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Validation of VITEK 2 Version 4.01 Software for Detection, Identification, and Classification of Glycopeptide-Resistant Enterococci

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We evaluated the ability of the new VITEK 2 version 4.01 software to identify and detect glycopeptide-resistant enterococci compared to that of the reference broth microdilution method and to classify them into the *vanA*, *vanB*, *vanC1*, and *vanC2* genotypes. Moreover, the accuracy of antimicrobial susceptibility testing with agents with improved potencies against glycopeptide-resistant enterococci was determined. A total of 121 enterococci were investigated. The new VITEK 2 software was able to identify 114 (94.2%) enterococcal strains correctly to the species level and to classify 119 (98.3%) enterococci correctly to the glycopeptide resistance genotype level. One *Enterococcus casseliflavus* strain and six *Enterococcus faecium vanA* strains with low-level resistance to vancomycin were identified with low discrimination, requiring additional tests. One of the *vanA* strains was misclassified as the *vanB* type, and one glycopeptide-susceptible *E. facium* wild type was misclassified as the *vanA* type. The overall essential agreements for antimicrobial susceptibility testing results were 94.2% for vancomycin, 95.9% for teicoplanin, 100% for quinupristin-dalfopristin and moxifloxacin, and 97.5% for linezolid. The rates of minor errors were 9% for teicoplanin and 5% for the other antibiotic agents. The identification and susceptibility data were produced within 4 h to 6 h 30 min and 8 h 15 min to 12 h 15 min. In conclusion, use of VITEK 2 version 4.01 software appears to be a reliable method for the identification and detection of glycopeptide-resistant enterococci as well as an improvement over the use of the former VITEK 2 database. However, a significant reduction in the detection time would be desirable.

The first glycopeptide-resistant enterococci (GRE) that harbored the *vanA* transposon were identified in 1987 in Europe (10). Within 10 years GRE represented >25% of the enterococci that cause bloodstream infections in hospitalized patients in the United States (2). The *vanA* and *vanB* genotypes (two genetically distinct forms of resistance) are recognized to be clinically important, whereas GRE strains harboring the intrinsic resistance genes *vanC1* and *vanC2* seem to play a less important clinical role. Contrary to the rates in other countries, the rates of GRE in German hospitals are low and GRE account for only about 1% of enterococcal isolates (8), but increasing rates in stool and clinical samples were reported recently (1, 17). Moreover, the numbers of nosocomial infections and the rates of transmission of GRE have increased (17). As GRE infections appear to be more deadly and more costly than infections caused by vancomycin-susceptible strains (15), rapid and reliable results of identification and antimicrobial susceptibility testing (AST) are necessary for the adequate treatment of infections caused by GRE and the prevention of transmission of GRE strains.

Many laboratories worldwide have adopted the VITEK automated system (bioMérieux, Nürtingen, Germany) for the detection of GRE strains in routine clinical microbiology. Earlier reports indicated that previous versions of the VITEK 2 software often had problems with the identification and AST of GRE, especially strains harboring the *vanB*, *vanC1*, and *vanC2* genes (11, 12). To overcome these problems the VITEK 2 system has successfully been improved. The recently introduced VITEK 2 version 4.01 software claims to be more sensitive than earlier versions of the software for the detection of enterococci with reduced susceptibilities to glycopeptide antibiotics. In this study, 121 selected isolates of enterococci were used to investigate whether the new VIITEK 2 software improved the ability to identify and detect enterococci with decreased susceptibilities to the glycopeptides vancomycin and teicoplanin. Moreover, the reliability of the VITEK 2 software for the testing of the newer antimicrobial agents with improved potencies against enterococci (quinupristin-dalfopristin, moxifloxacin, and linezolid) was determined.

MATERIALS AND METHODS

The study was conducted in three phases. In phases I and II, identification of enterococcal species and classification of the glycopeptide resistance genotype were investigated. Phase III compared the AST results determined by the VITEK 2 software and those determined by the reference broth microdilution (BMD) method.

Bacterial strains. The strains used in this study were isolated over 3 years in the context of a program of screening for GRE from different patients referred to hematologic or intensive care units. All GRE strains were stored at −70°C in the strain collection of the Institute for Hygiene and Microbiology of the University of Würzburg. For the study, the GRE strains were transferred from storage at −70°C, placed onto brain heart infusion plates, and subcultured a second time.

Identification of enterococcal species. The GRE isolates were identified to the species level on the basis of standard methods, like colony morphology, Gram stain, catalase and pyrase testing, the presence of the Lancefield group D antigen, pigment production, motility testing, methyl-alpha-D-glucopyranoside (MPG) acidification tests, and the API STREP system (bioMérieux, Nürtingen, Germany). Strains carrying the *vanC1* or the *vanC2* gene were identified as *Enterococcus gallinarum* or *Enterococcus casseliflavus* (4, 14). The standard iden-

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TABLE 1. Antimicrobial concentration ranges and breakpoints used in AST

Antimicrobial agent	MIC range (μg/ml)	
	Broth microdilution method	VITEK 2 method
Vancomycin	0.25–32	1–32
Teicoplanin	0.125–16	0.5–32
Quinupristin-dalfopristin	0.125–16	0.25–16
Linezolid	0.125–16	0.5–8
Moxifloxacin	0.063–8	0.25–8

tification results were compared with those obtained with the new VITEK 2 cards (GP identity card) for the identification gram-positive cocci, according to the manufacturer's instructions.

Classification of the enterococcal glycopeptide resistance genotypes. For the classification of the enterococcal resistance genotypes, PCR assays for the *vanA*, *vanB*, *vanC1*, and *vanC2* genes were used to assess the presence of the various glycopeptide resistance genes. The PCR results for the *vanA*, *vanB*, *vanC1*, and *vanC2* genotypes were compared with the phenotype-genotype classification obtained with the new VITEK 2 system.

AST. The susceptibilities of the isolates to vancomycin, teicoplanin, and antimicrobial agents with improved potencies against GRE strains were tested: quinupristin-dalfopristin for *E. faecium*, linezolid, and moxifloxacin. For the BMD method, 96-well MIC plates (Micronaut-S MRSA/IFSG GP panel; Merlin Diagnostika, Bornheim-Hersel, Germany) were used according to the manufacturer′s recommendations. The concentrations of the antibiotic agents used are shown in Table 1. Testing and interpretation of the results for vancomycin, teicoplanin, quinupristin-dalfopristin, and linezolid were performed according to the Clinical and Laboratory Standards Institute (CLSI) standards (3). MICs were interpreted as indicating susceptible, intermediate, or resistant categories according to the breakpoints recommended by the CLSI (3) or for moxifloxacin (susceptible, MIC $\leq$ 1 μg/ml; intermediate, MIC = 2 μg/ml; resistant, MIC $\geq$ 4 mg/ml) recommended by the pharmaceutical manufacturer (Bayer, Leverkusen, Germany, personal communication). For the VITEK 2 method, the new ATS-534 cards and the version 4.01 software were used. Testing was performed according to the manufacturer's instructions. To resolve discrepancies, the VITEK 2 and the reference tests were repeated in triplicate when discordant results occurred.

Interpretive category errors were assessed for each drug on the basis of the definitions given by van den Braak et al. (16). A very major error was defined as occurring when an isolate that was resistant by the BMD method appeared to be susceptible by the VITEK 2 test method. A major error was defined as occurring when an isolate that was susceptible by the BMD method scored resistant by the VITEK 2 test method. Thus, a lack of sensitivity of a given test was considered a more serious handicap than a lack of specificity. A minor error was defined as a discrepancy between the results of the BMD method and the VITEK 2 test method that differed by only one interpretation category. The sensitivity of the VITEK 2 test method was defined as the ability of the method to correctly distinguish the enterococci harboring the *vanA*, *vanB*, *vanC1*, or *vanC2* resistance determinants from susceptible strains not harboring these genes. However, among the 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 of the accuracy of VITEK 2 compared to the results of 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).

Quality control strains. The quality control strains used for the BMD method were *Enterococcus faecalis* ATCC 29212 and *E. faecalis* ATCC 51299. For the VITEK 2 method, the quality control strains used were *E. faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213. The control organisms for PCR were *E. faecalis* ATCC 51299 (*vanB* positive), *E. faecium* BM 4147 (*vanA* positive), *E. gallinarum* BM 4174 (*vanC1* positive), *E. casseliflavus* ATCC 25788 and *E. flavescens* CCM 439 (*vanC2* and *vanC3* positive, respectively), and *E. faecalis* ATCC 29212 (negative control).

RESULTS

Identification of enterococcal species by the new VITEK 2 identification card (GP identity card) versus that by standard methods. The VITEK 2 system correctly identified 114 of 121 (94.2%) strains to the species level (Table 2). For six *E. faecium vanA* strains, the species identification by the VITEK 2 method was discordant with the identification by the standard method (Table 2). Three of the *E. faecium vanA* strains were identified with low discrimination as *E. faecium-E. gallinarum* and two were identified as *E. faecium-E. gallinarum-E. casseliflavus*, because the software of the VITEK 2 system was not able to differentiate between the two or three species. One of the *E. faecium vanA* strains was identified as *E. faecium-E. hirae-E. gallinarum*, and the genotype was misidentified as *vanB* (Table 3). Most of the strains mentioned above and identified with low discrimination were due to *E. faecium* strains with low-level resistance to vancomycin ($\leq$32 μg/ml), in contrast to the correctly identified strains, whose vancomycin MICs were $\geq$256 μg/ml. Additional motility tests, pigmentation tests, as well as MPG acidification tests solved the discrepancies and increased the rate of agreement for the *E. faecium* isolates to 98.5%. In addition, one of the *E. casseliflavus* strains that was identified with low discrimination as *E. casseliflavus-E. gallinarum* could be correctly identified after a pigmentation

TABLE 2. Discrepancies between identification with the VITEK 2 system and by reference method

Species	No. of strains tested	Genotype determined by PCR assay	Species identification (no. [%] of strains)		Phenotype (no. of strains) by:	
			Correct	Discordant	Reference method	VITEK 2 method
E. faecium	1	Wild type	1	0		
E. faecium	66	*vanA*	60 (90.9)	6	*E. faecium* (3) *E. faecium* (2) *E. faecium* (1)	*E. faecium-E. gallinarum* *E. faecium-E. gallinarum-E. casseliflavus* *E. faecium-E. hirae-E. gallinarum*
E. faecium	14	*vanB*	14 (100)	0		
E. gallinarum	30	*vanC1*	30 (100)	0		
E. casseliflavus	10	*vanC2*	9 (90)	1	*E. casseliflavus* (1)	*E. casseliflavus-E. gallinarum*
Total	121		114 (94.2)	7 (5.8)		

TABLE 3. Discrepancies between classification of GRE genotypes with the VITEK 2 system and by PCR assays

Species	No. of strains tested	Genotype determined by PCR assay	Genotype identification (no. [%] of strains)		VITEK 2 genotype-phenotype
			Correct	Discordant	
E. faecium	1	Wild type	0	1	*vanA*
E. faecium	66	*vanA*	65 (98.5)	1	*vanB*
E. faecium	14	*vanB*	14 (100)	0	
E. gallinarum	30	*vanC1*	30 (100)	0	
E. casseliflavus	10	*vanC2*	10 (100)	0	
Total	121		119 (98.3)	2 (1.7)	

test, which increased the agreement for the *vanC2* group to 100% (Table 2).

Classification of enterococcal resistance genotypes with VITEK 2 system and by PCR. The VITEK 2 system classified all *E. faecium vanB* strains and all *E. gallinarum* and *E. casseliflavus* strains correctly as the *vanB*, *vanC1*, and *vanC2* types; and 65 of 66 (98.5%) *E. faecium vanA* strains were correctly detected as *vanA* types (Table 3). In one *van*A gene-positive *E. faecium* isolate, the MIC of vancomycin was 32 μg/ml by the BMD and VITEK 2 methods, but the MIC of teicoplanin showed discrepant results (MICs, 4 μg/ml by the BMD method and 1 μg/ml by the VITEK 2 method). According to this constellation, the VITEK 2 system misidentified this *vanA* type as a *vanB* type (Table 3). In addition, one *E. faecium* wild-type strain was misclassified by the VITEK 2 system as a glycopeptide-resistant *vanA* type. The PCR results for the *vanA*, *vanB*, and *vanC* genes were negative; and the strain was susceptible to vancomycin (MIC, 2 μg/ml) and teicoplanin (MIC, 1 μg/ml) by the BMD method but resistant to vancomycin (MIC, 16 μg/ml) and susceptible to teicoplanin (MIC, 4 μg/ml) by the VITEK 2 method (one minor error).

AST by MBD method versus VITEK 2 method. Tables 4 and 5 show the AST results generated by the BMD reference method and the VITEK 2 method with the new AST-534 cards (version 4.01 software). The sensitivity of vancomycin susceptibility testing by the VITEK 2 method for the detection of *E. faecium vanA* and *E. casseliflavus* strains was 100% (Table 5). In contrast, by the VITEK 2 method the *E. faecium* vancomycin-susceptible wild-type strain tested resistant to vancomycin (MIC, 16 mg/ml versus 4 μg/ml by the BMD method) but susceptible to teicoplanin (2 μg/ml versus 1 μg/ml by the BMD method). The sensitivities of the VITEK 2 system for the detection of *E. faecium vanB* strains and *E. gallinarum* were 85.7 and 86.7%, respectively (Table 5). When the vancomycin test results were analyzed, five minor errors were found among the *E. gallinarum* strains (5 of 30; 16.7%) and one minor error was found among the *E. casseliflavus* strains (1 of 10; 10%). No minor errors were encountered among the *vanA* or *vanB E. faecium* strains (Table 5).

The sensitivities of teicoplanin susceptibility testing were 97 and 85.7% for the *E. faecium vanA* and *vanB* strains, respectively, and 100% for the *E. gallinarum* and *E. casseliflavus* strains (Table 5). Eleven minor errors occurred among 66 samples (16.7%) when the teicoplanin susceptibility test results were analyzed for the detection of *E. faecium vanA* strains (Table 5). In one case, the MIC of vancomycin was 32 μg/ml by both methods, but the MIC of teicoplanin showed discrepant results (MICs, 4 μg/ml by the BMD method and 1 μg/ml by the VITEK 2 method). As mentioned above, this *vanA* strain was misclassified as a *vanB* strain by the VITEK 2 method (Table 3). No minor, major, or very major errors were encountered among the *E. faecium vanB*, *E. gallinarum*, or *E. casseliflavus* strains (Table 5).

AST of quinupristin-dalfopristin by the VITEK 2 method encountered four minor errors (4 of 30; 13.3%) among the *E. gallinarum* strains and two minor errors (2 of 10; 20%) among the *E. casseliflavus* strains (Table 5). In addition, AST with linezolid by the VITEK 2 method resulted in four minor errors (6.1%) among the 66 *E. faecium vanA* strains and one minor error among the *E. gallinarum* and *E. casseliflavus* strains. Finally, AST with moxifloxacin by the VITEK 2 method resulted in 6 minor errors (9%) among the 66 *vanA* enterococci (Table 5).

The overall rates of agreement of AST with the new VITEK 2 system compared with that by the reference BMD method for GRE are shown in Table 6. The rates of agreement were 94.2% (113 of 121) for the vancomycin test results, 95.9% (116 of 121) for the teicoplanin test results, 100% (121 of 121) for the quinupristin-dalfopristin and moxifloxacin test results, and 97.5% (118 of 121) for the linezolid test results.

The mean time required to obtain antimicrobial susceptibility test results for the enterococci tested in this study was 10 h (range, 8.25 to 14.75 h). The time for identification by the VITEK 2 system ranged from 4 h to 6.5 h, with a mean value of 5.25 h. For the additional tests (the motility, pigmentation, and MGP tests), 24 h to 48 h was necessary.

DISCUSSION

According to our knowledge, this is the first study in Germany that has assessed the ability of the VITEK 2 version 4.01 software to identify (GP identity card), classify, and detect (AST-534 card) enterococcal isolates with reduced susceptibilities to glycopeptides. Moreover, we also evaluated the accuracy of AST with newer agents with improved activities against GRE strains.

Our data show that the new VITEK 2 software (version 4.01) was able to identify and detect GRE and to classify them reliably as *vanA*, *vanB*, *vanC1*, and *vanC2* strains. Of 121 enterococcal strains, 114 (94.2%) were correctly identified to the species level as *E. faecium*, *E. gallinarum*, or *E. casseliflavus*. With the exception of one *E. casseliflavus* isolate, no problems with the identification of *E. faecium vanB*, *E. casseliflavus*, and *E. gallinarum* strains were observed. In contrast, some failures with the identification of *E. faecium vanA* strains occurred, where the correct identification rate was only 90.9% (Table 2). Six of the 66 isolates were phenotypically identified with low discrimination as *E. faecium-E. gallinarum* (three strains), *E. faecium-E. gallinarum-E. casseliflavus* (two strains), or *E. faecium-E. hirae-E. gallinarum* (one strain). The last strain was also misclassified as being of the *vanB* type. All these strains required additional tests (motility, pigmentation, and MPG acidification tests) for correct identification to the species level. The performance of further tests led to a correct identification rate of 100%, but the identification time increased by 24 h to 48 h, which is not acceptable from a clinical point of view. Nevertheless, our results with the new VITEK 2 software for the identification of *E. faecium vanB* strains are better than the

TABLE 4. MICs of antimicrobial agents against enterococci generated by broth microdilution and VITEK 2 methods

Agent	Species	Method	No. of isolates for which the MIC (μg/ml) is:						
			≤0.5	1	2	4	8	16	≥32
Vancomycin	*E. faecium* wild type (n = 1)	BMD			1				
		VITEK 2						1	
	E. faecium vanA (n = 66)	BMD							66
		VITEK 2							66
	E. faecium vanB (n = 14)	BMD					2	8	4
		VITEK 2						10	4
	E. gallinarum vanC1 (n = 30)	BMD					28	1	1
		VITEK 2					18	6	6
	E. casseliflavus vanC2 (n = 10)	BMD					10		
		VITEK 2				1	9		
Teicoplanin	*E. faecium* wild type (n = 1)	BMD		1					
		VITEK 2				1			
	E. faecium vanA (n = 66)	BMD				3	4	29	30
		VITEK 2		1		6	4	19	36
	E. faecium vanB (n = 14)	BMD	4	6	4				
		VITEK 2	8	6					
	E. gallinarum vanC1 (n = 30)	BMD	30						
		VITEK 2	30						
	E. casseliflavus vanC2 (n = 10)	BMD	8	2					
		VITEK 2	10						
Quinupristin-dalfopristin	*E. faecium vanA* (n = 66)	BMD	62	3		1			
		VITEK 2	62	3		1			
	E. faecium vanB (n = 14)	BMD	12	2					
		VITEK 2	12	2					
	E. gallinarum vanC1 (n = 30)	BMD	1	16	10	2	1		
		VITEK 2	1	15	10	4			
	E. casseliflavus vanC2 (n = 10)	BMD			3	7			
		VITEK 2			1	9			
Linezolid	*E. faecium vanA* (n = 66)	BMD		12	52	2			
		VITEK 2		2	59	5			
	E. faecium vanB (n = 14)	BMD		2	12				
		VITEK 2			14				
	E. gallinarum vanC1 (n = 30)	BMD		2	26	2			
		VITEK 2		2	26	2			
	E. casseliflavus vanC2 (n = 10)	BMD		1	8	1			
		VITEK 2		3	7				
Moxifloxacin	*E. faecium vanA* (n = 66)	BMD	3	38	9	3	13		
		VITEK 2	3	45	2	4	12		
	E. faecium vanB (n = 14)	BMD		8	2		4		
		VITEK 2		8	2		4		
	E. gallinarum vanC1 (n = 30)	BMD	26			4			
		VITEK 2	26			4			
	E. casseliflavus vanC2 (n = 10)	BMD	9	1					
		VITEK 2	10						

reported results obtained with the former VITEK 2 system. Concerning the *E. faecium vanA* strains, our results of 90.9% either fell within the range obtained by others, who have demonstrated a correlation between 83 and 95.3% for the results obtained with former VITEK 2 systems and those obtained by the reference method (7, 10; E. Halle, I. Klare, and U. B. Göbel, Abstr. 9th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. P147A, 1999), or are poorer in comparison to those of other authors using newer VITEK 2 software (16). Van den Braak et al. (16) investigated only *E. faecium vanA* strains with a high level of resistance to vancomycin (≥256 μg/ml) and found 100% agreement between the VITEK 2 and the reference methods. This is in accordance with our data, where all *E. faecium vanA* strains with high-level vancomycin resistance (MICs, ≥256 μg/ml) were correctly identified. On the other side, all isolates identified with low discrimination were *E. faecium vanA* strains (positive for *vanA* genes by PCR) with low-level resistance to vancomycin (MICs, ≤32 μg/ml) and teicoplanin (MICs, 4 to 16 μg/ml).

To our knowledge, very few studies have evaluated the VITEK 2 system for the identification of *E. gallinarum* and *E. casseliflavus*. In this study, all *E. gallinarum* strains and 90% of the *E. casseliflavus* strains were correctly identified. This is a significant improvement over the results obtained with the former VITEK 2 system and over the results obtained by other nonautomated methods. According to our experience (data not shown), the former VITEK 2 system database could not reliably differentiate between *E. casseliflavus* and *E. gallinarum*

TABLE 5. Comparison of MICs generated by the VITEK 2 method (P534 cards) with MICs generated by the reference microbroth dilution method for 121 enterococci[a]

Species	Drug	No. of VITEK 2 MICs that differed from reference MICs by the following dilution							EA (no. [%])	No. of errors		
		−3	−2	−1	Concordant	+1	+2	+3		Minor	Major	Very major
E. faecium wild type (n = 1)	Vancomycin				0			1		1		
	Teicoplanin				0		1					
E. faecium vanA (n = 66)	Vancomycin				66				66 (100)			
	Teicoplanin	1	1	5	52	7			64 (97)	11		
	Quin-dalfo				40	26			66 (100)			
	Linezolid		1		54	11			65 (98.5)	4		
	Moxifloxacin			9	56	1			66 (100)	6		
E. faecium vanB (n = 14)	Vancomycin			2	10		2		12 (85.7)			
	Teicoplanin		2	4	8				12 (85.7)			
	Quinu-dalfo				10	4			14 (100)			
	Linezolid				12	2			14 (100)			
	Moxifloxacin				14				14 (100)			
E. gallinarum vanC1 (n = 30)	Vancomycin				19	7	4		26 (86.7)	5		
	Teicoplanin				30				30 (100)			
	Quinu-dalfo			2	25	3			30 (100)	4		
	Linezolid				27	1	2		28 (93)	1		
	Moxifloxacin				30				30 (100)			
E. casseliflavus vanC2 (n = 10)	Vancomycin			1	9				10 (100)	1		
	Teicoplanin			1	9				10 (100)			
	Quinu-dalfo			2	8				10 (100)	2		
	Linezolid			3	7				10 (100)	1		
	Moxifloxacin			2	8				10 (100)			

[a] Dilutions indicate the number of VITEK 2 MIC dilutions compared to reference microbroth dilution MICs. EA, essential agreement (present VITEK 2 MICs within 1 dilution of reference MICs); minor error, intermediate by either the VITEK 2 or reference method and either susceptible or resistant by the other method. Quin-dalfo, quinupristin-dalfopristin.

but identified the majority of these strains as *E. gallinarum-E. casseliflavus*. This was confirmed in a study carried out by Ramotar et al. (13), where the VITEK GPI correctly identified only 13 of 115 (11.3%) *vanC1*-containing isolates of GRE as *E. gallinarum* and identified none of the *vanC2*-containing isolates as *E. casseliflavus*. In the same study, the authors demonstrated that the motility tests alone are not sufficient for the correct differentiation of *E. faecium* and *E. gallinarum*, since some of the *E. gallinarum* strains were nonmotile, and an additional MPG acidification test was necessary for discrimination.

No problems were observed with the genotypic classification of GRE. Of 121 enterococcal strains, 119 (98.3%) were correctly classified to the glycopeptide resistance genotype level by the new VITEK 2 software. One of these strains which was misclassified as a *vanB* type was clearly an *E. faecium vanA* strain with low-level vancomycin resistance (MIC, 32 μg/ml) by the BMD and the VITEK 2 methods but discordant susceptibility to teicoplanin by the BMD method (MIC, 4 μg/ml) and the VITEK 2 method (MIC, 1 μg/ml). This constellation may explain the difficulty with the correct identification and classification by the VITEK 2 method. In the second case, a glycopeptide-susceptible *E. faecium* wild-type strain with discordant teicoplanin susceptibilities by the BMD method (MIC, 1 μg/ml) and the VITEK 2 method (MIC, 4 μg/ml) and discordant vancomycin test results by the BMD method (MIC, 2 μg/ml)

TABLE 6. Comparison of MICs generated by the VITEK 2 method (P534 cards) with MICs generated by the reference microbroth dilution method for 121 enterococci[a]

Drug	No. of VITEK 2 MICs that differed from reference MICs by the following dilution:							EA (no. [%])	No. (%) of minor errors
	−3	−2	−1	Concordant	+1	+2	+3		
Vancomycin			3	104	7	6	1	113 (94.2)	6 (5)
Teicoplanin	1	3	10	99	7	1		116 (95.9)	11 (9)
Quin-dalfo			4	84	33			121 (100)	6 (5)
Linezolid		1	3	100	15	2		118 (97.5)	6 (5)
Moxifloxacin			12	108	1			121 (100)	6 (5)

[a] Dilutions indicate the number of VITEK 2 MIC dilutions compared to reference microbroth dilution MICs. EA, essential agreement (present VITEK 2 MICs within 1 dilution of reference MICs); minor error, intermediate by either the VITEK 2 or reference method and either susceptible or resistant by the other method. There were no major or very major errors. Quin-dalfo, quinupristin-dalfopristin.

and the VITEK 2 method (16 μg/ml) was, as mentioned above, misclassified as a vancomycin-resistant *vanA* strain. In this particular case, the vancomycin resistance obtained by the VITEK 2 method was confirmed several times, with MICs ranging from 16 to 32 μg/ml.

Several studies have been conducted to evaluate automated methods for the detection of GRE. Most studies identified major problems with the detection of enterococci harboring the *vanB*, *vanC1*, or *vanC2* gene (7, 11, 12). In the study of Ligozzi et al., the VITEK 2 system correctly detected all *vanA*-carrying resistant enterocooci but failed to detect 4 of 22 teicoplanin-resistant isolates (11). In a study of Garcia et al., the VITEK 2 system (AST-P516 card) failed to detect 2 of 57 (3.5%) *vanA* isolates, 3 of 16 (18.8%) *vanB* isolates, and 1 of 26 (3.8%) *vanC1* isolates (7). In this study, the sensitivities of vancomycin susceptibility testing with the new VITEK 2 software were 100% for *E. faecium vanA* and *E. casseliflavus vanC2* strains and 85.7 and 86.7% for *E. faecium vanB* and *E. gallinarum vanC1* strains, respectively. Concerning the enterococcal glycopeptide resistance genotypes of these strains, the VITEK 2 version 4.01 software correctly identified all *E. faecium vanB* strains and all *E. gallinarum* strains, despite the incorrect vancomycin MIC, as *vanB* and *vanC1* enterococci, respectively. All together, the new VITEK 2 system has a sensitivity of 98.5% for the detection of *vanA* strains and a sensitivity of 100% for the detection of *vanB*, *vanC1*, and *vanC2* enterococci. These results are better than those obtained by authors using the former VITEK 2 database (7, 10) and fell within the range obtained by others who have used the newer VITEK 2 software and who have demonstrated correlations of between 90 and 100% for *vanA*, *vanB*, and *vanC1* strains and about 79 to 80% for *vanC2* strains (9, 16). Compared to the Phoenix system, which detected 95% of the *vanA* strains and 77% of the *vanB* isolates tested, the new VITEK 2 software gave equal or better results (5; A. M. Butterworth, B. Turng, M. Votta, T. Wiles, J. Salomon, and J. Reuben, 12th Eur. Congr. Clin Microbiol. Infect. Dis., abstr. P 706, 2002).

Some minor errors ($n = 17$) occurred with the VITEK 2 system with the detection of GRE. However, the majority of the minor errors for the *vanA* group were for intermediate strains with teicoplanin ($n = 11$), which were reported to be resistant by the VITEK 2 system or vice versa. In the case of one minor error, a vancomycin-susceptible *E. faecium* wild type was, as mentioned above, misclassified as a vancomycin-resistant *vanA* strain. This minor error could not be explained. The minor errors for the *vanC1* group were mostly vancomycin-intermediate strains that were reported to be resistant by the VITEK 2 method.

Concerning the AST of the newer agents effective against enterococci (quinupristin-dalfopristin for the testing of *E. faecium* strains, linezolid, and moxifloxacin), the VITEK 2 system also showed excellent results, with essential agreement ranging from 97.5 to 100%. Only a few minor errors occurred.

The mean time to the retrieval of identification results for the enterococci was 5 h 15 min, with a range of from 4 h to 6 h 30 min. The VITEK 2 results for susceptibility testing takes 10 h, with a range of 8 h 15 min to 12 h 15 min. This is a disadvantage over the former software, which needed only 3 h for the identification of enterococci and about 8 h for susceptibility testing (6, 16). Although identification and AST results are obtained faster by the VITEK 2 method than by 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 is desirable.

In conclusion, new version 4.01 of the VITEK 2 system software appears to be a reliable method for the identification and classification of GRE and an improvement over the former VITEK 2 database and conventional methods. However, the detection of teicoplanin resistance and the detection of enterococcal strains containing the *vanA* gene with low-level vancomycin resistance should be improved. Moreover, further reduction of the detection time would be desirable.

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