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Identification of *Enterobacteriaceae* by MALDI-TOF Mass Spectrometry Using the VITEK MS System

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Amended Abstract

Background: This multi-center study evaluated the accuracy of matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry identifications from the VITEK MS system (bioMérieux, Marcy l'Etoile, France) for *Enterobacteriaceae* typically encountered in the clinical laboratory.
Methods: *Enterobacteriaceae* isolates (n=965) representing 17 genera and 40 species were analyzed on the VITEK MS system (database v2.0) in accordance with manufacturer instructions. Colony growth (≤ 72 h) was applied directly to the target slide. Matrix solution (α-cyano-4-hydroxycinnamic acid) was added and allowed to dry before mass spectrometry analysis. On the basis of confidence level, the VITEK MS provided a species, genus only, or no identification for each isolate. The accuracy of the mass spectrometric identification was compared to 16S rRNA gene sequencing performed at MIDI Labs (Newark, DE). Supplemental phenotypic testing was performed at bioMérieux when necessary.
Results: The VITEK MS result agreed with the reference method identification for 96.7% of 965 isolates tested with 83.8% correct to the species level and 12.8% limited to a genus level identification. There was no identification for 1.7% of the isolates. The VITEK MS misidentified 7 isolates (0.7%) as different genera. Three *Pantoea agglomerans* isolates were misidentified as *Enterobacter* spp. and single isolates of *Enterobacter cancerogenus, Escherichia hermannii, Hafnia alvei,* and *Raoultella ornitholytica* were misidentified *as Klebsiella oxytoca*, *Citrobacter koseri, Obesumbacterium proteus,* and *Enterobacter aerogenes*, respectively. Eight isolates (0.8%) were misidentified as a different species in the correct genus.
Conclusion: The VITEK MS system provides reliable mass spectrometric identifications for *Enterobacteriaceae*.

Introduction

Because organisms in the *Enterobacteriaceae* family are biochemically active, automated and manual phenotypic identification systems perform well, but require up to 48 h for results. Mass spectrometry can provide rapid identifications that are available within minutes. This multi-center study evaluated the accuracy of the VITEK MS system (bioMérieux, Marcy l'Etoile, France) for MALDI-TOF mass spectrometric identification of *Enterobacteriaceae* typically encountered in the clinical laboratory. This is the first report of VITEK MS system performance for *Enterobacteriaceae* identification using a new database (v2.0) and MYLA software developed for in vitro diagnostic (IVD) use.

Methods

Bacterial isolates. Study sites were asked to test a minimum of 10 *Enterobacteriaceae* isolates each for common species and six isolates representing less common *Enterobacteriaceae* species from unique patients. If an insufficient number of isolates were available at a study site, bioMérieux provided stock isolates. The five participating study sites were Cleveland Clinic (Cleveland, OH), Massachusetts General Hospital (Boston, MA), Barnes Jewish Hospital (St. Louis, MO), North Shore-LIJ Health System Laboratories (Lake Success, NY), and UCLA (Los Angeles, CA).
MALDI-TOF mass spectrometry. Clinical isolates were analyzed on the VITEK MS IVD system (database v2.0) in accordance with manufacturer instructions. The *E. coli* ATCC 8739 strain was used for every acquisition group on the target slide to calibrate the mass spectrometer. For a negative control, matrix solution (α-cyano-4-hydroxycinnamic acid; bioMérieux) was tested alone. In addition, one of four quality control organisms (*Enterobacter aerogenes* ATCC 13048, *K. oxytoca* ATCC 13182, *Pseudomonas aeruginosa* ATCC 10145, *Staphylococcus aureus* ATCC 29213) was tested with each new lot of target slides and matrix solution as well as on each day of clinical isolate testing. Most isolates were tested using ≤ 48 h growth from trypticase soy agar with 5% sheep blood (Remel, Lenexa, KS). Only nine cultures were > 48 h when tested. Thirty-eight isolates were tested from MacConkey II MUG agar (BBL, Sparks, MD). Frozen isolates were subcultured twice before mass spectrometric analysis. Colony growth (≤ 72 h) was applied directly to the target slide using a 1 µl loop. Matrix solution (1 µl) was added and allowed to dry before mass spectrometric analysis. On the basis of confidence level, the VITEK MS provided a species, genus only, or no identification for each isolate. Repeat VITEK MS testing was performed if there was quality control failure, calibration failure, poor or absent mass spectra, technical error, or a mixed culture.
Reference identification. Growth from the plate used for VITEK MS testing was inoculated to slants shipped for reference testing at MIDI Labs (Newark, DE) and bioMérieux. At MIDI Labs, 16S rRNA gene sequencing was performed using the MicroSEQ 500 16S rDNA Bacterial Identification kit (Applied Biosystems, Foster City, CA) with Sherlock DNA software (MIDI) analysis. The sequencing data were also analyzed by researchers at bioMérieux using the GenBank database [1] and BIBI (Bioinformatics Bacterial Identification) software [2]. Final 16S rRNA gene sequencing identifications were assigned according to Clinical Laboratory Standards Institute (CLSI) interpretive criteria [3]. Supplemental phenotypic testing was performed at bioMérieux with VITEK 2 GN card (bioMérieux), API 20E strips (bioMérieux), and/or classical biochemical tube or spot tests for organisms unidentified by 16S rRNA gene sequencing.
Data analysis. Each VITEK MS result (species or genus level only) was compared to the final reference identification and classified as correct if concordant or a misidentification if discordant. Isolates with a reference identification that is not claimed by VITEK MS were excluded from the study.

Table 1. Comparison of VITEK MS results to reference method identifications for 965 *Enterobacteriaceae* isolates

Organisms	No. isolates	No. (row %) isolates with VITEK MS result						
		Correct identification			Misidentification		No identification	
		Genus & species	Genus only	Total	Species	Genus	No result	Mixed genera
Citrobacter amalonaticus	30	27 (90.0)	2 (6.7)	29 (96.7)	-	-	1 (3.3)	0
Citrobacter braakii	18	6 (33.3)	8 (44.4)	14 (77.8)	1 (5.6)	-	2 (11.1)	1 (5.6)[a]
Citrobacter freundii	58	38 (65.5)	16 (27.6)	54 (93.1)	4 (6.9)	-	-	-
Citrobacter koseri	31	31 (100)	-	31(100)	-	-	-	-
Citrobacter youngae	13	5 (38.5)	8 (61.5)	13 (100)	-	-	-	-
Cronobacter sakazakii	10	6 (60)	4 (40)	10 (100)	-	-	-	-
Edwardsiella hoshinae	11	9 (81.8)	2 (18.2)	11 (100)	-	-	-	-
Edwardsiella tarda	9	8 (88.9)	1 (11.1)	9 (100)	-	-	-	-
Enterobacter aerogenes	52	52 (100)	-	52 (100)	-	-	-	-
Enterobacter asburiae	12	-	10 (83.3)	10 (83.3)	-	-	1 (8.3)	1 (8.3)[b]
Enterobacter cancerogenus	6	5 (83.3)	-	5 (83.3)	-	1 (16.7)		-
Enterobacter cloacae	27	-	26 (96.3)	26 (96.3)	-	-	1 (3.7)	-
Enterobacter gergoviae	10	10 (100)	-	10 (100)	-	-	-	-
Escherichia coli	65	65 (100)	-	65 (100)	-	-	-	-
Escherichia fergusonii	6	4 (66.7)	1 (16.7)	5 (83.3)	1 (16.7)	-	-	-
Escherichia hermannii	7	6 (85.7)	-	6 (85.7)	-	1 (14.3)	-	-
Ewingella americana	6	6 (100)	-	6 (100)	-	-	-	-
Hafnia alvei	19	16 (84.2)	-	16 (84.2)	-	1 (5.3)	-	2 (10.5)[c]
Klebsiella oxytoca	49	49 (100)	-	49 (100)	-	-	-	-
Klebsiella pneumoniae	58	58 (100)	-	58 (100)	-	-	-	-
Leclercia adecarboxylata	10	9 (90)	-	9 (90)	-	-	1 (10)	-
Morganella morganii	52	52 (100)	-	52 (100)	-	-	-	-
Pantoea agglomerans	22	19 (86.4)	-	19 (86.4)	-	3 (13.6)	-	-
Proteus mirabilis	58	57 (98.3)	-	57 (98.3)	-	-	1 (1.7)	-
Proteus penneri	19	-	18 (94.7)	18 (94.7)	-	-	1 (5.3)	-
Proteus vulgaris	23	-	23 (100)	23 (100)	-	-	-	-
Providencia rettgeri	33	32 (97)	-	32 (97)	-	-	1 (3.0)	-
Providencia stuartii	31	31 (100)	-	31 (100)	-	-	-	-
Raoultella ornithinolytica	11	9 (81.8)	1 (9.1)	10 (90.9)	-	1 (9.1)	-	-
Raoultella planticola	9	7 (77.8)	-	7 (77.8)	1 (11.1)	-	1 (11.1)	-
Salmonella enterica	35	33 (94.3)	2 (5.7)	35 (100)	-	-	-	-
Serratia fonticola	7	6 (85.7)	-	6 (85.7)	1 (14.3)	-	-	-
Serratia liquefaciens	23	22 (95.7)	1 (4.3)	23 (100)	-	-	-	-
Serratia marcescens	57	57 (100)	-	57 (100)	-	-	-	-
Serratia odorifera	30	30 (100)	-	30 (100)	-	-	-	-
Yersinia enterocolitica	14	14 (100)	-	14 (100)	-	-	-	-
Yersinia frederiksenii	10	8 (80)	-	8 (80)	-	-	-	2 (20)[d]
Yersinia intermedia	9	9 (100)	-	9 (100)	-	-	-	-
Yersinia kristensenii	7	5 (71.4)	1 (14.3)	6 (85.7)	-	-	-	1 (14.3)[e]
Yersinia pseudotuberculosis	8	8 (100)	-	8 (100)	-	-	-	-
Total	965	809 (83.8)	124 (12.8)	933 (96.7)	8 (0.8)	7 (0.7)	10 (1.0)	7 (0.7)

[a]*Citrobacter braakii / Citrobacter werkmanii / Enterobacter gergoviae / Citrobacter freundii.*
[b]*Pantoea dispersa / Enterobacter asburiae / Enterobacter cloacae.*
[c]*Finegoldia magna / Serratia odorifera; Obesumbacterium proteus / Hafnia alvei.*
[d]*Yersinia frederiksenii / Serratia odorifera.*
[e]*Kluyvera cryocrescens / Yersinia kristensenii / Yersinia enterocolitica.*

Results

Enterobacteriaceae isolates representing 17 genera and 40 species were included in the study (Table 1). Most of the 965 study isolates (73.1%) were recovered from patient cultures performed at one of the five study sites. The remaining 260 isolates (26.9%) were unique isolates provided by bioMérieux representing rare (17.9%) or uncommon (9%) strains. Supplemental phenotypic testing to determine the final reference identification was required for 167 isolates.

The accuracy of VITEK MS identifications in comparison to the reference method is shown in Table 1. The VITEK MS result agreed with the reference method for 96.7% of 965 isolates tested with 83.8% correct to the species level and 12.8% limited to a genus level identification. A small percentage of isolates (1.7%) were not identified by VITEK MS. Details of the 15 VITEK MS results (1.5%) classified as misidentifications are shown in Table 2. The species options included for genus level VITEK MS identifications are shown in Table 3.

Conclusions

The VITEK MS IVD system provided accurate genus or species level identifications for a large and diverse collection of *Enterobacteriaceae* clinical isolates. Implementation of MALDI-TOF mass spectrometric identification will allow laboratories to provide results in a more clinically relevant time frame than current commercial biochemical identification systems.

Table 2. Results for 15 *Enterobacteriaceae* isolates misidentified by VITEK MS

Reference method identification (no. isolates)	VITEK MS misidentification (no. isolates)	
	Species	Genus
Citrobacter braakii	*Citrobacter freundii*	-
Citrobacter freundii (4)	*Citrobacter youngae* (2)	-
	Citrobacter werkmanii (2)	-
Enterobacter cancerogenus	-	*Klebsiella oxytoca*
Escherichia fergusonii	*Escherichia coli*	-
Escherichia hermannii	-	*Citrobacter koseri*
Hafnia alvei	-	*Obesumbacterium proteus*
Pantoea agglomerans (3)	-	*Enterobacter asburiae/E. cloacae*
	-	*Enterobacter cancerogenus* (2)
Raoultella ornithinolytica	-	*Enterobacter aerogenes*
Raoultella planticola	*Raoultella ornithinolytica*	-
Serratia fonticola	*Serratia liquefaciens*	-

Table 3. Results for 124 *Enterobacteriaceae* isolates with VITEK MS identification limited to genus level

Reference identification (no. isolates)	Vitek MS result
Citrobacter amalonaticus (2)	*Citrobacter amalonaticus / Citrobacter farmeri*
Citrobacter braakii (4)	*Citrobacter braakii / Citrobacter farmeri*
Citrobacter braakii	*Citrobacter braakii / Citrobacter werkmanii*
Citrobacter braakii (2)	*Citrobacter braakii / Citrobacter werkmanii / Citrobacter youngae*
Citrobacter braakii	*Citrobacter braakii / Citrobacter youngae / Citrobacter freundii*
Citrobacter freundii (2)	*Citrobacter braakii / Citrobacter werkmanii / Citrobacter freundii*
Citrobacter freundii	*Citrobacter braakii / Citrobacter youngae*
Citrobacter freundii (11)	*Citrobacter werkmanii / Citrobacter freundii*
Citrobacter freundii (2)	*Citrobacter werkmanii / Citrobacter youngae / Citrobacter freundii*
Citrobacter youngae (2)	*Citrobacter werkmanii / Citrobacter youngae*
Citrobacter youngae (2)	*Citrobacter werkmanii / Citrobacter youngae / Citrobacter freundii*
Citrobacter youngae (4)	*Citrobacter youngae / Citrobacter freundii*
Cronobacter sakazakii (4)	*Cronobacter malonaticus / Cronobacter sakazakii*
Edwardsiella hoshinae (2)	*Edwardsiella tarda / Edwardsiella hoshinae*
Edwardsiella tarda	*Edwardsiella tarda / Edwardsiella hoshinae*
Enterobacter asburiae (10)	*Enterobacter asburiae / Enterobacter cloacae*
Enterobacter cloacae (26)	*Enterobacter asburiae / Enterobacter cloacae*
Escherichia fergusonii	*Escherichia coli / Escherichia fergusonii*
Proteus penneri (18)	*Proteus vulgaris / Proteus penneri*
Proteus vulgaris (23)	*Proteus vulgaris / Proteus penneri*
Raoultella ornithinolytica	*Raoultella ornithinolytica / Raoultella planticola*
Salmonella enterica	*Salmonella enterica ssp diarizonae / Salmonella enterica ssp arizonae*
Salmonella enterica	*Salmonella* ser. Paratyphi A / *Salmonella* group[a]
Serratia liquefaciens	*Serratia liquefaciens / Serratia odorifera*
Yersinia kristensenii	*Yersinia kristensenii / Yersinia enterocolitica*

[a]"*Salmonella* group" is comprised of *Salmonella enterica* ssp. *enterica, Salmonella* ser. Enteritidis, *Salmonella* ser. Paratyphi B, *Salmonella* ser. Paratyphi C, *Salmonella* ser. Typhimurium, and *Salmonella* spp.

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