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Comparative Evaluation of the BD Phoenix and VITEK 2 Automated Instruments for Identification of Isolates of the *Burkholderia cepacia* Complex

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We evaluated two new automated identification systems, the BD Phoenix (Becton Dickinson) and the VITEK 2 (bioMérieux), for identification of isolates of the *Burkholderia cepacia* complex (BCC). The test sample included 42 isolates of the highly virulent and epidemic genomovar III, 45 isolates of *B. multivorans*, and 47 isolates of other members of the BCC. Rates of correct identification by the BD Phoenix and VITEK 2 were similar when all BCC isolates were considered (50 and 53%, respectively) but differed markedly for genomovar III (71 and 38%; $P < 0.01$) and for *B. multivorans* (58 and 89%; $P < 0.001$). For the BD Phoenix as well as the VITEK 2, taking all 134 isolates of the BCC together, rates of correct identification of clinical isolates (56 and 55%, respectively; $n = 85$) were higher than those of environmental isolates (21 and 39%, respectively; $n = 28$). Clinical isolates of genomovar III ($n = 27$) showed correct identification rates of 81% (BD Phoenix) and 48% (VITEK 2) ($P < 0.01$). Rates of misidentification for BD Phoenix and VITEK 2 were 9 and 17% for genomovar III, 22 and 7% for *B. multivorans*, and 36 and 13% for the other BCC members ($P < 0.01$), respectively. More than half of the isolates misidentified by each instrument were identified as *Ralstonia pickettii*, *Ralstonia paucula* (CDC IV C-2 group), *Alcaligenes faecalis*, *Achromobacter* spp., or, for the VITEK 2, "various nonfermenters." This study reemphasizes that confirmatory identification of BCC, preferably by molecular methods, is highly recommended.

The *Burkholderia cepacia* complex (BCC) is composed of at least seven species of bacteria that occur in human clinical specimens and cause infections that are particularly life-threatening and difficult to treat in cystic fibrosis (CF) patients and other vulnerable individuals (13). Approximately 20 to 30% of *B. cepacia*-infected CF patients suffer from accelerated pulmonary deterioration or fulminant, necrotizing pneumonia with rapidly fatal bacteremia called the "cepacia syndrome" (18). In several parts of the world *B. cepacia* has been the cause of deadly outbreaks in the CF community, and the high risk of cross-contamination confronts physicians with the need to take drastic infection control measures which have dramatic social and psychological impacts on CF patients. Somewhat at odds with its clinical importance, *B. cepacia*, which was initially described as an environmentally occurring plant pathogen (4), has attracted intense interest from the agricultural industry as a possible agent for biodegradation and biocontrol (17). The potential risk posed by environmental *B. cepacia* is not currently well understood (17, 20), but it is troublesome that genomovar III, the subset of the BCC that is associated with most cases of cepacia syndrome and outbreaks of infection (25), is often associated with the roots of plants such as maize and wheat (1).

Routine diagnostic tests for reliable identification (ID) of *B. cepacia* organisms are still largely unsatisfactory. ID procedures include use of selective culture media (14, 15, 34), phenotypic methods (16), and semiautomated or automated commercial systems, but reliable and accurate ID of *B. cepacia* requires the use of molecular tools. Methods that have been validated include PCR-based assays (2, 5, 21, 35), whole-cell protein electrophoresis (31), PCR-restriction fragment length polymorphism (RFLP) (23, 28), amplified fragment length polymorphism (AFLP) (8), and ribotyping (3). A drawback of most of these methods is that they require specialized skills or equipment that are not widely available in the clinical microbiology laboratory.

The abilities of several commercial systems to identify *B. cepacia* have been evaluated, and they generally have been proven to have insufficient accuracy (19, 26, 29, 33). Nevertheless, fully automated systems are widely used in clinical microbiology laboratories for ID of bacteria from CF patients, sometimes as a first screening providing guidance for subsequent confirmatory ID (26, 29). Use of automated systems will continue because laboratories are often faced with the necessity of processing high volumes of tests with limited resources and personnel. In this context, it is important to evaluate automated systems for their abilities to detect the presence of dangerous pathogens such as *B. cepacia*. Here we evaluated two new automated systems, the Becton Dickinson Diagnostic Systems (Baltimore, Md.) Phoenix instrument and the VITEK 2 system (bioMérieux Benelux BV, s'Hertogenbosch, The Netherlands), for the ability to identify isolates of the BCC.

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MATERIALS AND METHODS

Bacterial isolates. A total of 134 isolates of the BCC were included in the study. Six *Ralstonia pickettii* and 13 *Burkholderia gladioli* isolates were also tested for comparison. *B. cepacia* isolates were derived from clinical samples ($n = 85$; from 64 patients, of whom 60 had CF) or from the environment (soil and plants outside the hospital) ($n = 28$) or were reference strains from culture collections (14 clinical isolates and 7 isolates from the environment, mainly from onions). Clinical isolates were mainly derived from Germany ($n = 34$), The Netherlands ($n = 26$), and Italy ($n = 21$). Isolates of BCC (including the reference strains) were all identified at the species or genomovar level by using a combination of ribotyping (3), 16S rRNA PCR-RFLP (28), protein profiling (31), and *recA* PCR and PCR-RFLP (23). All strains were analyzed by ribotyping, and representative strains of all ribotypes (defined as patterns distinguished by a single band difference) were analyzed with protein profiling and *recA* characterization. For some strains, identification was confirmed by using the additional methods. BCC isolates were identified as *Burkholderia multivorans* (31) ($n = 45$, including 34 clinical isolates, 3 environmental isolates, and 8 reference strains), genomovar III ($n = 42$, including 27 clinical isolates, 10 environmental isolates, and 5 reference strains), *Burkholderia stabilis* (formerly genomovar IV) (32) ($n = 21$, including 19 clinical and 2 environmental isolates), genomovar I ($n = 13$, including 2 clinical isolates, 5 environmental isolates, and 6 reference strains), *Burkholderia vietnamiensis* (formerly genomovar V) (12) ($n = 4$), genomovar VI ($n = 3$) (6), *Burkholderia ambifaria* ($n = 4$) (7), and *Burkholderia anthina* (genomovar VIII) (P. Vandamme, unpublished data) ($n = 3$). Results of the molecular identification were taken as the "gold standard" for this study.

Phoenix analysis. The ID part of the Phoenix panels (called "combi" panels) contains 45 wells with distinct substrates. ID is based on analysis of 20 enzymatic reactions, utilization of 16 carbohydrates and 7 other carbon sources, and resistance to 2 antibiotics (colistin and polymyxin B). The procedures recommended by the manufacturer were strictly followed. Strains were taken out of the freezer, grown on Colombia agar with 5% sheep red blood cells for 16 to 24 h at 37°C, replated, and grown again for 16 to 24 h at 37°C just before testing. A suspension of 0.5 McFarland (accepted range, 0.5 to 0.6) was prepared in the ID broth (Becton Dickinson, Erembodegem, Belgium) and poured within 30 min into the panel, which was then loaded into the instrument within 30 min. Four quality control strains (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *K. pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853) were loaded with each study batch, which always met quality control criteria. The Phoenix instrument gives an ID result when a species or group of species is identified with more than 90% confidence. The confidence value is a measure of the likelihood that the issued ID is the only correct ID. The average time required to reach an ID result ranges from 3 to 4 h.

VITEK 2 analysis. The ID card for gram-negative bacilli for the VITEK 2 system is a 64-well plastic card containing 41 fluorescent biochemical tests, including 18 enzymatic tests for aminopeptidases and -oxidases (10). Substrates used for detection of aminopeptidases are usually coupled with 7-amino-methylcoumarin; substrates for detection of aminooxidases are usually coupled with 4-methylumbelliferone. Furthermore, the ID card for gram-negative bacilli includes 18 fermentation tests and 3 miscellaneous tests. Suspensions and cards are put together with the "smart" tray into the VITEK 2 reader-incubator module, upon which the system fills and seals the cards automatically. Cards are automatically read every 15 min. Results were interpreted by the database after the incubation period of 3 h. The procedures recommended by the manufacturer were strictly followed. Strains were taken out of the freezer, grown on Colombia agar with 5% sheep red blood cells for 16 to 24 h at 37°C, replated, and grown again for 16 to 24 h at 37°C just before testing. A bacterial suspension was adjusted to a McFarland standard of 0.6 (range, 0.55 to 0.65) in 2.5 ml of 0.45% sodium chloride solution with a densitometer (bioMérieux). The time between preparation of the suspension and card filling was less than 30 min. Four quality control strains (*E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853) were loaded with each study batch. All quality control strains had to be identified correctly in order to allow ID of the test strains.

In the VITEK 2 system, the confidence value is expressed by seven different categories of results: "excellent ID," "very good ID," "good ID," or "acceptable ID" (each of these four categories shows only one ID result); "low discrimination" (more than one ID result is given, whereupon the software suggests performing additional tests such as oxidase, hemolysis, pigmentation, indole, and motility tests in order to obtain a correct ID); "inconclusive ID"; and "unidentified." The time required for VITEK 2 to arrive at a final ID result for all gram-negative bacilli is 3.5 h.

Data analysis. Results of the Phoenix analysis of BCC strains were ordered in the following four categories: "correct ID" (when the ID result was *B. cepacia*), "low discrimination" (when the result was "*B. cepacia* or *R. pickettii*" or "*B. cepacia* or *Ralstonia* sp."), "misidentified" (when an ID result that did not include *B. cepacia* was provided), and "no ID" (when no ID result was proposed by the instrument). Results of *B. gladioli* and *R. pickettii* IDs were classified accordingly. Results of the VITEK 2 analysis for BCC isolates were ordered in the following categories: "correct ID" (when the result was *B. cepacia*; this included ID results that were judged excellent, very good, good, or acceptable), "low discrimination" (when the result was *B. cepacia* plus one or two other organisms) (note that this category differs from the corresponding category of the Phoenix instrument), "misidentified" (when an ID result that did not include *B. cepacia* was provided), "inconclusive" (when the instrument could not decide because the number of positive reactions was too low for the database to give a conclusive ID) (note that this category does not exist in the Phoenix system), and "no ID" (when no ID result was proposed by the instrument). For comparison purposes, the "no ID" and "inconclusive" categories of the VITEK 2 were compared, in combination, to the "no ID" category of the Phoenix.

Comparisons of the rates of correct ID of the two instruments were performed by the chi-square method.

RESULTS

The ID results obtained for the 153 strains are presented in Table 1. When all isolates of the BCC were considered, correct ID results were found for 50% of isolates with the Phoenix and 53% with the VITEK 2 ($P = 0.624$). Taking into account the low-discrimination category, 73% (Phoenix) and 78% (VITEK 2) of the BCC isolates had a result that included *B. cepacia* ($P = 0.62$). The misidentification rate was higher with the Phoenix (23%) than with the VITEK 2 (12%) ($P = 0.016$). The rate of unidentified isolates was 3% with the Phoenix and 0.7% (1 isolate) with the VITEK 2 ($P = 0.17$). With the VITEK 2, an additional 10% of isolates had inconclusive ID results.

ID results were considered separately for genomovar III, the most frequent member of the BCC in CF patients and the one responsible for the majority of cepacia syndrome cases and outbreaks of infection; for *B. multivorans*, a member of the BCC that is frequently encountered in CF patients; for genomovar I and *B. stabilis*; and for the remaining BCC members taken together (9, 22, 24, 31) (Table 1). With each instrument, important differences could be observed among these groups of organisms. The Phoenix had a higher rate of correct ID results for genomovar III (71%) than for *B. multivorans* (58%) and other organisms (ranging from 15 to 31% depending on the organism [see Table 1]). The difference between genomovar III and *B. multivorans* was not significant ($P = 0.18$). The VITEK 2 showed a higher rate of correct ID results for *B. multivorans* (89%) than for genomovar III (38%) and other organisms (24 to 38% depending on the organism [Table 1]). The difference between genomovar III and *B. multivorans* results was highly significant ($P < 0.001$). Thus, the Phoenix identified genomovar III correctly (as *B. cepacia*) significantly more frequently than the VITEK 2 ($P < 0.01$), whereas the opposite was true for *B. multivorans* ($P < 0.001$). With both instruments, these two clinically highly relevant groups were better identified than the other members of the BCC complex.

When the ID results were analyzed according to the origin of the isolates (Table 1), it appeared that with both instruments, clinical isolates had a better rate of correct ID than environmental isolates. The difference was more pronounced with the Phoenix (56 versus 21%, respectively [$P = 0.0012$]). Conversely, with both instruments, the rate of low-discrimination

TABLE 1. ID results for BCC and related species with the Phoenix and VITEK 2 instruments

Organism (no. of isolates)	No. (%) of isolates with the following result:							
	Phoenix				VITEK 2			
	Correct ID	Low discrimination	Misidentified	No ID	Correct ID	Low discrimination	Misidentified	No ID or inconclusive
BCC (n = 134)	67 (50)	32 (24)	31 (23)	4 (3)	71 (53)	33 (25)	16 (12)	14 (10)
B. multivorans								
All (n = 45)	26 (58)	7 (15)	10 (22)	2 (5)	40 (89)	2 (5)	3 (7)	0 (0)
Clinical isolates (n = 34)	19 (56)	4 (12)	9 (26)	2 (6)	29 (85)	2 (6)	3 (9)	0 (0)
Genomovar III								
All (n = 42)	30 (71)	8 (19)	4 (9)	0 (0)	16 (38)	14 (33)	7 (17)	5 (12)
Clinical isolates (n = 27)	22 (81)	3 (11)	2 (7)	0 (0)	13 (48)	8 (30)	4 (15)	4 (15)
Other BCC[a] (n = 47)	11 (23)	17 (36)	17 (36)	2 (4)	15 (32)	17 (36)	6 (13)	9 (19)
Genomovar I (n = 13)	2 (15)	6 (46)	5 (38)	0 (0)	5 (38)	8 (62)	0 (0)	0 (0)
B. stabilis (n = 21)	5 (24)	8 (38)	7 (33)	1 (5)	5 (24)	6 (29)	3 (14)	7 (33)
Other groups[b] (n = 13)	4 (31)	3 (23)	5 (38)	1 (8)	5 (38)	3 (23)	3 (23)	2 (15)
Clinical (n = 85)	48 (56)	16 (19)	18 (21)	3 (4)	47 (55)	17 (20)	11 (13)	10 (12)
Environmental (n = 28)	6 (21)	9 (32)	12 (43)	1 (4)	11 (39)	11 (39)	3 (11)	3 (11)
Reference (n = 21)	13 (62)	7 (33)	1 (5)	0 (0)	13 (62)	5 (24)	2 (9)	1 (5)
B. gladioli (n = 13)	9 (69)	0 (0)	4 (31)	0 (0)	0 (0)	0 (0)	13 (100)	0 (0)
R. pickettii (n = 6)	0 (0)	2 (33)	4 (67)	0 (0)	5 (83)	1 (17)	0 (0)	0 (0)

[a] All BCC members except genomovar III and *B. multivorans*.
[b] Includes *B. ambifaria*, genomovar VI, *B. vietnamiensis*, and unclassified BCC members.

ID was higher for environmental isolates than for clinical isolates. Reference strains showed a correct-ID rate comparable to that of clinical isolates ($P = 0.65$ for Phoenix; $P = 0.584$ for VITEK 2). Importantly, the higher correct-ID rate for clinical isolates compared to environmental isolates was also observed within genomovar III: the Phoenix and VITEK 2 correctly identified 81 and 48% of the clinical isolates, respectively ($P = 0.01$) (Table 1). Considering the correct-ID and low-discrimination ID results together in the Phoenix and VITEK 2, 25 (93%) and 21 (78%) of genomovar III clinical isolates, respectively, had an ID result indicating *B. cepacia* ($P = 0.125$).

B. gladioli and *R. pickettii* are two species that are often encountered in the lungs of CF patients. Rates of correct ID of these species showed marked differences between the two instruments: *B. gladioli* was correctly identified in 69% of cases by the Phoenix but was always misidentified by the VITEK 2 ($P < 0.001$). This difference is obvious, as *B. gladioli* is not in the VITEK 2 database. In contrast, for the limited number of *R. pickettii* isolates tested ($n = 6$), the VITEK 2 had a much higher correct-ID rate (83%) than the Phoenix (0%) ($P < 0.01$).

Incomplete ID (the low-discrimination category) by the Phoenix consisted of "*B. cepacia* or *Ralstonia* sp." or "*B. cepacia* or *R. pickettii*," and the proposed differential test was the number of flagella (more than two for *B. cepacia*, two or fewer for *Ralstonia* spp.). Incomplete ID of BCC isolates with the VITEK 2 apparatus consisted of *B. cepacia* or *B. pseudomallei* ($n = 9$), *B. cepacia* or *P. aeruginosa* ($n = 1$), *B. cepacia* or *R. pickettii* or *B. pseudomallei* ($n = 1$), *B. cepacia* or *R. pickettii* ($n = 10$), *B. cepacia* or various nonfermenter gram-negative bacteria (VNFs) ($n = 10$), and *B. cepacia* or *R. pickettii* or VNFs ($n = 2$). Upon arriving at a low-discrimination result, the VITEK 2 system always proposes additional tests to perform, such as oxidase, hemolysis, motility, pigmentation, and indole tests.

The misidentified isolates were given ID results that are shown in Table 2. More than half of the misidentified isolates were identified as a limited number of species. *R. pickettii*, *Ralstonia paucula* (CDC group IV C-2 [30]), *Alcaligenes faecalis*, and *Achromobacter* spp. most frequently accounted for misidentification results with the Phoenix (52% of misidentifications altogether), whereas the VNF group (the composition of which is given in Table 2) most commonly accounted for misidentification results (69% of misidentification cases, including the "low-discrimination VNF" results) with the VITEK 2. Isolates with misidentification results consisted of species that, like *B. cepacia*, are generally characterized by a small number of positive biochemical tests among those available in the test panels (typically, 4 to 10 out of 45 tests were positive for the *B. cepacia* isolate in the Phoenix, for example). Among the *B. gladioli* and *R. pickettii* isolates that were misidentified, none was misidentified as *B. cepacia* and only one was identified as *B. cepacia* or *B. pseudomallei* (by the VITEK 2).

The reproducibility of the ID results was evaluated in the following way. First, we retested 30 isolates that were initially correctly identified by both instruments (Table 3). The proportions of reproducibly correct results were similar for the two instruments (83% for the Phoenix and 87% for the VITEK 2). Genomovar III ($n = 10$) correct-ID results were 100 and 80% reproducible with the Phoenix and VITEK 2, respectively ($P = 0.14$), whereas *B. multivorans* ($n = 15$) correct-ID results had reproducibility rates of 73 and 93%, respectively ($P = 0.14$). A second retesting of the same 30 isolates with the VITEK 2 gave results very similar to those of the first retesting (data not shown). Second, in order to evaluate whether the results for the misidentified and unidentified (or inconclusive) isolates were reproducible, all these isolates ($n = 35$ for the Phoenix; $n = 30$ for the VITEK 2) were retested (Table 3). Of the isolates that were initially misidentified, only approximately half (55% with the Phoenix, 56% with the VITEK 2) were still misidentified. With the Phoenix, 14 (45%) of these isolates showed a correct or low-discrimination ID, and this was the case for 6 (37%) of the isolates retested with the VITEK 2 ($P = 0.61$). Similarly, with both instruments, approximately half of the

TABLE 2. ID results of misidentified isolates

Organisms	Phoenix result		VITEK 2 result	
	Misidentified as:	No. of isolates (%)	Misidentified as:	No. of isolates (%)
BCC isolates ($n = 134$)	CDC IV C-2 *(Ralstonia paucula)*	9 (7)	VNFs[a]	5 (4)
	Ralstonia pickettii	5 (4)	Low-discrimination VNFs (VNFs or others)	6 (4)
	Alcaligenes faecalis	4 (3)	*Burkholderia pseudomallei*	4 (3)
	Achromobacter spp.	3 (2)	*Shigella* group or *Pseudomonas*	1 (1)
	Kingella denitrificans	2 (1)		
	Pseudomonas oryzihabitans	2 (1)		
	Eikenella corrodens	1 (1)		
	Pseudomonas fluorescens	1 (1)		
	Comamonas acidovorans	1 (1)		
	Pseudomonas putida	1 (1)		
	CDC EF-4a	1 (1)		
	Sphingomonas paucimobilis	1 (1)		
Total		31 (23)		16 (12)
B. gladioli ($n = 13$)	*Pseudomonas oryzihabitans*	3 (23)	VNFs	9 (69)
	Kingella kingae	1 (8)	Low-discrimination VNFs	2 (15)
			B. cepacia or *B. pseudomallei*	1 (8)
			Shewanella putrefaciens or *Brevundimonas vesicularis*	1 (8)
Total		4 (31)		13 (100)
R. pickettii ($n = 6$)	*Kingella kingae*	1 (17)		
	Pseudomonas sp.	1 (17)		
	B. gladioli	1 (17)		
	Pseudomonas oryzihabitans	1 (17)		
Total		4 (67)		0 (0)

[a] The category of VNFs includes *Acinetobacter haemolyticus*, *Acinetobacter johnsonii*, *Acinetobacter junii*, *Acinetobacter pwoffii*, *Alcaligenes faecalis*, *Alcaligenes xylosoxidans* subsp. *denitrificans*, *Alcaligenes xylosoxidans* subsp. *xylosoxidans*, *Bordetella avium*, *Bordetella bronchiseptica*, *Burkholderia mallei*, CDC group IV C-2 (*Ralstonia paucula*), *Comamonas acidovorans*, *Comamonas testeroni*, *Francisella* sp., *Moraxella lacunata*, *Moraxella nonliquefaciens*, *Moraxella osloensis*, *Oligella ureolyutica*, *Oligella urethralis*, *Pseudomonas alcaligenes*, *Pseudomonas fluorescens*, *Pseudomonas mendocina*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas putida*, *Pseudomonas stutzeri*, and *Psychrobacter phenylpyruvicus*.

isolates that were initially unidentified or showed an inconclusive ID were identified correctly or with low discrimination upon retesting.

DISCUSSION

In this evaluation, we used a set of strains representative of the taxonomic diversity of the BCC rather than a set of strains that would be representative of the relative frequencies of the members of the BCC complex in CF patients. Therefore, this evaluation is a "stress test," not a "weighted laboratory profile test" (27). Other commercial systems have been evaluated for the ability to identify isolates of the BCC (16, 29, 33), but none has compared the rates of correct ID of different species or genomovars within the BCC. Our results show that, interestingly, both instruments perform better with the two groups that are most frequently encountered in CF patients, genomovar III and *B. multivorans*, than they do with the other members of the BCC. Genomovar III is most frequently found as the cause of the cepacia syndrome, and many outbreaks described to date have been attributed to genomovar III strains (9, 22, 24, 31).

An important and unexpected result of this study was that the two instruments evaluated differed in their rates of correct ID of genomovar III, for which the Phoenix performed significantly better, and *B. multivorans*, for which the VITEK 2 performed significantly better. The same relationship between the two instruments was found (although the difference was not statistically significant) for the rate of reproducibility of correct IDs of genomovar III and *B. multivorans*. Shelly et al. (29) suggested that a high proportion of isolates misidentified as *B. cepacia* were *B. multivorans*, because many strains of this species have atypical phenotypes. In contrast, our results show that *B. multivorans* does not appear to be more difficult to identify by the Phoenix and VITEK 2 apparatuses than other genomovars or species of the BCC, with the exception of genomovar III for the Phoenix apparatus. *B. multivorans* was even identified better than genomovar III by the VITEK 2 apparatus ($P < 0.001$).

TABLE 3. ID results obtained after retesting of initially correctly identified isolates and of isolates misidentified or with no or inconclusive ID

Retesting result	No. (%) of isolates with the indicated retesting result		
	Initially correct	Initially misidentified	Initially with no ID or inconclusive ID
Phoenix			
Correct	25 (83)	9 (29)	1 (25)
Low discrimination	1 (3)	5 (16)	1 (25)
Misidentified	2 (7)	17 (55)	1 (25)
No ID	2 (7)	0 (0)	1 (25)
Total	30 (100)	31 (100)	4 (100)
VITEK 2			
Correct	26 (87)	2 (12)	4 (29)
Low discrimination	2 (7)	4 (25)	4 (29)
Misid.	0 (0)	9 (56)	2 (14)
No ID or inconclusive ID	2 (7)	1 (6)	4 (29)
Total	30 (100)	16 (100)	14 (100)[a]

[a] Include 13 isolates with inconclusive ID and one isolate with no ID.

Rates of accurate ID of BCC isolates by other systems have been reported. van Pelt et al. (33) evaluated the Vitek GNI, Vitek NFC, API 20NE, and MicroScan systems on two sets of *B. cepacia* isolates that differed in origin and method of initial identification. Rates of correct ID for these two sets (composed of 50 and 20 isolates, respectively) differed markedly; they were 90 and 45% with the Vitek GNI, 68 and 40% with the Vitek NFC, 90 and 80% with the API 20NE, and 68 and 25% with the MicroScan, respectively (33). In the same study, the correct-ID rate for *B. gladioli* (14 isolates tested) was 0% for all four systems, whereas the correct-ID rate for *R. pickettii* (6 isolates tested) ranged from 66% (API 20NE) to 100% (for the three other systems). In a study by Kiska et al. (19), 58 *B. cepacia* isolates were analyzed using the API rapid NF, the API rapid NF Plus, the Vitek GNI, and the Remel Uni-N/F Tek and N/F Screen. Rates of correct ID for BCC organisms ranged from 43% (API Rapid NFT) to 86% (Remel), with a rate of 50% for the Vitek GNI system.

Interstudies comparisons of the performances of commercial ID instruments are difficult for several reasons, including the criteria used to define a "correct ID" (27). In addition, the fact that different sets of test strains are used in different studies is a major confounding factor, given the genetic and phenotypic heterogeneity of the BCC, and given that the present results show significant differences in correct-ID rates for genomovars or species within the BCC, as well as for clinical versus environmental strains. We found an overall correct-ID rate of around 50% for both systems, but rates were higher for genomovar III (71% with the Phoenix and 38% with the VITEK 2) and *B. multivorans* (58 and 89%, respectively). Still higher rates were found for clinical isolates of genomovar III (81% with the Phoenix and 48% with the VITEK 2). Given that most strains in previous studies were clinical isolates and that the majority probably belonged to genomovar III or *B. multivorans*, a prudent conclusion from the present study is that the two new systems evaluated here have correct-ID rates that are in the upper range compared with those of other commercial automated systems. The absence of characterization at the genomovar or species level in previous studies does not allow a more affirmative statement.

A majority of the misidentified BCC isolates were given an ID result corresponding to a few species or groups of species (Table 2). As these species present biochemical profiles that are very similar to those of BCC isolates, it is not surprising that misidentification is (i) frequent and (ii) subject to interassay variation, as shown in our retesting study (Table 3), and it is unlikely that optimization of the substrate composition of test panels will soon result in major improvements in the ID rates. It is probable that the ID results are influenced by the densities of the inocula, which can only be standardized with a defined error margin. When an isolate from a CF patient is identified as one of the organisms listed in Table 2 by the Phoenix or VITEK 2, users should always be suspicious that misidentification of a *B. cepacia* isolate may have occurred. Identification as *B. pseudomallei*, which is normally not encountered in the northern United States or northern Europe, where most CF patients reside, should obviously stimulate confirmatory action.

It was not the aim of this study to estimate how frequently organisms other than *B. cepacia* are misidentified as *B. cepacia*, as was done by others (26, 29). However, we analyzed a few isolates from the species *B. gladioli* and *R. pickettii*, which are generally considered to be commensal organisms in CF patients (11) and are the organisms that have been found to be most frequently misidentified as *B. cepacia* by different commercial systems (19). In our study, none of these isolates was identified as *B. cepacia*, and only one *B. gladioli* isolate was identified as "*B. cepacia* or *B. pseudomallei*" by the VITEK 2 apparatus, even though *B. gladioli* is not present in the VITEK 2 database (and so should have a higher probability of misidentification). Still, given that other isolates of these species, or of other nonfermenters such as *Alcaligenes* or *Achromobacter*, could possibly be misidentified as *B. cepacia*, it is always advisable to confirm the ID result of *B. cepacia* by a molecular technique.

This study confirms the unsatisfactory performance of commercial systems based on biochemical reactions for ID of BCC isolates and expands this observation to two of the newest automated systems, even if each of these systems performed better on particular members of the BCC. Because correct ID of *B. cepacia* and related organisms is so critical for individuals with CF, we join the choir of investigators (19, 26, 29, 33) who have urged awareness of the limitations of the commercial systems for ID of BCC isolates and recommended confirmatory testing of *B. cepacia* isolates and related species. Although culture methods (14, 15, 34) and phenotypic characterization (16) can be useful, use of molecular methods (2, 3, 5, 8, 21, 23, 28, 31, 35) is considered necessary for reliable identification, especially for identification at the level of the genomovar or species within the BCC.

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