JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1996, p. 2042–2044 Vol. 34, No. 8
0095-1137/96/$04.00+0

Evaluation of Commercial Vancomycin Agar Screen Plates for Detection of Vancomycin-Resistant Enterococci

KENNETH G. VAN HORN,[1,2]* CHERYL A. GEDRIS,[1] KAREN M. RODNEY,[1] AND JOHN B. MITCHELL[1]

Department of Clinical Pathology, Westchester County Medical Center,[1] and Department of Pathology, New York Medical College,[2] Valhalla, New York 10595

Received 29 January 1996/Returned for modification 25 March 1996/Accepted 16 May 1996

Brain heart infusion–6-μg/ml vancomycin agar plates obtained from five commercial sources (B-D Microbiology Systems, Carr-Scarborough Microbiologicals, MicroBio Products, PML Microbiologicals, and REMEL) were evaluated with 714 enterococci for detection of vancomycin resistance. All 465 (100%) vancomycin-resistant enterococci (MIC ≥ 32 μg/ml) were detected by each manufacturer's agar screen plate, and each manufacturer's agar screen plate detected at least 99% of the 177 vancomycin-susceptible enterococci (MIC ≤ 4 μg/ml). Detection of the 72 vancomycin-intermediate enterococci (MIC = 6 to 16 μg/ml) ranged from 94% for B-D Microbiology Systems to 99% for PML Microbiologicals.

The enterococci have become major nosocomial pathogens, frequently causing urinary tract, surgical wound, and bloodstream infections (1, 6, 12). An increasing number of these infections are due to enterococci that are resistant to vancomycin (2, 5, 6). Accurate detection of vancomycin-resistant enterococci (VRE) is important so that appropriate therapy and infection control measures may be instituted. Current commercial antimicrobial susceptibility test systems and the disk diffusion method may not detect all VRE (16–19, 23–25).

An agar screen assay for detection of vancomycin resistance in enterococci was initially described by Willey et al. (23). Brain heart infusion agar (BHIA) plates with 6 μg of vancomycin per ml as recommended (9, 15) have recently become available from several commercial sources. The purpose of the present study was to evaluate the commercially available BHIA-vancomycin screen plates for detection of VRE.

(A preliminary report of this work was presented previously [20].)

A total of 714 recent clinical or rectal surveillance culture isolates of enterococci were tested for vancomycin resistance by five commercially prepared BHIA screen plate methods, with each plate supplemented with 6 μg of vancomycin per ml. The BHIA-vancomycin plates were obtained from B-D Microbiology Systems (BDMS) (Cockeysville, Md.), Carr-Scarborough Microbiologicals, Inc. (CSM) (Decatur, Ga.), MicroBio Products, Inc. (MBIO) (Tempe, Ariz.), PML Microbiologicals (Portland, Oreg.), and REMEL (Lenexa, Kans.). The BDMS plates were obtained as an investigational formulation. The test isolates were recent clinical isolates and included 177 vancomycin-susceptible enterococci (MIC ≤ 4 μg/ml); 465 VRE (MIC ≥ 32 μg/ml), including 436 *Enterococcus faecium*, 24 *Enterococcus faecalis*, and 5 *Enterococcus gallinarum* isolates; and 72 vancomycin-intermediate enterococci (MIC = 6 to 16 μg/ml), including 13 *E. faecalis*, 19 *Enterococcus casseliflavus*, and 40 *E. gallinarum* isolates.

The enterococci tested were all clinical isolates collected in this laboratory within the 12 months prior to testing. Each isolate was identified by conventional methods (3) and stored at 4°C until tested. Prior to testing, all isolates were subcultured at least twice on Trypticase soy–5% sheep blood agar (BDMS), and all tests were performed with isolates freshly grown (≤24 h) on Trypticase soy–5% blood agar. All BHIA-vancomycin plates were inoculated and incubated, and results were read according to the manufacturers' instructions. An inoculum suspension of each isolate equivalent to a 0.5 McFarland turbidity standard was prepared in sterile saline, and 10 μl (final inoculum, approximately 10^6 CFU/ml) was spot inoculated with a calibrated loop onto each of the five BHIA-vancomycin plates. Plates were incubated for a full 24 h in ambient air at 35°C. Growth of more than one colony indicated vancomycin resistance, and no growth indicated susceptibility. Quality control was performed with *E. faecalis* ATCC 29212, *E. faecium* WCMC strain 123 (vancomycin MIC > 256 μg/ml), and *E. gallinarum* WCMC strain R093 (vancomycin MIC = 8 μg/ml). A vancomycin E test (AB Biodisk, Culver City, Calif.), performed according to the manufacturer's instructions, was used to obtain all vancomycin MICs and was repeated when necessary to resolve any discrepancies.

Overall, the BHIA-vancomycin plates of the five manufacturers detected vancomycin-susceptible and -nonsusceptible (intermediate and resistant) enterococci with greater than 99% accuracy for the total 714 isolates tested (Table 1). All 465 (100%) of the VRE were accurately detected as resistant by each of the five BHIA-vancomycin plates. The BDMS, MBIO, and REMEL plates failed to grow each of the 177 (100%) vancomycin-susceptible enterococci. These results are similar to those of previous investigations of BHIA-vancomycin screen plates prepared in-house (15, 18). The REMEL BHIA-vancomycin formulation was previously investigated as part of the REMEL Synergy Quad plate (4) with results similar to those obtained in this study. There was only one discrepant vancomycin-susceptible isolate (Table 2), one of the two *E. casseliflavus* isolates (MIC = 4 μg/ml) which grew in small numbers, i.e., fewer than five colonies, on both the CSM and the PML plates. Growth of some enterococci, including *E. faecalis* and *vanC* enterococci for which MICs were near the susceptibility breakpoint of 4 μg of vancomycin per ml, on screen plates has been reported but appears to be rare (4, 15). The *vanC* enterococci, i.e., *E. casseliflavus* and *E. gallinarum*, have intrinsic resistance to vancomycin (7, 10), and most likely all strains should be considered vancomycin resistant regardless of the MIC and screen test results. No *E. faecalis* or *E. faecium* isolate for which the vancomycin MIC was ≤4 μg/ml grew on any BHIA-

* Corresponding author. Mailing address: Microbiology Laboratory, Department of Clinical Pathology, Westchester County Medical Center, Grasslands Rd., Valhalla, NY 10595. Phone: (914) 285-7502. Fax: (914) 285-1104. Electronic mail address: mail07931@pop.net.

TABLE 1. Detection of vancomycin resistance in *Enterococcus* spp. by five commercial vancomycin agar screen plates

MIC of vancomycin (μg/ml)	*Enterococcus* spp. (no. of isolates)	Total no. of isolates	Correct result (%) by plates from:				
			BDMS	CSM	MBIO	PML	REMEL
≤4	*Enterococcus* sp. (175), *E. casseliflavus* (2)	177	100	99	100	99	100
6–16	*E. faecalis* (13), *E. casseliflavus* (19), *E. gallinarum* (40)	72	94	97	96	99	97
≥32	*E. faecium* (436), *E. faecalis* (24), *E. gallinarum* (5)	465	100	100	100	100	100
Total		714	99.4	99.6	99.6	99.7	99.7

vancomycin plate. The enterococci used in this study were not tested for genetic relatedness or resistance markers, which could be a limitation of the study; however, previous studies have shown our population of VRE to be heterogeneous (8, 14).

The accuracy of detection of vancomycin-intermediate enterococci as resistant was somewhat lower, with a range of 94% for BDMS to 99% for PML (Table 1). One *E. gallinarum* isolate (MIC = 8 μg/ml) did not grow on the MBIO BHIA-vancomycin plate, and one (MIC = 6 μg/ml) did not grow on the BDMS BHIA-vancomycin plate. One *E. faecalis* isolate (MIC = 8 μg/ml) grew only on the MBIO BHIA-vancomycin plate, one (MIC = 6 μg/ml) grew only on the PML BHIA-vancomycin plate, and one (MIC = 6 μg/ml) grew only on the CSM, PML, and REMEL BHIA-vancomycin plates (Table 2). Errors at or near the resistance breakpoint of 6 μg of vancomycin per ml could be due to reader error with regard to either the BHIA-vancomycin plate or the E test MIC. The E test was used since vancomycin MICs obtained by this method compare favorably to MICs obtained by standard methods (13). Slight errors in interpretation of the E test MIC near the breakpoint might have led to some discrepant results, particularly with the two *E. faecalis* strains (MICs = 8 and 6 μg/ml) that each grew only on one commercial BHIA-vancomycin plate. The presence of *vanA* or *vanB* genes was not determined for any isolate in this study but might have helped to resolve these discrepancies. The performance of several formulations of BHIA from BDMS has been tested, with one modified formulation intended for industrial applications being found not as acceptable as others (15). The present BDMS plates do not use the referenced (15) modified BHIA formulation.

Fifty-nine of the 72 vancomycin-intermediate enterococci tested were *E. casseliflavus* and *E. gallinarum* isolates, which were detected as vancomycin resistant by most of the BHIA-vancomycin plates. These enterococci have not been associated with nosocomial outbreaks and are not yet considered an infection control problem, but they can be isolated from clinical specimens (3, 11) and from rectal surveillance cultures (21). Identification of these two enterococcal species is often necessary to prevent costly unwarranted infection control measures from being instituted, and yet several commercial systems fail to accurately identify them (21, 22). A simple test for motility at 30°C (3) should be performed on all enterococci resistant to vancomycin by a screen test method to rule out *E. casseliflavus* and *E. gallinarum*.

TABLE 2. Discrepancies with commercial BHIA-vancomycin screen plates

Organism	E test MIC (μg/ml)	Result[a] by plates from:				
		BDMS	CSM	MBIO	PML	REMEL
E. casseliflavus	4	S	R	S	R	S
E. faecalis	6	S	R	S	R	R
E. faecalis	6	S	S	S	R	S
E. gallinarum	6	S	R	R	R	R
E. faecalis	8	S	S	R	S	S
E. gallinarum	8	R	R	S	R	R
Total no. of discrepancies		4	3	3	2	2

[a] S, susceptible; R, resistant. Discrepant results are underlined.

While all five manufacturers recommend an inoculum of 1 to 10 μl, we inoculated all plates with 10 μl or approximately 10^6 CFU of enterococci per ml. The 1-μl (10^5 CFU/ml) and 10-μl inocula have been shown to yield equivalent results by vancomycin agar screen plate methods (4, 15, 18). A 10^6-CFU/ml inoculum may result in an inoculum haze that can be interpreted as growth, particularly when read by less-experienced technologists (18). This was not considered a problem in this study, since almost no false resistance was detected.

The BHIA-vancomycin screen plates manufactured by BDMS, CSM, MBIO, PML, and REMEL incorporate National Committee for Clinical Laboratory Standards recommendations (9) for screening of enterococci for vancomycin resistance and are accurate for detection of VRE. All enterococci that grow on the vancomycin screen plates should be identified at least by a motility test to rule out *vanC* isolates and confirmed as vancomycin resistant by a reliable susceptibility method.

We thank B-D Microbiology Systems; Carr-Scarborough Microbiologicals, Inc.; MicroBio Products, Inc.; PML Microbiologicals; and REMEL for supplying the BHIA-vancomycin agar screen plates.

REFERENCES

1. **Banerjee, S. N., T. G. Emori, D. H. Culver, R. P. Gaynes, W. R. Jarvis, T. Horna, J. R. Edwards, J. Tolson, T. Henderson, W. J. Martone, and the National Nosocomial Infections Surveillance System.** 1991. Secular trends in nosocomial primary bloodstream infections in the United States, 1980–1989. Am. J. Med. **91**(Suppl. 3B)**:**86s–89s.
2. **Centers for Disease Control.** 1993. Nosocomial enterococci resistant to vancomycin—United States, 1989–1993. Morbid. Mortal. Weekly Rep. **42:**597–599.
3. **Facklam, R. R., and M. D. Collins.** 1989. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. J. Clin. Microbiol. **27:**731–734.
4. **Free, L., and D. F. Sahm.** 1995. Investigation of the reformulated Remel Synergy Quad plate for detection of high-level aminoglycoside and vancomycin resistance among enterococci. J. Clin. Microbiol. **33:**1643–1645.
5. **Frieden, T. R., S. S. Munsiff, D. E. Low, B. M. Willey, G. Williams, Y. Faur, W. Eisner, S. Warren, and B. Kreiswirth.** 1993. Emergence of vancomycin-resistant enterococci in New York City. Lancet **342:**76–80.
6. **Grey, J. W., and S. J. Pedler.** 1992. Antibiotic-resistant enterococci. J. Hosp. Infect. **21:**1–14.
7. **Leclercq, R., S. Dutka-Malen, J. Duval, and P. Courvalin.** 1992. Vancomycin resistance gene *vanC* is specific to *Enterococcus gallinarum*. Antimicrob. Agents Chemother. **36:**2005–2008.
8. **Montecalvo, M. A., H. deLencastre, M. Carraher, C. Gedris, M. Chung, K. Van Horn, and G. P. Wormser.** 1995. Natural history of colonization with vancomycin-resistant *Enterococcus faecium*. Infect. Control Hosp. Epidemiol. **16:**680–685.
9. **National Committee for Clinical Laboratory Standards.** 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically,

3rd ed. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
10. **Navarro, F., and P. Courvalin.** 1994. Analysis of genes encoding D-alanine–D-alanine ligase-related enzymes in *Enterococcus casseliflavus* and *Enterococcus flavescens*. Antimicrob. Agents Chemother. **38:**1788–1793.
11. **Ruoff, K. L., L. de la Maza, M. J. Murtagh, J. D. Spargo, and M. J. Ferraro.** 1990. Species identities of enterococci isolated from clinical specimens. J. Clin. Microbiol. **28:**435–437.
12. **Schaberg, D. R., D. H. Culver, and R. P. Gaynes.** 1991. Major trends in the microbial etiology of nosocomial infections. Am. J. Med. **91**(Suppl. 3B)**:**72s–75s.
13. **Schulz, J. E., and D. F. Sahm.** 1993. Reliability of the E test for detection of ampicillin, vancomycin, and high-level aminoglycoside resistance in *Enterococcus* spp. J. Clin. Microbiol. **31:**3336–3339.
14. **Shay, D. K., S. A. Maloney, M. Montecalvo, S. Banerjee, G. P. Wormser, M. J. Arduino, L. A. Bland, and W. R. Jarvis.** 1995. Epidemiology and mortality risk of vancomycin-resistant enterococcal bloodstream infections. J. Infect. Dis. **172:**993–1000.
15. **Swenson, J. M., N. C. Clark, M. J. Ferraro, D. F. Sahm, G. Doern, M. A. Pfaller, L. B. Reller, M. P. Weinstein, R. J. Zabransky, and F. C. Tenover.** 1994. Development of a standardized screening method for detection of vancomycin-resistant enterococci. J. Clin. Microbiol. **30:**1700–1704.
16. **Swenson, J. M., M. J. Ferraro, D. F. Sahm, P. Charache, The National Committee for Clinical Laboratory Standards Working Group on Enterococci, and F. C. Tenover.** 1992. New vancomycin disk diffusion breakpoints for enterococci. J. Clin. Microbiol. **30:**2525–2528.
17. **Swenson, J. M., B. C. Hill, and C. Thornsberry.** 1989. Problems with the disk diffusion test for detection of vancomycin resistance in enterococci. J. Clin. Microbiol. **27:**2140–2142.
18. **Tenover, F. C., J. M. Swenson, C. M. O'Hara, and S. A. Stocker.** 1995. Ability of commercial and reference antimicrobial susceptibility testing methods to detect vancomycin resistance in enterococci. J. Clin. Microbiol. **33:**1524–1527.
19. **Tenover, F. C., J. Tokars, J. Swenson, S. Paul, K. Spitalny, and W. Jarvis.** 1993. Ability of clinical laboratories to detect antimicrobial agent-resistant enterococci. J. Clin. Microbiol. **31:**1695–1699.
20. **Van Horn, K., C. Gedris, and K. Rodney.** 1995. Evaluation of commercial vancomycin agar screen plates for detection of vancomycin-resistant *Enterococcus* species (VRE), abstr. D26, p. 71. *In* Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
21. **Van Horn, K., K. Rodney, L. Freundlich, and V. Aning.** 1995. Prevalence and microbiology of motile enterococci, *E. casseliflavus* (EC), and *E. gallinarum* (EG), abstr. C-294, p. 51. *In* Abstracts of the 95th General Meeting of the American Society for Microbiology. American Society for Microbiology, Washington, D.C.
22. **Willey, B. M., B. N. Kreiswirth, A. E. Simor, Y. Faur, M. Patel, G. Williams, and D. E. Low.** 1993. Identification and characterization of multiple species of vancomycin-resistant enterococci, including an evaluation of Vitek software version 7.1. J. Clin. Microbiol. **31:**2777–2779.
23. **Willey, B. M., B. N. Kreiswirth, A. E. Simor, G. Williams, S. R. Scriver, A. Philips, and D. E. Low.** 1992. Detection of vancomycin resistance in *Enterococcus* species. J. Clin. Microbiol. **30:**1621–1624.
24. **Zabransky, R. J., A. R. DiNuzzo, M. B. Huber, and G. L. Woods.** 1994. Detection of vancomycin resistance in enterococci by the Vitek AMS system. Diagn. Microbiol. Infect. Dis. **20:**113–116.
25. **Zabransky, R. J., A. R. DiNuzzo, and G. L. Woods.** 1995. Detection of vancomycin resistance in enterococci by the Alamar MIC system. J. Clin. Microbiol. **33:**791–793.