	2
<i>E. avium</i> (2)	1	1	2
<i>E. durans</i> (1)	0	0	1

cus was lowest with Brilliance VRE and the growth of yeasts was observed with chromID VRE and Brilliance VRE but not with VRESelect (Table 2). Among the breakthrough colonies, vancomycin resistant *Enterococcus* species other than *E. faecium* or *E. faecalis* were isolated in 5 specimens and they were 2 *E. gallinarum*, 2 *E. avium* and 1 *E. durans* (Table 3). Of these, only one *E. gallinarum* grew as a single organism and it grew on all the three chromogenic media and also from EB. One of *E. avium* and one *E. durans* were isolated only on VRESelect. The former grew with *E. faecalis* and *E. gallinarum* and the latter grew with *E. faecalis*. The colony color of *E. gallinarum* showed light green color on Brilliance VRE but it

showed cream color on chromID VRE and VRESelect. The colonies of *E. avium* revealed sky blue, cream and light green on Brilliance VRE, chromID VRE and VRESelect, respectively. One *E. durans* isolate which was identified only on VRESelect showed sky blue color. By Anyplex™ VanR Real-time PCR, all of them were found to harbor *vanA* gene.

DISCUSSION

Antibiotics resistant organisms, especially VRE are serious problem in health care institutions. For infection control and patient management, the rapid and reliable identification of VRE

is critical. Therefore, in this study, we compared the performance of the three different chromogenic VRE media using direct with the enterococcosel broth with 48h enrichment. Overall sensitivity of chromogenic media in this study was 77.8-81.9% and 88.9-91.7% at 24 h and 48 h of incubation. This is comparable with the study by Suwantararat et al. [2] where the overall sensitivity of the five chromogenic media was 89.9-94.9%. However, it is much lower than the study by Anderson et al. [9] which compared one chromogenic agar and BEAV and the sensitivity of chromogenic agar was 98.7% at 24-28 h of incubation. This difference in sensitivity might be caused by the number of compared chromogenic media, because more sporadic growths of VRE can be discovered among various media and it will lower the sensitivity of each chromogenic media. The other possible reason for the lower sensitivity in this study is that some of the VRE isolates might had a VanD phenotype (vancomycin MICs of 12 to 24 $\mu\text{g/mL}$, teicoplanin MICs of 4 to 8 $\mu\text{g/mL}$) despite of their *vanA* genotype as reported previously [4]. Because both the EB and chromogenic media showed low sensitivity for VRE with low MICs [10].

Taking into account that the additional 18-24 h required for subculture from the EB, the turn-around time (TAT) of 48 h incubation of chromogenic media is comparable to 24 h incubation of EB. Based on our results, the sensitivity of all three chromogenic media after 48 h incubation was much higher than that of EB at 24 h. This is in line with previous study which compared two chromogenic media (Brilliance VRE and VRESelect) with two non-chromogenic media, in the aspect that the implementation of either chromogenic media can contribute to the reduction of turn-around time for VRE detection without loss of sensitivity.

When the incubation time extended to 48 h, all the agars showed increased sensitivity (9.8-11.1%) and reduced specificity (5.9%, 11.8%, 1.9%, and 2.9% for EB, Brilliance VRE, chromID VRE, and VRESelect, respectively). The sensitivity of four methods was similar to EB with 48 h enrichment but the specificity of all the three chromogenic media was higher than EB and furthermore, the specificity of chromID VRE and VRESelect was higher than that of Brilliance VRE. It is difficult to directly compare the performance of chromogenic media using results of previous studies because each study used different media and/or criteria for false positives and negatives. The specificity of Brilliance VRE agar at 48 h correlated with that (86.2%) reported by Scopes and Henry [11] but are higher than that (80.4%) reported by Ongut et al. [12] or that (79.8%) reported by Willey

et al. (presented data at the 21st ECCMID/27th ICC (2011)).

In this study, several *vanA*-positive *E. gallinarum*, *E. avium* and *E. durans* were also isolated alone or mixed with *E. faecium*. Several cases of *vanA* and *vanB* genes acquired by *E. gallinarum* have been reported in many countries (Switzerland, Australia, Italy, Belgium, Taiwan and Brazil) [13]. In Brazilian case, the patient was colonized by not only *E. gallinarum* but also *E. faecalis*. It is reasonable to speculate that *vanA* gene cluster was transferred from *E. faecalis* to *E. gallinarum* *in vivo*. In addition, vancomycin resistant *E. raffinosus* was also isolated from fecal samples in USA [2]. Taken together, we also should pay attention to these vancomycin resistant *Enterococcus* species other than *E. faecium* or *E. faecalis*.

This study has a limitation in that all the VRE isolates only harbored the *vanA* gene. However *vanA* resistance gene is widespread in Korea and worldwide except Australia [14]. Our results showed that VRE isolation using chromID VRE and VRESelect is more reliable for the screening of vancomycin resistant *E. faecium* in rectal swab specimens. In our opinion, the color distinction was easier with VRESelect because the pink with blue were more contrasting than purple with blue-green. Also, the use of chromogenic medium has the advantage in detecting vancomycin resistant *Enterococcus* species other than *E. faecium* or *E. faecalis*.

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=국문초록=

반코마이신내성장알균 검출을 위한 발색배지의 비교

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배경: 반코마이신 내성 장알균의 감염감시 방법으로써, 직장 면봉 검체를 직접 접종한 세 개의 발색배지 배양법과 증균 배양과정을 포함하는 enterococcosel broth (EB) 이용법을 비교하였다.

방법: 반코마이신 내성 장알균의 감염감시를 위한 총 174개의 직장 면봉 검체가 본 연구에 포함되었다. 검체는 EB를 이용하여 검사실로 수송하였고 broth의 소분검체를 각각 Brilliance VRE (Oxoid, Canada), chromID VRE (bioMérieux, France)와 VRESelect (Bio-Rad, Marnes-la-Coquette, France)에 직접 접종한 후 최대 48시간까지 배양하였다. 각각의 발색배지와 EB는 배양 24시간/48시간에 확인하였다. 각 발색배지에서 적절한 색의 집락이 관찰된 경우 양성으로 판정하였으며, VITEK-2 system 혹은 VITEK MS로 균종을 동정하였다. 반코마이신 감수성 검사는 디스크 확산법을 이용하였다. 내성유전자의 존재 유무는 Anyplex VanR Real-time Detection (Seegene, Seoul, Korea)을 이용하여 확인하였다. 네 가지 방법 중 한 가지 이상에서 반코마이신 내성 장알균(*Enterococcus faecium* 또는 *Enterococcus faecalis*)이 확진되면 진양성으로 판정하였다.

결과: 174개의 직장 면봉 검체에서 73개의 반코마이신내성장알균이 분리되었다. 24시간/48시간에 EB, Brilliance VRE, chromID VRE와 VRESelect 각각의 예민도는 79.2%/91.7%, 83.3%/93.1%, 79.2%/91.4%와 79.2%/90.3%였고, 특이도는 85.3%/79.4%, 97.1%/85.3%, 98.0%/95.2%와 98.0%/95.1%였다. 24시간/48시간에 발색배지의 특이도는 EB보다 유의하게 높게 나타났다. 또한 48시간에 chromID VRE와 VRESelect의 특이도 Brilliance VRE보다 통계적으로 유의하게 높았다. 발색배지에서 반코마이신내성 *E. faecium*과 *E. faecalis* 집락의 색 구분은 VRESelect에서 보다 용이하였다.

결론: 이와 같은 결과로 미루어, chromID VRE와 VRESelect의 사용이 반코마이신내성장알균의 감시배양에 있어 보다 편리하고 신뢰할 만한 것으로 생각된다. 더불어 총 5개의 *vanA* 양성 *Enterococcus gallinarum*, *Enterococcus avium*과 *Enterococcus durans*가 분리되었고 그 중 2 균주(하나의 *E. avium*과 하나의 *E. durans*)는 VRESelect에서만 확인되었다. [Ann Clin Microbiol 2015;18:82-87]

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