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Low Vancomycin MICs and Fecal Densities Reduce the Sensitivity of Screening Methods for Vancomycin Resistance in Enterococci

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Active surveillance is part of a multifaceted approach used to prevent the spread of vancomycin-resistant enterococci (VRE). The impact of fecal density, the vancomycin MIC of the isolate, and the vancomycin concentration in liquid medium on test performance are uncertain. Using fecal specimens spiked with a collection of 18 VRE (predominantly *vanB*) with a wide vancomycin MIC range, we compared the performances of commercial chromogenic agars (CHROMagar VRE, chromID VRE, Brilliance VRE, and VRE Select) and 1 liquid medium (Enterococcosel enrichment broth) for VRE detection. The specificity of solid media was excellent; however, the sensitivity at 48 h varied from 78 to 94%. Screening using liquid medium was less sensitive than screening with solid media, particularly as the vancomycin content increased. Sensitivity declined (i) as the fecal VRE density decreased, (ii) when the media were assessed at 24 h (versus 48 h), and (iii) for isolates with a low vancomycin MIC (sensitivity, 25 to 75% versus 100% for isolates with vancomycin MIC of <16 mg/liter versus >32 mg/liter on solid medium using 10^6 CFU/ml of feces). Depending on local epidemiology and in particular VRE vancomycin MICs, the sensitivity of culture-based methods for VRE screening of stool or rectal specimens may be suboptimal, potentially facilitating secondary transmission.

Active surveillance is part of a multifaceted infection control approach used to prevent the spread of vancomycin-resistant enterococci (VRE) (1, 2). The ability to detect VRE-colonized patients allows prompt implementation of infection control measures to interrupt the transmission cycle, whereas exclusion of VRE colonization reduces the impact of such activity on patient care and hospital workflow.

A variety of in-house and commercial chromogenic solid and liquid media are available for VRE screening in stool or rectal swab specimens. Test performance depends upon a number of variables that may relate to the patient, the specimen, the assay, or the isolate. Previous studies have suggested that the vancomycin MIC of VRE is a determinant of test sensitivity and that the optimal screening method is hence likely to be dependent upon local VRE epidemiology (3, 4). Historically, Australian VRE epidemiology has differed from that in either North America or Europe (5–9), as it is dominated by *vanB Enterococcus faecium*, of which certain clones have low vancomycin MICs, creating unique challenges for detection during active surveillance (4, 10, 11). In the 2011 Australian Group on Antimicrobial Resistance *Enterococcus* Sepsis Surveillance program, which examined enterococci obtained from blood cultures from 29 institutions across Australia (12), 20 of 124 (16.1%) *vanB E. faecium* isolates had a vancomycin MIC at or below the Clinical and Laboratory Standards Institute (CLSI) susceptibility breakpoint of ≤4 mg/liter (13). A further 33 isolates (26.6%) had a vancomycin MIC within the CLSI intermediate category of 8 to 16 mg/liter. However, recent studies have demonstrated a significant presence of *vanB E. faecium* in both North America and Europe (3, 14, 15). *vanB* VRE are now more prevalent than *vanA* VRE in several European centers, including Sweden (16), Spain (17), and Germany (18), while recent Canadian national surveillance demonstrates that *vanB* strains constitute 10% of all their VRE (19).

The purpose of this study was to compare the performances of four commercial chromogenic VRE agars and a liquid medium for VRE screening of fecal specimens. CHROMagar VRE (CHROMagar, Paris, France), chromID VRE (bioMérieux, Marcy l'Étoile, France), Brilliance VRE (Oxoid, Basingstoke, United Kingdom), VRE Select (Bio-Rad Laboratories, Hercules, CA, USA), and Enterococcosel enrichment broth (EVB; Becton, Dickinson, Cockeysville, MD, USA) with various vancomycin concentrations were evaluated using fecal specimens spiked with various concentrations of a panel of 18 well-characterized *vanA*- or *vanB*-positive enterococcal isolates with a broad vancomycin MIC range.

MATERIALS AND METHODS

A VRE was defined as an enterococcal isolate that possessed either the *vanA* or *vanB* gene regardless of the vancomycin MIC and its relationship to susceptibility breakpoints. This definition has practical validity, as from an infection control perspective, the ability for the resistance mechanism to disseminate is dependent upon the presence of the gene, not the resistance phenotype. Additionally, the *vanA* and *vanB* genes are inducible, and hence, MIC expression may be variable (4).

Eighteen well-characterized enterococcal strains (14 *E. faecium* and 4 *Enterococcus faecalis* strains) that reflected contemporary Australian VRE epidemiology were selected from the Australian Collaborating Centre for *Enterococcus* and *Staphylococcus* Species (ACCESS) Typing and Research collection (Table 1). An enterococcal isolate possessing the *vanC* gene with a vancomycin MIC in the CLSI intermediate category was included in the study (*Enterococcus gallinarum* ATCC 49608), as was a fully susceptible enterococcal strain (*E. faecalis* ATCC 29212). The vancomycin MIC of each isolate was determined by Etest (bioMérieux, France). *vanA* and

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TABLE 1 Characteristics of the study isolates[a]

Enterococcal species	*vanA* or *vanB* genotype	MLST	Vancomycin MIC (mg/liter)
E. faecium	*vanB*	78	2
	vanB	78slv	16
	vanB	78	8
	vanB	78	16
	vanB	203	8
	vanB	203	12
	vanB	203	16
	vanB	203	24
	vanB	203	>256
	vanB	18	16
	vanB	17	>256
	vanB	173	>256
	vanA	17	24
	vanA	137	>256
E. faecalis	*vanB*	ND	32
	vanB	ND	>256
	vanB	ND	>256
	vanA	ND	>256
E faecalis (ATCC 29212)	Negative	ND	4
E. gallinarum (ATCC 49608)	Negative	ND	8

[a] MLST, multilocus sequence type; slv, single-locus variant; ND, not done.

vanB gene PCR and multilocus sequence typing (MLST) was performed using previously described methods (20, 21).

Randomly selected inpatient fecal samples were tested for the presence of *vanA* and *vanB* genes (22). Three negative samples were pooled to make a fecal suspension to which each test isolate was added to create working concentrations of 10^4 CFU/ml and 10^6 CFU/ml of feces, the latter being the usual VRE fecal density in patients colonized with VRE (23). Ten microliters of each spiked fecal suspension was directly inoculated onto four commercial chromogenic VRE agars (CHROMagar VRE, chromID VRE, Brilliance VRE, and VRE Select). All plates were incubated according to the manufacturers' recommendations. Growth was assessed at 24 and 48 h. Recovery of the original test isolate was established by identification of suspect isolates (based on their colony morphology as per the manufacturer's instructions) using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and PCR for *vanA* and *vanB* genes.

The performance of each direct plating method was assessed according to its sensitivity for recovery of the test VRE isolate, heterogeneity of recovered VRE colonies, ability to suppress fecal flora, and ability to suppress growth of the isolate possessing *vanC* with an elevated vancomycin MIC (*E. gallinarum* ATCC 49608). In order to facilitate the analysis of the impact of vancomycin MIC on the sensitivity of each method, isolates were arbitrarily categorized as having vancomycin MICs in the low (<16 mg/liter), medium (16 to 32 mg/liter), or high (>32 mg/liter) range.

Ten microliters of spiked fecal suspensions was also inoculated into EVB containing esculin, bile, sodium azide, and either 4, 6, or 8 mg/liter of vancomycin. Following 24 and 48 h of incubation at 35°C, broths that displayed evidence of esculin hydrolysis (i.e., turned black) were subcultured onto solid agar. The presence of the test VRE isolate was confirmed as previously. The vancomycin concentration of EVBs was assessed by high-performance liquid chromatography on a weekly basis without evidence of significant change in concentrations (data not provided).

RESULTS

Table 2 summarizes the sensitivity for recovery of the VRE isolates from the solid media using a fecal density of 10^4 CFU/ml. At 48 h, the sensitivity of CHROMagar VRE (94%) and that of chromID VRE (94%) were superior to that of either Brilliance VRE (83%) or VRE Select (78%). When the same analysis was done using a higher fecal density of 10^6 CFU/ml (Table 3), the only difference at 48 h was an improvement in the sensitivity of VRE Select to 94%.

Tables 4 and 5 demonstrate that regardless of the fecal density used, at 48 h the recovery of VRE from liquid medium was consistently lower than that for commercial chromogenic agars and was particularly poor when the EVB vancomycin concentration was highest (8 mg/liter).

The impact of the test isolate's vancomycin MIC category was remarkably consistent, with low-MIC isolates being recovered less frequently. The reduced sensitivity was evident across both fecal densities, regardless of the commercial chromogenic agar used or the vancomycin concentration of the EVBs (Tables 2 to 5).

All four commercial solid media suppressed fecal flora, resulting in no background growth on the plates. *E. faecalis* ATCC 29212 was suppressed on all four media. *E. gallinarum* ATCC 49608 growth was present after 24 h of incubation on CHROMagar VRE and VRE Select but was easily distinguished from either *E. faecalis* or *E. faecium* on the basis of colony color as per the manufacturer's instructions. Brilliance VRE and chromID VRE suppressed *E. gallinarum* at 48 h.

CHROMagar VRE gave minimal colony heterogeneity (size and color), whereas VRE Select gave marked colony variation (three types) that persisted even at 48 h. Brilliance VRE and chromID VRE gave a slight variation initially, consisting of one or two colonies of a lighter-than-expected color, which resolved after 48 h.

DISCUSSION

Numerous variables impact the performance of phenotypic screening methods, making comparisons difficult. By using a homogenous fecal suspension spiked with a large collection of well-characterized VRE isolates, our study allowed us to closely examine the impact of several of these variables on test performance,

TABLE 2 Recovery from solid media using an inoculum of 10^4 CFU/ml of test strain, categorized according to the vancomycin MIC of the test isolates

Vancomycin MIC (mg/liter)	No. of isolates	No. of isolates recovered (% sensitivity)							
		CHROMagar VRE		chromID VRE		Brilliance VRE		VRE Select	
		24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
<16	4	2 (50)	3 (75)	2 (50)	3 (75)	1 (25)	1 (25)	0	1 (25)
16–32	7	7 (100)	7 (100)	6 (85)	7 (100)	7 (100)	7 (100)	2 (28)	6 (85)
>32	7	7 (100)	7 (100)	7 (100)	7 (100)	7 (100)	7 (100)	7 (100)	7 (100)
Overall	18	16 (89)	17 (94)	15 (83)	17 (94)	15 (83)	15 (83)	9 (50)	14 (78)

TABLE 3 Recovery from solid media using an inoculum of 10^6 CFU/ml of test strain, categorized according to the vancomycin MIC of the test isolates

Vancomycin MIC (mg/liter)	No. of isolates	No. of isolates recovered (% sensitivity)							
		CHROMagar VRE		chromID VRE		Brilliance VRE		VRE Select	
		24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
<16	4	2 (50)	3 (75)	3 (75)	3 (75)	1 (25)	1 (25)	0	3 (75)
16–32	7	7 (100)	7 (100)	7 (100)	7 (100)	7 (100)	7 (100)	5 (71)	7 (100)
>32	7	7 (100)	7 (100)	7 (100)	7 (100)	7 (100)	7 (100)	7 (100)	7 (100)
Overall	18	16 (89)	17 (94)	17 (89)	17 (94)	15 (83)	15 (83)	12 (67)	17 (94)

including the vancomycin MIC of the isolate, the fecal density of the test isolate, and the vancomycin concentration of the liquid medium.

When using a fecal VRE density of 10^6 CFU/ml, and when results were read at 48 h, the sensitivities of CHROMagar VRE, chromID VRE, and VRE Select were excellent (94%). However, the sensitivity at 24 h ranged from 67 to 94%, which has important implications for hospitals that depend upon short turnaround times for results of VRE screening. According to the manufacturer's instructions, only VRE Select is recommended to be incubated for less than 48 h. Our findings suggest that chromogenic media may need to be incubated for 48 h to ensure adequate sensitivity.

In keeping with other studies, we found that the sensitivity of the liquid medium and some solid media was reduced as the fecal density of VRE was lowered, which may be influenced by concurrent antibiotic use by the patient (24). The advantage of identifying patients with low fecal VRE densities is uncertain, as such patients may be less likely to contribute to secondary VRE transmission.

In this study, we showed that all solid media were suboptimal for detection of enterococci with vancomycin MICs of <16 mg/liter. Low sensitivity for recovery of *vanB* VRE with low vancomycin MICs from commercial chromogenic agars has been demonstrated previously (3, 25). In both of these studies, the sensitivity ranged from 94 to 98%; however, these studies are not necessarily comparable to our study for several reasons. First, they inoculated plates with pure cultures (versus spiked fecal specimens); next, they did not attempt to use a standardized inoculum; and finally, they assessed a more limited number of chromogenic agars. The likely explanation for our study findings is that the vancomycin concentration used in the screening media was above the vancomycin MIC for a proportion of our test isolates. VRE screening media usually contain vancomycin concentrations ranging from 4 to 64 mg/liter (26). ChromID VRE contains 8 mg/liter vancomycin (27); however, we were unable to establish the glycopeptide content of the remaining three commercial solid media. Interestingly, supplementation of solid media with oxgall has been demonstrated to improve detection of *vanB* VRE and so may offer a means of improving test sensitivity (28). The reduced ability of screening media to detect isolates with low MICs is also an issue for detection of carbapenemase-producing *Enterobacteriaceae* (29) and methicillin-resistant *Staphylococcus aureus* (30).

Compared to screening with solid media, screening using EVB was less sensitive regardless of the vancomycin concentration in the broth. This is in contrast to results of Drews and colleagues (31), who reported 100% sensitivity for EVB when they tested 52 stool samples and rectal swabs. In keeping with North American VRE epidemiology, all their strains were *vanA* VRE, whereas we focused on *vanB* VRE, which usually have lower vancomycin MICs than *vanA* VRE, potentially explaining our contradictory findings. Not surprisingly, the combination of low VRE fecal density (10^4 CFU/ml) and high vancomycin concentration in broth (8 mg/liter) was associated with the lowest sensitivity (28% at 24 h and 67% at 48 h). A low vancomycin MIC of the test isolate appeared to adversely influence EVB test performance, with a sensitivity of ≤50% for detection of isolates with MIC of <16 mg/liter. The sensitivity of EVB improved with increased incubation time; thus, in the setting of VRE with low vancomycin MICs, EVB should be read at 48 h.

The specificity of the solid media was excellent, with complete suppression of all fecal microbiota members. Breakthrough growth of *E. gallinarum* ATCC 49608 on two of the media may have occurred, as this isolate has a vancomycin MIC that likely exceeded the vancomycin concentration on the screening media. However, the differing appearances of *E. gallinarum*, *E. faecium*, and *E. faecalis* on the chromogenic agars would prevent this from translating into increased laboratory workload. Previous studies of the commercial chromogenic agars used in our study had found significant breakthrough growth of yeasts and Gram-negative bacilli (27, 32, 33). However, these isolates were easily distinguish-

TABLE 4 Recovery from Enterococcosel enrichment broth using an inoculum of 10^4 CFU/ml of test strain, categorized according to the vancomycin concentration in the broth and the vancomycin MIC of the test isolates

Vancomycin MIC (mg/liter)	No. of isolates	No. of isolates recovered (% sensitivity) in EVB[a] with vancomycin concn of:					
		4 mg/liter		6 mg/liter		8 mg/liter	
		24 h	48 h	24 h	48 h	24 h	48 h
<16	4	1 (25)	2 (50)	1 (25)	2 (50)	1 (25)	2 (50)
16–32	7	3 (43)	5 (71)	2 (28)	5 (71)	0	5 (71)
>32	7	5 (71)	6 (85)	5 (71)	6 (85)	4 (57)	5 (71)
Overall	18	9 (50)	13 (72)	8 (44)	13 (72)	5 (28)	12 (67)

[a] EVB, Enterococcosel enrichment broth.

TABLE 5 Recovery from Enterococcosel enrichment broth using an inoculum of 10^6 CFU/ml of test strain, categorized according to the vancomycin concentration in the broth and the vancomycin MIC of the test isolates

Vancomycin MIC (mg/liter)	No. of isolates	No. of isolates recovered (% sensitivity) in EVB[a] with vancomycin concn of:					
		4 mg/liter		6 mg/liter		8 mg/liter	
		24 h	48 h	24 h	48 h	24 h	48 h
<16	4	2 (50)	2 (50)	2 (50)	2 (50)	1 (25)	2 (50)
16–32	7	5 (71)	7 (100)	5 (71)	7 (100)	4 (57)	7 (100)
>32	7	6 (85)	7 (100)	6 (85)	7 (100)	6 (85)	7 (100)
Overall	18	13 (72)	16 (89)	13 (72)	16 (89)	11 (61)	16 (89)

[a] EVB, Enterococcosel enrichment broth.

able from VRE, and thus no additional workup was required. This discrepancy could have arisen because of the organisms present in the fecal suspension used in our study.

The explanation for the colony variations observed with some solid media warrants further study, as it potentially creates an increased workload for laboratory staff when selecting colonies from the agar for additional workup. It may reflect the influence of proprietary ingredients within different chromogenic agars or heterogeneity that may be due to the presence of subpopulations with various vancomycin MICs.

In conclusion, while all four chromogenic media demonstrated excellent specificity, their ability to detect VRE with low vancomycin MICs is suboptimal. Screening using EVB was less sensitive than with any of the solid media, particularly when the broth contained high vancomycin concentrations. Our study highlights the limitations of phenotypic methods that rely upon their glycopeptide content to select for growth of VRE whose vancomycin MIC is variable. Genotypic methods that directly detect the *vanA* and/or *vanB* gene may circumvent this problem, although detection of the *vanB* gene should still be accompanied by confirmatory culture to ensure specificity, as other members of the bowel microbiota may also possess the *vanB* gene (34).

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