JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2001, p. 351–353 Vol. 39, No. 1
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.1.351–353.2001

Accuracy of the VITEK 2 System To Detect Glycopeptide Resistance in Enterococci

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Received 19 June 2000/Returned for modification 10 August 2000/Accepted 5 October 2000

We evaluated the accuracy of the VITEK 2 fully automated system to detect and identify glycopeptide-resistant enterococci (GRE) compared to a reference agar dilution method. The sensitivity of vancomycin susceptibility testing with VITEK 2 for the detection of *vanA*, *vanB*, and *vanC1* strains was 100%. The sensitivity of vancomycin susceptibility testing of *vanC2* strains was 77%. The sensitivity of teicoplanin susceptibility testing of *vanA* strains was 90%. Of 80 *vanC* enterococci, 78 (98%) were correctly identified by VITEK 2 as *Enterococcus gallinarum*/*Enterococcus casseliflavus*. Since the identification and susceptibility data are produced within 3 and 8 h, respectively, VITEK 2 appears a fast and reliable method for detection of GRE in microbiology laboratories.

The prevalence of glycopeptide resistance among clinical isolates of *Enterococcus* spp., first described in 1986 (11), is ever increasing, thereby limiting the treatment options for infections caused by glycopeptide-resistant enterococci (GRE). Molecular epidemiology has elucidated several determinants of glycopeptide resistance as well as gene reservoirs and has increased our awareness of the spread of GRE in hospitals and in the community (2, 5, 8). However, although microbiology laboratories have been delineated as the first line of defense to control the spread of GRE within our hospitals (9), many technical problems concerning the laboratory detection of GRE still exist. Previous studies have reported on problems with the detection of *vanB*-, *vanC1*-, and *vanC2*-type strains, in particular (6, 10, 14). Both convential and automated methods have problems in detecting these particular genotypes. The manufacturers of commercial susceptibility testing methods have joined in their efforts to contain the problem of increasing resistance by developing new and rapid susceptibility test methods. Our main objective in this study was to evaluate the ability of VITEK 2 to determine vancomycin and teicoplanin resistance in strains containing *vanA*, *vanB*, *vanC1*, or *vanC2*. The performance of VITEK GPI and VITEK 2 for the identification of *Enterococcus faecalis* and *E. faecium* has been evaluated by others (7, 12). Therefore, our second objective was to evaluate the performance of VITEK 2 for the identification of *vanC* enterococci to the species level, since most automated methods have problems with the identification of *E. gallinarum* and *E. casseliflavus* (6, 10, 14).

A collection of genetically distinct GRE and glycopeptide-susceptible enterococci (GSE) from diverse sources was used in this study. This collection was assembled and characterized by molecular methods in a previous study (6). A total of 195 enterococci, including *vanA* ($n = 50$), *vanB* ($n = 15$) *vanC1* ($n = 50$), *vanC2* ($n = 30$), and GSE ($n = 50$), were isolated from patients, pets, or poultry products in The Netherlands. All enterococci were identified to the species level on the basis of colony morphology, Gram stain, pyrase and catalase testing, pigment production, the presence of the Lancefield group D antigen, and Rapid ID32 Strep (bioMérieux, 's Hertogenbosch, The Netherlands). PCR assays for *vanA*, *vanB*, *vanC1*, and *vanC2* (4) were used to assess the presence of the various glycopeptide resistance genes. Strains carrying the *vanC1* or *vanC2* gene were identified as *E. gallinarum* and *E. casseliflavus*, respectively. The identification of *E. gallinarum* was confirmed by pulsed-field gel electrophoresis after digestion with *Sma*I, which led to the production of macrorestriction fragments of less than 200 kb only (5). All strains were genetically characterized by pulsed-field gel electrophoresis, and only unique strains were included in the study. Susceptibility results for vancomycin and teicoplanin obtained by agar dilution performed in accordance with the guidelines of the NCCLS (13) were used as a reference method. The VITEK 2 system was used as specified by the manufacturer (bioMérieux, Marcy 1'Etoile, France); ID-Gram Positive Cocci cards were used for identification. The Antimicrobial Susceptibility Testing P516 card was used for susceptibility testing. *E. faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 were used as quality control strains. MICs were interpreted as indicating susceptible, intermediate, or resistant categories according to the breakpoints recommended by the NCCLS. A very major error was defined as occurring when an isolate that was resistant by the agar dilution method appeared to be susceptible by the test method. A major error was defined as occurring when an isolate that was susceptible by the reference agar dilution method scored resistant by the test method. Thus, lack of sensitivity of a given test was considered to be a more serious handicap 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 that differed only by one interpretation category. The sensitivity of the test method was defined as the ability of the method to correctly distinguish the

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TABLE 1. Determination of vancomycin and teicoplanin MICs for 145 GRE and 50 GSE by genotype, using the VITEK 2 automated system versus standard agar dilution

Organism (no.)	Method	No. of isolates with the following vancomycin MIC (mg/liter):						No. of isolates with the following teicoplanin MIC (mg/liter):					
		≤1	2	4	8	16	≥32	≤1	2	4	8	16	≥32
vanA GRE (50)	VITEK 2						50				5	3	42
	Agar dilution						50						50
vanB GRE (15)	VITEK 2						15	14			1		
	Agar dilution				1	1	13	15					
vanC1 GRE (50)	VITEK 2				21	19	10	50					
	Agar dilution				30	19	1	50					
vanC2 GRE (30)	VITEK 2		1	6	23			30					
	Agar dilution				25	4	1	30					
GSE (50)	VITEK 2	42	6	1	1			49	1				
	Agar dilution	39	9	2				50					

vanA-, *vanB*-, *vanC1*-, or *vanC2*-harboring resistant enterococci from susceptible strains not harboring these genes. However, for enterococcal strains for which vancomycin MICs were 8 to 16 μg/ml, both intermediate and resistant results were considered correct, since both interpretation categories correctly distinguish these enterococci from fully susceptible strains (vancomycin MIC, 4 μg/ml; teicoplanin MIC, 8 μg/ml). For reporting the accuracy of VITEK 2 compared to the reference method, the overall percent agreement was defined as 100 × (number of strains with the reference drug MIC ± 1 dilution/total number of strains tested).

Table 1 shows the susceptibility results for the 195 enterococci obtained by VITEK 2 and the reference agar dilution method for vancomycin and teicoplanin. The sensitivity of vancomycin susceptibility testing by VITEK 2 for the detecting of *vanA*, *vanB*, and *vanC1* strains was 100%. However, it is important to note that the vancomycin MICs for all *vanA* strains in this study were >256 μg/ml. The sensitivity of the system for detecting *vanC2* strains was 77%. Several minor errors were found in the *vanB*, *vanC1*, and *vanC2* enterococci as well as in the GSE group: 1% (2 of 195), 5.6% (11 of 195), 4.1% (8 of 195), and 0.5% (1 of 195), respectively. No major or very major errors were encountered in the GRE or GSE group.

In contrast, minor errors in 50 samples (6%) and 5 very major errors in 50 samples (10%) occurred when the teicoplanin susceptibility test results were analyzed for detecting *vanA* strains. These five very major errors were confirmed several times, both by bioMérieux researchers and in our laboratory. However, the teicoplanin MICs for these five strains as determined by retesting with VITEK 2 ranged from 4 to >32 mg/liter on different testing days. Neither the isolation media used nor the inocula can explain these major errors (data not shown). All *vanB*, *vanC1*, and *vanC2* enterococci susceptible to teicoplanin were classified correctly by the VITEK 2 system.

The overall agreement of vancomycin susceptibility testing with the VITEK 2 system compared with the reference agar dilution method was 94% (184 of 195) (Table 2); the overall agreement of the teicoplanin testing results between the two methods was 97% (189 of 195).

Of 80 *vanC* enterococci, 78 (98%) were classified by VITEK 2 as *E. gallinarum*/*E. casseliflavus* and 2 were classified as unidentified by VITEK 2. This is a significant improvement over other nonautomated methods. Although VITEK 2 separates the *vanC1* and *vanC2* enterococci from the other enterococci, it cannot differentiate between *E. gallinarum* and *E. casseliflavus*. However, the clinical significance of separating these two species is doubtful.

The mean time for obtaining antimicrobial susceptibility results for the enterococci tested in this study was 8 h 6 min (range, 5 h 25 min to 14 h 30 min). All identification data were obtained within 3 h after starting the identification procedure, as guaranteed by the manufacturer.

Several studies have reported the accuracy of automated methods to detect GRE. Most of these studies identified major problems in the detection of enterococci harboring the *vanB*, *vanC1*, and *vanC2* genes (6, 10, 14). We previously reported very major errors which occurred with the VITEK GPS-TA card. However, most strains were correctly classified with the new VITEK GPS-101 card. The VITEK GPS-101 card had a sensitivity of 100% in detecting VanB phenotypes (6). In this study, no problems were found in detecting *vanB* strains. Minor errors ($n = 22$) occurred with the VITEK 2 system in detecting GRE. However, the 2 minor errors in the *vanB* group and the 10 minor errors in the *vanC1* group were intermediate strains reported as resistant. VITEK 2 is the first automated susceptibility method that tests both vancomycin and teicoplanin for antimicrobial susceptibility, which is important for the description of the resistance phenotype.

For identification and susceptibility testing, most conventional methods require a full 24 h of incubation; however, VITEK 2 provides susceptibility results in approximately 8 h. Barenfanger et al. (1) have demonstrated that rapid reporting

TABLE 2. Comparison of vancomycin MICs determined by VITEK 2 with MICs determined by the reference agar dilution method for 195 *Enterococcus* isolates

Organism (no.)	No. of VITEK 2 vancomycin MICs within indicated log of reference MIC							Agreement (%)[a]
	>−2	−2	−1	0	+1	+2	>+2	
vanA GRE (50)				50				100
vanB GRE (15)				13	1	1		93
vanC1 GRE (50)			8	22	14	6		88
vanC2 GRE (30)		2	10	18				93
GSE (50)			6	40	4			100
Total	2	2	24	143	19	7		95

[a] Agreement percentage is given by 100 × (number of strains with reference drug MIC ± 1 dilution/total number of strains).

of identification and susceptibility results may have important benefits in terms of patient outcome and cost-effectiveness. Moreover, Doern et al. reported that rapid identification and susceptibility test results reduced morbidity and mortality (3). VITEK 2 provides enterococcal susceptibility data in approximately 8 h. Although this is significant faster than overnight conventional methods, it implies that results can still not be obtained in one working shift. To maximize the impact of rapid testing, further improvement of the speed without compromising the accuracy of the test method is desired. In the meantime, we have found that prolonging the opening hours of the microbiology laboratory and adapting the work flow allow the production of earlier reports is an achievable goal.

In conclusion, the VITEK 2 system appears to be an improvement over conventional methods for the detection of vancomycin resistance in enterococci. However, detection of teicoplanin resistance in enterococci containing the *vanA* gene needs to be reassessed. Although the detection time was reduced to 8 h, further improvement of the algorithm and further reduction of the detection time may considerably increase the impact of rapid testing on patient care (1, 3).

We gratefully thank bioMérieux for supplying the VITEK 2 system and identification and susceptibility cards and Marian Humphrey for revision of the English text.

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