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Utility of Commercial Systems for Identification of *Burkholderia cepacia* Complex from Cystic Fibrosis Sputum Culture

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Performances of several commercial test systems were reviewed to determine their relative levels of accuracy in identifying *Burkholderia cepacia* complex isolates recovered from cystic fibrosis sputum culture. Positive predictive values ranged from 71 to 98%; negative predictive values ranged from 50 to 82%. All systems misidentified *B. cepacia* complex. The species most frequently misidentified as *B. cepacia* was *Burkholderia gladioli*. These data support the results of previous studies that recommend confirmatory testing, including the use of DNA-based methods, for sputum culture isolates presumptively identified as *B. cepacia*.

Certain of the species of the *Burkholderia cepacia* complex are important pathogens in persons with cystic fibrosis (CF) (7). Proper identification of these species from CF sputum culture underlies patient management and infection control measures and is enormously important to patient psychosocial well-being (8). *B. cepacia* complex bacteria typically exhibit broad-spectrum antimicrobial resistance, making infection refractory to therapy. Because some species may be transmitted between persons with CF, individuals who are diagnosed with *B. cepacia* complex infection may be precluded from participation in social programs that are an important part of their overall health care plan. Conversely, failure to properly detect *B. cepacia* complex in sputum culture poses a potential risk to CF contacts of colonized patients. The stringent infection control policies intended to limit interpatient spread place a tremendous social and economic burden on the CF community.

Unfortunately, accurate identification of *B. cepacia* and related species has been problematic since the recognition of these species as infectious agents in CF several years ago (1, 2, 11; J. J. LiPuma, D. Henry, F. Mehar, D. Speert, and L. Saiman, Pediatr. Res. **41:**304A, abstr. 1810, 1997). By using PCR-based assays and taxonomic evaluation, we recently demonstrated that 11% of a large set of isolates initially identified as *B. cepacia* based on phenotypic parameters had been misidentified (10). In this report, we assess the performance of the various commercial test systems used in the initial analyses of these isolates to determine their relative levels of accuracy in identifying members of the *B. cepacia* complex.

As described previously (10), a total of 1,051 bacterial isolates from CF sputum culture were received from 108 clinical microbiology laboratories from 91 cities in the United States. Among these, 770 were presumptively identified by the referring laboratories as "*B. cepacia*," "? *B. cepacia*," or "possible *B. cepacia*." The remaining 281 strains were submitted identified as another nonfermenting gram-negative species or were not specifically identified to the species level; this group included 40 isolates designated "*Burkholderia* spp." or "possible *Burkholderia* spp."

The method(s) of identification used by referring laboratories was requested for each isolate received. If the information provided was vague or incomplete, a questionnaire was sent to the referring laboratory requesting the primary method of identification and any secondary or supplemental protocol(s) used to identify nonfermenting gram-negative bacteria. For confirmation of species identification, all isolates underwent polyphasic phenotypic (selective media and biochemical testing) and genotypic (genus- and species-specific PCR) analyses as described previously (10). All isolates phenotypically identified as *Burkholderia gladioli* were confirmed as such by using species-specific PCR (15). Isolates for which species identification remained equivocal after biochemical and PCR analysis underwent additional taxonomic evaluation (10).

By using the results of confirmatory polyphasic analysis as the gold standard, the positive and negative predictive values (PPV and NPV, respectively) were calculated for each primary method of identification used by referring laboratories. PPV were calculated by using all isolates initially identified as *B. cepacia* or "possible *B. cepacia*" by referring laboratories as positive tests; NPV were calculated by using all isolates initially not specifically identified as *B. cepacia* as negative tests. To determine the accuracy of the PPV and NPV estimates, a 95% confidence interval was placed around each, except when the number of positive or negative tests was too small. A chi-square test to assess the positive and negative identifications of all methods was used separately to see if the methods differed nonrandomly in the occurrence of positive and negative identifications.

Nine different commercial systems were reported as primary methods of identification of *B. cepacia* by the 108 participating clinical microbiology laboratories (Table 1). Several laboratories reported the use of conventional biochemical panels (of unspecified content), and two laboratories reported that antibiograms were their primary means of identifying *B. cepacia*. In addition, the majority of laboratories reported using at least one other method as a secondary or supplemental test; a total of 57 different combinations of methods were reported by 75

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TABLE 1. Methods used by referring laboratories for identification of *B. cepacia*

Method[a]	No. of labs[b]	No. of isolates tested	PPV (%) (95% CI)[c]	NPV (%) (95% CI)
Vitek GNI Plus	24	178	94 (88–97)	63 (46–77)
Vitek GNI	15	86	83 (72–90)	50 (28–72)
MicroScan GNP	17	169	85 (77–91)	57 (44–70)
API 20NE	14	241	84 (78–89)	82 (72–89)
RapID NF Plus	10	102	87 (76–93)	63 (46–77)
MicroScan Rapid Neg ID	7	20	71 (46–87)	NA[d]
Crystal E/NF	5	29	92 (74–99)	NA
Remel N/F system	2	100	98 (92–99)	NA
Sherlock GLC	4	23	96 (78–99)	NA
Conventional biochemical tests	8	93	91 (78–97)	53 (39–66)
Antibiogram testing	2	10	88 (53–98)	NA

[a] Commercial systems included Vitek GNI Plus (bioMerieux, Hazlewood, Mo.); Vitek GNI (bioMerieux); MicroScan Conventional Gram Neg Panel (MicroScan GNP) (Dade International, West Sacramento, Calif.); API 20NE (bioMerieux); RapID NF Plus (Innovative Diagnostic Systems, Inc., Norcross, Ga.); MicroScan Rapid Neg ID (Dade International); Crystal Enteric/Nonfermenter ID (Crystal E/NF) (BD Biosciences, Sparks, Md.); Remel Uni-N/F Tek Plate and N/F Screen (Remel N/F system) (Remel, Lenexa, Kans.); and Sherlock gas-liquid chromatography (Sherlock GLC) (MIDI, Inc., Newark, Del.).

[b] Number of laboratories reporting the indicated method as the primary means of identification of *B. cepacia*.

[c] CI, confidence interval.

[d] NA, not available.

laboratories. Thirty-three laboratories reported using biochemical tests to augment commercial systems in evaluating *Burkholderia* and related species.

As reported previously, among the 770 isolates initially identified by referring laboratories as *B. cepacia*, 682 were confirmed to be members of the *B. cepacia* complex by polyphasic analysis (10). The species identification of the 88 remaining isolates and the primary methods used by referring laboratories for their initial evaluation are shown in Table 2. Twenty-eight (32%) were *B. gladioli*, 34 were identified as belonging to one of six other bacterial genera, and despite taxonomic evaluation, 26 isolates could not be placed definitively into a known bacterial species. Among the 281 isolates which were identified as a species other than *B. cepacia* or for which species was not specifically identified by the referring laboratory, 101 (36%) were identified as *B. cepacia* complex by polyphasic testing. The initial identification and methods used by referring laboratories in their evaluation of these 101 *B. cepacia* isolates are shown in Table 3. Twenty-nine (29%) had been presumptively identified as "*Burkholderia* species" or "possible *Burkholderia* species" by the referring laboratory. Comparison of the referring laboratories' initial identification results and the results of polyphasic testing allowed determination of the overall PPV and NPV for each test system (Table 1).

Recent efforts that have expanded our understanding of the taxonomy of the *B. cepacia* complex in part explain the difficulties with species identification. The *B. cepacia* complex consists of several distinct but closely related bacterial species (12). Some of these, in particular, *Burkholderia multivorans*, which is frequently recovered from CF sputum, have phenotypes that would be considered atypical for "*B. cepacia*" based on published criteria (4). In fact, in the present study, *B. multivorans* accounted for 47 of the 101 isolates not initially correctly identified as *B. cepacia* complex by referring laboratories; this exceeds the proportion of *B. multivorans* found among *B. cepacia* complex recovered from CF patients in general (unpublished observations).

van Pelt and colleagues recently assessed the performance of four biochemically based commercial systems in the identification of *Burkholderia* spp. (13). A set of 114 bacterial isolates that included 51 *B. cepacia*, each previously identified in reference laboratories, was tested by each method (Vitek GNI, Vitek NFC, API 20NE, and MicroScan) by a single researcher at a central laboratory. In general, the study demonstrated insufficient accuracy of these systems. The API 20NE test outperformed the other systems in identifying *B. cepacia*; none of the systems reliably identified *B. gladioli*. A similar study by Kiska et al. assessed the accuracy of the API Rapid NFT (since replaced by the API 20NE) and the Vitek GNI systems by studying 150 nonfermenting bacteria, including 58 isolates of *B. cepacia* (6). Their study included the RapID NF Plus and the Remel Uni-N/F Tek and N/F Screen, two systems used by several laboratories in the present study. Again, the overall performances of these systems were relatively poor. Accuracies ranged from 57 to 80%, with the RapID NF Plus being best for identifying nonfermenters in general; the Remel N/F system performed best for identification of *B. cepacia*.

TABLE 2. Eighty-eight isolates misidentified as *B. cepacia* by the referring laboratories

Method[b]	No. of isolates[a] whose final identification by polyphasic analysis was:				
	Burkholderia gladioli	Unidentified	*Stenotrophomonas maltophilia*	*Pseudomonas aeruginosa*	*Alcaligenes xylosoxidans*
Vitek GNI Plus	4	1	3	1	
Vitek GNI	2	9	1		
MicroScan GNP	2	3	5	5	2
API 20NE	9	7	2	3	2
RapID NF Plus	5	2		1	1
MicroScan Rapid Neg ID	1	2			2
Crystal E/NF		1			
Remel N/F system	2				
Sherlock GLC	1				
Conventional biochemical tests	2	1	1	1	
Antibiogram testing					
Total no. of isolates	28	26	12	11	7

[a] Include two *Ralstonia pickettii* isolates (initially identified as *B. cepacia* by Vitek GNI and Vitek GNI Plus) and one each *Flavobacterium* sp. and *Chryseobacterium* sp. (initially identified as *B. cepacia* by Crystal E/NF and antibiogram testing, respectively).

[b] Primary method (as defined in footnote *a* of Table 1) used by the referring laboratory to initially identify the isolates as *B. cepacia*.

TABLE 3. One hundred one isolates initially not identified as *B. cepacia* by the referring laboratories

Method[b]	No. of isolates[a] whose initial designation by the referring laboratory was:					
	Burkholderia spp.[c]	Unknown	NFGNR[d]	*Burkholderia gladioli*	*Ralstonia pickettii*	*Pseudomonas* spp.
Vitek GNI Plus	2	4	1		2	3
Vitek GNI		8				
MicroScan GNP	3	1	10	8		
API 20NE	6	5	2			1
RapID NF Plus	5	1	5		1	
MicroScan Rapid Neg ID						
Crystal E/NF	2	1				
Remel N/F system		1	1			
Sherlock GLC						
Conventional biochemical tests	11	6	1	1	4	
Antibiogram testing		1				
Total no. of isolates	29	28	20	9	7	4

[a] Include two *Stenotrophomonas maltophilia* isolates (initially evaluated by RapID NF Plus and MicroScan Rapid Neg ID) and two *Alcaligenes* spp. (initially evaluated by MicroScan GNP and Vitek GNI Plus).
[b] Primary method (as defined in footnote *a* of Table 1) used by the referring laboratory to initially evaluate isolates.
[c] Includes isolates referred as "possible *Burkholderia* species."
[d] NFGNR, nonfermenting gram-negative rod.

In the present study, we employed an approach complementary to that used by van Pelt et al. and Kiska et al. to assess the performance of commercial systems. A large number of CF sputum isolates (including 783 *B. cepacia* isolates) that had been evaluated in the course of routine operation of numerous clinical laboratories were studied to confirm their species identities. In an evaluation of this sort, interlaboratory variability has the potential to have an impact on overall test performance. Nevertheless, an assessment of real-life performance provides a useful means to determine the utility of systems currently employed by clinical laboratories. The identification results provided by participating laboratories were compared to the results of polyphasic confirmatory testing. The latter testing incorporated genus- and species-specific rRNA-based PCR assays that have previously been proven to be sensitive and specific for identification of *B. cepacia* complex (9) and *B. gladioli* (14).

Our results demonstrate that greater than 1 in 10 isolates identified as *B. cepacia* by referring laboratories were, in fact, not *B. cepacia* complex. The majority (70%) of misidentified isolates were actually members of other nonfermenting gram-negative species that also colonize the CF respiratory tract. It is not surprising that *B. gladioli* was most frequently misidentified as *B. cepacia*, considering that of the nine systems used by referring laboratories, only the RapID NF Plus and Crystal Enteric/Nonfermenter ID tests include this species in their databases. Most systems also identified as *B. cepacia* bacterial isolates that could not be placed into a known bacterial species, despite polyphasic taxonomic analyses. Some of these isolates have subsequently been defined as members of the novel genus *Pandoraea* (3); others are likely to represent novel taxa and will require additional taxonomic study.

Among the isolates not identified to the species level or identified by referring laboratories as a species other than *B. cepacia*, a surprising proportion (36%) were identified as *B. cepacia* complex by confirmatory testing. Although approximately 30% of these had been presumptively identified to the genus level and designated "*Burkholderia* spp." or "possible *Burkholderia* spp.," nearly one-half (48 isolates) were unidentified by the systems used and referred to us as "unknown" or "nonfermenting gram-negative" bacteria.

It is apparent from this study that laboratories vary considerably in the systems used to evaluate nonenteric, nonfermenting, gram-negative bacteria recovered from CF sputum culture. Nine different commercial systems were used as primary methods of identification, and their usage was well represented among the 108 participating laboratories; the test most commonly used (Vitek GNI Plus) was employed by only 22% of laboratories. Moreover, among the 76 laboratories reporting the use of secondary or supplemental tests, it is remarkable that 57 different combinations of tests were described. Unfortunately, because so many different combinations were used and the number of isolates evaluated by each was relatively small, it was not possible to determine the performance of all combinations. The statistical assessment of test performance was thus limited to those systems judged by each laboratory to be the primary method of identification.

The PPV of the systems used as primary identification methods ranged from 71 to 98%. For most systems, the lower limit of the 95% confidence interval was near 75%, indicating a relatively low degree of confidence that an isolate testing positive was likely to be *B. cepacia*. The exceptions to this were the Vitek GNI Plus and the Remel N/F systems; results from both indicated a high degree of confidence in a positive test for *B. cepacia*. The NPV for *B. cepacia* were quite low (range, 50 to 82%). Even the upper boundary of the 95% confidence interval did not exceed 90% for any test and was less than 80% for most systems. Thus, for most of these tests, a negative result still carries at least a 20% probability that the isolate being analyzed is *B. cepacia* complex. For several systems the number of non-*B. cepacia* isolates tested by referring laboratories was too low to allow a useful determination of NPV. Participating laboratories were invited to submit all *Burkholderia* isolates recovered from CF sputum culture; however, it is possible that some laboratories preferentially referred isolates that were particularly difficult to identify. This potential selection bias may have resulted in an underestimation of test accuracies. Nevertheless, by including only recent clinical isolates, this study provides an appropriate model for assessing test performance.

This study of several hundred clinical isolates confirms and expands previous observations of the poor performance of commercial systems in the identification of *B. cepacia* and related species. We document considerable variation among

laboratories in the methods used to evaluate such species and show that misidentification can be attributed to many systems in current use. Misidentification is also widespread; 55 (51%) of the 108 referring laboratories submitted at least one isolate that had been misidentified by phenotypic testing. The taxonomic complexities of *B. cepacia* and related nonfermenting species no doubt contribute to the difficulty with accurate identification. Because the implications of accurate identification are so important to individuals with CF, their families, and caregivers, we agree with previous investigators in recommending the use of confirmatory testing of any bacterial isolate presumptively identified as a *Burkholderia* or related species (e.g., *Ralstonia*, *Stenotrophomonas*, and *Alcaligenes* spp.). Selective media (5) and test kits with relatively high PPV (e.g., Vitek GNI Plus and Remel Uni-N/F Tek Plate and N/F Screen) should be employed and augmented by the use of conventional biochemical testing as described previously (10). *B. cepacia* complex species confirmation should be sought by use of DNA-based assays.

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