Validation of Vitek 2 Nonfermenting Gram-Negative Cards and Vitek 2 Version 4.02 Software for Identification and Antimicrobial Susceptibility Testing of Nonfermenting Gram-Negative Rods from Patients with Cystic Fibrosis[▽]

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Accurate identification and antimicrobial susceptibility testing (AST) of nonfermenters from cystic fibrosis patients are essential for appropriate antimicrobial treatment. This study examined the ability of the newly designed Vitek 2 nonfermenting gram-negative card (NGNC) (new gram-negative identification card; bioMérieux, Marcy-l'Étoile, France) to identify nonfermenting gram-negative rods from cystic fibrosis patients in comparison to reference methods and the accuracy of the new Vitek 2 version 4.02 software for AST compared to the broth microdilution method. Two hundred twenty-four strains for identification and 138 strains for AST were investigated. The Vitek 2 NGNC identified 211 (94.1%) of the nonfermenters correctly. Among morphologically atypical microorganisms, five strains were misidentified and eight strains were determined with low discrimination, requiring additional tests which raised the correct identification rate to 97.8%. Regarding AST, the overall essential agreement of Vitek 2 was 97.6%, and the overall categorical agreement was 92.9%. Minor errors were found in 5.1% of strains, and major and very major errors were found in 1.6% and 0.3% of strains, respectively. In conclusion, the Vitek 2 NGNC appears to be a reliable method for identification of morphologically typical nonfermenters and is an improvement over the API NE system and the Vitek 2 GNC database version 4.01. However, classification in morphologically atypical nonfermenters must be interpreted with care to avoid misidentification. Moreover, the new Vitek 2 version 4.02 software showed good results for AST and is suitable for routine clinical use. More work is needed for the reliable testing of strains whose MICs are close to the breakpoints.

Pseudomonas aeruginosa is the most important cause of lung infections in patients with cystic fibrosis (CF) (14). In our hospital, 50% of sputum-producing CF patients are colonized in their lower airways with *P. aeruginosa* or other nonfermenting bacteria. Accurate identification and antimicrobial susceptibility testing (AST) are essential for appropriate antimicrobial therapy.

A variety of automated commercial systems for identification and susceptibility testing of nonfermenting bacteria are available (2, 3, 12, 19, 20). They are widely used because of the increasing volumes of clinical specimens processed by clinical laboratories and perceived cost effectiveness. The automated systems decrease the in-laboratory turnaround time and enable a faster targeted antimicrobial therapy. Unfortunately, errors in classification and AST by any test system can have serious implications for the clinical outcome of patients. The most frequently reported errors have involved the inaccurate identification of nonfermenters due to their phenotypic variations and slower growth rates and the inconsistencies between the tested broad-spectrum β -lactam antibiotics. Because of the perceived inaccuracies of AST from CF isolates, a consensus

conference on CF microbiology recommended the use of the disk diffusion method for testing *P. aeruginosa* and other nonfermenters (13, 22).

To improve the identification rate of nonfermenting gram-negative bacilli, a new colorimetric Vitek 2 card (nonfermenting gram-negative card [NGNC]) with an enlarged database was recently introduced. To advance the accuracy of the AST results, a new software (version 4.02) was developed.

The aim of the present study was to evaluate the performance of the new NGNC for identification and the new software version 4.02 for AST of nonfermenters isolated from CF patients.

MATERIALS AND METHODS

The study was performed in two phases. In phase I, nonfermenting gram-negative bacilli isolated from the clinical samples were identified. Phase II compared the AST results determined by the Vitek 2 software and those determined by the reference broth microdilution (BMD) method.

Bacterial strains. Two hundred twenty-four strains which were isolated between January and December 2006 from 62 CF patients attending the CF center of the Children's Hospital of the University of Würzburg were investigated. All strains were stored at -70°C as glycerol stocks at the Institute for Hygiene and Microbiology of the University of Würzburg. In preparation for identification and AST, the strains were cultivated twice on blood agar plates.

Identification of gram-negative nonfermenting bacteria. The isolates were identified to the species level on the basis of standard methods, i.e., colony morphology, Gram staining, pigment production, growth at 37° and 42°C on cetrimide agar, oxidase testing, and susceptibility to C390, by the API 20 NE system (bioMérieux, Nürtingen, Germany), by Vitek 2 NGNC, and by partial 16S rRNA gene sequencing as a reference method (9). The number of bases analyzed

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was about 700 bp. All *Burkholderia cepacia* complex (BCC) and *Burkholderia gladioli* isolates were identified by partial 16S rRNA gene sequencing. The API 20 NE system was performed according to the instructions of the manufacturer. Substrate assimilations were read after 24 and 48 h. Interpretation of the results was done after 48 or 72 h using the interpretation software version 6.0.

The Vitek 2 system was also employed according to the instructions of the manufacturer. Data were analyzed using the new software of the NGNC. The interpretations provided by the software were as follows: (i) excellent species identification, (ii) very good species identification, (iii) good species identification, and (iv) acceptable species identification. In the case of acceptable identification, several possible species identifications, the correct species included, or the correct genus identification was reported.

For final identification of the isolates, the results of the API 20 NE system and the NGNC were compared. In the case of agreement, the Vitek 2 results were taken as correct unambiguous identification. In the following other cases, isolates were retested and identified by partial 16S rRNA gene sequencing as the reference method: (i) no identification with the Vitek 2 system, (ii) disagreement of the API 20 NE and the NGNC results, and (iii) acceptable identification results with the Vitek 2 system (genus identification or identification of several possible species).

The gene sequences were compared to entries in databases queried by NCBI BLAST (nucleotide sequence database; available at <http://www.ncbi.nlm.nih.gov/>). Furthermore, a comparison to sequences assembled by the ribosomal database project (RDP II) was performed (available at <http://rdp.cme.msu.edu/>). For definitive sequence identification, 99% or 100% identity was assumed.

Reporting of results. For interpretation of the identification results, the following four categories were taken into account: (i) correct identification (unambiguous correct species identification by Vitek 2), (ii) a low level of discrimination (either correct genus identification or acceptable species identification by Vitek 2 compared to the reference method), (iii) no identification (no results by Vitek 2), and (iv) misidentification (false identification by Vitek 2 compared to the reference method).

AST. The susceptibilities of the isolates to the following antimicrobial agents (Merck Company, Germany) were tested: cefepime, ceftazidime, piperacillin, imipenem, meropenem, ciprofloxacin, gentamicin, tobramycin, and trimethoprim-sulfamethoxazole (cotrimoxazole). The BMD method was performed according to the Clinical and Laboratory Standards Institute (CLSI) standards (5). MICs were interpreted as being in susceptible, intermediate, or resistant categories according to the breakpoints recommended by the CLSI standards (5). For the Vitek 2 method, the AST-NO21 cards and the new version 4.02 software were used for analysis. Testing was performed according to the manufacturer's instructions. To resolve discrepancies, the Vitek 2 method and the reference tests were repeated in triplicate and by Etest (AB Biodisk, Solna, Sweden) when discordant results occurred. We investigated a total of 138 strains with 885 assays.

The overall essential agreement (EA) was used to compare MICs obtained with the Vitek 2 system to those obtained with the BMD reference method. EA occurred when the Vitek 2 MIC was within one doubling dilution of the reference MIC. The percentage of EA was calculated by dividing the number of Vitek 2 MICs concordant with the reference method MICs ± 1 dilution by the total number of strains tested multiplied by 100. The results were considered in categorical agreement (CA) when the test (Vitek 2) and reference (BMD) MICs fell within the same evaluation category (i.e., susceptible, intermediate, or resistant, dependent on the agent tested). The percentage of CA agreement was calculated by dividing the number of tests with no category discrepancy by the number of organisms tested multiplied by 100. An evaluation of category errors was assessed for each drug as follows. (i) Very major errors (VME) occurred when an isolate that was determined as resistant by the BMD method appeared to be susceptible by the Vitek 2 test method (falsely susceptible). The percentage of VME was calculated by using the number of resistant isolates as the denominator. (ii) Major errors (ME) occurred when the BMD reference method categorized the isolate as susceptible, but the Vitek 2 test method categorized it as resistant (falsely resistant); these were calculated by using the number of susceptible isolates as the denominator. (iii) Minor errors (E) occurred when the BMD method categorized an organism as susceptible or resistant and the Vitek 2 test categorized it as intermediate or when the BMD method categorized it as intermediate and the Vitek 2 categorized it as susceptible or resistant. The percentage of E was calculated by using the total number of organisms tested as the denominator. Acceptable percentages of EA and CA for MICs were set at $\geq 90\%$, with VME of $\leq 1.5\%$ and ME of $\leq 3\%$ for each antimicrobial agent against all organisms tested. A CA of $< 90\%$ is acceptable if the EA is 100% and the majority of the discrepancies are E.

Control strains. During the study period, the following control strains were used: *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and ATCC 35218, *Stenotrophomonas maltophilia* ATCC 13636 and ATCC 51331,

Klebsiella pneumoniae ATCC 700603, *Acinetobacter lwoffii* ATCC 15309, *Brevundimonas diminuta* ATCC 11568, and *Burkholderia cepacia* ATCC 26416.

RESULTS

Identification of nonfermenting gram-negative bacteria by the Vitek 2 NGNC system versus the reference method. The new Vitek 2 system identified the control strains correctly to the species level in every case (data not shown). With regard to the CF isolates, 211 (94.1%) of 224 strains, including *P. aeruginosa*, *S. maltophilia*, *B. gladioli*, and bacteria of the BCC, were correctly identified to the species level; five strains (2.2%) were misidentified, and eight strains (3.6%) were identified with low discrimination. It was remarkable that the NGNC was able to identify unusual isolates like *Delftia acidovorans*, *Rhizobium radiobacter*, or *Ochrobactrum anthropi* correctly without any problems.

The number of correct species identification results increased to 219 strains (97.8%) when simple additional tests were applied for further classification of the strains with low discrimination (Table 1). As an example, the discrimination between *Acinetobacter* species (oxidase negative) and *Pseudomonas fluorescens* or *Moraxella* species (both oxidase positive) was readily achieved. Concerning the differentiation between *P. aeruginosa* and *P. fluorescens* or *Pseudomonas putida*, growth at 42°C on cetrimide agar and resistance to C390 confirmed the diagnosis of *P. aeruginosa*, while *P. fluorescens* and *P. putida* are not able to grow on cetrimide at 42°C and are susceptible to C390. All results obtained with additional tests could be confirmed by 16S rRNA gene sequencing. Table 2 shows in detail the results of the genotypic identification (reference method) and the phenotypic identification with the API 20 NE and Vitek 2 system for strains with low discrimination. The number of correct identification results generated by API 20 NE was poorer than that obtained by the new NGNC.

The time required for the identification by the Vitek 2 system ranged from 5 to 13.5 h, with a mean value of 6.25 h. For additional tests, some minutes (for oxidase tests) or 24 h (for growth at 42°C) were necessary. The time required for the API 20 NE results ranged from 24 to 72 h.

AST by the BMD method versus the Vitek 2 method. The new Vitek 2 system analyzed the control strains correctly in every case.

As mentioned above, a total of 138 strains with 885 assays were investigated. Resistance to one or more drugs occurred in 171 assays, and the Vitek 2 system failed to detect resistance in only three cases (two times when testing *Ochrobactrum anthropi* with cefepime and one case when testing *P. aeruginosa* with piperacillin).

Concerning susceptibility (determined by the BMD method), 62% of all isolates were susceptible to cefepime, 75% to ceftazidime, 79% to piperacillin, 73% to imipenem, 82% to meropenem, 55% to gentamicin, 73% to tobramycin, 75% to ciprofloxacin, and 81% to cotrimoxazole. In the case of *S. maltophilia*, 81% of the isolated strains were susceptible to cotrimoxazole (data not shown in detail).

Table 3 shows the AST results generated by the reference (BMD) and Vitek 2 method. For *S. maltophilia*, the Vitek 2 system provides data for only cotrimoxazole, so it was not possible to compare the results of susceptibility testing. In

TABLE 1. Discrepancies between identification with the Vitek 2 system and with the reference method (API NE and partial 16S rRNA gene sequencing)

Species identification by 16S rRNA gene sequencing	No. of strains tested	No. (%) of strains, species identified as indicated			No. (%) of strains correctly identified after additional tests
		Correct	Misidentified	Low discrimination	
<i>Achromobacter xylosoxidans</i>	13	12	1, <i>Sphingomonas</i> sp.		12
<i>Acinetobacter baumannii</i>	9	9	0		9
<i>Acinetobacter junii</i>	4	4	0		4
<i>Acinetobacter lwoffii</i>	4	3	0	1, <i>A. lwoffii</i> /Moraxella sp.	4
<i>Brevundimonas</i> sp.	4	4	0		4
<i>Burkholderia cepacia</i> group (BCC)	20	20	0		20
<i>Burkholderia gladioli</i>	6	6	0		6
<i>Chryseobacterium indologenes</i>	7	7	0		7
<i>Delftia acidovorans</i>	2	2	0		2
<i>Ochrobactrum anthropi</i>	3	3	0		3
<i>Pseudomonas aeruginosa</i> (mucoid)	34	34	0		34
<i>P. aeruginosa</i> (nonmucoid)	71	64 (90.1)	1, <i>P. fluorescens</i>	5, <i>P. fluorescens</i> / <i>P. aeruginosa</i> 1, <i>P. aeruginosa</i> / <i>P. putida</i> 1, <i>P. fluorescens</i> / <i>Acinetobacter</i> sp.	70 (98.5)
<i>Pseudomonas fluorescens</i>	6	5	0		6
<i>Pseudomonas putida</i>	6	6	0		6
<i>Pseudomonas stutzeri</i>	4	3	1, <i>Brucella</i> sp.		3
<i>Rhizobium radiobacter</i>	3	3	0		3
<i>Sphingomonas paucimobilis</i>	4	4	0		4
<i>Stenotrophomonas maltophilia</i>	24	22 (91.7)	2 (1), <i>Brevundimonas</i> sp./ <i>Sphingomonas</i> sp.		22
Total	224	211 (94.1)	5 (2.2)	8 (3.6)	219 (97.8)

addition, for AST of *B. cepacia*, CLSI takes into consideration MICs for only ceftazidime, meropenem, and cotrimoxazole.

The overall rates of agreement of AST with the new Vitek 2 system and the reference (BMD) method are shown in Table

4. The overall rates of EA and CA were 97.6% and 92.9% (range, 94 to 100% and 88 to 98%), respectively. The percentage of E was 5.1% (range, 1 to 11%), and those of ME and VME were 1.6% (range, 3 to 5%) and 0.3% (range, 1 to 2%),

TABLE 2. Phenotypic and genotypic identification results of Vitek 2 and API NE system

Species identified by 16S rRNA gene sequencing (no. of strains)	Species identified by indicated method (no. of strains)	
	Vitek 2	API NE
<i>Achromobacter xylosoxidans</i> (13)	<i>A. xylosoxidans</i> (12) <i>Sphingomonas paucimobilis</i> (1)	<i>A. xylosoxidans</i> (10) <i>Sphingomonas paucimobilis</i> (1) <i>Burkholderia cepacia</i> (1) <i>Achromobacter denitrificans</i> (1)
<i>Acinetobacter lwoffii</i> (4)	<i>A. lwoffii</i> (3) <i>A. lwoffii</i> /Moraxella lacunata (1) ^b	<i>A. lwoffii</i> (3) <i>Acinetobacter junii</i> (1)
<i>Pseudomonas aeruginosa</i> (71)	<i>P. aeruginosa</i> (64) <i>Pseudomonas fluorescens</i> (1) <i>P. aeruginosa</i> / <i>P. fluorescens</i> (5) ^a <i>P. aeruginosa</i> / <i>Pseudomonas putida</i> (1) ^a	<i>P. aeruginosa</i> (64) <i>Pseudomonas fluorescens</i> (1) <i>Shewanella putrefaciens</i> (2) <i>Comamonas testosteroni</i> (1) <i>Chromobacterium</i> sp. (2) <i>P. fluorescens</i> / <i>P. aeruginosa</i> (1)
<i>Pseudomonas fluorescens</i> (6)	<i>P. fluorescens</i> (5) <i>P. fluorescens</i> / <i>Acinetobacter</i> sp. (1) ^b	<i>P. fluorescens</i> (5) <i>P. fluorescens</i> / <i>Acinetobacter</i> sp. (1)
<i>Pseudomonas stutzeri</i> (4)	<i>P. stutzeri</i> (3) <i>Brucella</i> sp. (1)	<i>P. stutzeri</i> (4)
<i>Stenotrophomonas maltophilia</i> (24)	<i>S. maltophilia</i> (22) <i>Brevundimonas</i> sp. (1) <i>Sphingomonas paucimobilis</i> (1)	<i>S. maltophilia</i> (16) <i>Moraxella lacunata</i> (2) <i>Chryseobacterium indologenes</i> (1) <i>S. maltophilia</i> / <i>Chryseobacterium</i> sp. (4) <i>S. maltophilia</i> / <i>Brevundimonas</i> sp. (1)

^a Differentiation by growth on cetriride agar at 42°C and resistance to C390.

^b Differentiation by oxidase testing.

TABLE 3. Comparison of MICs generated by the Vitek 2 method (NGNC) with MICs generated by the reference BMD method for *Pseudomonas aeruginosa*, *Burkholderia cepacia* group, and other nonfermenters^a

Species (no. of strains)	Drug	No. (%) of Vitek 2 MICs that differed from reference MICs by the indicated dilution						No. (%) of results with indicated test parameter				
		-2	-1	Concordant	+1	+2	+3	EA	CA	E	ME	VME
<i>P. aeruginosa</i> (mucoid; 34)	Cefepime	2	6	22	2		2	30 (88)	32 (94)	2 (6)	2 (6)	
	Ceftazidime			32	2			34 (100)	33 (97)	1 (3)		
	Piperacillin		6	26	2			34 (100)	32 (100)		2 (6)	
	Imipenem			34				34 (100)	34 (100)			
	Meropenem		2	30	2			34 (100)	34 (100)			
	Gentamicin			26	8			34 (100)	28 (82)	6 (18)		
	Tobramycin		2	30	2			34 (100)	34 (100)			
	Ciprofloxacin		4	24	6			34 (100)	28 (82)	6 (18)		
<i>P. aeruginosa</i> (non-mucoid; 35)	Cefepime	1	7	22	5			34 (97)	32 (91)	3 (9)		
	Ceftazidime		2	18	11	1	3	31 (89)	27 (77)	6 (17)	2 (6)	
	Piperacillin	1	5	25	2	2		32 (91)	32 (91)		2 (6)	1 (3)
	Imipenem		2	32	1			35 (100)	35 (100)			
	Meropenem	2	2	29	2			33 (94)	35 (100)			
	Gentamicin		4	30	1			35 (100)	33 (94)	2 (6)		
	Tobramycin			34	1			35 (97)	35 (100)			
	Ciprofloxacin		2	26	6	1		35 (100)	34 (97)	1 (3)		
Nonfermentative bacteria (28)	Cefepime	1	8	17	2			27 (96)	23 (82)	2 (7)	1 (4)	2 (7)
	Ceftazidime		2	19	7			28 (100)	23 (82)	4 (14)	1 (4)	
	Piperacillin		4	24				28 (100)	25 (89)	2 (7)	1 (4)	
	Imipenem		5	23				28 (100)	26 (93)	2 (7)		
	Meropenem		3	25				28 (100)	27 (96)	1 (4)		
	Gentamicin		4	24				28 (100)	25 (89)	3 (11)		
	Tobramycin		2	26				28 (100)	26 (93)	2 (7)		
	Ciprofloxacin			23	5			28 (100)	26 (93)	2 (7)		
	Cotrimoxazole			27	1			28 (100)	28 (100)			
<i>Burkholderia cepacia</i> group	Ceftazidime			12	8			20 (100)	20 (100)			
	Meropenem	2	2	16				18 (90)	20 (100)			
BCC (20)	Cotrimoxazole			18		2		18 (90)	18 (90)		2 (10)	
<i>S. maltophilia</i> (21)	Cotrimoxazole			18	2	1		20 (95)	20 (95)		1 (5)	

^a Dilutions (-1, -2, +1, +2, +3) indicate the number of Vitek 2 MIC dilutions compared to reference BMD MICs. EA, present Vitek 2 MICs within ± 1 dilution of reference MICs; CA, Vitek 2 MICs and the reference MICs that fall within the same evaluation category; E, intermediate by either the Vitek 2 or reference method and either susceptible or resistant by the other method; ME, resistant by the Vitek 2 method but susceptible by the reference method; VME, susceptible by the Vitek 2 method but resistant by the reference method.

respectively. The antibiotics cefepime, ceftazidime, and gentamicin did not reach an acceptable CA of $\geq 90\%$. The mean time required to obtain AST results was 14 h (range, 7 to 17 h).

DISCUSSION

The aim of this study was to validate the new Vitek 2 NGNC for identification and the new software version 4.02 for AST of nonfermenting gram-negative bacteria from CF patients. The database of the new colorimetric NGNC has been enlarged and allows the identification of 159 different taxa.

The NGNC achieved a high level of accuracy in the identification of nonfermenting gram-negative rods. It was remarkable that the NGNC was able to identify unusual isolates like *Delftia acidovorans*, *Rhizobium radiobacter*, or *Ochrobactrum anthropi* correctly without any problems. These results are similar to those of Funke et al., who have investigated 144 strains of nonfermenting gram-negative rods with the new NGNC and have demonstrated a correct identification rate of 94.2% (6.3% with low discrimination) and a misidentification rate of 1.4% (7). These and our data demonstrate an improved quality of

the revised Vitek 2 system in comparison to other automated systems, the former Vitek 2 ID-GNB card version 4.01 system included.

Concerning *P. aeruginosa*, Joyanes et al. tested 146 routinely isolated strains, and no CF isolates, with the Vitek 2 system and ID-GNB cards and found correct identification rates of 91.6% (12). Results from other investigators indicate that the Vitek ID-GNB cards correctly identified 85.3 to 100% of *P. aeruginosa* strains routinely isolated from no CF patients (8, 11, 16). Finally, Saiman et al. examined 189 mucoid and nonmucoid strains of *P. aeruginosa* from CF patients by MicroScan Autoscan and received correct identification rates of only 83% and 86%, respectively (20).

In the present study, correct identification rates of *P. aeruginosa* were 90.1 and 98.5% without or with additional tests for nonmucoid strains and 100% for mucoid phenotypes. The new NGNC identified only six nonmucoid strains with low discrimination as a mixed taxon of *P. aeruginosa* and *P. fluorescens* or as *P. aeruginosa* and *P. putida*; one strain was misidentified as *P. fluorescens*. All problematic strains were morphologically

TABLE 4. Comparison of MICs generated by the Vitek 2 method (NGNC) with MICs generated by the reference BMD method for nonfermenters^a

Drug (no. of assays)	No. of Vitek 2 MICs that differed from reference MICs by the indicated dilution						No. (%) of results with indicated test parameter				
	-2	-1	Concordant	+1	+2	+3	EA	CA	E	ME	VME
Cefepime (97)	4	21	61	9		2	91 (94)	85 (88)	7 (7)	3 (5)	2 (2)
Ceftazidime (117)		4	81	28	1	3	113 (97)	103 (88)	11 (9)	3 (3)	
Piperacillin (97)	1	15	75	4	2		94 (97)	89 (92)	2 (2)	5 (5)	1 (1)
Imipenem (97)		7	89	1			97 (100)	95 (98)	2 (2)		
Meropenem (117)	4	9	100	4			113 (97)	116 (99)	1 (1)		
Gentamicin (97)		8	80	9			97 (100)	86 (89)	11 (11)		
Tobramycin (97)		4	90	3			97 (100)	95 (98)	2 (2)		
Ciprofloxacin (97)		6	73	17	1		96 (99)	88 (91)	9 (9)		
Cotrimoxazole (69)			63	3	3		66 (96)	66 (93)		3 (3)	
Total (885)	9	74	712	78	7	5	864 (97.6)	823 (92.9)	45 (5.1)	14 (1.6)	3 (0.3)

^a Dilutions (-1, -2, +1, +2, +3) indicate the number of Vitek 2 MIC dilutions compared to reference BMD MICs. EA, present Vitek 2 MICs within ± 1 dilution of reference MICs; CA, Vitek 2 MICs and reference MICs that fall within the same evaluation category; E, intermediate by either the Vitek 2 or reference method and either susceptible or resistant by the other method; ME, resistant by the Vitek 2 method but susceptible by the reference method; VME, susceptible by the Vitek 2 method but resistant by the reference method. The percentages of VME and ME were calculated by using the number of resistant and susceptible isolates as the denominator, respectively. The percentage of E was calculated by using the total number of organisms tested as the denominator.

atypical and showed special features. They were isolated from adult CF patients, who had received various antibiotic agents in regular intervals. Moreover, the strains were unpigmented, nonmucoid, showed slow growth without metallic sheen and were phenotypically not easily recognizable as *P. aeruginosa* but rather appeared as harmless nonfermenters. The API 20 NE system was also not able to classify the atypical strains, and the number of incorrectly identified isolates was higher in the API 20 NE than in the Vitek 2 group. As shown in the literature, there is little probability that morphologically nontypical *P. aeruginosa* strains will be correctly identified by current phenotypic test systems (20, 24). In contrast, it is more likely that such strains could be determined only with molecular methods. The determination of morphologically atypical strains in laboratory practice remains problematic. The microbiological knowledge to use simple additional tests, for example, the ability of *P. aeruginosa* to grow at 42°C on cetrimide agar, to differentiate between *P. aeruginosa* and other *Pseudomonas* spp. provides in our experience a worthwhile tool in these cases.

In our opinion, the advantage of the Vitek 2 system was that the instrument mostly did not indicate a false species identification but listed several possible taxa, including the correct one. The user could achieve the correct differentiation by application of additional tests. For example, the differentiation between *P. fluorescens* and *P. aeruginosa* could easily be achieved by using the ability of *P. aeruginosa* to grow on cetrimide agar at 42°C to make a diagnosis, and the correctness of this diagnosis could be confirmed by 16S rRNA gene sequencing.

Satisfactory results were achieved for the identification of BCC (100%), *B. gladioli* (100%), *S. maltophilia* (92%), and *Achromobacter* sp. (92%). Although a species-specific identification of BCC is not possible with the new Vitek 2 system, the NGNC identified all BCC strains correctly to the genus level and was able to differentiate reliably between the pathogenic BCC and the usually clinically not significant *B. gladioli* strains. This is a great improvement of the new Vitek 2 database.

The accurate identification of BCC has been problematic since the recognition of this species as an infectious agent in CF patients. As shown recently, the majority of organisms

within the BCC and related organisms could not be accurately identified by phenotypic investigations (Vitek 2; API NE, Phoenix, AZ). The published identification rates were poor and ranged from 55 to 90% (1, 2, 23). Therefore, it has been recommended to identify nonfermenting gram-negative rods from CF patients with PCR-based methods. Despite the exactness, 16S rRNA sequencing of all CF isolates is not suitable for routine use because of its high costs and due to limitations in the case of BCC in particular. As found by Bosshard et al., 35% of BCC strains could not be unambiguously assigned to a single species by 16S rRNA gene sequencing (1). Therefore, it seems advantageous and cheaper to screen BCC isolates with an automated system and to confirm the results with species-specific PCR-based assays. In the case of BCC, a *recA* gene PCR shows better specificity than the 16S rRNA gene sequencing method (15) and is, therefore, the method of choice for the diagnosis of BCC.

S. maltophilia can be independently identified from the Vitek 2 method results by a negative oxidase reaction, a positive DNase reaction, no growth on cetrimide agar at 37 and 42°C, and resistance against carbapenems.

Concerning AST, this is to our knowledge the first study which assessed the ability of the new Vitek 2 software version 4.02 for AST of nonfermenting gram-negative rods isolated from CF patients. The overall percentage of EA for the reference method and the Vitek 2 system was 97.6%, and the overall CA was 92.9% with 0.3% VME, 1.6% ME, and 5.1% E rates. The criteria for category errors used by the FDA in considering a susceptibility test system for clearance specify EAs and CAs of $\geq 90\%$ as well as VMEs of $\leq 1.5\%$ and MEs of $\leq 3\%$ (6, 11). CAs of $< 90\%$, observed for cefepime, ceftazidime, and gentamicin, were also accepted if the EAs are $\geq 90\%$ and if the majority of the errors are E. With the exception of cefepime and piperacillin, these results fulfill the FDA criteria for clearance and are convincing.

Nevertheless, for some phenotypes of *P. aeruginosa* and for some nonfermenters, the percentage of E was high and ranged up to 18%. Furthermore, the percentages of ME and VME exceeded 1.5% and 3%, respectively. In these cases, MICs of

all strains were close to the breakpoints of the antibiotics tested, and the Vitek 2 system tended to yield either higher MICs (for ceftazidime and gentamicin, false intermediate instead of susceptible in both cases) or lower MICs (for ciprofloxacin, false susceptible instead of intermediate; for piperacillin and cefepime, false susceptible instead of resistant) in comparison to the BMD method. Moreover, AST of these drugs was associated with the lowest rates of overall CA, but overall EAs were $\geq 94\%$.

These results are consistent with others. Joyanes and coworkers determined the AST of 198 clinical isolates of nonfermenters (no CF isolates) with the Vitek AT-NO11 cards and found EAs between 88 and 100%; the VMEs and MEs were $<5\%$, but the E were $>30\%$ for some phenotypes resistant to ciprofloxacin, imipenem, and ceftazidime (12). Burns et al. tested 99 clinical strains of *P. aeruginosa* from CF patients and received EAs between 87.4 and 99% (3). Sader and coworkers investigated 100 non-CF strains of *P. aeruginosa* isolated from hospitals worldwide with three automated systems, including Vitek 2 (GN09 susceptibility cards), and demonstrated poor rates of CA ranging from 44 to 71% and high rates (19 to 27%) of VMEs for piperacillin-tazobactam (19). These poor results may be related to the special strains tested in their study. While the other authors used susceptible or resistant clinical strains, Sader et al. investigated worldwide isolated strains that fall within $\pm 2 \log_2$ dilutions of current CLSI susceptible and resistant breakpoints, with the consequence that the deviation of 1 log dilution (128 instead of 64 $\mu\text{g/ml}$), which is inside the error rate of the method, leads to false-susceptible values (19). For AST of such strains, the Etest method should be preferred (3, 4, 18, 21).

In conclusion, the new Vitek NGNC system appears to be a reliable method for rapid identification of typical nonfermenting gram-negative CF isolates and is an improvement over the API 20 NE system and the former Vitek 2 database. However, classification of morphologically atypical *P. aeruginosa* strains must be interpreted with great care to avoid misidentification. Moreover, the data indicate that the Vitek 2 version 4.02 software offers great reliability for AST of unambiguous resistant or susceptible organisms but may fail in the AST of strains with MICs close to the breakpoints. This proves that the Vitek 2 system is suitable for routine clinical use, but more effort should be taken when testing strains whose MICs are close to the breakpoints.

At present, those strains should be retested by disk diffusion, and this is an additional workload to already cumbersome cultures. But as CF guidelines for microbiology laboratories have not changed in terms of the recommendation to use non-automated susceptibility tests for CF nonfermenter isolates, unreliable results must be repeated by reliable AST methods.

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