JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2011, p. 3947–3949 Vol. 49, No. 11
0095-1137/11/$12.00 doi:10.1128/JCM.00180-11

Comparison of Performance of the Novel Chromogenic Spectra VRE Agar to That of Bile Esculin Azide and *Campylobacter* Agars for Detection of Vancomycin-Resistant Enterococci in Fecal Samples[▽]

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Received 28 January 2011/Returned for modification 3 March 2011/Accepted 21 August 2011

A total of 142 stool specimens were evaluated for vancomycin-resistant enterococcus (VRE). Twenty-four-hour sensitivities and specificities, respectively, were 98% and 95% for Spectra VRE chromogenic agar (Remel, Lenexa, KS), 86% and 92% for bile esculin azide with vancomycin (BEAV; Remel), and 96.5% and 92% for *Campylobacter* agar (CAMPY; Remel). Spectra VRE and CAMPY are significantly more sensitive at 24 h than BEAV.

Vancomycin-resistant enterococci (VRE) have emerged as important pathogens in many health care facilities (8). Although common gastrointestinal (GI) tract colonizers, VRE may also lead to extraintestinal infections, such as bacteremia and peritonitis (8, 9). An obstacle to control of the spread of VRE is the large, unrecognized cohort of patients with GI tract VRE colonization (12). Prompt, accurate identification of patients with VRE is imperative.

Various chromogenic VRE agars appear promising for use in VRE stool screening (1, 3, 4–6, 11). Chromogenic VRE media can reduce turnaround time to results through early visual colony identification. *Campylobacter* medium (CAMPY) with 5 antibiotics, including 10 μg/ml of vancomycin, is also a sensitive stool screening tool for VRE (8). CAMPY agar and bile esculin azide agar with 6 μg/ml of vancomycin (BEAV) usually require 24 to 48 h to preliminarily identify colonies, with additional time for confirmatory identification and susceptibility testing. Molecular VRE screening methods decrease the time to identification but are costly. Culture remains the screening method of choice for VRE stool screening, but molecular methods are becoming increasingly recognized (10).

The purpose of this study was to compare the performance of the new commercially available Spectra VRE agar (Spectra VRE; Remel, Thermo Fisher Scientific, Lenexa, KS) to that of bile esculin azide agar with 6 μg/ml of vancomycin (BEAV; Remel, Thermo Fisher Scientific, Lenexa, KS). The performance of *Campylobacter* agar with 10 μg/ml of vancomycin (CAMPY; Remel, Thermo Fisher Scientific, Lenexa, KS), which is our current VRE screening medium, was also compared. Ours is the second publication on the use of Spectra VRE with surveillance cultures, but it is the first to compare the performance of these 3 VRE screening media.

Fresh stool specimens submitted for unrelated *Clostridium difficile* testing from 142 unique intensive care unit (ICU) patients were directly inoculated to Spectra VRE, BEAV, and CAMPY, without broth enrichment. Each plate was inoculated with a separate swab which had been immersed in the stool specimen for several seconds. Excess stool was lightly tapped off the swab before inoculation, in order to maintain the same inoculum size. Cultures were incubated at 35°C in ambient air. On Spectra VRE, light blue to blue colonies were presumptively identified as *Enterococcus faecalis*, according to the package insert. Pink to light purple or navy blue colonies were presumptive *Enterococcus faecium*. Presumptive *Enterococcus* spp. appeared as black-pigmented colonies on BEAV and as small to medium colonies with alpha- or gamma-hemolysis on CAMPY. All plates were examined for growth after 18, 24, and 48 h. The plates were inspected independently by 2 different observers, whose results correlated with each other. In order to avoid bias in colony color interpretation, the Spectra VRE plates were read first, followed by BEAV and CAMPY. Suspect colonies were subcultured after 48 h to 5% sheep blood agar (BBL, BD, Franklin Lakes, NJ). Isolates were identified to the species level using the Vitek2 (bioMérieux, Durham, NC), as well as by Gram stain and classic biochemical testing (catalase, pyrrolidonyl arylamidase [PYR], arabinose, and methyl-α-D-glucopyranoside). Vancomycin MICs were determined by Etest (bioMérieux, Durham, NC) and interpreted by Clinical Laboratory and Standards Institute (CLSI) guidelines (2). Control organisms, including *E. faecalis* ATCC 51299, *E. faecium* ATCC 51559, and *E. faecalis* ATCC 29212, were tested each day of use; all ATCC strains performed as expected. Specimens with discrepant results, such as growth on one medium and not another, were not replated, as they had been previously deidentified.

VRE were recovered from 58/142 (40.8%) patients. Fifty-five patients harbored vancomycin-resistant (VR) *E. faecium*, and 4 patients harbored VR *E. faecalis.* (Two patients were colonized with both VR *E. faecium* and VR *E. faecalis.*) Vancomycin MICs exceeded 256 μg/ml for all *Enterococcus* spp. isolates tested. Sensitivities and specificities at 18 h were not calculated, as the colonies were too small for adequate assessment. Spectra VRE detected 57/58 VRE isolates by 24 h and

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▽ Published ahead of print on 31 August 2011.

TABLE 1. Performance characteristics of 3 media for detection of vancomycin-resistant *Enterococcus* spp.

Incubation period	Medium[a]	% sensitivity (95% CI)[b]	% specificity (95% CI)	% PPV (95% CI)	% NPV (95% CI)
24 h	Spectra VRE	98.3 (89.5–100)	95.2 (87.6–98.5)	93.4 (83.2–97.9)	98.8 (92.4–100)
	BEAV	86.2 (74.0–93.4)	91.7 (83.0–96.3)	87.7 (75.7–94.5)	90.6 (81.8–95.5)
	CAMPY	96.5 (87.0–99.4)	91.7 (83.0–96.3)	88.9 (77.8–95.0)	97.5 (90.3–99.6)
48 h	Spectra VRE	100 (92.3–100)	95.2 (87.6–98.4)	93.5 (83.5–97.9)	100 (94.3–100)
	BEAV	96.5 (87.0–99.4)	84.5 (74.6–91.2)	81.1 (69.6–89.2)	97.3 (89.6–99.5)
	CAMPY	100 (92.3–100)	89.3 (80.2–94.7)	86.6 (75.5–93.3)	100 (93.9–100)

[a] BEAV, bile esculin azide agar with 6 μg/ml of vancomycin; CAMPY, *Campylobacter* agar with 10 μg/ml of vancomycin; PPV, positive predictive value; NPV, negative predictive value.
[b] 95% CI, 95% confidence interval.

58/58 by 48 h. Four patient cultures exhibited growth of organisms other than VRE by 24 h on Spectra VRE. Such colonies included yeast from 2 patients (tiny, slightly colored at 24 h and blue at 48 h), and non-*Pseudomonas* Gram-negative bacilli from 2 patients (one purple at 24 h and both purple at 48 h). Most *E. faecium* isolates were purple; however, one-third were navy blue. The blue colonies of *E. faecium* at 24 and 48 h were difficult to differentiate from *E. faecalis*, as both were a darker shade of blue. Two *E. faecalis* isolates appeared light blue at 24 h but dark blue at 48 h. On BEAV, 50/58 VRE isolates were recovered by 24 h and 56/58 by 48 h. Organisms other than VRE grew on 13 patient cultures by 48 h. Such organisms included *Pseudomonas* spp. (black in color), various yeasts, *Pediococcus* spp., and enteric Gram-negative bacilli which were not further identified. On CAMPY, 56/58 VRE were detected by 24 h, compared to 58/58 detected at 48 h. Isolates other than VRE recovered from 9 specimens at 48 h included various yeasts, *Pseudomonas* spp., *Pediococcus* spp., and enteric Gram-negative bacilli which were not further identified.

The following 24 h sensitivities were obtained, using VRE positivity confirmed by Vitek2 on any medium at 48 h as the "gold standard": Spectra VRE, 98.3%; BEAV, 86.2%; and CAMPY, 96.5% (Table 1). The sensitivities of Spectra VRE and CAMPY agars increased to 100% at 48 h. The 24-h sensitivities of Spectra VRE and CAMPY were each significantly higher than the 24-h sensitivity of BEAV ($P < 0.05$, McNemar χ^2 two-sided test). However, the difference in sensitivities between Spectra VRE and CAMPY at 24 h was not significant ($P > 0.05$, McNemar χ^2 two-sided test). The sensitivity differences among media at 48 h were not significant. The single false-negative culture at 24 h by Spectra VRE was also negative at 24 h by the other methods and only demonstrated a few colonies at 48 h. One of the false-negative CAMPY plate specimens at 24 h was due to a low colony count; however, the false-negative BEAV cultures were not generally associated with low colony counts. Spectra VRE maintained a specificity of 95.2% at both 24 and 48 h, while the specificities for the other two agar types decreased by 48 h. In 5 cases, VRE isolates detected on both CAMPY and Spectra VRE at 24 h of incubation exhibited delayed growth on BEAV. VRE colonies were more easily visualized on Spectra VRE and BEAV than on CAMPY. There were no quantifiable differences in colony size for Spectra VRE compared to the other two agars.

The sensitivities of Spectra VRE and CAMPY were significantly higher than that of BEAV for detection of VRE from fecal specimens at 24 h, which is the manufacturer's recommended incubation time for Spectra VRE. Our data are similar to the sensitivities and specificities demonstrated for chromogenic VRE media in the literature, although the data vary according to whether prior broth enrichment was performed. Studies included use of Spectra VRE (Remel) (7), ChromID VRE (bioMérieux, Marcy l'Etoile, France) (3, 5), BBL CHROMagar VanRE (BD Diagnostics, Sparks, MD) (4, 11), and Chromogenic VRE (AES Laboratoire, Bruz Cedex, France) (1). Peterson and colleagues demonstrated a slightly higher specificity of Spectra VRE at 24 h. However, our study population of ICU patients was likely predisposed to a higher number of false-positive cultures, such as the Gram-negative rods seen on Spectra VRE in our study. Gram-negative rods were not reported to be contaminants in the Peterson study.

Some studies have suggested the addition of prior broth enrichment to improve sensitivity and specificity of chromogenic VRE agar (6). Directly plated stool specimens occasionally impart an inoculum color onto chromogenic media, aside from the colors due to colony growth. However, we encountered few problems due to inoculum effect when reading the chromogenic plates. Additionally, broth enrichment increases the time to identification.

Colonies of VRE were more easily visualized on Spectra VRE than on CAMPY. However, the distinction between the dark blue colors of VR *E. faecalis* and some of the VR *E. faecium* isolates was occasionally difficult to appreciate at or beyond 24 h. Approximately one-third of the VR *E. faecium* isolates in our study were dark blue and would require further identification, such as PYR and a Vitek identification card. A pink to light purple colony is easily recognizable as *E. faecium*. One limitation of our study was the low number of *E. faecalis* isolates with which to evaluate colony color. BEAV and CAMPY agars have no differential capabilities in distinguishing *E. faecalis* from *E. faecium*. Thus, further workup and identification must be performed on all suspicious isolates from BEAV and CAMPY plates.

The performance characteristics of CAMPY were similar to those reported for the *Campylobacter* medium manufactured by BD (Becton, Dickinson, and Company, Franklin Lakes, NJ) (8). To our knowledge, this is the first study reporting on the use of CAMPY agar by Remel for VRE screening. In approximately 10% of cases, VRE were detected 24 h earlier on Spectra VRE and CAMPY than on BEAV. BEAV plates should be read at 48 h of incubation to maximize sensitivity.

In conclusion, Spectra VRE demonstrated adequate sensitivity and specificity at 24 h for the detection of VRE from

stool specimens. The sensitivities of Spectra VRE and CAMPY at 24 h were not significantly different from each other but were higher than that of BEAV. Suspicious colonies were more easily visualized on Spectra VRE than on CAMPY and were sometimes evident 24 h earlier than on BEAV. Based on our data, due to the overlap in color of some isolates on Spectra VRE, it is suggested that dark blue colonies receive further workup and identification.

This study was funded in part by Remel, Thermo Fisher Scientific, Lenexa, KS.

REFERENCES

1. **Asir, K., K. Wilkinson, J. D. Perry, R. H. Reed, and F. K. Gould.** 2009. Evaluation of chromogenic media for the isolation of vancomycin-resistant enterococci from stool samples. Lett. Appl. Microbiol. **48:**230–233.
2. **Clinical and Laboratory Standards Institute.** 2010. Performance standards for antimicrobial susceptibility testing: 20th informational supplement M100-S20. NCCLS, Wayne, PA.
3. **Cuzon, G., T. Naas, N. Fortineau, and P. Nordmann.** 2008. Novel chromogenic medium for detection of vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis*. J. Clin. Microbiol. **46:**2442–2444.
4. **Kallstrom, G., C. D. Doern, and W. M. Dunne.** 2010. Evaluation of a chromogenic agar under development to screen for VRE colonization. J. Clin. Microbiol. **48:**999–1001.
5. **Kuch, A., E. Stefaniuk, T. Ozorowski, and W. Hryniewicz.** 2009. New selective and differential chromogenic agar medium, chromID VRE, for screening vancomycin-resistant *Enterococcus* species. J. Microbiol. Methods **77:** 124–126.
6. **Peltroche-Llacsahuanga, H., J. Top, J. Weber-Heynemann, R. Lutticken, and G. Haase.** 2009. Comparison of two chromogenic media for selective isolation of vancomycin-resistant enterococci from stool specimens. J. Clin. Microbiol. **47:**4113–4116.
7. **Peterson, J. F., et al.** 2010. Evaluation of Spectra VRE, a new chromogenic agar medium designed to screen for vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium*. J. Clin. Microbiol. **48:**4627–4629.
8. **Shigei, J., G. Tan, A. Shiao, L. M. de la Maza, and E. M. Peterson.** 2002. Comparison of two commercially available selective media to screen for vancomycin-resistant enterococci. Am. J. Clin. Pathol. **117:**152–155.
9. **Sood, S., M. Malhotra, B. K. Das, and A. Kapil.** 2008. Enterococcal infections and antimicrobial resistance. Indian J. Med. Res. **128:**111–121.
10. **Stamper, P. D., M. Cai, C. Lema, K. Eskey, and K. C. Carroll.** 2007. Comparison of the BD GeneOhm VanR assay to culture for identification of vancomycin-resistant enterococci in rectal and stool specimens. J. Clin. Microbiol. **45:**3360–3365.
11. **Stamper, P. D., et al.** 2010. Evaluation of BBL CHROMagar VanRE for detection of vancomycin-resistant enterococci in rectal swab specimens. J. Clin. Microbiol. **48:**4294–4297.
12. **Tacconelli, E., and M. A. Cataldo.** 2008. Vancomycin-resistant enterococci (VRE): transmission and control. Int. J. Antimicrob. Agents **31:**99–106.