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NOTES

Comparative Study of Selective Chromogenic (chromID VRE) and Bile Esculin Agars for Isolation and Identification of *vanB*-Containing Vancomycin-Resistant Enterococci from Feces and Rectal Swabs[▽]

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The new chromogenic agar chromID VRE (cIDVRE; bioMérieux) was compared with bile esculin agar (BD) containing 6 mg/liter vancomycin for the detection of colonization with *vanB*-containing vancomycin-resistant enterococci (VRE). At 48 h of incubation, the results obtained with both media were comparable. However, cIDVRE detected significantly more VRE at 24 h (39.3% versus 21.3%, $P = 0.003$), and its use may facilitate the timely implementation of infection control procedures.

Vancomycin-resistant enterococci (VRE) are significant nosocomial pathogens, and both *vanA*- and *vanB*-containing strains of VRE may cause serious infections, including bacteremia (10, 17). In Australia, unlike the United States and Europe, *vanB*-containing VRE strains predominate (2, 6). The early detection of VRE may facilitate the timely implementation of infection control measures, and surveillance by examination of fecal and rectal swab specimens has been adopted as a means of reducing outbreaks caused by VRE (16). Recently, it has been reported from Europe and the United States that the use of new chromogenic agars improves the ability to detect VRE isolates from fecal and rectal swab specimens (5, 11, 12). ChromID VRE agar (cIDVRE; bioMérieux, Marcy-l'Etoile, France), which contains 8 mg/liter vancomycin, also has the advantage of differentiating *Enterococcus faecalis* and *Enterococcus faecium*, as it detects the β-glucosidase and the β-galactosidase produced by the two species respectively (5, 11, 12; cIDVRE product insert, reference no. 43 002, 2007; bioMérieux). *vanA*-containing VRE typically have vancomycin MICs of ≥64 mg/liter (4, 15) and would be expected to grow well in the presence of 8 mg/liter vancomycin. However, *vanB*-containing VRE strains, which may exhibit much lower vancomycin MICs, including MICs of ≤4 mg/liter (4, 8), may not be as readily isolated on cIDVRE as they are on bile esculin agar (Enterococcosel; BD, Sparks, MD) containing 6 mg/liter vancomycin (EVA). At Austin Health, where *vanB*-containing VRE predominate, we compared cIDVRE for the detection of vancomycin-resistant strains of *E. faecalis* and *E. faecium* with EVA for the isolation of VRE from fecal and rectal swab specimens.

Feces and rectal swabs were inoculated directly onto both cIDVRE and EVA. The media were inoculated with separate cotton-tipped swab sticks for fecal specimens. As only one rectal swab specimen was collected from each patient, rectal swabs with laboratory accession numbers that were an odd number were inoculated onto cIDVRE first and then onto EVA. Inoculation of the media was in the reverse order for even-numbered specimens. The cultures were incubated at 35°C in ambient air and were examined daily, with cIDVRE incubated for 48 h, according to the manufacturer's recommendations (cIDVRE product insert; bioMérieux), and EVA plates were incubated for 72 h, as reported previously (13). Strains producing β-galactosidase (violet color) and β-glucosidase (blue-green color) on cIDVRE and esculin-positive isolates (black color) on EVA were considered possible *E. faecium* or *E. faecalis* isolates. Only suspected colonies (three colonies of each suspicious morphological appearance; violet or blue-green on cIDVRE or black on EVA) that grew on either medium were investigated by using routine methods for the identification of enterococci (15). Isolates provisionally identified as *E. faecium* or *E. faecalis* (gram-positive cocci, pyrrolidonyl-β-naphthylamide hydrolase [PYR] positive, nonmotile, catalase negative, and pigment negative) were tested by methyl-α-D-glucopyranoside (MGP), *ddl* gene, and *van* gene (*vanA*, *vanB*, *vanC1*, *vanC2*, *vanC3*) PCRs (3, 7, 15) and vancomycin and teicoplanin Etest (AB Biodisk, Solna, Sweden) with a 0.5 McFarland inoculum, according to the manufacturer's instructions (1; Etest MIC determination product insert, 2007; AB Biodisk). Statistical analysis was performed by Fisher's exact test, with *P* values of less than 0.05 considered significant.

A total of 610 specimens (363 rectal swab specimens, 247

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TABLE 1. Specimen VRE culture results on cIDVRE at 48 h incubation versus specimen VRE culture results on EVA at 72 h

VRE culture result on cIDVRE at 48 h	No. of specimens with the following VRE culture result on EVA at 72 h:		
	Positive[a]	Negative	Total
Positive[a]	94	16	110
Negative	12	488	500
Total	106	504	610

[a] VRE-positive specimens were those with *vanB*-containing *E. faecalis* isolates and/or *vanB*-containing *E. faecium* isolates.

fecal specimens) were collected from 509 patients on predominantly high-risk wards (the renal and liver transplant wards and the intensive care unit [ICU] accounted for 69% of the specimens). A total of 122/610 (20%) specimens were VRE positive on one or both media. As both *E. faecium vanB* and *E. faecalis vanB* species were isolated from some of the VRE-positive specimens, a VRE-positive specimen was defined as any specimen from which *vanB*-containing *E. faecalis* and/or *vanB*-containing *E. faecium* was isolated. The culture results for the specimens are presented in Table 1. Overall, of all positive specimens, cIDVRE (48 h) and EVA (72 h) detected 110/122 (90.2%) and 106/122 (86.8%), respectively. The final VRE isolation rates were comparable ($P = 0.76$), with cIDVRE (48 h) identifying 110/610 (18.0%) VRE-positive specimens (95 with *E. faecium vanB* isolates, 9 with *E. faecalis vanB* isolates, and 6 with both *E. faecium vanB* and *E. faecalis vanB* isolates) and EVA (72 h) detecting 106/610 (17.3%) VRE-positive specimens (93 with *E. faecium vanB* isolates, 10 with *E. faecalis vanB* isolates, and 3 with both *E. faecium vanB* and *E. faecalis vanB* isolates). However, at 24 h of incubation, cIDVRE identified 48/122 (39.3%) of all the VRE-positive specimens, which was significantly higher than the rate of detection on EVA (26/122 [21.3%]; $P = 0.003$) but significantly less than that on cIDVRE at 48 h ($P < 0.001$). As shown in Table 2, VRE isolates from both media had a range of vancomycin MICs, including a small number with MICs of ≤4 mg/liter.

The growth of isolates other than *vanA*- or *vanB*-containing VRE was common on both media. In all, from 204 specimens cultured on cIDVRE, there were 271 suspicious colonial morphologies (violet or blue-green) on cIDVRE that were not confirmed to be VRE (yeasts, 126/271 [46.5%]; gram-negative bacilli, 56/271 [20.7%]; *van*-negative *E. faecalis*, 44/271 [16.2%], catalase-positive gram-positive cocci, 38/271 [14.0%], *van*-negative *E. faecium*, 4/271 [1.5%]; gram-positive bacilli, 3/271 [1.1%]). However, motile VRE were not isolated on cIDVRE, and yeast and gram-negative organisms demonstrated subtle differences in their colonial morphologies, which made identification easier. It is of interest that cIDVRE was significantly more specific at 24 h, with only 74 specimens found to have 80 colonies with suspicious colonial morphologies which were not confirmed to be VRE; 64/80 (80%) of these were yeasts or gram-negative bacilli. On EVA at 48 h, from 113 specimens there were 120 colonies with suspicious (black) colonial morphologies that were not VRE. These consisted of PYR-negative isolates (predominantly gram-positive cocci or gram-positive bacilli; 65/120 [54.2%]), enterococci other than *E. faecalis* and *E. faecium* (motile, MGP positive, pigment positive, and/or *vanC1* gene positive or *E. faecium* and *E. faecalis ddl* negative; 39/120 [32.5%]), catalase-positive gram-positive cocci (2/120 [1.7%]), *van*-negative *E. faecalis* isolates (12/120 [10.0%]), and *van*-negative *E. faecium* isolates (2/120 [1.7%]).

TABLE 2. Vancomycin MICs of all VRE isolates[a] from cIDVRE and EVA

Medium and isolate	No. (%) of isolates	No. of isolates with the following vancomycin MIC (mg/liter):		
		≤4	8–16	≥32
cIDVRE				
E. faecium	101 (87.1)	2	12	87
E. faecalis	15 (12.9)	1	0	14
Total	116	3	12	101
EVA				
E. faecium	96 (88.1)	1	17	78
E. faecalis	13 (11.9)	2	0	11
Total	109	3	17	89

[a] All VRE-positive isolates (*vanB*-containing *E. faecalis* or *vanB*-containing *E. faecium* isolates) also includes both species when more than one species was isolated from the same specimen.

This study has demonstrated that overall cIDVRE is comparable to EVA for the detection of *vanB*-containing VRE, but the rate of isolation of VRE on cIDVRE is significantly higher at 24 h of incubation. This outcome did not change if only one specimen (either the first or the last specimen) per patient was analyzed (data not shown). Notably, the rate of isolation of positive specimens on cIDVRE at 24 h (48/122 [39.3%]) was less than the 96.4% previously reported by Ledeboer et al. (11), who isolated predominantly *vanA*-containing VRE strains. However, *vanB*-containing VRE strains may exhibit much lower vancomycin MICs than *vanA*-containing VRE strains (4, 15), and thus, a longer incubation may be required. Interestingly, as shown in Table 1, a comparable number of isolates which had vancomycin MICs of ≤16 mg/liter were isolated from VRE-positive specimens on both media, even though cIDVRE contained a higher concentration of vancomycin (8 mg/liter in cIDVRE versus 6 mg/liter in EVA).

There was a high rate of isolation of non-VRE isolates on cIDVRE (271 isolates from 204 specimens), but importantly, 67.2% of the false-positive isolates were yeasts (126/271 [46.5%]) and gram-negative bacilli (56/271 [20.7%]). These isolates had subtle difference in their colonial morphologies compared to those of suspected VRE isolates, and their identities could easily be confirmed by microscopy with little delay. The members of our study population, which included a high proportion of transplant, dialysis, and ICU patients, were likely to be receiving antibiotic therapy; and this may explain the higher rates of isolation of yeasts and gram-negative bacilli than those reported by Delmas et al. (5). However, in our study the numbers of *van*-negative *Enterococcus* species were unexplainably higher than those found by Delmas et al. (23 from 1,007 specimens) (5). In our study, 48 false-positive isolates (from 610 specimens) on cIDVRE were either *van*-negative *E. faecium* or *van*-negative *E. faecalis*, which indicates that confirmatory molecular or phenotypic testing is necessary, contrary to the conclusions of Ledeboer et al. (11, 12). As confir-

matory tests were required, the comparative analysis of the yield of VRE was limited in this study. A molecular "gold standard" for the direct detection of VRE in fecal specimens would have been a useful comparator, but it would have been problematic because of the high rate of carriage of *vanB* in nonenterococcal isolates, even in healthy adults (9, 14, 18).

In conclusion, for the detection of *vanB*-containing VRE, cIDVRE is comparable to EVA, and cultures need to be incubated for only 48 h; however, the identification of isolates as VRE must still be confirmed. A key advantage of cIDVRE is that a significantly higher rate of isolation of VRE was detected after 24 h on cIDVRE than on EVA. This factor would allow the earlier notification of VRE colonization and facilitate the implementation of infection control strategies, thus minimizing the risk of exposure for VRE-negative patients.

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