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Application of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Identification of the Fastidious Pediatric Pathogens *Aggregatibacter*, *Eikenella*, *Haemophilus*, and *Kingella*

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The accuracy of matrix-assisted laser desorption–ionization time of flight mass spectrometry (MALDI-TOF MS) in the identification of *Haemophilus*, *Aggregatibacter*, *Cardiobacterium*, *Eikenella*, and *Kingella* (HACEK) species was compared to that of phenotypic methods (Remel RapID and Vitek 2). Overall, Vitek MS correctly identified more isolates, incorrectly identified fewer isolates, and failed to identify fewer isolates than both phenotypic methods.

Haemophilus, *Aggregatibacter*, *Cardiobacterium*, *Eikenella*, and *Kingella* organisms have been placed in the HACEK group because of their slow growth, nutritional needs, and role as oropharyngeal flora (3). These organisms are responsible for approximately 3% of the pediatric infectious endocarditis cases in which a causative agent is isolated and also cause other significant diseases, including pneumonia, meningitis, septic arthritis, osteomyelitis, abdominal abscesses, and urinary tract infections (2, 3). Though the ability to detect these species has improved greatly (9), it is still difficult to correctly identify these organisms. Identification methods typically use biochemical tests to determine organism phenotypes. Despite the relatively new status of matrix-assisted laser desorption–ionization time of flight mass spectrometry (MALDI-TOF MS) as a diagnostic technique for bacterial identification, its rapid turnaround time and high specificity and sensitivity could prove quite useful in addressing the challenges associated with the identification of these organisms.

MALDI-TOF MS identifies bacterial isolates by comparing the protein spectra of isolates to a database of spectra. MALDI-TOF MS technology has previously been shown to successfully identify a wide range of clinically relevant bacteria (4). Several studies have also applied MALDI-TOF MS technology to fastidious pediatric pathogens, though no large-scale comprehensive studies have evaluated Vitek MS for use with these genera (1, 5–8). The aim of the present study was to evaluate the use of the Vitek MALDI-TOF mass spectrometer for the identification of members of the genera *Haemophilus*, *Aggregatibacter*, *Eikenella*, and *Kingella* compared to Remel RapID NH tests and Vitek 2 NH cards (identification systems currently FDA approved), with 16S ribosomal sequencing as the reference method.

(This work was previously presented and published in abstract form [E. A. Powell, D. Blecker-Shelly, S. Montgomery, and J. E. Mortensen, Abstr. Annu. Meet. Am. Soc. Microbiol. 2013, abstr. C-120].)

The set of organisms used in this study was composed of American Type Culture Collection (ATCC) isolates, recent clinical isolates, and laboratory stock strains of *Haemophilus*, *Aggregatibacter*, *Eikenella*, and *Kingella* (Table 1). A total of 140 isolates representing 10 species were analyzed: 55 clinical isolates from the Cincinnati Children's Hospital Medical Center, 2 isolates from the University of Cincinnati, 7 isolates from the Nationwide Children's Hospital, 37 ATCC strains, and 39 isolates from bioMérieux.

Organisms were cultured on chocolate agar plates at 37°C in 5% CO_2 according to laboratory procedure. Remel RapID NH tests (Thermo Fischer Scientific, Lenexa, KS) were performed with each organism in accordance with the manufacturer's instructions. For Vitek 2 NH card (bioMérieux, Durham, NC) analysis, isolates were tested according to standard laboratory policies and procedures for the Vitek2 XL instrument. Identification was considered definitive when a result was obtained with at least 85% confidence. Isolates were sent to the Genetic Variation and Gene Discovery Core Facility at the Cincinnati Children's Hospital Medical Center, a Clinical Laboratory Improvement Amendments of 1988-approved laboratory, for identification by 16S rRNA sequencing. 16S RNA sequencing was performed for all isolates as the reference method.

MALDI-TOF analysis was performed with a bioMérieux Vitek MALDI-TOF mass spectrometer (bioMérieux, Durham, NC), and spectra were compared against Vitek MS SARAMIS research use only (RUO) database version 4.09 by using the SuperSpectra algorithm (referred to here as MALDI-TOF MS). Identification was considered definitive when the probability provided was greater than 70%. For MALDI-TOF MS analysis, one colony was applied to one spot of the test slide with a disposable inoculating loop, overlaid with 1 μl of matrix solution (α-cyano-4-hydroxycinnamic acid), and dried completely before analysis. MALDI-TOF MS analysis was performed in duplicate, with tests performed simultaneously on the same slide. If the duplicates gave different results, the analysis was repeated in triplicate. If methods contradicted one another, both were repeated.

As shown in Table 1, MALDI-TOF MS correctly identified the most isolates. Additionally, this method incorrectly identified the

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TABLE 2 Incorrect identifications by each method[a]

Method	Isolate identity	Incorrect identification(s)
MALDI-TOF MS	*H. haemolyticus* (11)	*H. influenzae* (11)
	A. aphrophilus (2)	*A. segnis* (1), *H. parainfluenzae* (1)
Remel RapID NH	*H. haemolyticus* (7)	*H. influenzae* (6), *H. parainfluenzae* (1)
	H. parahaemolyticus (2)	*H. influenzae* (1), *H. parainfluenzae* (1)
	A. aphrophilus (2)	*H. influenzae* (1), *H. parainfluenzae* (1)
	A. segnis (1)	*H. influenzae* (1)
	K. denitrificans (4)	*Gardnerella vaginalis* (3), *H. parainfluenzae* (1)
	K. kingae (20)	*Gardnerella vaginalis* (18), *H. ducreyi* (1), *H. parainfluenzae* (1)
Vitek 2 NH	*H. influenzae* (1)	*Actinobacillus ureae* (1)
	H. parainfluenzae (1)	*Actinobacillus ureae* (1)
	H. haemolyticus (4)	*H. influenzae* (3), *H. parainfluenzae* (1)
	H. parahaemolyticus (2)	*H. influenzae* (2)
	A. aphrophilus (2)	*H. influenzae* (1), *A. segnis* (1)
	K. denitrificans (1)	*H. parainfluenzae* (1)
	K. kingae (3)	*H. parainfluenzae* (1), *Moraxella catarrhalis* (1), *Neisseria cinerea* (1)

[a] Isolate identity is that determined by 16S rRNA sequencing, while incorrect identification refers to the identity obtained by the method in the leftmost column. Values in parentheses are the numbers of isolates identified as the species shown.

TABLE 1 Number of isolates identified by each method[a]

Organism	Total no. of isolates	MALDI-TOF MS				Remel RapID NH system				Vitek 2 NH system			
		Correctly identified to:		Not identified	Incorrectly identified	Correctly identified to:		Not identified	Incorrectly identified	Tests required	Correctly identified to species level	Not identified	Incorrectly identified	Tests required
		Genus level	Species level			Genus level	Species level							
Haemophilus spp.														
H. influenzae	26		26 (100)				25 (96.2)			1 (3.8)	23 (88.5)		1 (3.8)	2 (7.7)
H. parainfluenzae	20	1 (5)	18 (90)	1 (5)		1 (5)	10 (50)			9 (45)	15[b] (78.9)	2[b] (10.5)	1[b] (5.3)	1[b] (5.3)
H. haemolyticus	11				11 (100)			1 (9.1)		3 (27.2)		2 (18.2)	4 (36.4)	5 (45.4)
H. parahaemolyticus	6		6 (100)							4 (66.7)	3[b] (60)		2[b] (40)	
Aggregatibacter spp.														
A. actinomycetemcomitans	10		10 (100)				10 (100)				10 (100)			
A. aphrophilus	13	1 (7.7)	10 (76.9)		2 (15.4)		11 (84.6)			2 (18.1)	8 (72.7)	3 (27.3)	2 (18.2)	
A. segnis	1		1 (100)							1 (100)	1 (100)			
Eikenella corrodens	29		29 (100)				29 (100)				29 (100)			
Kingella spp.														
K. denitrificans	4		3 (75)	1 (25)						4 (100)	2 (50)		1 (25)	
K. kingae	20		18 (90)	2 (10)						20 (100)	15[b] (78.9)	1[b] (5.3)	3[b] (15.8)	1 (25)
Total	140	2 (1.5)	121 (86.4)	4 (2.9)	13 (9.3)	1 (0.7)	85 (60.7)	1 (0.7)	36 (25.7)	17 (12.1)	106 (77.4)	8 (5.8)	14 (10.2)	9 (6.6)

[a] Values represent the absolute numbers of identifications in each category for each species by a particular method. Values in parentheses are the percentages of isolate identifications made for each species by each method that fall into each category.

[b] Not all isolates were tested with the Vitek 2 system. One isolate each of *H. parainfluenzae*, *H. parahaemolyticus*, and *K. kingae* was not available for testing with the Vitek 2 method.

fewest isolates (Table 2). While MALDI-TOF MS correctly identified isolates of most species, no isolates of *Haemophilus haemolyticus* were correctly identified, likely because this version of the database does not include any *H. haemolyticus* SuperSpectra.

Most commonly used methods for identifying members of the genera *Aggregatibacter*, *Haemophilus*, *Eikenella*, and *Kingella* in routine clinical laboratories are based on the phenotype of the isolate. These tests generally require minimal incubation times of 4 to 8 hours and may require subjective interpretation based on a colorimetric change. 16S rRNA sequencing, in contrast, is extremely accurate but typically is not done by clinical microbiology laboratories and is very costly.

The advent of MALDI-TOF MS for bacterial isolate identification allows a more accurate means of identification without the inconvenience and cost associated with 16S rRNA sequencing. However, previous studies of MALDI-TOF MS with the genera included in this study have been limited. One study (8) analyzed a limited number of HACEK species isolates and reported that 98% of the 51 isolates representing four species were correctly identified by MALDI-TOF MS. The use of 16S rRNA sequencing only for discrepant results, protein extraction, and a different MALDTI-TOF and database in the previous study likely contributed to the discrepant results with respect to those of this study.

Another study used more isolates and a greater variety of species but was performed with the Bruker MS system and used a custom-made database that is not commercially available (5). Similar to the present study, Couturier et al. used 16S rRNA sequencing as the definitive identification method; however, no comparisons were made to identification methods conventionally used in clinical microbiology laboratories. Several other studies included only a small numbers of HACEK organisms or included only members of the genus *Haemophilus* (1, 6, 7).

To our knowledge, this is the first large-scale study of HACEK organisms or fastidious pediatric pathogens to be undertaken with Vitek MS. Additionally, it is the first to use 16S rRNA sequencing to verify the accuracy of a commercially available database for these organisms. Comparisons of the identifications obtained with Vitek MS and two commercially available systems—Vitek 2 NH and Remel RapID NH—reveal the robustness of Vitek MS. Vitek MS with

SARAMIS RUO database version 4.09 correctly identified the most organisms of the three methods. As Vitek MS is more accurate than either of the commercially available methods tested here, it is appropriate for use with fastidious pediatric pathogens.

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