Identification of *Burkholderia* and Uncommon Glucose-Nonfermenting Gram-Negative Bacilli Isolated from Patients with Cystic Fibrosis by Use of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS)

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Environmental glucose-nonfermenting Gram-negative bacilli other than *Pseudomonas aeruginosa* are frequently isolated from respiratory specimens of patients with cystic fibrosis (CF) (1). A limitation in the use of matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) for the identification of glucose nonfermenters, including *Burkholderia* species, has been a lack of inclusion of these specific species in reference libraries (2, 3). We evaluated the performance of updated databases for the bioMérieux Vitek MS *in vitro* diagnostic (IVD) system for the identification of these organisms.

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A set of 195 archived glucose nonfermenters (Table 1) recovered from CF respiratory specimens between January 2005 and December 2015 were grown on Trypticase soy agar with 5% sheep blood (SBA) and MacConkey (MAC) agar (Becton Dickinson, Franklin Lakes, NJ) at 35°C with 5% CO₂ and on *Burkholderia cepacia* selective agar (BCSA; Remel, Lenexa, KS) at 35°C in air. MALDI-TOF MS (bioMérieux, Inc., Durham, NC) was performed according to the manufacturer's protocol after 24 h, 48 h, and 72 h of incubation. Briefly, a well-isolated colony was directly spotted and overlaid with α -cyano-4-hydroxycinnamic acid (CHCA) matrix solution without formic acid extraction. Spectra were analyzed using the bioMérieux Vitek MS research use-only system (RUO) with the SARAMIS v4.12 database and the IVD system with the current v2.0 and the new v3.0 databases and the draft of v3.1 Knowledge Base (KB) database. Satisfactory results were defined as identifications matched to the species level for *Burkholderia* species and to the genus level for other glucose nonfermenters between MALDI-TOF MS and reference methods, including identification of *Burkholderia* by the *Burkholderia cepacia* Research Laboratory and Repository at the University of Michigan or identification of other glucose nonfermenters using partial 16S rRNA gene sequencing. Predefined criteria were applied according to the Clinical Microbiology-Immunology Laboratories (CMIL) protocol for interpretation of MALDI-TOF MS: identifications were valid if SARAMIS scores were $\geq 75\%$; IVD KB scores were considered valid at $\geq 90\%$ for *Burkholderia* isolates and $\geq 80\%$ for other isolates (2).

Overall satisfactory results were achieved at 58.5%, 46.2%, 83.6%, and 89.2% using the SARAMIS RUO database and the v2.0, v3.0, and v3.1 KB databases on isolates grown on SBA at 24 h

(Table 1). There were similar trends seen on BCSA and MAC agar but with fewer satisfactory results on these media than on SBA (for BCSA, 51.5%, 40.0%, 77.0%, and 84.2%; for MAC agar, 38.7%, 39.3%, 63.1%, and 71.4%). Marked decreases in satisfactory results were observed in older isolates using the RUO database (Table 2). Table 1 shows percentages of satisfactory results for different organisms grown on SBA at 24 h. The newer databases improved identification of *B. cenocepacia*, *B. contaminans*, *Pandora* species, and *Inquilinus* species (95.2%, 100%, 87.5%, and 100% on v3.1). Identification of *B. cepacia*, *Ralstonia* species, *Cupriavidus* species, and *Herbaspirillum* species was obtained for 63.6%, 85.7%, 66.7%, and 66.7% of the isolates using v3.1. All of 4 *Bordetella bronchiseptica* isolates were identified correctly, whereas there was no identification for other *Bordetella* species. Identification of *B. multivorans*, *B. vietnamiensis*, *B. gladioli*, *Achromobacter* species, *Ochrobactrum* species, *Sphingobacterium* species, and *Elizabethkingia* species remained at 100%. Interestingly, the percentage of satisfactory results decreased using the new databases for 10 isolates of *Chryseobacterium* species tested.

Our data showed improvement in identification of uncommon glucose-nonfermenting Gram-negative rods from CF patients, especially *B. cenocepacia* and *B. contaminans*. Infections with *B. cenocepacia* in patients with cystic fibrosis are known to be associated with a rapid decline in lung function and require strict isolation practices (4). Versions 3.0 and 3.1 greatly improve the identification of *B. cenocepacia* while allowing continued accurate identification of *B. vietnamiensis* and *B. multivorans*. Eighty-six percent of *B. cepacia* complex isolates referred to the *Burkholderia cepacia* Research Laboratory and Repository at the University of Michigan belong to these species (5). Paradoxically, the performances of v3.0 and v3.1 are still as unfavorable as that of v2.0 at identifying *B. cepacia*, a species that makes up less than 5% of *B. cepacia* complex organisms recov-

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TABLE 1 Performance of SARAMIS RUO and Vitek MS IVD v2.0, v3.0, and v3.1 Knowledge Base databases on organisms grown on sheep blood agar at 24 h of incubation

Organism	No. of isolates	Satisfactory results (%) ^a			
		SARAMIS RUO	v2.0 IVD	v3.0 IVD	v3.1 IVD
<i>Burkholderia cenocepacia</i> ^b	42	50.0	0	90.5	95.2
<i>Burkholderia contaminans</i> ^b	12	0	0	100	100
<i>Burkholderia cepacia</i>	11	0	81.8	0	63.6
<i>Burkholderia multivorans</i>	10	100	100	100	100
<i>Burkholderia vietnamiensis</i>	8	87.5	100	100	100
<i>Burkholderia gladioli</i>	10	90	100	100	100
Other <i>Burkholderia</i> ^{b,c}	9	11.1	0	88.9	88.9
<i>Pandoraea</i> spp. ^b	24	50.0	0	87.5	87.5
<i>Ralstonia</i> spp.	14	85.7	85.7	78.6	85.7
<i>Chryseobacterium</i> spp.	10	80.0	90.0	80.0	70.0
<i>Achromobacter</i> spp.	10	70.0	100	100	100
<i>Bordetella</i> spp.	7	57.1	57.1	57.1	57.1
<i>Ochrobactrum</i> spp.	6	100	100	100	100
<i>Cupriavidus</i> spp.	6	50.0	33.3	66.7	66.7
<i>Sphingobacterium</i> spp.	6	100	100	100	100
<i>Elizabethkingia</i> spp.	4	100	100	100	100
<i>Herbaspirillum</i> spp. ^b	3	33.3	0	0	66.7
<i>Inquilinus</i> spp. ^b	3	33.3	0	100	100
All <i>Burkholderia</i> spp.	102	47.1	36.3	84.3	93.1
All glucose nonfermenters other than <i>Burkholderia</i>	93	70.9	56.9	82.8	84.9
Total	195	58.5	46.2	83.6	89.2

^a RUO, research use-only system; IVD, *in vitro* diagnostic system.^b *B. cepacia* complex isolate(s) not in the current v2.0 KB database.^c *Burkholderia ambifaria* (1 isolate), *B. anthina* (1 isolate), *B. arboris* (1 isolate), *B. diffusa* (1 isolate), *B. dolosa* (2 isolates), *B. metallica* (1 isolate), *B. stabilis* (1 isolate), *B. seminalis* (1 isolate).

ered from CF patients (5). Improvement in identification of other glucose fermenters was seen with both v3.0 and v3.1 although gaps in identification still remain. MALDI-TOF MS may reduce the need for molecular identification and offer more-rapid diagnosis. SBA and BCSA are preferable to MAC agar as media. Laboratories should develop identification algorithms using MALDI-TOF MS and sequencing/use of reference laboratory to most accurately identify problem isolates.

TABLE 2 Percentages of satisfactory results for 195 isolates from different databases on various culture media and time of incubation

Database	Satisfactory results (%) ^a								
	Sheep blood agar			MacConkey agar			BCSA		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
SARAMIS RUO	58.5	52.8	44.1	38.7	32.4	16.6	51.5	49.4	40.8
v2.0 IVD	46.2	46.7	45.6	39.3	38.7	40.6	40.0	42.9	41.4
v3.0 IVD	83.6	85.1	83.1	63.1	59.5	56.6	77.0	76.8	79.3
v3.1 IVD	89.2	89.2	87.7	71.4	71.1	66.3	84.2	86.3	82.8

^a BCSA, *Burkholderia cepacia* selective agar; RUO, research use-only system; IVD, *in vitro* diagnostic system.

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