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Comparison of Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) Mass Spectrometry Platforms for the Identification of Gram-Negative Rods from Patients with Cystic Fibrosis

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We evaluated the performance of the Bruker Biotyper and the bioMérieux Vitek MS with both the SARAMIS v4.09 and Knowledge Base v2.0 databases for the identification of 203 non-glucose-fermenting Gram-negative rods that had previously been identified by 16S rRNA gene sequencing. Including those that underwent repeat testing, 96.6%, 90.1%, and 93.6% of isolates, respectively, had identifications that agreed with the previous identification.

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is quickly becoming the primary means of identification of bacteria in the clinical microbiology laboratory. The performance of MALDI-TOF for identification of routine organisms is well documented (reviewed in reference 1), but few studies have been done on more atypical, environmental organisms. Patients with cystic fibrosis (CF) become chronically infected with a variety of environmental Gram-negative organisms that are difficult to identify using phenotypic methods, often requiring molecular methods, such as 16S rRNA gene sequencing. In this study, we evaluated the performance of three mass spectrometry platforms for use in place of 16S rRNA identification of organisms isolated from our CF patients.

We utilized 203 archived isolates collected from CF patients between 2009 and 2011 that could not be identified using traditional phenotypic methods. Isolates were initially identified by 16S RNA gene sequencing using MicroSeq reagents (Life Technologies, Carlsbad, CA) and the SmartGene IDNS database (Raleigh, NC). *Burkholderia cepacia* complex members were further identified at the *Burkholderia cepacia* Research Laboratory and Repository at the University of Michigan. Archived isolates were grown on sheep blood agar at 35°C with 5% CO_2 for 48 h before evaluation on the MALDI-TOF platforms. The MALDI-TOF platforms evaluated were the Bruker (Billerica, MA) Microflex with the Biotyper database (v3.2.1.1; 4,110 isolates), the bioMérieux (Durham, NC) Vitek MS research-use-only (RUO) system with the SARAMIS SuperSpectra (software v3.5) v4.09 database, and the bioMérieux Vitek MS *in vitro* diagnostic (IVD) system with the v2.0 Knowledge Base database. All isolates were spotted once initially, and if no satisfactory identification was achieved, they were spotted again with and without a 70% formic acid overlay. Isolates that were repeated included those that did not generate an identification, those that generated multiple identifications that contained both the target identification and an unrelated organism, and those that were undercalled (e.g., *Burkholderia* spp. as opposed to *Burkholderia gladioli* or family *Alcaligenaceae* instead of *Achromobacter* spp.). Satisfactory MALDI-TOF identifications were defined as those that agreed with the sequence-based organism identification (Table 1). We note that many of the organisms tested cannot be reliably identified to species level based on the 16S rRNA sequencing

TABLE 1 Organisms and identifications used in this study

Identification	No. of isolates
Achromobacter spp.	44
Acidovorax spp.	1
Acinetobacter calcoaceticus	1
Acinetobacter ursingii	1
Bordetella spp.	6
Burkholderia cepacia complex	
B. cenocepacia	6
B. cepacia	3
B. contaminans	4
B. multivorans	16
B. vietnamiensis	4
Not otherwise specified[a]	4
Burkholderia gladioli	41
Chryseobacterium spp.	20
Cupriavidas spp.	3
Elizabethkingia meningoseptica	1
Herbaspirillum spp.	1
Ochrobactrum spp.	6
Pandoraea spp.	4
Pseudomonas aeruginosa	8
Pseudomonas spp. (not *P. aeruginosa*)	5
Ralstonia spp.	11
Sphingobacterium spp.	2
Stenotrophomonas maltophilia	10
Xanthomonas spp.	1

[a] These organisms were not able to be identified beyond being members of the *Burkholderia cepacia* complex.

we perform and are therefore evaluated on the ability of the MALDI-TOF instruments to provide an accurate identification to the genus level only. Biotyper identification scores needed to be >2.0 for a species-level identification. SARAMIS scores of <75%

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TABLE 2 Performance of the MALDI-TOF systems

Result	Vitek MS SARAMIS (RUO)				Vitek MS v2.0 (IVD)				Bruker Biotyper			
	Initial		Postrepeat		Initial		Postrepeat		Initial		Postrepeat	
	No. of identifications	%	No. of identifications	%	No. of identifications	%	No. of identifications	%	No. of identifications	%	No. of identifications	%
Correct	171	84.2	183	90.2	180	88.7	190	93.6	181	89.2	196	96.6
Undercalled	20	9.9	10	4.9	1	0.5	1	0.5	5	2.5	2	1.0
No ID	12	5.9	10	4.9	20	9.9	12	5.9	17	8.4	0	0.0
Indeterminate[a]	0	0.0	0	0.0	2	1.0	0	0.0	0	0.0	5	2.5

[a] Multiple identifications that contained both the target identification and an unrelated organism.

and Knowledge Base scores of <80% (90% for *Burkholderia*) were considered nonidentifications.

Upon initial testing, using the Vitek MS SARAMIS database, 171 of the 203 (84.2%) isolates matched the satisfactory identification, 180 (88.7%) matched using the Vitek MS v2.0, and 181 (89.2%) matched using the Biotyper (Table 2). After initial testing, 32 isolates were repeat tested using the Vitek MS SARAMIS, yielding an additional 12 satisfactory identifications, for an overall satisfactory identification rate of 90.2% including repeats. There were 23 isolates that were repeat tested using the Vitek MS v2.0 platform, yielding 10 additional satisfactory identifications for an overall satisfactory identification rate of 93.6%. The two isolates that initially generated indeterminate identifications were satisfactorily identified via repeat testing. Finally, 22 isolates were repeat tested using the Biotyper; 15 additional correct identifications were achieved, resulting in an overall satisfactory identification rate of 96.6%. For all three systems, the addition of formic acid to the repeat spots did not significantly improve identification, contrary to what is seen with yeast and some Gram-positive organisms (2, 3). Table 3 summarizes the performance of each database for the most common organisms requiring 16S rRNA identification at our institution.

One of the more difficult yet most important genera to identify to species level from CF patient cultures is *Burkholderia*, especially members of the *Burkholderia cepacia* complex. Historically, our laboratory has used a combination of 16S rRNA gene sequencing and referral testing to identify these organisms. We therefore evaluated the ability of the three MALDI-TOF databases to identify *Burkholderia* spp., especially members of the *Burkholderia cepacia* complex and related species (Table 3). All databases identified all isolates to at least the genus level. The Vitek MS SARAMIS database correctly identified all 41 isolates of *B. gladioli*, a species phenotypically similar to members of the *B. cepacia* complex (4), and 28 (75.7%) *B. cepacia* complex isolates to the species level. The Vitek MS v2.0 identified 22 (59.5%) of the *B. cepacia* complex isolates to the species level, while correctly identifying all 41 *B. gladioli* isolates. Finally, the Biotyper identified 39 (95.1%) of the *B. gladioli* isolates correctly to the species level and 26 (70.3%) of the 37 *B. cepacia* complex isolates to species level. *Burkholderia multivorans* and *Burkholderia vietnamiensis* were the members of the complex best identified by all systems, while *Burkholderia cenocepacia* was identified to the species level only by the Vitek MS SARAMIS and the Biotyper. *Burkholderia dolosa* was not evaluated in this study. Other members of the *B. cepacia* complex will require better representation in the databases for consistent species-level identification.

We also evaluated the ability of MALDI-TOF MS to identify a subset of organisms (21 *Burkholderia cepacia* complex, 10 *Chryseobacterium*, 7 *Achromobacter*, 5 *B. gladioli*, and 4 *Ralstonia* isolates) from selective media used in the isolation of *Burkholderia cepacia* complex. We determined the performance of the Vitek MS v2.0, for isolates grown on MacConkey agar (MAC), *Burkholderia cepacia* agar (BC), *Burkholderia cepacia* selective agar (BCSA), and oxidation/fermentation polymyxin-bacitracin-lactose (OFPBL) medium for 48 h. We note that not all organisms grew on all media tested, which is consistent with known growth characteristics. In-

TABLE 3 Performance of the MALDI-TOF systems for the identification of selected organisms, including *Burkholderia* spp.

Organism	No. of isolates									
		Vitek MS SARAMIS			Vitek MS v2.0			BrukerBiotyper		
	Total	Genus	Family	No ID	Genus	Family	No ID	Genus	Family	No ID
Achromobacter spp.	44	43	1	0	44	0	0	44	0	0
Chryseobacterium spp.	20	16	0	4	18	0	2	19	0	1
Ralstonia spp.	11	11	0	0	11	0	0	11	0	0
		Species	Complex	Genus	Species	Complex	Genus	Species	Complex	Genus
Burkholderia cenocepacia	6	6	0	0	0	5	1	5	1	0
Burkholderia cepacia	3	2	0	1	2	1	0	1	2	0
Burkholderia contaminans	4	0	0	4	0	4	0	0	4	0
Burkholderia multivorans	16	16	0	0	16	0	0	16	0	0
Burkholderia vietnamensis	4	4	0	0	4	0	0	4	0	0
Burkholderia cepacia complex, not further identified[a]	4	0	0	4	0	4	0	0	4	0
Burkholderia gladioli	41	41	0	0	41	0	0	39	0	2

[a] These organisms were not able to be identified beyond being members of the *Burkholderia cepacia* complex.

terestingly, very little difference was seen when the selective media were used as opposed to blood agar. Thirty-one isolates grew on the MAC plates; all 7 *Achromobacter* isolates maintained satisfactory identifications, as did all 19 members of the *Burkholderia cepacia* complex that grew and 4 of the 5 *Burkholderia gladioli* isolates. From the BC plate, 38 isolates grew (8 *Chryseobacterium*, 4 *Ralstonia*, 5 *B. gladioli*, and 21 *Burkholderia cepacia* complex isolates), and all had satisfactory identifications. For both BCSA and OFPBL medium, 40 isolates (10 *Chryseobacterium*, 4 *Ralstonia*, 5 *B. gladioli*, and 21 *Burkholderia cepacia* complex isolates) grew, with all but one *Chryseobacterium* isolate being satisfactorily identified by Vitek MS v2.0.

One of the benefits of identifying organisms by MALDI-TOF MS is the potential cost savings that can be achieved. We therefore calculated the cost to identify these 203 organisms on the various platforms. For the calculations, we assumed that only 65% of any one slide would be used at any one time and used our costs for Vitek MS reagents and extrapolated costs for Bruker reagents from the report of Tan et al. (5). We used the same labor costs for both systems. We calculated a cost of $112.93 for sequencing and a shipping cost of $24.72 for isolates referred for further identification. Importantly, these calculations do not include the instrument cost for either sequencing or MALDI-TOF. Therefore, based on previous methods, the total cost for identification of the 203 isolates in this study was $23,839.43. The costs per isolate identified by MS, including repeats, were $0.82, $0.76, and $0.50 for the Vitek MS SARAMIS, Vitek MS v2.0, and Biotyper, respectively. The cost to use either of the MALDI-TOF platforms was significantly less, saving over $20,000 to achieve all 203 identifications, including repeat spotting and sequencing/referral identification for those not adequately identified by MALDI-TOF.

The results of our study augment those presented previously (6–8) by further challenging these MALDI-TOF systems with large numbers of isolates not previously thoroughly evaluated, including 78 *Burkholderia*, 44 *Achromobacter*, 20 *Chryseobacterium*, and 11 *Ralstonia* isolates (Tables 1 and 3). All the isolates tested in this study required molecular identification initially, due to the inaccuracy of phenotypic identification. Additionally, our study evaluated all three databases side by side and, importantly, found that the three databases performed comparably, all identifying >90% of the isolates correctly. Importantly, it has been previously shown that custom databases can achieve species-level identification for >98% (9) of isolates such as members of the *Burkholderia cepacia* complex, suggesting the impact of having an extensive and appropriate database. It should be noted that the Bruker Biotyper did identify the most organisms and had the lowest associated total reagent cost for the 203 isolates in this study. Based on our data, MALDI-TOF MS using either available platform and any of the three databases is an accurate and cost-efficient replacement for 16S rRNA gene sequencing for the identification of our difficult CF isolates. The biggest impact of implementing MALDI-TOF MS, however, is the potential change in workflow. A delay in identification with *Burkholderia cepacia* complex members in particular may result in patients being placed in isolation unnecessarily if the isolate does not belong to this complex or, more concerning, not being placed in isolation when they have an organism that is eventually identified as belonging to this complex. Additionally, CF patients may not be eligible for lung transplantation when they are infected with this organism complex (10, 11), further emphasizing the significant impact on patient care that utilization of MALDI-TOF MS can have in this setting: achieving reliable identifications for all of these organisms the same day with minutes of hands-on time compared to days for sequencing and weeks for referral identification.

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