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ARTICLE

Identification of *Enterobacteriaceae* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using the VITEK MS system

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Abstract This multicenter 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. *Enterobacteriaceae* isolates (n=965) representing 17 genera and 40 species were analyzed on the VITEK MS system (database v2.0), in accordance with the manufacturer's 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 the confidence level, the VITEK MS system 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. The VITEK MS result agreed with the reference method identification for 96.7 % of the 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 system 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 ornithinolytica* 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. The VITEK MS system provides reliable mass spectrometric identifications for *Enterobacteriaceae*.

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Introduction

Development of the "soft" ionization technique called matrix-assisted laser desorption/ionization (MALDI) in 1985 allowed the mass spectrometric detection of macromolecules without fragmentation [1–3]. A new approach to matrix composition with time-of-flight (TOF) mass spectrometry in 1988 enabled

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large protein molecules to be ionized [4]. Advances in microbial genomic research led to the recognition that many mass spectral peaks represent ribosomal proteins, and this proteomic technology could be used to identify clinically important bacteria such as *Enterobacteriaceae* [5, 6]. Reference mass spectra libraries and software developed for data analysis have been incorporated into commercial MALDI-TOF mass spectrometry identification systems for use in clinical laboratories.

This multicenter study evaluated the accuracy of the VITEK MS system (bioMérieux, Marcy l'Etoile, France) for the mass spectrometric identification of *Enterobacteriaceae* typically encountered in the clinical laboratory. Because organisms in the *Enterobacteriaceae* family are biochemically active, automated and manual phenotypic identification systems perform well, but require up to 48 h for the results to be available. Mass spectrometry can provide rapid identifications that are available within minutes [3, 5].

The *Enterobacteriaceae* family is a heterogeneous group of bacteria ranging from species that are part of normal intestinal flora to organisms that are always considered pathogens. Diseases associated with *Enterobacteriaceae* include urinary tract, intestinal, respiratory, wound, and bloodstream infections [7]. Antimicrobial-resistant *Enterobacteriaceae* that produce carbapenemases or extended-spectrum β-lactamases (ESBLs) (e.g., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter* spp., *Escherichia coli*) have become a public health concern [7, 8].

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. The VITEK MS software has been improved by the selective weighting of mass spectral peaks, a feature that is especially helpful for distinguishing between species. The laser scanning function of the IVD system has been optimized to allow more variability in the amount of organism applied to the target slide.

Materials and methods

Bacterial isolates

Study sites were asked to test a minimum of ten *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). Each study site obtained institutional review board approval prior to the initiation of the clinical trial.

MALDI-TOF mass spectrometry

Clinical isolates were analyzed on the VITEK MS IVD system (database v2.0), in accordance with manufacturer's 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.

The protocol allowed isolates to be tested as long as 72 h after subculture. 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 growth when tested. Thirty-eight isolates were tested from MacConkey II MUG agar (BBL, Sparks, MD). Frozen isolates were subcultured twice before mass spectrometric analysis. A thin layer of colony growth 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 the confidence level, the VITEK MS system 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 [9] and BIBI (Bioinformatics Bacterial Identification) software [10]. Final 16S rRNA gene sequencing identifications were assigned according to Clinical and Laboratory Standards Institute (CLSI) interpretive criteria [11]. Supplemental phenotypic testing was performed at bioMérieux with VITEK 2 GN cards (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.

Results

Enterobacteriaceae isolates representing 17 genera and 40 species were included in the study (Table 1). The majority 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.

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 the 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.

Optimal performance (100 % of isolates assigned a VITEK MS species-level identification concordant with the reference method) occurred for *Citrobacter koseri* (*n*=31), *E. aerogenes* (*n*=52), *Enterobacter gergoviae* (*n*=10), *Ewingella americana* (*n*=6), *K. oxytoca* (*n*=49), *K. pneumoniae* (*n*=58), *Morganella morganii* (*n*=52), *Providencia stuartii* (*n*=31), *Serratia marcescens* (*n*=57), *Serratia odorifera* (*n*=30), *Yersinia enterocolitica* (*n*=14), *Yersinia intermedia* (*n*=9), and *Yersinia pseudotuberculosis* (*n*=8). There was also 100 % species-level agreement for the 65 *E. coli* tested, but this result includes a limitation that MALDI-TOF mass spectrometry cannot differentiate between *E. coli* and *Shigella* spp. and, for this reason, *Shigella* spp. were not included in the study. Five additional organisms had species-level identifications concordant with the reference method for at least 90 % of isolates: *Citrobacter amalonaticus* (90 %), *Leclercia adecarboxylata* (90 %), *Proteus mirabilis* (98.3 %), *Providencia rettgeri* (97 %), *Salmonella enterica* (94.3 %), and *Serratia liquefaciens* (95.7 %).

Seven organisms were more likely to have a genus- rather than species-level VITEK MS identification: *Citrobacter braakii* (33.3 % species, 44.4 % genus), *Citrobacter youngae* (38.5 % species, 61.5 % genus), *Enterobacter asburiae* (0 % species, 83.3 % genus), *E. cloacae* (0 % species, 96.3 % genus), *Proteus penneri* (0 % species, 94.7 % genus), and *Proteus vulgaris* (0 % species, 100 % genus). The species options included for genus-level VITEK MS identifications are shown in Table 3.

There were only two organisms, *Citrobacter freundii* and *Pantoea agglomerans*, with multiple VITEK MS misidentifications. Four *C. freundii* isolates had an incorrect VITEK MS species identification of *C. youngae* (*n*=2) or *Citrobacter werkmanii* (*n*=2). Three *P. agglomerans* isolates were misidentified by VITEK MS as *Enterobacter cancero genus* (*n*=2) or *Enterobacter* spp. (Table 2).

Supplemental phenotypic testing to determine the final reference identification was required for 167 of the 964 isolates. In addition to 16S rRNA gene sequencing, biochemical testing using VITEK 2 GN cards, API 20E strips, and/or classical tube or spot tests was performed on eight *C. amalonaticus*, eight *C. braakii*, 19 *C. freundii*, three *C. koseri*, four *C. youngae*, 29 *E. aerogenes*, three *E. asburiae*, one *E. cancerogenus*, 18 *E. cloacae*, six *Escherichia coli*, one *E. hermannii*, seven *Hafnia alvei*, 20 *K. oxytoca*, two *K. pneumoniae*, one *Morganella morganii*, four *P. agglomerans*, one *Proteus mirabilis*, 17 *P. vulgaris*, three *Providencia rettgeri*, one *Providencia stuartii*, one *Raoultella ornithinolytica*, one *Raoultella planticola*, three *Salmonella enterica*, three *Serratia liquefaciens*, two *S. marcescens*, and one *Yersinia enterocolitica* isolate.

Discussion

This multicenter study demonstrated that identifications provided by the VITEK MS IVD system for *Enterobacteriaceae* are highly accurate in comparison to a molecular reference method. The *Enterobacteriaceae* family includes genera and species that can be difficult to identify using 16S rRNA gene sequencing, so supplemental phenotypic testing was required for many isolates [11–13]. Some limitations of 16S rRNA sequencing identification have also been observed with mass spectrometry methods. For example, *E. coli* and *Shigella* spp. are indistinguishable by 16S rRNA gene sequencing and the VITEK MS system. It has been suggested that *Shigella* and *E. coli* should be reclassified as the same species [14, 15]. Mass spectrometric identifications of *E. coli* can be accepted for isolates showing lactose fermentation and indole production or motility, but serotyping, lysine decarboxylation, and other phenotypic tests are required in order to differentiate *Shigella* from inactive (lactose-nonfer menting) *E. coli* [16]. The recovery of *Shigella* from an extraintestinal site is uncommon [16].

Genera such as *Citrobacter*, *Enterobacter*, *Klebsiella*, and *Pantoea* belong to multiple genogroups and their taxonomic classification is based on biochemical testing [11]. Despite this genetic heterogeneity, the overall accuracy of VITEK MS identifications for these genera was excellent with only a small number of misidentifications. For multiple *Citro bacter* and *Enterobacter* species, VITEK MS identifications limited to the genus level were common.

The most frequent VITEK MS genus misidentification was for *P. agglomerans*: 13.6 % of isolates were reported as species in the closely related genus *Enterobacter*. Yellow

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

Organisms	No. of isolates	No. (row %) of isolates with VITEK MS result						
		Correct identification			Misidentification		No identification	
		Genus and 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)	–
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)	–	–	–

Table 1 (continued)

Organisms	No. of isolates	No. (row %) of isolates with VITEK MS result						
		Correct identification			Misidentification		No identification	
		Genus and species	Genus only	Total	Species	Genus	No result	Mixed genera
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*

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

Reference method identification (no. of isolates)	VITEK MS misidentification (no. of 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*	–

pigmented colonies with a mass spectrometric identification of *Enterobacter* spp. should be assessed for the biochemical characteristics of *P. agglomerans* (negative reactions for ornithine decarboxylase and arginine dihydrolase) and *Cronobacter sakazakii* [7]. Although most *P. agglomerans* are usually less antimicrobial resistant than *Enterobacter* spp., a recent report of an ESBL-producing strain highlights the need for susceptibility testing of clinically significant *Enterobacteriaceae* [17].

The majority of VITEK MS species misidentifications were within the *Citrobacter* genus. Four *C. freundii* isolates

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

Reference identification (no. of 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.

were reported as *C. youngae* or *C. werkmanii*, and one *C. braakii* was misidentified as *C. freundii*. Most *C. youngae* and *C. braakii* were genus- rather than species-level identifications, with the result split between multiple species. No isolates with a reference identification of *C. werkmanii* were included in this study. Use of the term "*Citrobacter freundii* complex" for *C. freundii*, *C. youngae*, *C. braakii*, *C. werkmanii*, and *C. sedlakii* [18] may be the best approach to reporting mass spectrometry results for these species. The *C. amalonaticus* and *C. koseri* isolates studied were reliably identified to the species level by VITEK MS and tend to be more susceptible to cephalosporins than *C. freundii*.

Six species are included in the *Enterobacter cloacae* complex (*E. asburiae*, *E. cloacae*, *E. hormaechei*, *E. kobei*, *E. ludwigii*, *E. nimipressuralis*) [19]. The only *E. cloacae* complex species included in this study were *E. asburiae* (n=10) and *E. cloacae* (n=26). Since the VITEK MS system provided genus-level identifications for all 36 isolates as a split between these two species, laboratories may choose to report "*E. cloacae* complex" rather than "*Enterobacter* spp."

The VITEK MS results for *C. koseri*, *E. aerogenes*, *E. gergoviae*, *E. coli*, *E. americana*, *K. oxytoca*, *K. pneumoniae*, *M. morganii*, *P. mirabilis*, *P. rettgeri*, *P. stuartii*, *S. marcescens*, *S. odorifera*, *Y. enterocolitica*, *Y. intermedia*, and *Y. pseudotuberculosis* were excellent, with 97–100 % of isolates correctly identified to the species level. The inability of VITEK MS to discriminate between *P. vulgaris* and *P. penneri* can be resolved with supplemental indole testing.

There are five options for *Salmonella enterica* reporting by the VITEK MS v2.0 database: *Salmonella* serotype Typhi, *Salmonella* serotype Paratyphi A, *Salmonella* serotype Gallinarum, *Salmonella enterica* subsp. *arizonae*/*Salmonella enterica* subsp. *diarizonae*, and *Salmonella* group (*Salmonella enterica* subsp. *enterica*, *Salmonella* serotype Enteritidis, *Salmonella* serotype Paratyphi B, *Salmonella* serotype Paratyphi C, *Salmonella* serotype Typhimurium). The limited variety of *Salmonella* isolates included in this study did not allow VITEK MS identification of specific serotypes to be assessed. The utility of mass spectrometry to prescreen for some important *Salmonella enterica* serovars and reduce the number of isolates requiring traditional serotyping at public health laboratories has been demonstrated in other studies [20, 21].

Because the clinical significance and susceptibility test methods are similar for *Enterobacteriaceae*, the impact of the VITEK MS misidentifications reported in this study on patient care would be minimal. Most laboratories have routine procedures in place requiring any unusual identification (such as the *Obesumbacterium proteus* result that occurred for the *H. alvei* isolate) to be followed up with additional biochemical testing.

Cherkaoui et al. reported a higher percentage of correct mass spectrometric *Enterobacteriaceae* identifications provided by the Bruker Biotyper (Bruker Daltonics) in comparison to the Shimadzu system (current VITEK MS RUO) [22]. In the current study, the accuracy of *K. pneumoniae*, *E. cloacae*, and *S. marcescens* identifications provided by the VITEK MS IVD system were better than the prior report of Shimadzu system performance and similar to the performance of the Bruker Biotyper [22].

Saffert et al. reported a lower percentage of *S. marcescens*, *K. oxytoca*, and *P. agglomerans* isolates identified to the genus or species level by the Bruker Biotyper (v2.0 software) in comparison to the current VITEK MS study [23]. The Bruker Biotyper had difficulty differentiating *Klebsiella* from the closely related genus *Raoultella* [23, 24].

A prospective Bruker Biotyper study of all clinical isolates identified during a four-week period included 15 *Entero bacteriaceae* species (~50 % were *E. coli*) [25]. Bruker (v3.0 software) misidentifications for *Shigella*, *Citrobacter*, *Enterobacter*, and *Klebsiella* species, similar to the present VITEK MS study, were noted [25].

Martiny et al. compared the VITEK MS RUO, VITEK MS IVD, and Microflex LT/Bruker Biotyper (v3.0 software) systems available in Europe by testing many *E. coli* and small numbers of other species in the *Enterobacteriaceae* family [26]. The IVD version of each system performed well, but higher errors for the species-level identification of *Serratia* species were noted with the Bruker database [26].

Strengths of our study include the large number of *Enterobacteriaceae* isolates from different geographic regions of the U.S. and the use of a reference molecular method for all isolates. A study limitation is the lack of clinically important species such as *Shigella* spp. and a variety of *Salmonella* serotypes (e.g., Typhi, Paratyphi A).

The efficient workflow used in our study (single spotting of isolates, no extraction step) minimizes the hands-on time required for mass spectrometric testing. Although not measured in this study, the faster identification available with mass spectrometry should improve patient care [22, 27–30]. Lower reagent and labor costs for organism identification is another benefit of MALDI-TOF mass spectrometry [22, 27–30].

In conclusion, the VITEK MS IVD system provided accurate genus- or species-level identifications for a large and diverse collection of *Enterobacteriaceae* clinical isolates. The 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.

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