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Accuracy of Four Commercial Systems for Identification of *Burkholderia cepacia* and Other Gram-Negative Nonfermenting Bacilli Recovered from Patients with Cystic Fibrosis

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Burkholderia cepacia **has recently been recognized as an important pathogen in chronic lung disease in patients with cystic fibrosis (CF). Because of the social, psychological, and medical implications of the isolation of *B. cepacia* from CF patients, accurate identification of this organism is essential. We compared the accuracies of four commercial systems developed for the identification of nonfermenting, gram-negative bacilli with that of conventional biochemical testing for 150 nonfermenters including 58 isolates of *B. cepacia* recovered from respiratory secretions from CF patients. The accuracies of the four systems for identifying all nonfermenters ranged from 57 to 80%, with the RapID NF Plus system being most accurate. The accuracies of these systems for identifying *B. cepacia* ranged from 43 to 86%, with the Remel system being most accurate. Depending on the commercial system, from two to seven isolates were misidentified as *B. cepacia*. The relatively poor performance of the commercial systems requires that identification of certain nonfermenters be confirmed by conventional biochemical testing. These organisms include *B. cepacia*, *Burkholderia* sp. other than *B. cepacia*, and infrequently encountered environmental species (*Pseudomonas* and *Flavobacterium* species). In addition, conventional biochemical testing should be done if a commercial system fails to assign an identification to an organism. Confirmatory testing should preferably be performed by a reference laboratory with experience in working with organisms isolated from CF patients.**

Chronic lung infection caused by a limited variety of microorganisms is responsible for greater than 90% of the premature deaths seen in individuals with cystic fibrosis (CF) (7). The most important cause of chronic lung infection in these patients is an unusual phenotype of *Pseudomonas aeruginosa* referred to as mucoid. These organisms are easily recognized by their typical colonial morphology (7). During the past decade, another nonfermenter, *Burkholderia cepacia*, has been recognized as an important agent of pulmonary disease in these patients (7, 12, 20). Two factors are important when considering this organism. First, approximately 20% of patients who became infected with this organism will have a rapid decline in their pulmonary function, some will become bacteremic, and death will occur in 1 to 6 months. Patients fitting this clinical course are said to have the "cepacia syndrome" (7, 20). Serious infection with this organism has not been reported in patients with other types of chronic pulmonary diseases. Second, there is accumulating evidence that at least some clones of *B. cepacia* can be transmitted from person to person (10, 16, 19). The practice of cohorting *B. cepacia*-infected patients from all other CF patients has been done to prevent the spread of this organism (21). This practice has clear social and psychological impacts on the infected patients. In addition, *B. cepacia*-infected patients are not considered candidates for lung transplants at most centers, although this exclusion is controversial (6, 18, 19).

Because of the medical, social, and psychological impacts of the recovery of *B. cepacia* from a CF patient, accurate identification of this organism is critical. Many laboratories use commercial identification systems to identify nonfermenting gram-negative bacilli, even though there are no published evaluations of the accuracies of these systems in identifying this group of organisms recovered specifically from CF patients. Often, these evaluations are not undertaken because of problems encountered with CF patient-derived nonfermenting isolates. These include phenotypic variation and slower growth rates because of the significant antimicrobial pressure that these organisms face in the lungs of CF patients.

In the study described here, we compared the accuracies of four commercially available identification systems with that of conventional biochemical testing to identify nonfermenting gram-negative bacilli recovered from respiratory secretions from CF patients.

MATERIALS AND METHODS

Test organisms. A total of 150 clinical isolates of nonfermenting gram-negative bacilli recovered from the respiratory tracts of CF patients were tested in the study. One hundred nine of these strains were collected and tested prospectively beginning in January 1994 and continuing to January 1995. The remaining 41 strains, including 37 *B. cepacia* isolates, were clinical isolates obtained from frozen strain collections of the Microbiology Laboratories of the University of North Carolina Hospitals and Columbia-Presbyterian Medical Center, New

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York, N.Y. Multiple isolates of the same species from an individual patient were not tested. The *P. aeruginosa* strains included in the study were only those isolates which did not demonstrate characteristic colonial morphology on primary isolation plates; i.e., they were not mucoid and/or did not produce characteristic pigment and therefore were not readily recognized as *P. aeruginosa.*

Isolate preparation. Fresh clinical isolates were subcultured onto Trypticase soy agar with 5% sheep blood (SBA) and were incubated at 35°C in an ambient atmosphere for 24 h. Most *B. cepacia* strains required 48 h for sufficient growth. Frozen stocks of clinical isolates were stored in skim milk at −70°C and were subcultured twice onto SBA before inoculation of the test systems. Prior to inoculation, the SBA plates were inspected for purity, and oxidase testing of each organism was performed with 1% tetramethyl *p*-phenylenediamine dihydrochloride. This oxidase result was used for all of the identification systems except the RapID NF Plus system, which provided an oxidase reagent.

Conventional biochemical testing. The following tests and media were prepared in house: triple sugar iron (TSI); citrate; urea; lysine decarboxylase; ornithine decarboxylase; arginine dihydrolase; oxidation-fermentation (OF) sugars (glucose, xylose, lactose, mannitol, sucrose, maltose, fructose, galactose, mannose) with bromthymol blue as a pH indicator; *o*-nitrophenyl-β-D-galactoside (ONPG); nitrate reduction and gas; gelatin; esculin; egg yolk agar for lecithinase activity; litmus milk; cetrimide; flo; tech; growth at 42°C; hemolysis; motility; and growth on MacConkey, PC, and OFPBL agars. PC and OFPBL agars are selective media used in the isolation of *B. cepacia* from clinical specimens (9, 24). Medium components and reagents were purchased from the following manufacturers: Difco, Detroit, Mich.; Becton-Dickinson MicroSystems, Cockeysville, Md.; or Sigma Chemical, St. Louis, Mo.

All biochemical tests and media were incubated at 35°C in an ambient atmosphere and were examined at 2, 3, 5, and 7 days, with the exceptions of TSI slants, which were examined at 24 h; tests with ONPG, which was examined at 48 h; and tests with nitrate, *Pseudomonas cepacia*, and OFPBL (oxidative fermentation base-polymyxin B-bacitracin-lactose) media, which were examined at 72 hours. Organisms routinely used for quality control of the media included *Enterobacter aerogenes*, *Oligella urealyticum*, *Flavobacterium meningosepticum*, *Acinetobacter calcoaceticus* var. *anitratus*, and *Aeromonas hydrophila*. Flagellar staining (Carr Scarborough Microbiologicals, Decatur, Ga.) was performed when necessary to supplement conventional biochemical testing. Organism identification was based on a comparison of the biochemical reactions obtained at the completion of each test with tables found in the *Pseudomonas* and *Burkholderia* chapter in the 6th edition of the *Manual of Clinical Microbiology* (8).

Commercial identification systems. The commercial systems used in the present study included RapID NF Plus (Innovative Diagnostic Systems, Norcross, Ga.), API Rapid NFT (bioMérieux-Vitek, Hazelwood, Mo.), Vitek Auto/Microbic System GNI (bioMérieux-Vitek) and Uni-N/F Tek and N/F Screen (Remel, Lenexa, Kans.). Preparation of organism suspensions, inoculation, incubation times and temperatures, interpretation of reactions, and quality control were according to the manufacturers' recommendations for each system. Technologists performing organism identifications did not know the identity of the organism prior to inoculation or the interpretation of the test results. Ten isolates identified by conventional biochemical testing as *B. cepacia* were selected at random and were tested three different times to determine the reproducibilities of the commercial systems for identifying *B. cepacia.*

Data analysis. The identifications obtained with the four commercial systems were compared with the identifications obtained by the conventional biochemical reference method. The identifications with the commercial systems were considered correct if they agreed with the reference method to the species level without the need for supplemental testing. RapID NF Plus identifications were accepted as correct if the biocode designation was implicit, satisfactory, or adequate, corresponding to a likelihood of >95%. API Rapid NFT biocode designations of excellent, very good, or good were accepted as correct, representing a likelihood of ≥90%. Correct identification with the Vitek system required a likelihood of ≥90%. Biocode designations of excellent or good with the Remel system were accepted as correct, representing a likelihood of ≥90%.

The identifications obtained with the commercial systems were considered to be incomplete if the identification obtained by the reference method was listed among two or more choices. Such identifications were referred to as probability overlaps by the RapID NF Plus system, a good identification to the genus level or a low level of discrimination by the API Rapid NFT system, good confidence or marginal separation by the Vitek system, and unacceptable by the Remel system. Any organism that gave a discrepant identification with one or more of the commercial systems was referred for confirmatory identification to the Special Bacteriology Laboratory of the North Carolina State Laboratory of Public Health. A small percentage of strains were referred to the Centers for Disease Control and Prevention, Atlanta, Ga., for confirmatory identification. Both reference laboratories used conventional biochemical testing for organism identification. Comparison of system performances was assessed by the χ^2 test. For overall performance comparisons with the API/Rapid NFT, Vitek, and Remel systems, the data for *Burkholderia gladioli* were not included since this organism was not in the databases for these systems.

RESULTS

The results of the comparisons of the identifications obtained with the four commercial systems with those obtained with conventional biochemicals are given in Tables 1 to 4. There was no difference in the performance of the systems when identifications of frozen isolates were compared with those of prospectively collected isolates (data not shown). Overall, the RapID NF Plus system was the most accurate commercial system for identifying nonfermenters (Table 1). However, the RapID NF Plus system accurately identified only 47 of 58 (81%) *B. cepacia* isolates. Eight isolates were identified as belonging to the *Alcaligenes faecalis-Alcaligenes odorans* group (Table 2). Biocodes for all eight isolates were similar, and all eight isolates were negative for γ-glutamyl-β-naphthylamide (GGT), a key test for the identification of *B. cepacia* by this system. Six of eight isolates would have been correctly identified if the GGT result had been positive. In addition, three isolates required supplemental testing to allow *B. cepacia* to be accurately distinguished from other species of nonfermenters (Table 4). Seven of the 150 isolates were misidentified as *B. cepacia* (Table 3). Five of those seven misidentified isolates were *B. gladioli* (two isolates) and *Burkholderia pickettii* (three isolates). Particularly problematic for this system was the identification of other species of *Burkholderia* other than *B. cepacia*, some of which were identified as *B. cepacia* (Table 3).

Of the 10 isolates selected for reproducibility studies by the RapID NF Plus system, 7 were initially identified correctly. Six isolates were correctly identified on both retests, one isolate was identified correctly once and incorrectly once on retesting, and two isolates were misidentified all three times that they were retested. One isolate identified incorrectly twice as *A. faecalis* was correctly identified as *B. cepacia* on the third attempt.

The API Rapid NFT system accurately identified 25 of 58 (43%) of the *B. cepacia* isolates (Table 1). Of the five incorrect identifications, two were identified as *B. pickettii* and three were identified as fluorescent pseudomonads (Table 2). In addition, seven isolates (five *B. gladioli* and two *B. pickettii* isolates) were misidentified as *B. cepacia* (Table 3). There were 18 incomplete identifications of *B. cepacia* with the API Rapid NFT system. The majority of these were due to the inability of this system to differentiate *B. cepacia* from *Pseudomonas aureofaciens*. An additional xylose oxidation test would allow these two organisms to be separated and would improve the accuracy of the API Rapid NFT system for identifying *B. cepacia* to 74%. Ten biocodes were not found in either the code book or the company's phone access computer database. *B. gladioli* was not in the API Rapid NFT system's database, but this organism was frequently identified as some other species, particularly *B. cepacia* (five of nine isolates). Only one isolate of *B. gladioli* was not assigned an identification by the system.

Reproducibility studies of the API Rapid NFT system showed that six *B. cepacia* isolates were correctly identified initially. Only one of those six isolates was correctly identified on two retests. Four isolates either were not identified (two isolates) or were identified as non-*Burkholderia* species (two isolates). Two isolates which were misidentified originally were identified as *B. cepacia* on both retests. No identification was obtained for two organisms in all three trials of the NFT system.

The API Rapid NFT system performed inadequately for the identification of *Alcaligenes xylosoxidans* and *P. aeruginosa* (Table 1). The problems encountered in the identification of *A. xylosoxidans* were generally due to strains with negative glucose

TABLE 1. Identification of glucose-nonfermenting bacilli by commercial systems

Organism	No. of isolates	System	No. (%) of isolates with the following identification:			
			Correct[a]	Incomplete[b]	Incorrect	Unidentified
B. cepacia	58	RapID NF Plus	47 (81)	3	8	0
		API Rapid NFT	25 (43)	18	5	10
		Vitek	29 (50)	12	7	10
		Remel	50 (86)	5	0	3
S. maltophilia	30	RapID NF Plus	30 (100)	0	0	0
		API Rapid NFT	26 (87)	1	1	2
		Vitek	28 (93)	0	1	1
		Remel	30 (100)	0	0	0
A. xylosoxidans subsp. *xylos*	24	RapID NF Plus	21 (88)	0	3	0
		API Rapid NFT	17 (71)	2	1	4
		Vitek	13 (54)	4	5	2
		Remel	5 (21)	13	6	0
P. aeruginosa	14	RapID NF Plus	13 (93)	1	0	0
		API Rapid NFT	8 (57)	1	2	3
		Vitek	9 (64)	2	2	1
		Remel	7 (50)	2	5	0
B. gladioli	9	RapID NF Plus	2	0	7	0
		API Rapid NFT[c]	0	0	8	1
		Vitek[c]	0	0	6	3
		Remel[c]	0	0	8	1
B. pickettii (biovar 1)	3	RapID NF Plus	1	0	2	0
		API Rapid NFT	0	1	1	1
		Vitek	0	1	1	1
		Remel	1	0	2	0
B. pickettii (biovar 3)	3	RapID NF Plus	1	0	2	0
		API Rapid NFT	0	0	3	0
		Vitek	1	1	1	0
		Remel	3	0	0	0
Other[d]	9	RapID NF Plus	5	1	3	0
		API Rapid NFT	4	2	2	1
		Vitek	4	2	3	0
		Remel	5	0	4	0
Total	150	RapID NF Plus	120 (80)	5	25	0
	141	API Rapid NFT	80 (57)	25	15	21
	141	Vitek	84 (60)	22	20	15
	141	Remel	101 (72)	20	17	3

[a] Identification to the species level without supplemental testing.
[b] Low level of discrimination; the correct identification was listed among two or more choices.
[c] *B. gladioli* not in the databases of the API Rapid NFT, Vitek, and Remel systems.
[d] *A. calcoaceticus* var. *anitratus* ($n = 2$), *A. calcoaceticus* var. *lwoffii* ($n = 1$), *A. xylosoxidans denitrificans* ($n = 2$), *Flavobacterium oryzihabitans* ($n = 1$), *Pseudomonas diminuta* ($n = 1$) (not in Vitek database), and *Pseudomonas putida* ($n = 2$) (*Pseudomonas putida*/*Pseudomonas fluorescens* considered correct for Rapid NF Plus, Vitek, and Remel systems).

assimilation test results. These strains also tested negative by conventional OF glucose oxidation tests. The poor performance of the API Rapid NFT system in identifying *P. aeruginosa* was not due to the failure of any characteristic reaction(s). It should be noted that three strains identified by conventional biochemical testing as atypical *P. aeruginosa* were incorrectly identified by the API Rapid NFT system.

Only 50% of the *B. cepacia* strains were correctly identified by using the Vitek system (Table 1). Seven *B. cepacia* isolates were incorrectly identified as *B. pickettii* (Table 2). Ten *B. cepacia* isolates were not identified by the Vitek system. Only two isolates, a *B. gladioli* isolate and a *B. pickettii* isolate, were misidentified as *B. cepacia*. Twelve *B. cepacia* isolates required supplemental testing to differentiate them from *B. pickettii*. The addition of lysine decarboxylase, ONPG, and growth on cetrimide tests improved the accuracy of the Vitek system to 71% for *B. cepacia*. The majority of strains requiring supplemental testing exhibited negative lysine decarboxylase test results with the Vitek system. When the result of this test was keyed into the Vitek system as positive, the likelihood of identification as *B. cepacia* became >99% for 10 of the 12 isolates. The Vitek system correctly identified only 13 of 24 *A. xylosoxidans* strains. This low level of performance was due mainly to negative OF glucose and xylose test results. The corresponding conventional OF glucose and OF xylose test results were either negative or required 5 to 7 days for the development of a positive reaction.

Five of 10 *B. cepacia* isolates were correctly identified ini-

TABLE 2. Incorrect identification of *B. cepacia*

System	Total no. of isolates	Incorrect identification (no. of isolates)
RapID NF Plus	8	*Alcaligenes faecalis-Alcaligenes odorans* (8)
API Rapid NFT	5	*Burkholderia pickettii* (2) *Pseudomonas fluorescens* (2) *Pseudomonas chlororaphis-Pseudomonas fluorescens* (1)
Vitek	7	*Burkholderia pickettii* (biovar 3) (4) *Burkholderia pickettii* (biovar 1) (2) *Burkholderia pickettii* (biovar 3)/*Flavobacterium oryzihabitans* (1)
Remel	0	

tially in reproducibility studies with the Vitek system. Three of the five isolates were correctly identified on both retests; the other two isolates were correctly identified on one retest, and no identification was obtained for the other isolate. One isolate which gave no identification on initial testing was correctly identified on two retests. Four isolates were misidentified or gave no identification all three times that they were tested.

The Remel system was the most accurate commercial system for the identification of *B. cepacia*. This system correctly identified 50 of 58 (86%) *B. cepacia* strains (Table 1). No *B. cepacia* isolate was incorrectly identified. Four isolates, two *B. gladioli* isolates and two *B. pickettii* isolates, were misidentified as *B. cepacia* (Table 3). In addition, five *B. cepacia* isolates required further testing to distinguish them from *Flavobacterium* spp. (Table 4).

Eight of 10 *B. cepacia* isolates were identified correctly on initial testing in the reproducibility studies with the Remel system. Four isolates were identified correctly on both retests; four isolates were incorrectly identified as other organisms on both retests. Two isolates which were given no identification initially were identified correctly as *B. cepacia* on both retests.

The major weakness of the Remel system was the difficulty encountered in identifying both *A. xylosoxidans* and nonpigmented *P. aeruginosa* isolates. Fifteen of the *A. xylosoxidans* isolates exhibited a positive glucose reaction at 24 h, which resulted in the inability of the system to distinguish this organism from *B. pickettii*. A negative glucose reaction would have generated a correct identification of *A. xylosoxidans*. This system also failed to identify 50% of the *P. aeruginosa* isolates. This failure was due in part to the fact that several strains were negative for pigment production, a key characteristic in the Remel identification scheme for this organism. In addition, three strains giving either incorrect or incomplete results were identified as atypical *P. aeruginosa* isolates by conventional biochemical tests.

DISCUSSION

At least in some clinical situations, and some might argue in many clinical situations, the accurate identification of microorganisms to the species level is of limited importance in the clinical care of a patient. It can be argued that accurate antimicrobial susceptibility test results for organisms which are not reliably susceptible to specific groups of antimicrobial agents have greater clinical utility. Species-level identification becomes more important when identifying infection outbreaks or attempting to understand the pathologic potential of infrequently or newly encountered organisms.

Accurate isolation and identification of *B. cepacia* from the respiratory tracts of patients with CF is critical for the care of not only the individual patient but also the community of individuals with CF. This is due to the transmissibility of at least some *B. cepacia* clones in this population (10, 16) and the significant mortality associated with infection with this organism (7, 20). Cohorting CF patients infected with *B. cepacia* has been shown to be effective in reducing the transmission of this organism (21). Cohorting may result in the infected individual being separated from close friends. Cases in which siblings with CF in which one child is infected and the others are not or cases in which individuals with CF who are involved in amorous relationships in which one member is infected and the other is not are two of the more difficult situations facing these patients' caregivers. This is especially true because *B. cepacia* transmission has occurred in the second situation cited and has resulted in the death of the previously uninfected partner (10).

Published evaluations exist for each of the four commercial systems evaluated in the present study (1, 2, 4, 11, 14, 15, 17, 22, 23). However, only one of those studies, the one by Kitch et al. (11), specifically states if any of their study isolates were recovered from CF patients. Kitch et al. (11) evaluated the NF RapID Plus system and found that 88% ($n = 27$) of *B. cepacia* isolates were identified accurately, which was consistent with our finding of 81% accuracy. In their study, 85% of the *B. cepacia* isolates were from CF patients. All eight isolates of *B. cepacia* incorrectly identified in our study were identified as belonging to the *A. faecalis-A. odorans* group, an organism group which was not found in our CF population. An identification of a member of the *A. faecalis-A. odorans* group with the NF RapID Plus system should be confirmed by an alternative method to ensure that a *B. cepacia* isolate is not being incorrectly identified.

In the study by Kitch et al. (11), too few isolates of other *Burkholderia* spp. ($n = 2$) were studied to determine the frequency with which those organisms were misidentified as *B. cepacia*. In the current study, 5 of 15 *B. gladioli* or *B. pickettii* isolates were misidentified as *B. cepacia* by the NF RapID Plus system. Both *B. gladioli* and *B. pickettii* appear to be commensal organisms in CF patients (5, 8).

Two groups have previously evaluated earlier versions of the API Rapid NFT system (1, 23). They found the system to be very accurate in identifying *B. cepacia*. A total of only 28

TABLE 3. Organisms misidentified as *B. cepacia*

System	Total no. of isolates	Organism identification (no. of isolates)
RapID NF Plus	7	*Burkholderia gladioli* (2) *Burkholderia pickettii* (biovar 3) (2) *Burkholderia pickettii* (biovar 1) (1) *Pseudomonas diminuta* (1) *Pseudomonas putida*/*Pseudomonas fluorescens* (1)
API Rapid NFT	7	*Burkholderia gladioli* (5) *Burkholderia pickettii* (biovar 3) (2)
Vitek	2	*Burkholderia gladioli* (1) *Burkholderia pickettii* (biovar 3) (1)
Remel	4	*Burkholderia gladioli* (2) *Burkholderia pickettii* (biovar 1) (2)

TABLE 4. Supplemental tests required for the identification of *B. cepacia*

System	Total no. of isolates	Identification (no. of isolates)	Supplemental tests[a]
RapID NF Plus	3	*Chromobacterium violaceum* versus *Burkholderia cepacia* (1)	Pigmentation
		Pseudomonas alcaligenes versus *Moraxella liquefaciens* versus *Moraxella osloensis* versus *Burkholderia cepacia* (1)	Motility, glu, xyl
		Burkholderia cepacia versus *Acinetobacter* sp. (1)	Motility, mann
API Rapid NFT	18	*Burkholderia cepacia* versus *Pseudomonas aureofaciens* (13)	xyl
		Burkholderia cepacia versus *Pseudomonas aureofaciens* versus *Pseudomonas fluorescens* (4)	xyl, lys, 42°C
		Burkholderia cepacia versus *Pseudomonas aureofaciens* versus *Pseudomonas fluorescens* versus *Pseudomonas aeruginosa* (1)	xyl, lys, 42°C, lac
Vitek	12	*Burkholderia cepacia* versus *Burkholderia pickettii* (biovar 3) (11)	lys, ONPG, cet
		Burkholderia cepacia versus *Burkholderia pickettii* (biovar 1) (1)	lys, ONPG, cet
Remel	5	*Burkholderia cepacia* versus *Flavobacterium oryzihabitans* (3)	lys
		Burkholderia cepacia versus *Flavobacterium indologenes* (2)	motility, lac, mann

[a] glu, OF glucose; xyl, OF xylose; mann, OF mannitol; lys, lysine decarboxylase; lac, OF lactose; cet, cetrimide.

isolates of *B. cepacia* were studied and were correctly identified by the two groups. In the two studies, the system correctly identified all 20 *B. pickettii* isolates tested. Two isolates of *Burkholderia pseudomallei* were identified as *B. cepacia* (23). Since *B. pseudomallei* is not encountered in North America or northern Europe, the area where most CF patients reside, these misidentifications are not likely to occur with isolates from CF patients. Their data are in direct contrast to our findings. We found that less then half of the *B. cepacia* isolates tested were correctly identified as *B. cepacia*. The API Rapid NFT system often did not distinguish *B. cepacia* from *Pseudomonas aureofaciens. P. aureofaciens* is an environmental organism which has not been isolated from clinical specimens (13). Therefore, the inability of this system to distinguish between *B. cepacia* and *P. aureofaciens* isolates is of little practical clinical importance. If those 13 isolates were considered as being correctly identified, the accuracy of the API Rapid NFT system would improve to 66%. Seven of 15 other *Burkholderia* isolates were identified as *B. cepacia* by the API Rapid NFT system. One of the problems with this system was that *B. gladioli* was not in the API Rapid NFT system's database. Instead of not giving an identification for this organism, the system assigned an incorrect identification to eight of nine *B. gladioli* isolates, including five as *B. cepacia.*

The Vitek card has been evaluated in several laboratories (14, 15, 17, 22). However, only two studies have evaluated the system with multiple isolates of *B. cepacia* and related organisms. In an early evaluation (17), 29 of 31 isolates of *B. cepacia* were correctly identified. However, only nine of the isolates were from clinical sources. Other organisms including *P. aeruginosa*, *Pseudomonas putida*, and *Stenotrophomonas maltophilia* were misidentified as *B. cepacia*. In the second evaluation (15), 27 of 32 *B. cepacia* isolates were correctly identified. The problem of misidentification appears to have been addressed in the database since no isolates were misidentified in the study (15). In our study, 29 of 58 *B. cepacia* strains were accurately identified by the Vitek system. A major problem with the Vitek system was its inability to distinguish *B. cepacia* from *B. pickettii*. Seven isolates of *B. cepacia* were identified as *B. pickettii* and 12 isolates were incompletely identified. Interestingly, only 2 of 15 other *Burkholderia* spp. were misidentified as *B. cepacia*. This verifies previous findings that this system only infrequently misidentifies these organisms as *B. cepacia*. It would be prudent for users of the Vitek system to confirm the identities of all isolates identified as *B. pickettii* by alternate means to ensure that these isolates are not *B. cepacia.*

Of the commercial nonfermenting gram-negative bacillus identification systems evaluated in the present study, the Remel system has been used for the longest period of time. Results of early studies showed that the Remel system accurately identified all *B. cepacia* isolates tested, although only a small number of isolates ($n = 20$) were studied (2, 4). In our study, the Remel system was the most accurate system for identifying *B. cepacia*. It did not incorrectly identify any of the 58 isolates. Five *B. cepacia* isolates could not be distinguished from two different *Flavobacterium* spp. As was the case with all of the other systems tested, distinguishing the closely related *Burkholderia* spp. from *B. cepacia* was problematic.

The answer to the question of what approach should be used to identify members of the *Burkholderia* genus is not clear. Commonly used commercial identification systems are flawed for the identification of *B. cepacia*, although the RapID NF Plus and Remel systems were accurate ($P < 0.001$) and the results were relatively reproducible when compared with those of the API Rapid NFT and Vitek systems prior to supplemental testing. Because the RapID NF Plus system is enzyme based, its ability to identify weakly oxidizing *B. cepacia* isolates and atypical *P. aeruginosa* isolates may be enhanced compared with those of the other commercial systems. It is clear that difficulty in differentiating members of the *Burkholderia* genus was encountered with all systems. Conventional biochemical batteries incubated for 7 days appear to be the most accurate method for identifying members of the *Burkholderia* genus. Even this approach to the determination of species has not been validated by using isolates whose species were determined either by DNA hybridization or by sequence analysis, both of which are highly specific but not widely available techniques (3). Until easily applied genetic techniques which can discriminate between species of *Burkholderia* are available, these organisms will continue to be difficult to distinguish. The initial isolate from a CF patient identified as a *Burkholderia* sp. with a commercial identification system should be sent to a reference laboratory which uses conventional biochemical testing to confirm the identity of that organism. Laboratories should also be cognizant of the fact that they may experience difficulty in distinguishing *B. cepacia* from other organisms when using currently available commercial ID systems. If an identification system lists organisms which are primarily envi-

ronmental organisms (*P. aureofaciens*) for an isolate recovered from a CF patient, the laboratory should be highly suspicious that the isolate may be *B. cepacia* and that such isolates should be sent for conventional testing as well. Finally, isolates which do not appear in the database of the commercial systems should also be identified by conventional biochemical tests.

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