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# Comparison of Medium, Temperature, and Length of Incubation for Detection of Vancomycin-Resistant *Enterococcus*

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***Campylobacter* (Campy; BD Diagnostics, Sparks, MD), Spectra VRE (Remel, Lenexa, KS), and bile-esculin-azide-vancomycin (BEAV; Remel) agars were compared for their ability to detect vancomycin-resistant enterococci (VRE) in 750 stool specimens. The media were compared at 24 h and 48 h of incubation at 35°C and 42°C. When incubated for 24 h at 35°C, Campy was the most sensitive (97.8%) and specific (99.9%) but was comparable to Spectra, which has a sensitivity of 95.6% and a specificity of 99.1%, whereas BEAV was significantly less sensitive (90%) and specific (96.1%). Incubation at 42°C or extended incubation at 35°C for 48 h yielded no advantage over incubation at 35°C for 24 h.**

Screening for vancomycin-resistant enterococci (VRE) has been suggested as a method for reducing the nosocomial transmission of this organism. VRE are more commonly found in patients that are in critical care units and those who have been on antibiotics. The most common specimens to screen for colonization with VRE are rectal swabs or stool specimens. Several selective and differential media have been developed and evaluated specifically for the purpose of screening for VRE (2–8, 10). *Campylobacter* (Campy) agar, which supports the growth of VRE and contains 10 μg/ml vancomycin, is readily available in most clinical laboratories due to its use in the plating of routine stool cultures to isolate *Campylobacter jejuni*. We have previously reported on the use of this medium for the detection of VRE (9). Due to its sensitivity and relatively low cost and the fact that it is already incorporated into most laboratory routines, it is a reasonable choice that has served as a cost-effective way to screen for this potential pathogen. In this present study, we compared Campy medium, which contains 10 μg/ml of vancomycin (BD Diagnostics, Sparks, MD) to two media designed for the detection of VRE, bile-esculin-azide-vancomycin agar (BEAV; Remel) and the newer chromogenic agar Spectra VRE Agar (Remel, Lenexa, KS), both of which contain 6 μg/ml of vancomycin. In addition, since incubation of stools at a higher temperature, as done for *C. jejuni* isolation, also selects for enterococci due to its ability to grow at this elevated temperature, the media were further evaluated at different temperatures, 35°C and 42°C, as well as at 24 h and 48 h of incubation.

Rectal swabs ($n = 730$) and stool specimens ($n = 20$) from 750 patients that were submitted to the Medical Microbiology Laboratory at the University of California, Irvine, Medical Center for VRE screening were included in this evaluation. Rectal swabs were collected using either a single or a double BBL CultureSwab (BD), while feces were submitted in a sterile container. All specimens were plated upon receipt or refrigerated at 4 to 8°C for up to 24 h prior to being cultured. In order to ensure an even distribution of the specimen for the culture media, swabs were placed into 0.5 ml of sterile saline (BD) and vortexed for 5 s. This suspension was then used to inoculate two sets of media, each consisting of the Campy, Spectra, and BEAV agars. Feces were inoculated directly onto the media. One set of plates was incubated at 35°C and the other was incubated at 42°C. In order to examine the value of incubating the plates for an additional 24 h, a subset of 544 cultures was examined after 24 h and 48 h of incubation.

All culture plates were screened for the presence of organisms resembling *Enterococcus* spp. Growth was recorded from 1+ to 4+, and cultures with ≤10 colonies/ml were noted. Colony morphology consistent with enterococci was used when evaluating Campy agar, while the Spectra and BEAV agars were evaluated based on typical colony appearance and color, as recommended by the manufacturer. Characteristic colors of VRE colonies were black on BEAV and light blue (*Enterococcus faecalis*) and navy blue to a pink-purple (*Enterococcus faecium*) on Spectra. Isolates that were confirmed to be Gram-positive cocci by a Gram stain were further tested for L-pyrrolidonyl arylamidase (PYR), arabinose utilization, and production of acid from methyl-α-D-glucopyranoside (MGP). The MIC to vancomycin was established for all *Enterococcus* spp. by the Etest (AB bioMérieux, Durham, NC) (1).

For the purpose of data analysis, a consensus gold standard to which all methods were compared was used to define a positive specimen. A positive specimen was one from which VRE was detected and confirmed using any of the three media at the stated incubation time and temperature. A true positive was defined as an isolate that phenotypically resembled *Enterococcus* on the media and was confirmed to be Gram positive, PYR positive, MGP negative, and intermediate or resistant to vancomycin (1). A false positive was defined as an isolate that phenotypically resembled *Enterococcus* and was PYR and MGP positive but was found to be vancomycin susceptible, with a MIC of ≤4 μg/ml.

Of the 750 specimens evaluated using the consensus gold standard for VRE detected after 24 h of incubation at 35°C, there were 90 (12.0%) specimens positive for VRE. From these specimens, 82 were confirmed to be *E. faecium*, 1 was *E. faecalis*, and 7 specimens contained both species. Campy agar was the most sensitive in that it detected 88/90 (97.8%) of the true-positive specimens, followed by Spectra agar, which detected 86/90 (95.6%), and BEAV agar

**Received** 23 February 2012 **Returned for modification** 19 March 2012
**Accepted** 18 April 2012
**Published ahead of print** 25 April 2012
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doi:10.1128/JCM.00479-12

**TABLE 1** Comparison of the three media examined after 24 h for detection of VRE from 750 rectal specimens[a]

| Medium | Incubation temp (°C) | No. of: | | | Sensitivity (%) | Specificity (%) | Predictive value (%) | |
|---|---|---|---|---|---|---|---|---|
| | | True positives | False negatives | False positives | | | Positive | Negative |
| Campy[b] | 35 | 88 | 2 | 1 | 97.8 | 99.9 | 99.0 | 99.7 |
| Spectra[b] | 35 | 86 | 4 | 6 | 95.6 | 99.1 | 93.5 | 99.4 |
| BEAV[b] | 35 | 81 | 9 | 26[c] | 90.0 | 96.1 | 75.7 | 98.6 |
| Campy[d] | 42 | 84 | 5 | 1 | 94.4 | 99.9 | 98.2 | 99.3 |
| Spectra[d] | 42 | 84 | 5 | 6 | 94.4 | 99.1 | 93.3 | 99.2 |
| BEAV[d] | 42 | 83 | 6 | 32[e] | 93.3 | 95.2 | 72.2 | 99.1 |

[a] There were 20 stool specimens and 730 rectal swabs.
[b] There were a total of 90 true positives by the consensus standard at 35°C incubation for 24 h.
[c] $P = 0.057$ by Fischer's exact test for the comparison of BEAV and Campy.
[d] There were a total of 89 true positives by the consensus standard at 42°C incubation for 24 h.
[e] $P = 0.0001$ and $P = 0.005$ for BEAV compared to Campy and Spectra, respectively.

was the least sensitive, detecting 81/90 (90%) of the true positives (Table 1).

To examine whether incubation for 24 h at 42°C would increase the detection of VRE over that after incubation at 35°C due to the selective advantage of the higher temperature, thereby suppressing other normal stool flora, the three agar plates were compared (Table 1). Taking into account only the consensus result from the three media incubated for 24 h at 42°C, there were 89 (11.9%) specimens positive for VRE, one less than when the media were compared at 35°C for 24 h. Campy and Spectra each detected 84 specimens positive for VRE at the higher incubation temperature, less than the numbers detected by each medium at 35°C for 24 h. As with incubation at 35°C, BEAV agar was less sensitive and specific in detecting VRE. Therefore, we found no advantage in using the higher temperature of incubation.

In general, when a VRE isolate was not detected by all three media, the growth of VRE on the positive medium was <10 colonies/plate. Therefore, most likely, the majority of the discrepant results were due to the low numbers of organisms in a given specimen. There was one exception in which there was 1+ to 2+ growth of VRE on both Spectra and BEAV yet VRE was not recovered on Campy. There were a total of 10 VRE isolates that were not detected by at least one of the three media on the initial cultures. Of these, nine were available for repeat testing using a standardized inoculum of 100 CFU/plate. Six of the isolates grew the expected number of colonies on all three media upon repeat testing. Of the remaining three isolates, each medium failed to support the growth of one.

Campy agar had the highest specificity, 99.9%, detecting only one false positive at both temperatures, followed by Spectra, which had a specificity of 99.1%, detecting six false positives at both temperatures. BEAV agar grew significantly more false positives, 26 and 32 at 35°C and 42°C, respectively, with specificities of 96.1% and 95.2% at each of the temperatures, respectively. Therefore, BEAV agar recovered fewer VRE, and there was considerable breakthrough of MGP-positive, non-*E. faecium* and non-*E. faecalis* enterococci that were not resistant to vancomycin.

To address whether extended incubation would recover more VRE, a subset of 544 specimens was incubated at 35°C and 42°C and read both at 24 h and 48 h (Table 2). There were 69/544 (12.7%) specimens positive for VRE when both incubation periods were considered to make up the consensus standard. Of these VRE isolates, 62 were *E. faecium*, 1 was *E. faecalis*, and 6 were mixed with both species. While the predictive values were slightly different from those obtained for the 750 specimens discussed above, the overall result was the same in that Campy agar was the most sensitive and specific, followed by Spectra agar, with the least sensitive and specific being BEAV agar. Extended incubation did result in 2 more VRE detected by BEAV and 1 more VRE detected by Spectra. All specimens that were discrepant between the 24-h and 48-h plates were those in which there were <10 CFU/plate. Therefore, similar to previous reports, there was no significant difference in overall results when incubation was extended to 48 h beyond the initial 24 h of incubation (4, 5, 6, 10).

Another factor that needs to be taken into consideration when choosing a medium for the routine screening of specimens for VRE is cost. Campy medium is approximately 5 and 10 times less expensive than Spectra and BEAV, respectively. However, when examining Campy agar, more technical experience is required to recognize colonies that phenotypically resemble enterococci. In addition, when using Campy agar, a Gram stain and PYR test are performed to confirm that the isolates are enterococci, while the appearance of the typical blue colony, light blue or navy blue for *E. faecalis* or *E. faecium*, respectively, on Spectra agar does not require further confirmation in most cases. Since the vancomycin concentration in the Campy agar is 10 μg/ml, the potential that a *vanB*-carrying enterococcal isolate may be missed exists; however, in this evaluation and those of others, isolates in this MIC range were not detected (5, 8).

In summary, this study is the first to have compared the three media Campy, Spectra VRE, and BEAV for the ability to identify specimens positive for VRE when incubated at both 35°C and 42°C. Corroborating previous reports, BEAV was found to be the

**TABLE 2** Predictive values of three media for the detection of VRE from 544 rectal specimens[a]

| Medium | Incubation time (h) | Sensitivity (%) | Specificity (%) | Predictive value (%) | |
|---|---|---|---|---|---|
| | | | | Positive | Negative |
| Campy | 24 | 94.2 | 100 | 100 | 99.1 |
| | 48 | 94.2 | 100 | 100 | 99.1 |
| Spectra | 24 | 91.3 | 98.7 | 91.3 | 98.7 |
| | 48 | 92.8 | 99.3 | 95.3 | 98.2 |
| BEAV | 24 | 85.5 | 96.8 | 79.7 | 97.9 |
| | 48 | 88.4 | 96.2 | 76.9 | 98.1 |

[a] There were 17 stool specimens and 527 rectal swabs. All plates were incubated at 35°C.

least sensitive and specific of the three media evaluated (5, 10). Extended incubation of the plates beyond 24 h and incubation of the plates at 42°C were of no benefit over 24 h of incubation at 35°C. The Campy and Spectra agars were similar in their performance for detection of VRE but differ considerably in cost.

## ACKNOWLEDGMENTS

We want to thank Remel, Thermal Fisher Scientific, for providing the Spectra and BEAV agar plates used in this study.

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