

Evaluation of the VITEK 2 System for Identification and Antimicrobial Susceptibility Testing of Medically Relevant Gram-Positive Cocci

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A study was conducted to evaluate the new VITEK 2 system (bioMérieux) for identification and antibiotic susceptibility testing of gram-positive cocci. Clinical isolates of *Staphylococcus aureus* ($n = 100$), coagulase-negative staphylococci (CNS) ($n = 100$), *Enterococcus* spp. ($n = 89$), *Streptococcus agalactiae* ($n = 29$), and *Streptococcus pneumoniae* ($n = 66$) were examined with the ID-GPC identification card and with the AST-P515 (for staphylococci), AST-P516 (for enterococci and *S. agalactiae*) and AST-P506 (for pneumococci) susceptibility cards. The identification comparison methods were the API Staph for staphylococci and the API 20 Strep for streptococci and enterococci; for antimicrobial susceptibility testing, the agar dilution method according to the procedure of the National Committee for Clinical Laboratory Standards (NCCLS) was used. The VITEK 2 system correctly identified to the species level (only one choice or after simple supplementary tests) 99% of *S. aureus*, 96.5% of *S. agalactiae*, 96.9% of *S. pneumoniae*, 92.7% of *Enterococcus faecalis*, 91.3% of *Staphylococcus haemolyticus*, and 88% of *Staphylococcus epidermidis* but was least able to identify *Enterococcus faecium* (71.4% correct). More than 90% of gram-positive cocci were identified within 3 h. According to the NCCLS break-points, antimicrobial susceptibility testing with the VITEK 2 system gave 96% correct category agreement, 0.82% very major errors, 0.17% major errors, and 2.7% minor errors. Antimicrobial susceptibility testing showed category agreement from 94 to 100% for *S. aureus*, from 90 to 100% for CNS, from 91 to 100% for enterococci, from 96 to 100% for *S. agalactiae*, and from 91 to 100% for *S. pneumoniae*. Microorganism-antibiotic combinations that gave very major errors were CNS-erythromycin, CNS-oxacillin, enterococci-teicoplanin, and enterococci-high-concentration gentamicin. Major errors were observed for CNS-oxacillin and *S. agalactiae*-tetracycline combinations. In conclusion the results of this study indicate that the VITEK 2 system represents an accurate and acceptable means for performing identification and antibiotic susceptibility tests with medically relevant gram-positive cocci.

Automation in clinical microbiology is still in a very early stage of development compared with the level of automation that has been achieved in clinical chemistry, hematology, and immunology laboratories.

In the last 20 years, a variety of automated systems for the identification and antimicrobial susceptibility testing (AST) of microorganisms has been developed based on automated interpretation of the results of biochemical tests or using microdilution trays following overnight incubation and photometric determination of growth (6, 18, 20, 21). Advances in technology that may provide rapid bacterial identification and AST are now recognized as having both clinical and financial benefits (2).

The VITEK system originated in the 1970s as an automated system for identification and AST and has evolved today into the VITEK 2 system, which automatically performs all of the steps required for identification and AST after a primary inoculum has been prepared and standardized (9). This system allows kinetic analysis by reading each test every 15 min. The optical system combines multichannel fluorimeter and pho-

tometer readings to record fluorescence, turbidity, and colorimetric signals.

The purpose of this study was to evaluate the VITEK 2 system (software version VT2-R01.02) for identification and AST of gram-positive cocci belonging to *Staphylococcus* spp., *Enterococcus* spp., *Streptococcus agalactiae*, and *Streptococcus pneumoniae*. Because of the increased incidence of diseases caused by these microorganisms and the emergence of resistance to several antimicrobial agents (3, 7, 8, 12, 15, 23), rapid and accurate identification as well as MIC evaluation for these pathogens has become increasingly important.

MATERIALS AND METHODS

Bacterial strains. A total of 384 strains of gram-positive cocci were studied. Three hundred were clinical isolates consecutively collected in our clinical microbiology laboratory over a 1-year period (single-patient isolates), and 84 were strains with well-characterized mechanisms of resistance collected in our laboratory or sent from other institutions. Species and numbers of strains tested are reported in Table 1. Isolates were stored at -70°C in Trypticase soy broth-glycerol, subcultured twice on Columbia agar with 5% sheep blood, and grown overnight at 35°C prior to testing.

Inoculum preparation. Suspensions were prepared by emulsifying bacterial isolates in 0.45% saline to the equivalent of a 0.5 McFarland turbidity standard. The same suspension was used for identification and AST for the VITEK 2 system. Suspensions for the comparative identification method were made according to the manufacturer's recommendations.

Comparative identification methods. All isolates were tested on appropriate API strips (bioMérieux), including API Staph for members of the *Micrococceae*

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TABLE 1. Performance of the VITEK 2 system for identification of gram-positive cocci compared to that of the API method

Taxon	No. of strains tested	No. (%) ^a of strains:			
		Correctly identified	Identified with low discrimination	Misidentified	Not identified
Staphylococci					
<i>S. aureus</i>	100	99 (99)		1 (1)	
<i>S. epidermidis</i>	50	45 (90)	2 (4)		3 (6)
<i>S. haemolyticus</i>	23	21 (91.3)	2 (8.6)		
<i>S. hominis</i>	7	4	3 (42.8)		
<i>S. saprophyticus</i>	4	4			
<i>S. xylosus</i>	2	2			
<i>S. capitis</i>	5	4	1		
<i>S. simulans</i>	5	3	1	1	
<i>S. cohnii</i>	1	1			
<i>S. warneri</i>	1	1			
<i>S. lugdunensis</i>	1	0	1		
<i>S. sciuri</i>	1	1			
Total	200	185 (92.5)	10 (5.0)	2 (1.0)	3 (1.5)
Enterococci					
<i>E. faecalis</i>	55	51 (92.7)	1 (1.8)	1 (1.8)	2 (3.6)
<i>E. faecium</i>	28	20 (71.4)	4 (14.3)	2 (7.1)	2 (7.1)
<i>E. durans</i>	4	1	2	1	
<i>E. gallinarum</i>	2	2			
Total	89	74 (83.1)	7 (7.9)	4 (4.5)	4 (4.5)
Streptococci					
<i>S. agalactiae</i>	29	28 (96.5)			1 (3.4)
<i>S. pneumoniae</i>	66	64 ^b (96.9)			2 (3.0)
Total for all gram-positive strains	384	351 (91.4)	17 (4.4)	6 (1.6)	10 (2.6)

^a Percentages were calculated for species for which more than 20 strains were tested.

^b Fourteen strains were identified as "low discrimination resolved" by a simple test (optochin test).

family and API 20 Strep for members of the *Streptococcaceae* family. Other testing performed included serologic typing for beta-hemolytic streptococci, coagulase test for *Staphylococcus aureus*, motility test and yellow pigment production for enterococci, and optochin susceptibility for *S. pneumoniae* (5, 11, 17).

Identification with VITEK 2. The test panels (ID-GPC) contained 46 fluorimetric tests that included pH change tests and derivatives to detect aminopeptidases and -osidases. Substrates used for detection of aminopeptidases are coupled with 7-amino-methylcoumarin (7AMC); substrates for the detection of -osidases are usually coupled with 4-methylumbelliferone (4MU). The 21 test substrates are as follows: 4MU- α -L-arabinofuranoside, 4MU- α -D-galactoside, 4MU- α -D-glucoside, 4MU- α -D-N-acetylneuraminic acid, 4MU- β -D-galactoside, 4MU- β -D-glucoside, 4MU- β -D-glucuronide, 4MU- β -D-mannoside, 4MU-*n*-acetyl- β -D-glucosaminide, 4MU-phosphate, alanine-7AMC, arginine-7AMC, urease (butiloxycarbonyl-Val-Pro-Arg-AMC), histidine-7AMC, α -glutamic acid-7AMC, threonine-7AMC, lysine-7AMC, phenylalanine-7AMC, proline-7AMC, pyroglutamic acid-7AMC, and tyrosine-7AMC. Furthermore, the ID-GPC card includes 16 fermentation tests (for D-raffinose, amygdaline, arbutine, D-galactose, glycerol, D-glucose, L-arabinose, lactose, D-maltose, D-mannitol, N-acetylglucosamine, salicin, D-sorbitol, D-trehalose, D-melibiose, and D-xylose), two decarboxylase tests (for ornithine and arginine), and six miscellaneous tests (for urease, pyruvate, optochin, novobiocin, polymyxin B sulfate, and 6% NaCl).

The card was automatically filled by a vacuum device, sealed and inserted into the VITEK 2 reader-incubator module (incubation temperature, 35.5°C), and subjected to a kinetic fluorescence measurement every 15 min. The results were interpreted by the ID-GPC database, and final results were obtained automatically. All cards used were automatically discarded into a waste container.

Agar dilution reference method. This method was performed according to the NCCLS guidelines (13, 14). A 0.5 McFarland bacterial suspension was used directly in this test. Antimicrobial concentration ranges used and breakpoints are shown in Table 2.

Oxacillin screen. Oxacillin screen agar testing was performed for *S. aureus* according to the NCCLS methodology (13, 14) using Mueller-Hinton agar with 4% NaCl and oxacillin (6 μ g/ml). Plates were inoculated with the 0.5 McFarland bacterial suspension and incubated at 35°C for 24 h.

High-concentration aminoglycoside testing. The agar screen reference method for gentamicin and streptomycin was performed according to NCCLS methods (13, 14) in brain heart infusion agar and using gentamicin at 500 μ g/ml and streptomycin at 2,000 μ g/ml.

Genetic methods. The presence of *mecA* (staphylococci) and *vanA* and *vanB* (enterococci) genes, as well as genes for high-level aminoglycoside resistance in enterococci, was confirmed by PCR as previously described (1, 4, 16, 22).

AST with VITEK 2. The 0.5 McFarland bacterial suspension was diluted to 1.5×10^7 CFU/ml in 0.45% saline. Cards were automatically filled, sealed, and loaded into the VITEK 2 instrument for incubation and reading. The AST-P515 card used for staphylococci contained benzylpenicillin, clindamycin, erythromycin, fusidic acid, gentamicin, kanamycin, lincomycin, minocycline, nitrofurantoin, norfloxacin, ofloxacin, oxacillin, pristinamycin, rifampin, teicoplanin, tetracycline, tobramycin, trimethoprim-sulfamethoxazole, and vancomycin; the AST-P516 card used for enterococci and *S. agalactiae* contained ampicillin, ampicillin-sulbactam, cefuroxime, ciprofloxacin, clindamycin, erythromycin, high-concentration (HC) gentamicin, imipenem, HC kanamycin, levofloxacin, nitrofurantoin, norfloxacin, ofloxacin, quinupristin-dalfopristin, HC streptomycin, teicoplanin, tetracycline, trimethoprim-sulfamethoxazole, and vancomycin; the AST-P506 card used for pneumococci contained amoxicillin, benzylpenicillin, cefotaxime, ceftriaxone, chloramphenicol, erythromycin, imipenem, ofloxacin, pristinamycin, tetracycline, trimethoprim-sulfamethoxazole, and vancomycin. The antibiotic-microorganism combinations selected for evaluating the performance of the VITEK 2 system in this study are shown in Table 2.

Analysis of identification results. There are four possibilities for analysis of identification results: (i) correct identification, in which strains are correctly

TABLE 2. Antimicrobial concentration ranges and breakpoints used in AST

Microorganism(s) and antimicrobial agent	MIC ($\mu\text{g/ml}$) range determined by method		Breakpoint ^a ($\mu\text{g/ml}$)	
	VITEK 2	Reference ^b	Susceptible	Resistant
<i>Staphylococcus</i> spp.				
Clindamycin	0.25–8	0.015–32	0.5	4
Erythromycin	0.25–8	0.015–32	0.5	8
Gentamicin	0.5–16	0.03–64	4	16
Norfloxacin	0.25–16	0.03–16	2	16
Oxacillin	0.5–8	0.03–64	2 (0.25 ^c)	4 (0.5 ^c)
Rifampin	0.5–32	0.015–32	1	4
Teicoplanin	0.5–32	0.015–16	8	32
Vancomycin	1–32	0.05–16	4	32
<i>Enterococcus</i> spp.				
Ampicillin	2–32	0.015–32	8	16
Teicoplanin	0.5–16	0.015–16	8	32
Vancomycin	1–16	0.015–16	4	32
HC Gentamicin	120	500	500	
HC Streptomycin	200	2,000	2,000	
<i>S. agalactiae</i>				
Ampicillin	0.25–32	0.015–32	0.25	
Clindamycin	0.25–8	0.015–32	0.25	1
Erythromycin	0.25–8	0.015–32	0.25	1
Teicoplanin	0.5–16	0.015–16		
Tetracycline	1–16	0.03–16	2	8
Vancomycin	1–16	0.015–16	1	
<i>S. pneumoniae</i>				
Penicillin	0.06–2	0.015–32	0.06	2
Cefotaxime	0.06–4	0.12–32	0.5	2
Erythromycin	0.06–1	0.015–32	0.25	1
Ofloxacin	1–8	0.03–16	2	8

^a Breakpoints as given in reference 14.

^b Agar dilution.

^c Breakpoint for CNS.

identified to the species level or strains with low discrimination are resolved (two or more species are proposed by the VITEK 2 system [one being identical to the reference method], and the identification can be resolved by simple additional tests); (ii) low discrimination, in which strains with low discrimination are not resolved (two or more species are proposed by the VITEK 2 system [one being identical to the reference method], and the identification cannot be resolved by simple additional tests); (iii) misidentification, in which discrepant results are obtained for strains (one or more species proposed by the VITEK 2 system are different from those identified by the reference method); and (iv) no identification, in which no identification was provided. The mean time for result generation was also calculated for all identifications.

Analysis of susceptibility testing. There are two possibilities for analysis of susceptibility testing. (i) Category agreement (CA) may occur. In CA, the MICs determined by both methods identified the category of microbial susceptibility as susceptible, intermediate, or resistant according to NCCLS interpretative criteria (13, 14). (ii) Discrepancies may occur. Discrepancies were considered very major errors (VME) when the VITEK 2 system indicated susceptibility and the reference method indicated resistance, were considered major errors (ME) when the VITEK 2 system indicated resistance and the reference method indicated susceptibility, and were considered minor errors (mE) when the VITEK 2 system indicated intermediate susceptibility and the reference method indicated susceptibility or resistance or when the VITEK 2 system indicated susceptibility or resistance and the reference method indicated intermediate susceptibility.

Quality control. The quality control strains *Enterococcus casseliflavus* ATCC 700327, *Kocuria rosea* ATCC 186, *S. aureus* ATCC 29213, *Staphylococcus lugdunensis* ATCC 700328, *Staphylococcus sciuri* ATCC 29061, *S. pneumoniae* ATCC 6301, and *Streptococcus uberis* ATCC 9927 were included for identification; *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *E. faecalis* ATCC 51299, *Escherichia coli* ATCC 35218, and *S. pneumoniae* ATCC 49619 were included for AST.

RESULTS

Identification of gram-positive cocci with the VITEK 2 system. Table 1 shows the performance of the VITEK 2 system for the identification of individual species or bacteria groups.

Low-discrimination identifications were frequent among coagulase-negative staphylococci (CNS) (10%), whereas only one misidentification occurred for *S. aureus* and one misidentification occurred for CNS. Three *Staphylococcus epidermidis* isolates were not identified. The only *S. aureus* isolate misidentified by the VITEK 2 system was identified as *Staphylococcus warneri*.

Five *Enterococcus faecium* isolates were not discriminated from other enterococcal species: four were not discriminated from *Enterococcus hirae* and *Enterococcus durans*, and one was not discriminated from *E. casseliflavus-Enterococcus gallinarum*. The latter discrepancy was resolved by motility test and yellow pigment production, whereas the others were not resolved since no simple test is available to discriminate among *E. faecium*, *E. hirae*, and *E. durans*. Three *E. durans* isolates were not discriminated from or were misidentified as *E. hirae*. Four strains (two *E. faecalis* strains and two *E. faecium* strains) were not identified.

One strain of *S. agalactiae* was not discriminated from *Streptococcus acidominimus*, but the discrepancy was resolved by immunological testing, and one strain was not identified.

For 14 *S. pneumoniae* isolates the correct identification was proposed by the VITEK 2 system as an alternative to other streptococcal species. All of the low discrimination identifications were resolved by the performance of the optochin test. Two strains were not identified.

Of the total gram-positive strains, discordant identifications were seen with 1.6% of the isolates and no identification was observed for 2.6% of the isolates. All quality control strains were correctly identified by the VITEK 2 system.

Time to obtain identification. The VITEK 2 system identified all *S. agalactiae* and all *S. pneumoniae* isolates within 2 h; 98% of *S. aureus* isolates and 71% of CNS were identified in 3 h. Six hours was required to identify the remaining isolates, i.e., two *S. aureus* isolates and 29 CNS.

AST. MICs of each antimicrobial agent generated by the VITEK 2 system were compared with each MIC determined by the NCCLS reference procedure (13, 14). A susceptibility category was also assigned to each MIC based on the current NCCLS breakpoint criteria (13, 14). Since there were a number of off-scale MICs that could not be compared, the agreement was defined by CA and not by essential agreement (VITEK 2 system MIC results within 1 doubling dilution from the reference result). Discrepant results for oxacillin (staphylococci), high-concentration gentamicin and streptomycin (enterococci), and vancomycin and teicoplanin (enterococci) were resolved by genetic methods which identified the gene(s) responsible for resistance. The final error rates were calculated using the values obtained when errors were resolved.

In total, 2,483 drug-organism combinations were tested. Performance compared to reference standard testing is shown in Table 3.

No ME and VME were found with *S. aureus* AST, but mE were frequent with norfloxacin. One erythromycin-resistant and one oxacillin-resistant CNS were identified as susceptible

TABLE 3. Performance of the VITEK 2 system for AST of gram-positive cocci compared to that of the reference method

Microorganism(s) and antimicrobial agent	No. of isolates with susceptibility ^a			No. of errors			CA (%)
	S	I	R	VME	ME	mE	
<i>S. aureus</i> (n = 100)							
Clindamycin	50	0	50	0	0	0	100
Erythromycin	40	1	59	0	0	0	100
Gentamicin	50	0	50	0	0	0	100
Norfloxacin	38	7	55	0	0	6	94
Oxacillin	39	0	61	0	0	0	100
Rifampin	92	2	6	0	0	2	98
Teicoplanin	100	0	0	0	0	0	100
Vancomycin	100	0	0	0	0	0	100
CNS (n = 100)							
Clindamycin	59	6	35	0	0	6	94
Erythromycin	40	7	53	1	0	9	90
Gentamicin	38	3	59	0	0	9	91
Norfloxacin	46	9	45	0	0	8	92
Oxacillin	32	0	68	1	2	0	97
Rifampin	74	7	19	0	0	3	97
Teicoplanin	95	5	0	0	0	7	93
Vancomycin	100	0	0	0	0	0	100
<i>Enterococcus</i> spp. (n = 89)							
Ampicillin	69	1	19	0	0	1	99
Teicoplanin	67	0	22	4	0	4	91
Vancomycin	67	0	22	0	0	1	99
HC Gentamicin	69		20	1	0	0	99
HC Streptomycin	44		45	0	0	0	100
<i>S. agalactiae</i> (n = 29)							
Ampicillin	29	0	0	0	0	0	100
Clindamycin	28	0	1	0	0	0	100
Erythromycin	28	0	1	0	0	0	100
Tetracycline	12	0	17	0	1	0	96
Teicoplanin	29	0	0	0	0	0	100
Vancomycin	29	0	0	0	0	0	100
<i>S. pneumoniae</i> (n = 66)							
Penicillin	60	5	1	0	0	6	91
Cefotaxime	65	1	0	0	0	2	97
Erythromycin	49	0	17	0	0	0	100
Ofloxacin	66	4	0	0	0	3	95
% for all individual species tested ^b				0.24	0.12	2.7	97
% according to FDA-NCCLS criteria ^c				0.82	0.17	2.7	96

^a Abbreviations; S, susceptible; I, intermediate; R, resistant.

^b Calculated from results for 2,483 drug-organism combinations.

^c Percent VME = number of VME × 100/total number of resistant strains (730); percent ME = number of ME × 100/total number of susceptible strains (1,753); percent mE = number of mE × 100/total number of strain tested (2,483). FDA, Food and Drug Administration.

by the VITEK 2 system. For this group of microorganisms mE were frequently observed with nearly all the antibiotics.

For enterococci, VME and mE were found with teicoplanin in particular. Table 4 shows that the VITEK 2 system correctly identified the glycopeptide resistance phenotype for the five reference strains and that most VME were clustered at the susceptible breakpoint.

The CA for *S. agalactiae* was 100% for all the antibiotics

used for comparison with the reference method, except tetracycline, which gave one ME.

Four penicillin-intermediate and two penicillin-susceptible strains of *S. pneumoniae* were identified by the VITEK 2 system as resistant and intermediate, respectively. Two cefotaxime-susceptible strains were identified as intermediate. The only penicillin-resistant strain in our collection was correctly identified.

Time to obtain MIC. The time to obtain MICs ranged from 6 h (20% of *S. aureus*, 5% of CNS, 49% of enterococci, 33% of *S. agalactiae*, and 0% of *S. pneumoniae*) to 17 h (time required to complete all CNS AST). Ninety percent of AST was completed in 8 h for *S. aureus*, in 11 h for CNS, in 9 h for enterococci, in 7 h for *S. agalactiae*, and in 9 h for *S. pneumoniae*.

DISCUSSION

Identification. The ability of the VITEK 2 system and ID-GPC card to accurately give a rapid identification of clinically significant gram-positive cocci was first assessed by Bassel et al. (Abstr. 8th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. P255, 1997), who showed, with isolates of staphylococci, streptococci, and enterococci, 98.0% overall agreement (86.8% agreement to species level without supplemental testing required and 11.2% agreement to species level after supplemental testing), whereas 1.7% of isolates were misidentified and 0.3% of isolates were unidentified. In particular, stock isolates and fresh isolates of *S. aureus* were identified with an accuracy of 98.8 and 96%, respectively. Stock and fresh isolates of the following organisms were identified with the indicated accuracies, respectively: *S. epidermidis*, 92.7 and 97%; *Staphylococcus haemolyticus*, 83.8 and 97%; *E. faecalis*, 97.7 and 97%; *E. faecium* 94.6 and 91%; *S. agalactiae*, 97.7 and 95%; and *S. pneumoniae*, 95.8 and 76%. With the VITEK 1 system and GPC cards, the range of correct identification among CNS was 67 to 83% and that among *S. epidermidis* isolates was 88 to 95% (15).

In our study, the VITEK 2 system demonstrated similar rates of accuracy in identification of staphylococci. Taxa with low-discrimination identification results included a very limited number of strains to provide useful information for improve-

TABLE 4. Glycopeptide MICs of reference strains and glycopeptide-resistant clinical isolates with discrepant results

Isolate	Geno-type	MIC (μg/ml) of antimicrobial as determined by method			
		Vancomycin		Teicoplanin	
		VITEK 2	Reference	VITEK 2	Reference
<i>E. faecium</i> BM4147 ^a	<i>vanA</i>	≥32	≥32	≥32	32
<i>E. faecium</i> NJ1 ^b	<i>vanA</i>	≥32	≥32	16	16
<i>E. faecium</i> NJ2 ^b	<i>vanB</i>	≥32	≥32	≤0.5	≤0.5
<i>E. faecalis</i> NJ3 ^b	<i>vanB</i>	≥32	≥32	≤0.5	≤0.5
<i>E. gallinarum</i> NJ4 ^b	<i>vanC</i>	8	8	≤0.5	1
<i>E. faecalis</i> VI538 ^c	<i>vanA</i>	≥32	≥32	8	≥32
<i>E. faecalis</i> VI535 ^c	<i>vanA</i>	≥32	≥32	8	≥32
<i>E. faecium</i> VI511 ^c	<i>vanA</i>	≥32	≥32	2	≥32
<i>E. faecalis</i> VI534 ^c	<i>vanA</i>	≥32	≥32	8	≥32

^a Reference strain values as given in reference 1.

^b Reference strain values as given in reference 19.

^c Clinical isolate.

ments in the VITEK 2 system, and none of the problematic reactions for the misidentified strains was significantly more frequently observed than others. The fact that CNS were not identified as well as *S. aureus* could be explained by the slow metabolism of some CNS strains, leading to ambiguous results in the reaction wells. This was also observed in evaluation of other automated identification systems with CNS (15).

A relatively low rate of correct identifications was observed in our study with *E. faecium* (71.4%). More recently Garcia-Garrote et al. (10) reported the accuracy of the VITEK 2 system as 98.3 and 76.3% in the identification of *E. faecalis* and *E. faecium*, respectively; in that study the latter species was mostly misidentified as *E. casseliflavus*-*E. gallinarum*. In our study most *E. faecium* isolates were misidentified as *E. hirae* or *E. durans*, and these discrepancies could not be resolved since simple tests are not available to discriminate among these species, whereas a simple motility test can discriminate *E. faecium* from *E. casseliflavus*-*E. gallinarum*. We have no explanations for accuracy shown by the VITEK 2 system in our study and for the prevalence of misidentification with *E. hirae* or *E. durans*. Nevertheless, in the clinical setting, reasons for species identification of enterococci are very limited (serious infections, such as endocarditis, or epidemiological surveillance within hospitals). In general, presumptive identification to the genus level together with determination of susceptibility is considered to be sufficient (12). Several taxonomy changes have been introduced in the *Enterococcus* genus, mainly involving species other than *E. faecalis* (5). The difficulties of commercial tests in the identification of species other than *E. faecalis* probably reflects problems with the new species definition or in the criteria available for identifying them.

Antimicrobial susceptibility test. The Food and Drug Administration has established minimal performance characteristics to assess antimicrobial susceptibility tests (20). These guidelines indicate that CA should be >90%, ME should be <3%, and VME should be ≤1.5%. Our investigation indicates that the VITEK 2 system provides accurate susceptibility test results, since the agreement with the reference method was well above, and the rates of discrepancies (VME and ME) were far below, the limit for all the antibiotic-microorganism combinations.

The detection of oxacillin-resistant staphylococci with the VITEK 2 system was very accurate. Even if VME or ME were observed in CNS, the rates were below the recommended limit. Our results agreed with those reported in the few studies which have assessed the performance of the VITEK 2 system in AST of staphylococci, reporting for oxacillin resistance detection, with agreement rates ranging from 95 to 100% (M. E. Reverdy, L. Carret, and A. Quaglia, Abstr. 8th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. P284, 1997; R. Gross, B. Grünastel, K. Becker, and G. Peters, Abstr. 8th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. P657, 1997; C. Bradford, L. Meeh, D. Freiner, J. Rader, and J. Gerst, Abstr. 98th Gen. Met. Am. Soc. Microbiol., abstr. C479, 1998; C. Shubert, R. Griffith, W. McLaughlin, M. Ullery, and M. Peyret, Abstr. 98th Gen. Met. Am. Soc. Microbiol., abstr. C478, 1998; J. Krzyszton-Russjan, J. Walory, and K. Nowak, Abstr. 9th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. P827, 1999).

In our study the VITEK 2 system correctly detected all vancomycin-resistant enterococci but failed to identify 4 of the

22 teicoplanin-resistant isolates. Failures in detection of glycopeptide resistance were also reported by others (10), who, however, reported a VME rate for vancomycin higher than that for teicoplanin. In our study, vancomycin resistance was correctly identified in all strains, and the VME with teicoplanin would have as a consequence the misidentification of the precise glycopeptide resistance phenotype. Correct identification of the phenotype or genotype can be subsequently performed with supplementary tests. In addition, it should be stressed that the high VME rate observed for the teicoplanin-enterococci combination is due in part to the fact that the selection of organisms included in our study favored resistant strains. These problems may not be seen in the largely glycopeptide-susceptible enterococci population commonly encountered in clinical microbiology laboratories at this time in Italy (7).

Agreement among results for ampicillin, HC gentamicin, and HC streptomycin resistance was found to be better in our study than in a previous one (10), but we examined a lower number of resistant strains.

The results of VITEK 2 system AST for *S. agalactiae* correlated highly with those of the reference method and are similar to those reported by others (Ghanem et al., Abstr. 98th Gen. Met. Am. Soc. Microbiol., abstr. C484, 1998). In this species, the resistance usually encountered is that to tetracycline, and the VITEK 2 system correctly identified tetracycline resistance in all strains. One susceptible strain was identified as resistant. This ME may not be significant, since tetracycline is not the drug of choice for treatment of infections caused by *S. agalactiae*.

Finally, no VME or ME were observed in the AST results for *S. pneumoniae*, but higher MICs of penicillin, cefotaxime, and ofloxacin causing mE rates were seen. The VITEK 2 system performed very well for erythromycin-resistant strains, which are encountered in Italy more frequently than penicillin-resistant strains (3).

In conclusion, this study validates the VITEK 2 system as an easy-to-use system that provides a rapid (4 to 15 h) and accurate means for identification and AST of most commonly isolated species of gram-positive cocci.

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REFERENCES

1. Arthur, M., C. Molinas, F. Depardieu, and P. Courvalin. 1993. Characterization of Tn1546, a Tn3-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. *J. Bacteriol.* **175**:117-127.
2. Barenfanger, J., C. Drake, and G. Kacich. 1999. Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing. *J. Clin. Microbiol.* **37**:1415-1418.
3. Cornaglia, G., G. Lo Cascio, L. Masala, The Italian Surveillance Group for Antimicrobial Resistance, and R. Fontana. 2000. Macrolide resistance among *S. pneumoniae* isolates in Italy, p. 250-254. In S. H. Zinner, L. S. Young, J. F. Acar, and C. Ortiz-Neu (ed.), New considerations for macrolides, azalides, streptogramins, and ketolidides. M. Dekker, Inc., New York, N.Y.
4. Dukta-Malen, S., S. Evers, and P. Courvalin. 1995. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. *J. Clin. Microbiol.* **33**:24-27.
5. Facklam, R. R., D. F. Sahm, and L. Martins Teixeira. 1999. Enterococcus, p. 297-305. In P. R. Murray, E. J. Baron, M. A. Tenover, F. C. Tenover, and R. H. Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

6. Ferraro, M. J., and J. H. Jorgensen. 1995. Instrument-based antibacterial susceptibility testing, p. 1375–1384. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
7. Fontana, R., M. Ligozzi, A. Mazzariol, G. Veneri, The Italian Surveillance Group for Antimicrobial Resistance, and G. Cornaglia. 1998. Resistance of enterococci to ampicillin and glycopeptide antibiotics in Italy. *Clin. Infect. Dis.* **27**:84–86.
8. Fontana, R. 1985. Leading article: penicillin-binding proteins and the intrinsic resistance to beta-lactams in Gram-positive cocci. *J. Antimicrob. Chemother.* **16**:412–416.
9. Funke, G., Monnet, C. deBernardis, A. von Graevenitz, and J. Freney. 1998. Evaluation of the VITEK 2 system for rapid identification of medically relevant gram-negative rods. *J. Clin. Microbiol.* **36**:1948–1952.
10. Garcia-Garrote, F., E. Cercenado, and E. Bouza. 2000. Evaluation of a new system, VITEK 2, for identification and antimicrobial susceptibility testing of enterococci. *J. Clin. Microbiol.* **38**:2108–2111.
11. Kloos, W. E., and T. L. Bannerman. 1999. *Staphylococcus* and *Micrococcus*, p. 264–282. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 7th ed. American Society for Microbiology, Washington, D.C.
12. Murray, B. E. 1990. The life and times of the enterococcus. *Clin. Microbiol. Rev.* **3**:46–65.
13. National Committee for Clinical Laboratory Standards. 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 5th ed. M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
14. National Committee for Clinical Laboratory Standards. 2001. Performance standards for antimicrobial susceptibility testing: ninth informational supplement. M100-S11. National Committee for Clinical Laboratory Standards, Wayne, Pa.
15. Pfaller, M. A., and L. A. Herwaldt. 1988. Laboratory, clinical, and epidemiological aspects of coagulase-negative staphylococci. *Clin. Microbiol. Rev.* **1**: 281–299.
16. Predari, S. C., M. Ligozzi, and R. Fontana. 1991. Genotypic identification of methicillin-resistant coagulase-negative staphylococci by polymerase chain reaction. *Antimicrob. Agents Chemother.* **35**:2568–2573.
17. Ruoff, K. L., R. A. Whitley, and D. Beighton. 1999. *Streptococcus*, p. 283–296. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 7th ed. American Society for Microbiology, Washington, D.C.
18. Stager, C. E., and J. R. Davis. 1992. Automated systems for identification of microorganisms. *Clin. Microbiol. Rev.* **5**:302–327.
19. Tenover, F. C., J. Tokars, J. Swenson, S. Paul, K. Spitanly, and W. Jarvis. 1993. Ability of clinical laboratories to detect antimicrobial agent-resistant enterococci. *J. Clin. Microbiol.* **31**:1695–1699.
20. Thornsberry, C. 1985. Automated procedures for antimicrobial susceptibility tests, p. 1015–1018. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 4th ed. American Society for Microbiology, Washington, D.C.
21. Tomfohrde, K. M. 1991. Review criteria for assessment of antimicrobial susceptibility devices: what do they mean? *Clin. Microbiol. Newsl.* **8**:1–8.
22. Van de Klundert, J. A. M., and J. S. Vliegthart. 1993. PCR detection of genes coding for aminoglycoside modifying enzymes, p. 547–552. In D. H. Persing, T. E. Smith, F. C. Tenover, and T. J. White (ed.), *Diagnostic molecular microbiology: principles and applications*. Mayo Foundation, Rochester, Minn.
23. Woodford, N., A. P. Johnson, D. Morrison, and D. C. E. Speller. 1995. Current perspective on glycopeptide resistance. *Clin. Microbiol. Rev.* **8**:585–615.