

## Evaluation of the VITEK 2 System for the Identification and Susceptibility Testing of Three Species of Nonfermenting Gram-Negative Rods Frequently Isolated from Clinical Samples

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VITEK 2 is a new automatic system for the identification and susceptibility testing of the most clinically important bacteria. In the present study 198 clinical isolates, including *Pseudomonas aeruginosa* ( $n = 146$ ), *Acinetobacter baumannii* ( $n = 25$ ), and *Stenotrophomonas maltophilia* ( $n = 27$ ) were evaluated. Reference susceptibility testing of cefepime, cefotaxime, ceftazidime, ciprofloxacin, gentamicin, imipenem, meropenem, piperacillin, tobramycin, levofloxacin (only for *P. aeruginosa*), co-trimoxazole (only for *S. maltophilia*), and ampicillin-sulbactam and tetracycline (only for *A. baumannii*) was performed by microdilution (NCCLS guidelines). The VITEK 2 system correctly identified 91.6, 100, and 76% of *P. aeruginosa*, *S. maltophilia*, and *A. baumannii* isolates, respectively, within 3 h. The respective percentages of essential agreement (to within 1 twofold dilution) for *P. aeruginosa* and *A. baumannii* were 89.0 and 88.0% (cefepime), 91.1 and 100% (cefotaxime), 95.2 and 96.0% (ceftazidime), 98.6 and 100% (ciprofloxacin), 88.4 and 100% (gentamicin), 87.0 and 92.0% (imipenem), 85.0 and 88.0% (meropenem), 84.2 and 96.0% (piperacillin), and 97.3 and 80% (tobramycin). The essential agreement for levofloxacin against *P. aeruginosa* was 86.3%. The percentages of essential agreement for ampicillin-sulbactam and tetracycline against *A. baumannii* were 88.0 and 100%, respectively. Very major errors for *P. aeruginosa* (resistant by the reference method, susceptible with the VITEK 2 system [resistant to susceptible]) were noted for cefepime (0.7%), cefotaxime (0.7%), gentamicin (0.7%), imipenem (1.4%), levofloxacin (2.7%), and piperacillin (2.7%) and, for one strain of *A. baumannii*, for imipenem. Major errors (susceptible to resistant) were noted only for *P. aeruginosa* and cefepime (2.0%), ceftazidime (0.7%), and piperacillin (3.4%). Minor errors ranged from 0.0% for piperacillin to 22.6% for cefotaxime against *P. aeruginosa* and from 0.0% for piperacillin and ciprofloxacin to 20.0% for cefepime against *A. baumannii*. The VITEK 2 system provided co-trimoxazole MICs only for *S. maltophilia*; no very major or major errors were obtained for co-trimoxazole against this species. It is concluded that the VITEK 2 system allows the rapid identification of *S. maltophilia* and most *P. aeruginosa* and *A. baumannii* isolates. The VITEK 2 system can perform reliable susceptibility testing of many of the antimicrobial agents used against *P. aeruginosa* and *A. baumannii*. It would be desirable if new versions of the VITEK 2 software were able to determine MICs and the corresponding clinical categories of agents active against *S. maltophilia*.

Several automated systems are available for the identification and susceptibility testing of the clinically most important bacteria (21). The VITEK system (bioMérieux-Vitek, Hazelwood, Mo.) was originally designed as an onboard system for the detection and identification of urinary tract pathogens from astronauts in spacecraft. It was first introduced in clinical laboratories in 1979 and has since been evaluated extensively (6). More recently, the new VITEK 2 system (bioMérieux-Vitek) was introduced [J. P. Gayral, R. Robinson, and D. Sandstedt, Clin. Microbiol. Infect. 3(Suppl. 2):53, abstr. P254, 1997; V. Rekasius, R. B. Carey, L. Lee, M. Motyl, L. Chen, J. Montgomery, and R. Horvat, Abstr. 98th Gen. Meet. Am. Soc. Microbiol. 1998, abstr. A17, 1998]. The VITEK 2 system detects metabolic changes by fluorescence-based methods which facilitate the identification of gram-negative bacteria within 3 h. This system monitors the kinetics of bacterial growth and

calculates MICs using a unique algorithm. In addition, the VITEK 2 system incorporates several technical improvements which automate many procedures that were performed manually with the previous VITEK system (Gayral et al., Clin. Microbiol. Infect., abstr. P254).

Many studies have shown various drawbacks of automated systems associated with the reliable identification of gram-negative rods, particularly nonfermenting ones, and with the determination of susceptibility to several groups of antimicrobial agents, some  $\beta$ -lactams in particular (4, 8, 11, 17, 20, 22–25). Several systems, including the VITEK 2 system, incorporate expert systems to control the results of identification and susceptibility tests by applying a series of predefined rules which detect infrequent or impossible phenotypes.

In a previous study (9), the VITEK 2 system was shown to identify correctly within 3 h 84.7% of selected species of members of the family *Enterobacteriaceae* and nonenteric bacilli covering 70 different taxa, with 0.8% being misidentified strains and 1.2% being nonidentified strains. After a few rapid manual tests were performed, an additional 3.8% strains were identified. For 9.5% of strains, these tests did not enable iden-

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TABLE 1. Phenotypes of resistance of 146 strains of *P. aeruginosa*, as defined by clinical categories, to ceftazidime and ticarcillin, imipenem, meropenem, and ciprofloxacin<sup>a</sup>

Phenotype	CAZ, TIC	IPM	MEM	CIP	No. (%) of strains
Basal resistance	S	S	S	S	48 (32.9)
CIP <sup>r</sup>	S	S	S	R	26 (17.8)
CAZ <sup>r</sup>	R	S	S	S	19 (13.0)
IPM <sup>r</sup>	S	R	S, I, R <sup>b</sup>	S	22 (15.1)
CAZ-IPM <sup>r</sup>	R	R	S, I, R <sup>c</sup>	S, R <sup>d</sup>	21 (14.4)
Not named	R	S	S	R	7 (4.8)
Not named	S	R	S	R	3 (2.0)

<sup>a</sup> Abbreviations: CAZ, ceftazidime; TIC, ticarcillin; IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; CIP<sup>r</sup>, ciprofloxacin resistant; CAZ<sup>r</sup>, ceftazidime resistant; IPM<sup>r</sup>, imipenem resistant; S, susceptible; I, intermediate; R, resistant.

<sup>b</sup> Susceptible, *n* = 7; intermediate, *n* = 11; resistant, *n* = 4.

<sup>c</sup> Susceptible, *n* = 2; intermediate, *n* = 10; resistant, *n* = 9.

<sup>d</sup> Susceptible, *n* = 10; resistant, *n* = 11.

tification at the species level, even though a correct identification was given among the organisms listed by the VITEK 2 system. Preliminary reports have also evaluated the ability of the VITEK 2 system to identify and determine the susceptibilities of gram-negative rods, including nonfermenting organisms, to antimicrobial agents [C. Jay, C. Schubert, D. Parreno, S. Pétré, M. T. Albertini, and M. Peyret, Clin. Microbiol. Infect. 3(Suppl. 2):62, abstr. P287, 1997; M.-F. Jossart and R. J. Courcol, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. D53, 1997; D. Monnet and J. Freney, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. D54, 1997].

The objective of the present study was to evaluate the VITEK 2 system for the identification and susceptibility testing of three species of gram-negative rods of major clinical importance: *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia*.

#### MATERIALS AND METHODS

**Bacteria.** One hundred ninety-eight organisms (one per patient) were studied, including 146 *P. aeruginosa*, 25 *A. baumannii*, and 27 *S. maltophilia* isolates. Bacteria were isolated at the Clinical Microbiology Laboratory, Hospital Universitario Virgen Macarena, Seville, Spain, during the period from January 1997 to November 1998 (*P. aeruginosa* and *S. maltophilia*) and between January 1995 and December 1997 (*A. baumannii*).

The results of susceptibility testing of *P. aeruginosa* with the VITEK 2 system were evaluated as a function of the results of reference susceptibility tests (see below) with ceftazidime, ciprofloxacin, imipenem, meropenem, and ticarcillin. Seven phenotypes were defined among the 146 isolates of *P. aeruginosa*, as shown in Table 1. The 25 isolates of *A. baumannii*, isolated from blood cultures during the indicated period, represent different clones (as defined by pulsed-field gel electrophoresis of *Sma*I-digested total DNA [manuscript in preparation]) and, within the same clone, isolates with different patterns of resistance to antimicrobial agents. In total, 16 clones of *A. baumannii* were studied.

*Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as control strains in susceptibility testing assays.

**Identification.** Identification of *P. aeruginosa* was performed with the API 20NE system (BioMérieux, La Balme les Grottes, France) and by conventional phenotypic tests, including colony morphology, culture odor, and pigment production (13). Reference identification of *S. maltophilia* was also performed with the API 20NE system, while identification of *A. baumannii* was performed in accordance with the scheme of Bouvet and colleagues (2, 3).

Identification with the VITEK 2 system was performed with ID-GNB cards, according to the manufacturer's instructions. The 64-well plastic ID-GNB cards contain 41 tests, including 18 tests for sugar assimilation, 18 tests for sugar fermentation, 2 decarboxylase tests, and 3 miscellaneous tests (for urease, utilization of malonate, and tryptophane deaminase). With a vacuum device, the

cards are inoculated with a 0.5 McFarland suspension of the organism prepared from a 18- to 20-h-old Columbia sheep blood agar plate (bioMérieux) and are then automatically sealed and manually inserted inside the VITEK 2 reader-inoculator module. Fluorescence is measured every 15 min, and the results of identification are determined after 3 h.

**Antimicrobial susceptibility testing.** A microdilution assay with homemade panels was used as the reference method, according to NCCLS guidelines (15). The following antimicrobial agents were tested: cefepime (Bristol-Myers Squibb, Madrid, Spain), cefotaxime (Sigma, Madrid, Spain), ceftazidime (Glaxo, Barcelona, Spain), ciprofloxacin (Sigma), gentamicin (Sigma), imipenem (Merck Sharp & Dohme, Madrid, Spain), levofloxacin (Hoechst Marion Roussel, Ro-mainville, France), meropenem (Zeneca, Madrid, Spain), piperacillin (Sigma), ticarcillin (Sigma), tobramycin (Sigma), co-trimoxazole (trimethoprim-sulfamethoxazole [1:19]; Sigma) (only for *S. maltophilia*), ampicillin-sulbactam (2:1; ampicillin [Sigma] and sulbactam [Pfizer, Madrid, Spain]), and tetracycline (Sigma) (only for *A. baumannii*). The ranges of concentrations used were 512 to 4 μg/ml (piperacillin, ticarcillin), 256 to 2 μg/ml (tetracycline), 128 to 1 μg/ml (cefepime, ceftazidime, cefotaxime), 64 to 0.5 μg/ml (imipenem, gentamicin, meropenem, tobramycin), 32 to 0.25 μg/ml (ciprofloxacin, levofloxacin), and 32/608 to 0.25/4.75 μg/ml (co-trimoxazole).

Susceptibility tests with the VITEK 2 system were performed with AT-N011 cards, according to the manufacturer's instructions. The 64-well AT-N011 card contains the following antimicrobial agents (as dehydrated substances) at the indicated concentrations: ampicillin, 1, 16, and 32 μg/ml; ampicillin-sulbactam, 4, 16, and 32 μg/ml; cefazolin, 4, 16, and 64 μg/ml; cefepime, 1, 8, and 16 μg/ml; cefixime, 0.25, 1, and 2 μg/ml; cefotaxime, 1, 4, 16, and 32 μg/ml; ceftazidime, 1, 2, 8, and 32 μg/ml; cefuroxime, 2, 8, and 32 μg/ml; cefuroxime axetil, 2, 4, and 32 μg/ml; ciprofloxacin, 0.5, 2, and 4 μg/ml; gentamicin, 4, 16, and 32 μg/ml; imipenem, 2, 4, and 16 μg/ml; levofloxacin, 0.5, 4, and 8 μg/ml; meropenem, 0.5, 4, and 16 μg/ml; mezlocillin, 4, 16, and 64 μg/ml; norfloxacin, 1, 8, and 32 μg/ml; piperacillin, 4, 16, and 64 μg/ml; piperacillin-tazobactam, 4, 16, and 128 μg/ml; tetracycline, 2, 4, and 8 μg/ml; tobramycin, 8, 16, and 64 μg/ml; and co-trimoxazole, 10, 40, and 320 μg/ml (the trimethoprim concentration). The results obtained with several antimicrobial agents included in the AT-N011 card (ampicillin, cefazolin, cefixime, cefuroxime, cefuroxime axetil, mezlocillin, norfloxacin, and piperacillin-tazobactam) were not evaluated either because they are not normally used in clinics against the species tested or because the mechanisms of resistance against them are similar to those against other drugs evaluated in the study. Ticarcillin was not included on the AT-N011 card but was considered a reference antimicrobial agent against *P. aeruginosa* for the evaluation of mechanisms of resistance of the isolates included in the present study.

The cards were filled with an inoculum of ca.  $8 \times 10^6$  CFU/ml (prepared from the 0.5 McFarland suspension used with the identification cards) and then sealed and read. The VITEK 2 system automatically processes the antimicrobial susceptibility cards until the MICs are obtained. The VITEK 2 expert system subsequently corrects, where necessary, for MICs or clinical category, in accordance with an internal database of possible phenotypes for microorganism-antimicrobial agent combinations. Finally, the MICs and clinical categories defined by the expert system (to simulate an actual situation in the clinical laboratory) were considered and compared with those obtained by the reference method.

**Analysis of results.** Compared with the results of the reference method, the identifications obtained with the VITEK 2 system were divided into four categories: correct identification (unambiguous correct identification at the species level), low level of discrimination (either identification at the genus level or low level of discrimination between several species, including the correct species), no identification (a doubtful, unacceptable, or unreliable identification), and misidentification (the species identified with the VITEK 2 system was different from that identified by the reference method).

Essential agreement (EA) was used to compare MICs obtained with the VITEK 2 system with those obtained by the reference microdilution method. EA occurs when the VITEK 2 MIC was within 1 twofold dilution of the reference MIC. MICs obtained either by microdilution or with the VITEK 2 system were translated into clinical categories (susceptible, intermediate, or resistant) according to the interpretive criteria of NCCLS (15). Very major errors were considered when an organism was defined as resistant by the reference method but was categorized as susceptible with the VITEK 2 system. Major errors were defined when an organism found to be susceptible by the reference method was considered resistant with the VITEK 2 system. Minor errors occurred when an organism was considered susceptible or resistant either by the reference microdilution method or with the VITEK 2 system but intermediate by the other method.

TABLE 2. EA, ACC, and errors in clinical categories between VITEK 2 and reference microdilution method for 146 strains of *P. aeruginosa*

Antimicrobial agent	Percent				
	EA	ACC	Minor error	Major error	Very major error
Cefepime	89.0	82.9	14.4	2.0	0.7
Cefotaxime	91.1	76.7	22.6	0.0	0.7
Ceftazidime	95.2	94.5	4.8	0.7	0.0
Ciprofloxacin	98.6	96.6	3.4	0.0	0.0
Gentamicin	88.4	88.4	10.9	0.0	0.7
Imipenem	87.0	91.8	6.8	0.0	1.4
Levofloxacin	86.3	91.1	6.2	0.0	2.7
Meropenem	85.0	90.4	9.6	0.0	0.0
Piperacillin	84.2	93.9	0.0	3.4	2.7
Tobramycin	97.3	98.6	1.4	0.0	0.0
All agents	90.2	90.7	7.7	0.7	0.9

## RESULTS

The VITEK 2 system correctly identified 88 of 146 (60.3%) strains of *P. aeruginosa*, 17 of 25 (68.0%) strains of *A. baumannii*, and all 27 (100%) strains of *S. maltophilia*. Forty-six (31.5%), 1 (0.7%), and 11 (7.5%) strains of *P. aeruginosa* were identified at a low level of discrimination, misidentified, or not identified, respectively. Two (8%) and six (24%) strains of *A. baumannii* were identified at a low level of discrimination or were not identified. In all cases, results were available within 3 h. In total and in consideration of the fact that a low level of discrimination is an acceptable identification from a clinical point of view, 91.8% of *P. aeruginosa* strains and 76% of *A. baumannii* strains were correctly identified.

The susceptibility testing results for *P. aeruginosa* and *A. baumannii* are shown in Tables 2 to 8 and Table 9, respectively. It was not possible to compare the results of susceptibility testing of *S. maltophilia* obtained by the microdilution method with those obtained with the VITEK 2 system because the automated method was unable to provide quantitative data for any antimicrobial agent except co-trimoxazole. For co-trimoxazole, EA was 100%, and no category errors were observed between the reference microdilution method and the VITEK 2 system.

Percentages of resistance to the antimicrobial agents among the 146 strains of *P. aeruginosa* studied were 32.2% (ceftazidime, ticarcillin, ciprofloxacin), 31.5% (imipenem), 10.3% (meropenem), 12.3% (gentamicin), and 7.5% (tobramycin). Because of the complexity of the resistance mechanisms in *P. aeruginosa*, the susceptibility testing results obtained with the VITEK 2 system for the 146 isolates of this species as a whole and for the five most frequent phenotypes of resistance, as set out in Table 1, were evaluated. Considering the 146 isolates as a whole, 15 (10.3%) and 11 (7.5%) strains were intermediate and resistant to meropenem, respectively. With respect to aminoglycosides, 18 (12.3%) and 13 (8.9%) of the strains were resistant and intermediate to gentamicin, respectively, and 11 (7.5%) and 3 (2.1%) of the strains were resistant and intermediate to tobramycin, respectively. Seven (4.8%) strains were simultaneously resistant to both gentamicin and tobramycin.

For the 146 strains of *P. aeruginosa*, EA ranged from 84.2% (piperacillin) to 98.6% (ciprofloxacin). Essential agreement for 4 of the 10 agents evaluated was >90%, and for 9 of the 10

TABLE 3. EA, ACC, and discrepancies between VITEK 2 and reference microdilution method for 48 strains of *P. aeruginosa* susceptible to ceftazidime, ticarcillin, imipenem, meropenem, and ciprofloxacin

Antimicrobial agent	Percent				
	EA	ACC	Minor error	Major error	Very major error
Cefepime	95.8	97.9	2.1	0.0	0.0
Cefotaxime	83.3	66.7	33.3	0.0	0.0
Ceftazidime	95.8	97.9	2.1	0.0	0.0
Ciprofloxacin	100.0	97.9	2.1	0.0	0.0
Gentamicin	83.3	93.8	6.2	0.0	0.0
Imipenem	93.8	100.0	0.0	0.0	0.0
Levofloxacin	91.7	97.9	2.1	0.0	0.0
Meropenem	81.2	100.0	0.0	0.0	0.0
Piperacillin	87.5	100.0	0.0	0.0	0.0
Tobramycin	97.9	100.0	0.0	0.0	0.0
All agents	91.0	95.2	4.8	0.0	0.0

agents this value was  $\geq 85\%$  (Table 2). Agreement in clinical category (ACC) ranged from 76.7% (cefotaxime) to 98.6% (tobramycin), and for 7 of the 10 antimicrobial agents tested, ACC was >90%. Only for cefepime and gentamicin were both EA and ACC simultaneously lower than 90%. Essential agreement, ACC, and the distribution of errors by clinical category for the different phenotypes evaluated are shown in Tables 3, 4, 5, 6, and 7.

In the case of cefepime, EA ranged from 80.8% (ciprofloxacin-resistant strains) to 95.8% (strains with the basal resistance phenotype). Discrepancies in clinical category for cefepime were caused by 1 very major error (in a ciprofloxacin-resistant strain), 3 major errors (in one imipenem-resistant strain, one ceftazidime- and imipenem-resistant strain, and one strain resistant to both ceftazidime and ciprofloxacin), and 21 minor errors (ranging from 2.1% for strains with the basal resistance phenotype to 30.8% for ciprofloxacin-resistant strains). Table 8 shows the percentages of strains of *P. aeruginosa* for which the cefepime MICs, as determined with the VITEK 2 system, were  $\geq 2$  dilutions higher or  $\geq 2$  dilutions lower than the corresponding reference values. It can be observed that when all the strains are taken into account, discrepancies between MICs

TABLE 4. EA, ACC, and discrepancies between VITEK 2 and reference microdilution method for 19 ceftazidime-resistant<sup>a</sup> *P. aeruginosa* strains

Antimicrobial agent	Percent				
	EA	ACC	Minor error	Major error	Very major error
Cefepime	89.5	78.9	21.1	0.0	0.0
Cefotaxime	100.0	94.7	5.3	0.0	0.0
Ceftazidime	94.7	94.7	5.3	0.0	0.0
Ciprofloxacin	94.7	100.0	0.0	0.0	0.0
Gentamicin	100.0	89.5	10.5	0.0	0.0
Imipenem	100.0	100.0	0.0	0.0	0.0
Levofloxacin	84.2	100.0	0.0	0.0	0.0
Meropenem	84.2	100.0	0.0	0.0	0.0
Piperacillin	89.5	89.4	0.0	5.3	5.3
Tobramycin	100.0	100.0	0.0	0.0	0.0
All agents	93.7	94.8	4.2	0.5	0.5

<sup>a</sup> Strains resistant to ceftazidime (and to ticarcillin and piperacillin) and susceptible to the carbapenems and fluoroquinolones tested.



TABLE 5. EA, ACC, and discrepancies between VITEK 2 and reference microdilution method for 26 isolates of *P. aeruginosa* resistant to ciprofloxacin and susceptible to  $\beta$ -lactams

Antimicrobial agent	Percent				
	EA	ACC	Minor error	Major error	Very major error
Cefepime	80.8	65.4	30.8	0.0	3.8
Cefotaxime	80.8	50.0	46.2	0.0	3.8
Ceftazidime	96.2	92.3	7.7	0.0	0.0
Ciprofloxacin	96.2	92.3	7.7	0.0	0.0
Gentamicin	84.6	76.9	23.1	0.0	0.0
Imipenem	88.5	100.0	0.0	0.0	0.0
Levofloxacin	88.5	76.9	23.1	0.0	0.0
Meropenem	88.5	100.0	0.0	0.0	0.0
Piperacillin	84.6	100.0	0.0	0.0	0.0
Tobramycin	88.5	96.2	3.8	0.0	0.0
All agents	87.7	85.0	14.3	0.0	0.7

were likewise caused by either higher or lower values than the reference MICs, but significant differences were obtained for some particular phenotypes. In the case of cefotaxime, the best EA and ACC results (both 100%) were obtained for ceftazidime- and imipenem-resistant strains. For other groups of strains, EAs were 80.8% (ciprofloxacin resistance phenotype) and 100% (ceftazidime-resistant and imipenem-resistant strains), while ACCs ranged from 50.0% (ciprofloxacin-resistant strains) to 94.7% (ceftazidime-resistant strains). In contrast to the results for cefotaxime, the EA and ACC values for ceftazidime were similar except for the 21 ceftazidime- and imipenem-resistant strains, for which the EA was 95.2% but for which the ACC was only 85.7%. The EA for piperacillin was lower than the ACC except in the cases of ceftazidime-resistant or ceftazidime- and imipenem-resistant strains.

The MICs of imipenem and meropenem for the *P. aeruginosa* strains determined with the VITEK 2 system are highly related to their phenotype of carbapenem resistance. In imipenem-susceptible strains, the EA for imipenem ranged from 88.5% (ciprofloxacin-resistant strains) to 100% (ceftazidime-resistant strains), and the EA for meropenem ranged from 81.2% (strains with the basal resistance phenotype) to 88.5%

TABLE 6. EA, ACC, and discrepancies between VITEK 2 and reference microdilution method for 22 imipenem-resistant<sup>a</sup> *P. aeruginosa* isolates

Antimicrobial agent	Percent				
	EA	ACC	Minor error	Major error	Very major error
Cefepime	86.4	91.0	4.5	4.5	0.0
Cefotaxime	100.0	91.0	9.0	0.0	0.0
Ceftazidime	90.9	95.5	0.0	4.5	0.0
Ciprofloxacin	100.0	100.0	0.0	0.0	0.0
Gentamicin	90.9	100.0	0.0	0.0	0.0
Imipenem	95.4	81.8	13.7	0.0	4.5
Levofloxacin	95.4	100.0	0.0	0.0	0.0
Meropenem	95.4	86.3	13.7	0.0	0.0
Piperacillin	68.2	95.5	0.0	4.5	0.0
Tobramycin	100.0	100.0	0.0	0.0	0.0
All agents	92.3	94.1	4.1	1.4	0.4

<sup>a</sup> Isolates resistant to imipenem and susceptible to the cephalosporins and fluoroquinolones tested.

TABLE 7. EA, ACC, and discrepancies between VITEK 2 and reference microdilution method for 21 *P. aeruginosa* strains resistant to ceftazidime and imipenem

Antimicrobial agent	Percent				
	EA	ACC	Minor error	Major error	Very major error
Cefepime	80.9	71.4	23.8	4.8	0.0
Cefotaxime	100.0	100.0	0.0	0.0	0.0
Ceftazidime	95.2	85.7	14.3	0.0	0.0
Ciprofloxacin	100.0	95.2	4.8	0.0	0.0
Gentamicin	95.2	76.2	23.8	0.0	0.0
Imipenem	90.5	66.7	28.5	0.0	4.8
Levofloxacin	66.7	85.7	0.0	0.0	14.3
Meropenem	76.2	47.6	52.4	0.0	0.0
Piperacillin	85.7	81	0.0	9.5	9.5
Tobramycin	100.0	95.2	4.8	0.0	0.0
All agents	89.1	80.5	15.2	1.4	2.9

(ciprofloxacin-resistant strains), while the ACC was 100% for both agents. However, for strains whose phenotypes included imipenem resistance, the ACCs were 81.8% (imipenem) and 86.3% (meropenem) for imipenem-resistant strains but only 66.7% (imipenem) and 47.6% (meropenem) for ceftazidime- and imipenem-resistant strains. Two very major errors (one for an imipenem-resistant strain and one for a ceftazidime- and imipenem-resistant strain) were obtained for imipenem.

EA results for ciprofloxacin against *P. aeruginosa* were always better than those for levofloxacin, while the ACC results for ciprofloxacin were equal to (basal resistance phenotype, ceftazidime-resistant and imipenem-resistant strains) or better than (ciprofloxacin-resistant and ceftazidime- and imipenem-resistant strains) those for levofloxacin. Very major errors were observed for levofloxacin with the VITEK 2 system: 2.7% for all 146 isolates and 14.3% for ceftazidime- and imipenem-resistant strains.

Very good EA and ACC results for tobramycin MICs for *P. aeruginosa* were obtained with the VITEK 2 system. For the 146 isolates, the EA was 97.3% and the ACC was 98.6%. The only case in which the EA or the ACC for tobramycin was <90% was noted for ciprofloxacin-resistant strains (Table 5). Results with gentamicin, however, were worse: both the EA and the ACC for all 146 strains were 88.4%. There were wide variations in the EAs of gentamicin for isolates included in different phenotypic groups of resistance: 83.3% (basal resis-

TABLE 8. Percentages of *P. aeruginosa* strains for which the MICs of cefepime obtained with VITEK 2 differed from the values obtained by the reference method

Phenotype (no. of strains)	% Strains with the following dilution difference:		
	$\geq 2$	$\leq 2$	$0 \pm 1$
All strains (146) <sup>a</sup>	5.5	6.2	88.3
Basal resistance (48)	2.1	2.1	95.8
Ceftazidime resistant (19)	5.3	5.3	89.4
Imipenem resistant (22)	4.5	9.1	86.4
Ciprofloxacin resistant (26)	3.8	15.4	80.8
Ceftazidime and imipenem resistant (21)	14.3	4.8	80.9

<sup>a</sup> Ten strains (corresponding to two minor phenotypes) are not indicated separately.

TABLE 9. EA, ACC, and discrepancies between VITEK 2 and reference microdilution method for 25 strains of *A. baumannii*

Antimicrobial agent	Percent				
	EA	ACC	Minor error	Major error	Very major error
Ampicillin-sulbactam	88.0	84.0	16.0	0.0	0.0
Cefepime	88.0	80.0	20.0	0.0	0.0
Cefotaxime	100.0	96.0	4.0	0.0	0.0
Ceftazidime	96.0	92.0	8.0	0.0	0.0
Ciprofloxacin	100.0	100.0	0.0	0.0	0.0
Gentamicin	100.0	96.0	4.0	0.0	0.0
Imipenem	92.0	88.0	8.0	0.0	4.0
Meropenem	88.0	88.0	12.0	0.0	0.0
Piperacillin	96.0	88.0	12.0	0.0	0.0
Tetracycline	100.0	100.0	0.0	0.0	0.0
Tobramycin	80.0	88.0	12.0	0.0	0.0
All agents	93.4	90.9	8.7	0.0	0.4

tance phenotype) to 100% (ceftazidime-resistant strains). A similar finding was noted for ACCs: 76.2% (ceftazidime- and imipenem-resistant strains) to 100% (imipenem-resistant strains). Disagreements in EAs for gentamicin were observed for 17 of the 146 strains, including 12 susceptible strains, 3 intermediate strains, and 2 strains resistant to gentamicin. This translates into only one very major error. Most minor errors for gentamicin were obtained with strains for which the reference MIC was 4 to 8  $\mu\text{g/ml}$ , for which a disagreement in the MIC of 1 dilution step caused a change in clinical category. In the case of tobramycin, disagreements in EA (four strains) or ACC (two strains) were noted for tobramycin-susceptible strains, but not for strains which were intermediate or resistant to tobramycin. For none of the strains resistant to both gentamicin and tobramycin were there any observable disagreements in EA or clinical category.

The EAs for the 25 strains of *A. baumannii* tested (Table 9) ranged from 80% (tobramycin) to 100% (cefotaxime, ciprofloxacin, tetracycline, gentamicin). In the case of carbapenems, EAs were 92% (imipenem) and 88% (meropenem). No major errors were noted for any of the antimicrobial agents tested, and only one very major error (for imipenem) was obtained; this was for an *A. baumannii* strain for which the reference MIC was 16  $\mu\text{g/ml}$ , whereas the MIC obtained with the VITEK 2 system was 4  $\mu\text{g/ml}$ .

## DISCUSSION

The VITEK 2 system combines several advantages that may be of clinical interest for routine testing of gram-negative rods isolated from clinical samples: rapid identification (3 h), a simple methodology, a high level of automation, and taxonomically updated databases. Results from previous studies indicate that the VITEK 2 system correctly identified 85.3 to 100% of *P. aeruginosa* strains (9; Jossart and Courcol, 37th ICAAC; Monnet and Freney, 37th ICAAC; Rekasius et al., Abstr. 98th Gen. Meet. Am. Soc. Microbiol. 1998). In our study the VITEK 2 system identified all *S. maltophilia* isolates, 91.8% of *P. aeruginosa* isolates, and 76% of *A. baumannii* isolates. Among the *P. aeruginosa* isolates, 31.5% were identified with a low level of discrimination, but from a clinical point of view, it should be considered that most *P. aeruginosa* isolates can be

easily distinguished from related organisms by simple additional tests. The VITEK 2 system identified a significant number of nonfermenting gram-negative rods within 3 h, which may be clinically relevant, because rapid reporting of microbiology results to physicians has been shown to significantly reduce the mortality rate and to favor earlier initiation of appropriate antimicrobial therapy, a shorter hospital stay, and a lower average variable cost per patient (1, 7).

There is no obvious explanation for the discrepancy between our results and those of other investigators, who have reported the ability of the VITEK 2 system to identify *A. baumannii* correctly. The unambiguous separation of *A. baumannii* from related *Acinetobacter* species requires a complex battery of phenotypic tests or, ideally, genotypic methods which are beyond the capabilities of practically all routine laboratories. The methodology for the identification of this species is not clearly stated in other reports, making it difficult to evaluate the actual taxonomic status of the organisms studied. Most *A. baumannii* isolates within an institution are commonly related epidemiologically, and it may be supposed that the isolates evaluated in other studies corresponded to a single or a limited number of clones. In the present study a large number (16) of clones (as defined by pulsed-field gel electrophoresis [manuscript in preparation]) were included, challenging the ability of the VITEK 2 system to identify this microorganism. It is possible that the VITEK 2 system is able to identify some clones more easily than others, but this seems unlikely because isolates belonging to the same clone were correctly identified in some cases but were incorrectly identified in others (data not shown).

We evaluated the results of susceptibility testing with the VITEK 2 system taking into account the clinical categories defined by the expert system in order to simulate, as much as possible, the performance of the system in the routine work of a clinical laboratory. Sanders et al. (19) have recently evaluated the expert system of the VITEK 2 system in the identification of  $\beta$ -lactam resistance in members of the family *Enterobacteriaceae* and *P. aeruginosa* but included only 16 strains of four different phenotypes of the latter species. It is, however, difficult to define reference phenotypes of resistance to  $\beta$ -lactams in *P. aeruginosa*, because, in addition to the loss of porin OprD and the expression of chromosomal  $\beta$ -lactamase (5, 12), the expression of efflux pumps (particularly MexA-MexB-OprM) greatly influences the MICs of penicillins, cephalosporins, and carbapenems (16, 18), while the importance of other factors (for example, altered penicillin-binding proteins) is largely unknown. Similar problems exist when one is evaluating other antimicrobial agents such as fluoroquinolones or aminoglycosides. Our indirect approach to defining phenotypes of resistance by considering resistance to a few marker antimicrobial agents seems adequate for a preliminary evaluation of the results of susceptibility testing of *P. aeruginosa* with the VITEK 2 system.

It has been reported (14) that an overall category error rate of <10% should be obtained for an acceptable performance of susceptibility tests, including  $\leq 1.5\%$  of very major errors and  $\leq 3.0\%$  major errors. According to these values, it can be stated that, overall, the VITEK 2 system reliably carries out susceptibility testing for many of the antimicrobial agents included in the present study against *P. aeruginosa*. Only in the case of ceftazidime- and imipenem-resistant strains was the percent-

age of very major errors >1.5%, and this was due to a single agent (levofloxacin). Additionally, for some phenotypes (ciprofloxacin-resistant and ceftazidime- and imipenem-resistant strains), the percentages of minor errors were unacceptably high. These results indicate that in order to improve the VITEK 2 system it would be interesting to further study strains with defined mechanisms of resistance.

A previous study tested the susceptibilities of 122 strains of *P. aeruginosa* with the VITEK 2 system (Jay et al., Clin. Microbiol. Infect., abstr. P287), which showed EAs ranging from 89.9% (ceftazidime) to 99.2% (gentamicin). Cefepime, levofloxacin, meropenem, and cefotaxime were not evaluated in that study. EAs were higher than those obtained in the present study for piperacillin (96.6 versus 84.2%), imipenem (96.6 versus 87.0%), ciprofloxacin (98.6 versus 98.3%), and gentamicin (99.2 versus 88.4%). These differences may be related to the different strains used in each study.

It is to be expected that for many of the antimicrobial agents to which *P. aeruginosa* is normally highly resistant but that were not included in the present study (that is, ampicillin, cefazolin, and cefuroxime), the results are at least as good as those for the agents that we did evaluate, because data from the present study indicate that the VITEK 2 system offers greater reliability for organisms with high levels of resistance. It should also be noted that the distribution of phenotypes among the strains tested in the present study does not reflect the distribution of phenotypes among routine clinical isolates. In our institution and during the period of study, the percentages of strains with the major phenotypes of resistance defined for use in the study were significantly ( $P < 0.05$  in all cases) lower than the percentages of strains with the major phenotypes of resistance actually included in the study (data not shown). As the best results with the VITEK 2 system were observed for strains of *P. aeruginosa* with the basal resistance phenotype (particularly when ACCs are considered), it is presumed, from a clinical point of view, that the system performs better in routine work than can be inferred from the results obtained in the present study.

For piperacillin, variations in MICs determined with the VITEK 2 system in comparison with those determined by the reference method did not frequently determine a change in clinical category. A low EA along with a high ACC is related to the fact that MICs are far from the breakpoints for susceptibility and resistance and to the absence of an intermediate category for this agent.

In the case of cefotaxime, the apparently paradoxical discrepancy between EA and ACC is due to the fact that a 1-dilution-step variation in MICs determined with the VITEK 2 system compared to those obtained by the reference microdilution method does not (by definition) affect the EA, while a small quantitative variation in MIC may determine a change in clinical category (commonly a minor error) when the MIC is close to the breakpoint for clinical categorization. It is assumed that clinical discrepancies for cefotaxime are of low clinical significance, as this drug is not commonly used to treat *P. aeruginosa* infections. Interestingly, the results obtained with the VITEK 2 system for the more clinically useful oxyiminocephalosporin, ceftazidime, were excellent for *P. aeruginosa* in terms of both EA and ACC.

A 25.5% error rate in the clinical categorization of cefepime

and *P. aeruginosa* was reported with the previous version of the VITEK system (10). This was caused by the higher MICs obtained with the VITEK system than by the reference method. In the present study both the EA and the ACC of cefepime were >95% only for strains with a basal resistance phenotype. For all other groups there were at least 2 dilution steps of difference in the MICs of this drug determined with the VITEK 2 system compared with those determined by the reference microdilution method for a significant number of strains (Table 8). Nevertheless, in contrast to the results obtained by other investigators with the previous version of the VITEK system, discrepancies in MICs determined with the VITEK 2 system were caused by either higher (ceftazidime- and imipenem-resistant strains) or lower (imipenem-resistant and ciprofloxacin-resistant strains) values compared with those obtained by the reference method. Most disagreements in MICs caused minor errors and only one very major error (0.7%; in a ciprofloxacin-resistant strain), in contrast to 5.3% very major errors reported with the previous VITEK system.

Overall, ACCs were higher for the phenotypic groups containing imipenem-susceptible strains than for those containing imipenem-resistant strains. The MICs of imipenem for 43 of 46 (93.5%) imipenem-resistant strains were in the range of 16 to 32  $\mu\text{g/ml}$ , and for these strains, minor changes in MICs may not affect EA but may determine a change to the intermediate category, which translates into a high percentage (13.7 to 28.5%) of minor errors. The results obtained with the VITEK 2 system for meropenem were similar to those obtained for imipenem in terms of both EA and ACC except for the ceftazidime- and imipenem-resistant subgroup, for which the ACC was the lowest among all the antimicrobial agent-phenotype group combinations evaluated. Once again, this occurs because of a significant number of minor errors for strains for which the meropenem MICs were in the range of 4 to 16  $\mu\text{g/ml}$ , just 1 dilution step higher or lower than the breakpoint for the intermediate category.

The EA between the VITEK 2 system and the reference microdilution method for ciprofloxacin can be considered adequate; and the same can be concluded for ACC, with only minor errors observed, as before, for strains for which the MIC of ciprofloxacin was near the intermediate breakpoint (2  $\mu\text{g/ml}$ ). Overall, the MICs of levofloxacin for *P. aeruginosa* were higher than those of ciprofloxacin. This makes the percentage of strains for which the levofloxacin MICs were in the range of 2 to 8  $\mu\text{g/ml}$  higher than the percentage of strains for which the ciprofloxacin MICs were in the range 1 to 4  $\mu\text{g/ml}$ . As has been discussed in connection with other agents, this caused more errors with levofloxacin than with ciprofloxacin.

Among the aminoglycosides tested, the results obtained with the VITEK 2 system were better for tobramycin than for gentamicin. There was a single very major error for gentamicin (for one of the strains resistant to ciprofloxacin and imipenem and susceptible to ceftazidime), but there were none for tobramycin. The worst results were obtained for the group of strains with the basal resistance phenotype and for the EA with gentamicin, because for 15.3% of the strains, the MICs obtained with the VITEK 2 system were lower than those obtained by the reference microdilution method.

The results obtained by determination of the MIC for *A. baumannii* with the VITEK 2 system follow pattern a very



similar to those observed for *P. aeruginosa*: no major errors and only one very major error were noted. The latter was observed for one strain for which the imipenem MICs were 4 µg/ml and, by the reference method, 16 µg/ml. Again, for this species ACC translated into minor errors, in most cases due to a relatively large number of strains for which the MIC obtained by the reference method was close to the breakpoint for intermediate. Our current knowledge of the mechanisms of resistance in *A. baumannii* is even more limited than our current knowledge of the mechanisms of resistance in *P. aeruginosa*. For the same reason offered for the latter species, we think that new studies should be performed with strains whose mechanisms of resistance have been determined before a reliable expert system becomes available for evaluation of the susceptibility of *A. baumannii* with the VITEK 2 system.

One major limitation of the VITEK 2 system in evaluating the susceptibilities of gram-negative bacteria is its inability to provide the MICs of any agent other than co-trimoxazole for *S. maltophilia*. There is controversy surrounding the usefulness of MICs as a guide to the treatment of infections caused by *S. maltophilia*, but in our opinion, it would be better for clinical microbiologists to evaluate their own MICs rather than trust the limitations imposed by the expert system of the VITEK 2 system.

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