JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1998, p. 592–594 Vol. 36, No. 2
0095-1137/98/$04.00+0

Comparison of Eight Methods To Detect Vancomycin Resistance in Enterococci

HUBERT P. ENDTZ,[1]* NICOLE VAN DEN BRAAK,[1] ALEX VAN BELKUM,[1] WIL H. GOESSENS,[1] DEBORAH KREFT,[1] A. BARNARD STROEBEL,[2] AND HENRI A. VERBRUGH[1]

Erasmus University Medical Center Rotterdam[1] and St. Clara Hospital,[2] Rotterdam, The Netherlands

Received 21 August 1997/Returned for modification 10 October 1997/Accepted 13 November 1997

A collection of genetically unrelated vancomycin-resistant enterococci (VRE) including 50 *vanA*, 15 *vanB*, 50 *vanC1*, and 30 *vanC2* VRE were used to evaluate the accuracy of eight currently available susceptibility test methods (agar dilution, disk diffusion, E-test, agar screen plate, Vitek GPS-TA and GPS-101, and MicroScan overnight and rapid panels). *vanA* VRE were detected by all methods. *vanB* VRE were often not detected by Vitek GPS-TA and MicroScan rapid (sensitivities, 47 and 53%, respectively), though the new Vitek GPS-101 was found to be a significant improvement. E-test and the agar screen were the only two methods detecting all VRE, including the *vanC1*/*C2* VRE.

The rapid increase in the incidence of infections with vancomycin-resistant enterococci (VRE) in the western hemisphere is reason for great concern (8). The Hospital Infection Control Practices Advisory Committee recently published recommendations for preventing the spread of vancomycin resistance (4). An important role is sought for the microbiology laboratories as they, through accurate and timely detection of resistance, are the first line of defense. To date, several studies have been done assessing the accuracy of various antimicrobial susceptibility methods in detecting vancomycin resistance in enterococci (11–17). Since the occurrence of VRE is increasing in the United States (1) and is likely to increase in Europe as well, it is crucial to optimize the laboratory's ability to detect vancomycin resistance.

Three different genotypes (*vanA*, *vanB*, and *vanD*) have been described that encode either high-, intermediate-, or low-level acquired glycopeptide resistance, mainly in *Enterococcus faecium* and *Enterococcus faecalis* (6). In addition, a fourth genotype (*vanC*) has been found in *Enterococcus gallinarum* and *Enterococcus casseliflavus*. This genotype encodes intrinsic, low-level resistance to vancomycin but not to teicoplanin. Antimicrobial susceptibility tests may have problems detecting the low-level glycopeptide resistance phenotype (VanB or VanC). To date, some reports have shown failure of several automated susceptibility tests to detect vancomycin resistance (16, 17). In response, the manufacturers of the Vitek system (BioMerieux, Marcy l'Etoile, France) developed a new gram-positive susceptibility card (GPS-101) and updated the software to overcome this problem. Thus, the objective of this study was to evaluate the accuracy of seven currently available commercial methods, including the Vitek GPS-101 card, to detect VRE compared to a reference agar dilution method (9). A collection of fully characterized VRE strains, representing all the above-mentioned genotypes and phenotypes, was used in this study. One hundred and ninety-five enterococci, including 50 *vanA*, 15 *vanB*, and 50 *vanC1* VRE (*E. gallinarum*) and 30 *vanC2* VRE (*E. casseliflavus*) were isolated from patients or poultry products in Europe; the remaining 50 strains lacked these resistance markers and were fully susceptible to vancomycin. Identification of *Enterococcus* spp. was made on the basis of colonial morphology, pigment production, Gram stain, catalase, pyrrolidonyl arylamidase, and Lancefield group D antigen and by the API 32 rapid system. *E. gallinarum* was identified upon digestion of DNA with *Sma*I and pulsed-field gel electrophoresis showing all fragments to be <200 kb and by the presence of the *vanC1* gene (3, 5). The test strains were carefully selected in order to maximize the variety of resistance genotypes and phenotypes (16). Identical strains were excluded. All had unique pulsed-field gel electrophoresis patterns and were, therefore, genetically unrelated (data not shown). PCR assays for *vanA*, *vanB*, *vanC1*, and *vanC2* genes were performed as described by Dutka-Malen et al. (2). Agar dilution and disk diffusion were performed in accordance with the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (9, 10) on cation-adjusted Mueller-Hinton (MH) agar (Difco Laboratories, Detroit, Mich.). E-test (AB Biodisk, Solna, Sweden) was done on MH agar in accordance with the instructions of the manufacturer. The results were read after a 24-h incubation at 37°C. An agar screen containing 6 μg of vancomycin (BBL Microbiology Systems, Cockeysville, Md.) per ml was used as described by Tenover et al. (16) with an inoculum of 10 μl (approximately 10^6 CFU) of a 0.5 McFarland standard suspension. The 30-well Vitek GPS-TA, the 45-well Vitek GPS-101 with the updated GUI software, MicroScan conventional overnight Pos Combo type 6 panels, and MicroScan Rapid Pos Combo type 1 panels with V.20.30 software (Dade International, West Sacramento, Calif.) were used as recommended by their respective manufacturers. *E. faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 were used as quality control strains. The NCCLS breakpoints were used for interpretation of the results (9). A very major error was defined as an isolate that was resistant by the reference agar dilution method but susceptible with the test method. A major error was defined as an isolate that was susceptible by the reference agar dilution method but resistant with the test method. Thus, lack of sensitivity of a given test was deemed to be more serious clinically than lack of specificity. A minor error was defined as a discrepancy between the results of the reference agar dilution method and the test method corresponding to one interpretation category. However, for the *E. gallinarum* and *E. casseliflavus* strains for which MICs were 8 to 16 μg/ml, both intermediate- and resistant-

* Corresponding author. Mailing address: Department of Medical Microbiology & Infectious Diseases, Erasmus University Medical Center Rotterdam, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands. Phone: (31)10 4635820. Fax: (31)10 4633875. E-mail: ENDTZ@BACL.AZR.NL.

TABLE 1. MH agar determination of MICs for 145 VRE and 50 VSE[a] by genotype

Organism (*n*)	No. of isolates for which the MIC (μg/ml) was:										
	0.25	0.5	1	2	4	8	16	32	64	128	≥256
VRE											
vanA (50)											50
vanB (15)						1	1	3	1	5	4
vanC1 (50)						30	19	1			
vanC2 (30)						25	4	1			
VSE (50)	1	6	32	9	2						

[a] VSE, vancomycin-susceptible enterococci.

phenotype results were considered correct, since both interpretation categories correctly distinguish these *vanC1*- or *vanC2*-harboring enterococci from fully susceptible strains (MIC ≤ 4 μg/ml). Similarly, sensitivity was defined as the ability of the test method to correctly distinguish the *vanA*-, *vanB*-, *vanC1*-, or *vanC2*-harboring resistant enterococci from susceptible strains not harboring these genes. Therefore, for strains with intermediate results with the reference agar dilution (MIC, 8 to 16 μg/ml), both intermediate- and resistant-phenotype test results were considered correct.

The MICs of vancomycin with the reference agar dilution method are shown by genotype in Table 1. Table 2 presents the percentages of very major, major, and minor errors of the different tests compared with the reference agar dilution method. The comparative sensitivities of seven methods for the detection of *vanA*, *vanB*, and *vanC1/C2* VRE are shown in Table 3. All methods were 100% sensitive for the detection of *vanA*-mediated vancomycin resistance. However, it is important to note that for all of the 50 *vanA* VRE MICs of vancomycin were ≥256 μg/ml, and these strains were therefore detected easily. For *vanB* VRE, the sensitivity dropped to 47, 53, and 93% with Vitek GPS-TA, the MicroScan rapid panel, and disk diffusion, respectively. In contrast, Vitek GPS-101, the MicroScan conventional panel, the agar screen, and E-test were 100% sensitive for detecting *vanB* VRE. For *vanC1/C2* VRE, E-test and the agar screen were the only methods that correctly identified all resistant strains as such. High error rates were produced by disk diffusion and by all automated methods (Table 2). The MicroScan conventional panel detected only 7% of the *vanC2 E. casseliflavus*. The sensitivities of the other automated methods were 67 to 90% (Table 3). The specificities of the different methods were 96 to 100%.

Earlier studies have reported on the performance of commercial and reference methods for the detection of vancomycin resistance in enterococci (11–17). Surprisingly, none of these studies were performed in Europe. Some of the studies reported on the difficulties of automated methods in detecting low-level or intermediate-level vancomycin resistance (16, 17). In the study by Tenover et al., the performance of the MicroScan rapid panel and the Vitek GPS-TA card were problematic, with very major error rates of 20.7 and 10.3%, respectively. Many errors occurred with *E. casseliflavus*, *E. gallinarum*, and *vanB* VRE. We confirm the failure of these two methods. The MicroScan rapid panel and Vitek GPS-TA had 33 and 40% very major errors with *vanB* strains, respectively (Table 2). However, no very major errors occurred with the MicroScan conventional panel or with Vitek GPS-101. No susceptible (vancomycin MIC ≤ 4μg/l) *E. gallinarum* or *E. casseliflavus* was found, possibly due to the fact that the strains were initially isolated with the use of a selective broth medium containing 6 μg of vancomycin per liter. Since for 78 of the 80 *E. gallinarum* and *E. casseliflavus* strains vancomycin MICs were in the intermediate category (8 to 16 μg/ml), most errors in these species were, by definition, minor errors. For one *vanC1 E. gallinarum* strain and one *vanC2 E. casseliflavus* strain, the MIC of vancomycin was 32 μg/ml. The latter strain was incorrectly reported as susceptible by the MicroScan conventional panel, and this result was scored as a very major error (Table 2). The MicroScan conventional panel and MicroScan rapid panel had 24 and 14% minor errors, respectively, with *vanC1 E. gallinarum* but 90 and 10%, respectively, with *vanC2 E. casseliflavus*. Vitek GPS-TA and Vitek GPS-101 had 28 and 12% minor errors, respectively, with *vanC1 E. gallinarum* and 37 and 30%, respectively, with *vanC2 E. casseliflavus*. The minor error rates of the disk diffusion with *E. gallinarum* and *E. casseliflavus* were 50 and 37%, respectively. Swenson et al. reported minor error rates of 14.5% of total values. However, their collection of 100 VRE included only 10 *E. gallinarum* or *E. casseliflavus* isolates, and the most significant errors in detection were in fact obtained mainly with these strains (14). E-test and the agar screen were the only methods

TABLE 2. Error rates of seven methods for detection of vancomycin resistance in enterococci

Method	Error rate (%)[a]								
	Very major				Major (*n* = 50)	Minor			
	vanA (*n* = 50)	*vanB* (*n* = 15)	*vanC1* (*n* = 50)	*vanC2* (*n* = 30)		*vanA* (*n* = 50)	*vanB* (*n* = 15)	*vanC1* (*n* = 50)	*vanC2* (*n* = 30)
E-test	0	0	0	0	0	0	13	2	0
Disk diffusion	0	0	0	0	2	0	27	50	37
Agar screen	0	0	0	0	4				
MicroScan									
Conventional	0	0	0	3	0	0	0	24	90
Rapid	0	33	0	0	0	0	27	14	10
Vitek									
GPS-TA	0	40	0	0	4	0	13	28	37
GPS-101	0	0	0	0	4	2	7	12	30

[a] Relative to the NCCLS reference agar dilution assay. Error types are defined in the text.

TABLE 3. Sensitivities of seven methods for detection of *vanA*, *vanB*, and *vanC1*/*C2* enterococci[a]

Method	Sensitivity (%) for VRE			
	vanA (n = 50)	*vanB* (n = 15)	*vanC1* (n = 50)	*vanC2* (n = 30)
E-test	100	100	100	100
Disk diffusion	100	93	52	63
Agar screen	100	100	100	100
MicroScan				
Conventional	100	100	76	7
Rapid	100	53	86	90
Vitek				
GPS-TA	100	47	72	67
GPS-101	100	100	88	73

[a] For *vanB*, *vanC1*, and *vanC2* strains with MICs of 8 to 16 μg/ml, both intermediate- and resistant-phenotype results were considered correct.

that correctly detected all VRE in our study. Light growth was observed on the agar screen with two vancomycin-susceptible strains (MIC, 4 μg/ml). This high sensitivity is in concordance with recent data reported by Willey et al. (17). They found the agar screen plate (using the same vancomycin concentration as that used in our study) to be 100% sensitive and specific. In another study, which included only a small number of strains with MICs in the 8- to 16-μg/ml range, E-test proved to be a reliable method compared to agar dilution (12).

The prevalence and the clinical relevance of *E. casseliflavus* and *E. gallinarum* remain to be elucidated. These VRE are often misidentified by commercial identification systems (data not shown) (11), and their intermediate level of resistance may not be detected. It is likely that these two species are being underreported in the literature (7, 11).

In conclusion, *vanA* VRE are detected by all methods. *vanB* VRE are often not detected by Vitek GPS-TA and the MicroScan rapid panel, though the new Vitek GPS-101 appears to be a significant improvement. All methods except E-test and the agar screen continue to show problems in the detection of *vanC1*/*C2* VRE. The agar screen appears to be the most reliable and easy method for routine screening, if detection of *vanA*-, *vanB*-, and *vanC1*/*C2*-mediated resistance in enterococci is required. The new 45-well Vitek GPS-101 shows improved sensitivity, compared to the Vitek GPS-TA, without significant loss of specificity.

We gratefully acknowledge M. Humphrey for reading the English version of the manuscript.

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