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ARTICLE

Multi-center evaluation of the VITEK® MS system for mass spectrometric identification of non-*Enterobacteriaceae* Gram-negative bacilli

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Abstract Studies have demonstrated that matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a rapid, accurate method for the identification of clinically relevant bacteria. The purpose of this study was to evaluate the performance of the VITEK MS v2.0 system (bioMérieux) for the identification of the non-*Enterobacteriaceae* Gram-negative bacilli (NEGNB). This multi-center study tested 558 unique NEGNB clinical isolates, representing 18 genera and 33 species. Results obtained with the VITEK MS v2.0 were compared with reference 16S rRNA gene sequencing and when indicated *rec*A sequencing and phenotypic analysis. VITEK MS v2.0 provided an identification for 92.5 % of the NEGNB isolates (516 out of 558). VITEK MS v2.0 correctly identified 90.9 % of NEGNB (507 out of 558), 77.8 % to species level and 13.1 % to genus level with multiple species. There were four isolates (0.7 %) incorrectly identified to genus level and five isolates (0.9 %), with one incorrect identification to species level. The remaining 42 isolates (7.5 %) were either reported as no identification (5.0 %) or called "mixed genera" (2.5 %) since two or more different genera were identified as possible identifications for the test organism. These findings demonstrate that the VITEK MS v2.0 system provides accurate results for the identification of a challenging and diverse group of Gram-negative bacteria.

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Introduction

The non-*Enterobacteriaceae* Gram-negative bacilli (NEGNB) comprise a diverse group of pathogens that cause a variety of infections, primarily pulmonary, urinary, skin and soft tissue, catheter-related, bloodstream, and gastrointestinal [1–8]. *Achromobacter* spp., *Acinetobacter* spp., *Burkholderia* spp., *Pseudomonas* spp., and *Stenotrophomonas* spp., as well as additional rare NEGNB, are significant causes of acute care-associated pneumonia or respiratory disease in patients with underlying comorbidities such as cystic fibrosis (CF) [1, 5, 6, 8–14]. *Burkholderia cepacia* complex (Bcc) infections in CF patients are especially problematic owing to increased morbidity and premature mortality [9, 10, 12, 13], risk of transmission to other CF patients [12], and potential exclusion from lung transplantation [13]. *Bordetella pertussis* and occasionally *Bordetella parapertussis* cause "whooping cough" [2, 3],

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while *Bordetella bronchiseptica* may cause respiratory disease of varying severity in immune-compromised patients or in patients with chronic lung disease.

Vibrio spp. and *Aeromonas* spp. are associated primarily with water- and/or foodborne disease [4, 7, 15–17]. Since the 2010 earthquake in Haiti and cholera epidemic, the United States (US) incidence of travel-related *V. cholera* infections has significantly increased [15, 17], whereas *V. parahaemolyticus* is endemic in US coastal regions, with approximately 4,500 cases/year. *Vibrio* spp. identification is essential for surveillance, outbreak investigations, initiation of public health measures, and for treatment, as therapy varies by species. Immune-compromised persons, especially those with chronic liver disease, are at risk of *Vibrio vulnificus* infections [4]. *Aeromonas hydrophila* can cause wound infections, septicemia, pneumonia, and meningitis [7]. *Pasteurella* spp., in particular *P. multocida*, are a common cause of aggressive wound infections and sometimes septicemia associated with animal bites, especially dog- and cat-related [18].

Non-*Enterobacteriaceae* Gram-negative bacilli can be challenging from diagnostic and therapeutic standpoints. The accuracy of NEGNB identification using biochemical test methods can vary [19–23]. Alternatively, molecular methods, such as 16S rRNA gene sequencing, have been used to identify NEGNB [24–27], but are not available in most clinical laboratories. Finally, NEGNB antibiotic susceptibility profiles and interpretation of susceptibility test results can be highly divergent depending on genus and sometimes species [1, 5–7, 9, 11, 18]. Consequently, for specific NEGNB incorrect identification could result in inappropriate therapy and increased morbidity and mortality.

Studies have shown that improved NEGNB identification can be accomplished using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) [14, 16, 24, 26–49]. The bioMérieux MALDI-TOF MS system (VITEK MS v2.0, Marcy l'Etoile, France), examines the molecular masses of high abundance bacterial proteins that have been ionized from intact bacterial cells [50–56]. Spectral analysis is performed across a mass/charge ratio (m/z) range of 2,000–20,000 Da. The VITEK MS v2.0 knowledge base does not consist of a library of spectra, but instead utilizes a bin matrix, which is a table consisting of specificity values for mass signals per bin (bins are portions of spectra divided into small mass ranges; each spectrum is divided into 1,300 bins) for each species present in the knowledge base [56]. Advanced Spectra Classifiers identify the mass signals, compare them with the bin matrix, and incorporate the peak intensity of each mass signal. The 1,300 m/z bins were selected based on their discriminatory power over the entire knowledge base. Bins in the lower m/z range are smaller, potentially accommodating fewer mass peaks while bins in the higher m/z ranges can potentially accommodate more mass peaks. The use of the bins is first qualitative, in that each taxon is evaluated for the presence or absence of any peaks in the bin's m/z range and then quantitatively for the specificity of the presence or absence for each taxon. The quantitative aspect uses a numerical weight per bin that is subsequently used to calculate a composite number for each of the knowledge base taxa for a given unknown spectrum. Composite numbers are applied to an overall threshold to determine if the spectrum is a valid match to the knowledge base, and then a second threshold is used to compare all viable choices and determine if there is a single or a multiple choice result. Composite values are compared with each other. The confidence value is calculated and applied to a threshold to determine whether the result is either one choice or too similar to differentiate among two to four choices (low discrimination [LD] result). Thresholds retain only the more significant organisms or organism groups. When more than four organisms or organism groups are found, the organism is considered not identified.

This multi-center study evaluated the performance of the VITEK MS v2.0 for identifying clinically relevant NEGNB. These data were presented in part at the 113th General Meeting of the American Society for Microbiology, Denver, CO, , USA, May 2013.

Materials and methods

Clinical trial sites

The VITEK MS v2.0 System clinical trial was performed at five US sites (California, Massachusetts, Missouri, New York, and Ohio). Ethical approval was obtained according to the Institution Review Board requirements of each testing site.

Bacterial isolates

The test group consisted of 558 unique NEGNB isolates representing 33 different species from 18 genera (Table 1). Fresh clinical isolates (never frozen) derived from a variety of clinical samples submitted for routine testing at each of the five clinical trial sites were collected from agar plate and/or agar slant cultures. Each isolate was tested once at the trial site. There were a total of 95 unique frozen clinical isolates tested, 65 of which were provided and tested by the clinical trial sites, and 30 frozen isolates were provided by bioMérieux and tested at one clinical trial site. Additionally, uncommon or rare strains were obtained as frozen isolates from the culture collections of the five trial sites or provided by the study sponsor. Of the 558 isolates included in this study, a total of 187 rare isolates (identification confirmed by sequencing) were provided by bioMérieux and were used to develop the knowledge base. All clinical isolates of *Bordetella parapertussis*, *Brevundimonas diminuta*, *Sphingobacterium multivorum*, *Sphingobacterium spiritivorum*, *Vibrio cholerae*,

Table 1 Performance of the VITEK MS for identification of clinical isolates of non-*Enterobacteriaceae* Gram-negative bacilli

Species	Isolates tested, *n*	Correct to genus and species levels, *n* (%)[c]	Correct to genus level only,[d] *n* (%)	Total correct results,[e] *n* (%)	MisID to genus and species level, *n* (%)	MisID to species level only,[f] *n* (%)	Total MisID,[g] *n* (%)	Total no ID,[h] *n* (%)
Achromobacter denitrificans	17	0 (0.0)	15 (88.2)	15 (88.2)	0 (0)	0 (0)	0 (0)	2 (11.8)
Achromobacter xylosoxidans	24	0 (0.0)	22 (91.7)	22 (91.7)	0 (0)	0 (0)	0 (0)	2 (8.3)
Acinetobacter baumannii complex[a]	65	56 (86.2)	0 (0)	56 (86.2)	0 (0)	0 (0)	0 (0)	9 (13.8)
Acinetobacter haemolyticus	6	6 (100)	0 (0)	6 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Acinetobacter junii	11	6 (54.5)	3 (27.3)	9 (81.8)	0 (0)	1 (9.1)	1	2 (18.2)
Acinetobacter lwoffii	26	22 (84.6)	1 (3.8)	23 (88.5)	0 (0)	0 (0)	0 (0)	3 (11.5)
Aeromonas hydrophila/caviae[b]	25	16 (64.0)	8 (32.0)	24 (96.0)	0 (0)	2 (8.0)	2 (8.0)	1 (4.0)
Aeromonas sobria	10	4 (40.0)	6 (60.0)	10 (100)	0 (0)	1 (10.0)	1 (10.0)	0 (0)
Alcaligenes faecalis ssp. *faecalis*	12	11 (91.7)	0 (0)	11 (91.7)	1 (8.3)	0 (0)	1 (8.3)	0 (0)
Bordetella bronchiseptica	10	2 (20.0)	3 (30.0)	5 (50.0)	0 (0)	0 (0)	0 (0)	5 (50.0)
Bordetella parapertussis	6	6 (100.0)	0 (0)	6 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Bordetella pertussis	9	6 (66.7)	3 (33.3)	9 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Brevundimonas diminuta	7	7 (100)	0 (0)	7 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Burkholderia cepacia	9	3 (33.3)	5 (55.6)	8 (88.9)	0 (0)	0 (0)	0 (0)	1 (11.1)
Burkholderia multivorans	25	24 (96.0)	0 (0)	24 (96.0)	0 (0)	0 (0)	0 (0)	1 (4.0)
Burkholderia stabilis	6	0 (0)	6 (100)	6 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Chryseobacterium indologenes	8	7 (87.5)	0 (0)	7 (87.5)	0 (0)	0 (0)	0 (0)	1 (12.5)
Elizabethkingia meningoseptica	10	10 (100)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Ochrobactrum anthropi	10	10 (100)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Pasteurella multocida	14	14 (100)	0 (0)	14 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Pseudomonas aeruginosa	57	55 (96.5)	0 (0)	55 (96.5)	0 (0)	0 (0)	0 (0)	2 (3.5)
Pseudomonas fluorescens	19	15 (78.9)	3 (15.8)	18 (94.7)	0 (0)	0 (0)	0 (0)	1 (5.3)
Pseudomonas putida	25	20 (80.0)	2 (8.0)	22 (88.0)	0 (0)	1 (4.0)	1 (4.0)	3 (12.0)
Pseudomonas stutzeri	8	8 (100)	0 (0)	8 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Ralstonia pickettii	10	8 (80.0)	0 (0)	8 (80.0)	0 (0)	0 (0)	0 (0)	2 (20.0)
Rhizobium radiobacter	14	10 (71.4)	0 (0)	10 (71.4)	2 (14.3)	0 (0)	2 (14.3)	2 (14.3)
Sphingobacterium multivorum	5	4 (80.0)	0 (0)	4 (80.0)	0 (0)	0 (0)	0 (0)	1 (20.0)
Sphingobacterium spiritivorum	10	10 (100)	0 (0)	10 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Sphingomonas paucimobilis	9	9 (100)	0 (0)	9 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Stenotrophomonas maltophilia	53	51 (96.2)	0 (0)	51 (96.2)	1 (1.9)	0 (0)	1 (1.9)	1 (1.9)
Vibrio cholerae	11	10 (90.9)	0 (0)	10 (90.9)	0 (0)	0 (0)	0 (0)	1 (9.1)
Vibrio parahaemolyticus	16	14 (87.5)	1 (6.3)	15 (93.8)	0 (0)	0 (0)	0 (0)	1 (6.3)

Table 1 (continued)

Species	Isolates tested, *n*	Correct to genus and species levels, *n* (%)[c]	Correct to genus level only,[d] *n* (%)	Total correct results,[e] *n* (%)	MisID to genus and species level, *n* (%)	MisID to species level only,[f] *n* (%)	Total MisID,[g] *n* (%)	Total no ID,[h] *n* (%)
Vibrio vulnificus	11	10 (90.9)	0 (0)	10 (90.9)	0 (0)	0 (0)	0 (0)	1 (9.1)
Total	558	434 (77.8)	78 (14.0)	512 (91.8)	4 (0.7)	5 (0.9)	9 (1.6)	42 (7.5)

n number, *%* percentage, *MisID* incorrect identification, *ID* identification

[a] *A. baumannii* complex isolates included *A. baumannii* ($n=19$), *A. baumannii* complex ($n=34$), *A. calcoaceticus* ($n=8$), and *A. nosocomialis* ($n=4$)

[b] *A. hydrophila*/*caviae* isolates included *A. hydrophila* ($n=13$), *A. caviae* ($n=11$), and *A. hydrophila*/*caviae* ($n=1$)

[c] Number (percentage of total tested)

[d] Correct identification to genus with either two or three species listed including correct species ($n=73$) or without correct species ($n=5$); or single identification correct to genus level, but incorrect to species level ($n=5$)

[e] Total identifications correct to genus level only ($n=78$) or to genus and species levels ($n=434$)

[f] Single identification correct to genus level, but incorrect to species level ($n=5$). Results are also included in the correct to genus level only category

[g] Total with incorrect identification to genus and species levels ($n=4$) or single identification correct to genus level, but incorrect to species level ($n=5$)

[h] Total with no identification owing to either mixed genera ($n=14$) or "no ID" ($n=28$)

and Vibrio vulnificus tested in this study were also used to develop the knowledge base. The remaining 371 isolates were unique to the study and not used to develop the knowledge base. The overall data presented represent only unique isolates.

Calibrators, controls

Each VITEK MS disposable bar-coded target slide (VITEK MS-DS, bioMérieux) is divided into three sections (known as an acquisition group), each section consisting of 16 sample wells and one well for the calibrant strain (*Escherichia coli* ATCC 8739). The calibrant strain in each acquisition group is tested first to ensure that VITEK MS is calibrated and retested after the isolates within the acquisition group. Results from isolates and controls within an acquisition group that failed calibration were considered invalid and testing was repeated. VITEK MS control organisms included *Enterobacter aerogenes* (ATCC 13048), *Klebsiella oxytoca* (ATCC 13182), *Pseudomonas aeruginosa* (ATCC 10145), and *Staphylococcus aureus* (ATCC 29213). Since different groups of organisms (as represented by the controls) were tested in this study, and often on the same VITEK MS run, the controls were rotated throughout the study. A negative control, consisting of matrix only applied to a well of the target slide, was included in each run.

Preparation of isolates, controls, calibrant

Fresh isolates were subcultured to trypticase soy agar plates with 5 % sheep blood (TSAB, Remel, Lenexa, KS) for 18–72 h at 35–37 °C in aerobic conditions. Frozen isolates, controls, and calibrant were cultured under the same conditions, but were subcultured twice on TSAB prior to testing. A small portion of a colony of NEGNB, calibrant, and control(s) was applied to VITEK MS slide wells. Subsequently, 1.0 μl of matrix solution (α-cyano-4-hydroxycinnamic acid in 50 % acetonitrile and 2.5 % trifluoroacetic acid; VITEK MS-CHCA, bioMérieux) was added to each well and dried at room temperature. VITEK MS testing was performed according to the manufacturer's instructions.

Identification of NEGNB

A correct VITEK MS result was denoted as either correct to genus and species levels when a single species result matched the reference species result or correct to genus level only when there were multiple choices (LD identifications) all correct to the reference genus level, with more than one species as a possible identification. Species identification was based on species sensu strictu with two exceptions, *A. hydrophila*/*caviae* and *A. baumannii* complex. A discordant VITEK MS result was denoted when either a single species or genus result was discordant with the reference result, or more than

one species in the same genus was identified, but the genus did not match the reference genus. A no identification ("no ID") result was indicated when either the VITEK MS result had two or more genera (denoted as mixed genera) listed as possible identifications for the target organism, or a VITEK MS result of "no ID" was provided owing to no identification present in the knowledge base. Samples and controls were repeated only if the initial result was "no ID." The repeat result was used if the initial "no ID" result had a message of "bad spectrum during acquisition" or "not enough peaks." If a "no ID" result was obtained with both the initial and the repeat tests, regardless of the message, the result was considered "no ID."

Data analysis

VITEK MS v2.0 results were compared with results obtained from amplification and sequencing (MicroSeq System, Applied Biosystems, Foster City, CA, USA) of a 500-bp region of the 16S rRNA gene, performed using MIDI (Newark, DE, USA) [57, 58]. Sherlock® DNA software (MIDI) was used for sequence analysis and bacterial identification. If no match or an LD result was obtained using this method, phenotypic testing (traditional biochemical tests or VITEK GN card [bioMérieux]) and/or sequencing of *rec*A [59] was performed by the study sponsor. Isolates tested by the study sponsor for reference results were blind coded for the tester who had no prior knowledge of VITEK MS results.

Results

Performance of VITEK MS v2.0

VITEK MS v2.0 provided an identification for 516 out of 558 of the NEGNB tested (92.5 %; Table 1). VITEK MS v2.0 correctly reported 507 out of 558 NEGNB (90.9 %) to species level (77.8 %) or to genus level (13.1 %). With the exception of one sample, all other samples with a correct genus level-only result included the correct species as one of two or three choices. The remaining 42 isolates (7.5 %) were either reported as "no ID" ($n = 28$; 5.0 %) or were called mixed genera ($n = 14$; 2.5 %). Of the 14 isolates called mixed genera, 13 had the target organism as one of the identifications. When the isolates used to develop the bioMérieux knowledge base were excluded, 16 genera and 27 species of NEGNB were represented (Table 2). Of these 371 isolates, 290 (78.2 %) were correctly identified to the species level and an additional 51 (13.7 %) were identified correctly to the genus level. There were 29 isolates (7.8 %) either reported as "no ID" or called mixed genera.

Overall, there were 9 out of 558 isolates (1.6 %) with an incorrect identification. Five isolates (0.9 %) had a single result correct to genus level and incorrect to species level (Table 3; one *Acinetobacter junii* identified as *Acinetobacter haemolyticus*, two *Aeromonas caviae* identified as *Aeromonas sobria*, one *A. sobria* identified as *Aeromonas hydrophila/caviae*, one *Pseudomonas putida* identified as *Pseudomonas viridiflava*). Therefore, 512 out of 558 (91.8 %) of NEGNB isolates were identified correctly to genus level. There were four isolates (0.7 %) incorrectly identified to genus level (Table 3). One *Alcaligenes faecalis* subsp. *faecalis* was identified as *Staphylococcus aureus* and this incorrect result was most likely a technical error or contamination. One *Rhizobium radiobacter* was identified as *Obesumbacterium proteus* and one *R. radiobacter* identified as *A. denitrificans/xylosoxidans*. Of the remaining 12 *R. radiobacter* isolates, 10 (71.4 %) were correct to species level and no identification was provided for two isolates. One *Stenotrophomonas maltophilia* was identified as *Ochrobactrum anthropi*. The remaining 51 out of 53 *S. maltophilia* isolates (96.2 %) were identified correctly to species level and no identification was provided for one isolate.

Bordetella bronchiseptica had the highest "no ID" rate (5 out of 10 isolates; 50.0 %) owing to the detection of mixed genera that included *B. bronchiseptica*. Overall, 14 out of 25 *Bordetella* spp. isolates (56.0 %) were correctly identified to species level and six additional isolates (24.0 %) correctly identified to genus level. All six *B. parapertussis* and 6 out of 9 (66.7 %) *B. pertussis* isolates were identified correctly to species level.

There were 98 out of 109 isolates of *Pseudomonas* spp. (89.9 %) correctly identified to species level and five additional isolates (4.6 %) correctly identified to genus level. The *Acinetobacter* spp. were correctly identified at the species level for 90 out of 108 isolates (83.3 %) and 4 out of 108 isolates (3.7 %) at the genus level. For 14 isolates (13.0 %) no identification was provided. All *A. denitrificans* and *A. xylosoxidans* were identified to genus level only, but VITEK MS v2.0 listed both species as identified.

Overall, 95.0 % of VITEK MS v2.0 tests performed on Bcc strains produced results correct to complex level (*B. cepacia*: 88.9 %, *B. multivorans*: 96.0 %, *B. stabilis*: 100 %). At the species level, 33.3 % of *B. cepacia* and 96.0 % of *B. multivorans* were correctly identified. Only two tests (5.0 %) did not provide an identification, one *B. multivorans* reported as "no ID,",and one reported as mixed genera, including the correct isolate identification (*B. cepacia*).

Identification of *Vibrio* spp., was consistent among the three strains tested. Species-level results were correct in 90.9 % for *V. cholerae*, 87.5 % for *V. parahaemolyticus*, and 90.9 % for *V. vulnificus*. One additional isolate of *V. parahaemolyticus* was identified to genus level and three isolates (one of each species) gave "no ID." Overall identification of *Aeromonas* spp. was

Table 2 Performance of the VITEK MS for identification of clinical isolates of non-*Enterobacteriaceae* Gram-negative bacilli for isolates not used to develop the knowledge base

Species	Isolates tested, *n*	Correct to genus and species, *n* (%)[c]	Correct to genus only,[d] *n* (%)	Total correct results,[e] *n* (%)	MisID to genus and species level, *n* (%)	MisID to species level only,[f] *n* (%)	Total MisID,[g] *n* (%)	Total no ID,[h] *n* (%)
Achromobacter denitrificans	7	0 (0)	5 (71.4)	5 (71.4)	0 (0)	0 (0)	0 (0)	2 (28.6)
Achromobacter xylosoxidans	24	0 (0)	22 (91.7)	22 (91.7)	0 (0)	0 (0)	0 (0)	2 (8.3)
Acinetobacter baumannii complex[a]	62	53 (85.5)	0 (0)	53 (85.5)	0 (0)	0 (0)	0 (0)	9 (14.5)
Acinetobacter haemolyticus	3	3 (100)	0 (0)	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Acinetobacter junii	3	1 (33.3)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)	2 (66.7)
Acinetobacter lwoffii	26	22 (84.6)	1 (3.8)	23 (88.5)	0 (0)	0 (0)	0 (0)	3 (11.5)
Aeromonas hydrophila/caviae[b]	25	16 (64.0)	8 (32.0)	24 (96.0)	0 (0)	2 (8.0)	2 (8.0)	1 (4.0)
Aeromonas sobria	2	0 (0)	2 (100)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Alcaligenes faecalis ssp. *faecalis*	4	4 (100)	0 (0)	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Bordetella bronchiseptica	2	2 (100)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Bordetella pertussis	5	4 (80.0)	1 (20.0)	5 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Burkholderia cepacia	9	3 (33.3)	5 (55.6)	8 (88.9)	0 (0)	0 (0)	0 (0)	1 (11.1)
Burkholderia multivorans	16	15 (93.8)	0 (0)	15 (93.8)	0 (0)	0 (0)	0 (0)	1 (6.3)
Burkholderia stabilis	1	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Chryseobacterium indologenes	3	3 (100)	0 (0)	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Elizabethkingia meningoseptica	2	2 (100)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Ochrobactrum anthropi	1	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Pasteurella multocida	6	6 (100)	0 (0)	6 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Pseudomonas aeruginosa	57	55 (96.5)	0 (0)	55 (96.5)	0 (0)	0 (0)	0 (0)	2 (3.5)
Pseudomonas fluorescens	19	15 (78.9)	3 (15.8)	18 (94.7)	0 (0)	0 (0)	0 (0)	1 (5.3)
Pseudomonas putida	25	20 (80.0)	2 (8.0)	22 (88.0)	0 (0)	1 (4.0)	1 (4.0)	3 (12.0)
Pseudomonas stutzeri	3	3 (100)	0 (0)	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Ralstonia pickettii	2	2 (100)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Rhizobium radiobacter	4	3 (75.0)	0 (0)	3 (75.0)	0 (0)	0 (0)	0 (0)	1 (25.0)
Sphingomonas paucimobilis	1	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Stenotrophomonas maltophilia	53	51 (96.2)	0 (0)	51 (96.2)	1 (1.9)	0 (0)	1 (1.9)	1 (1.9)
Vibrio parahaemolyticus	6	5 (83.3)	1 (16.7)	6 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Total	371	290 (78.2)	51 (13.7)	341 (91.9)	1 (0.3)	3 (0.8)	4 (1.1)	29 (7.8)

n number, *%* percentage, *MisID* incorrect identification, *ID* identification

[a] *A. baumannii* complex isolates included *A. baumannii* (*n*=19), *A. baumannii* complex (*n*=34), *A. calcoaceticus* (*n*=5), and *A. nosocomialis* (*n*=4)

[b] *A. hydrophila/caviae* isolates included *A. hydrophila* (*n*=13), *A. caviae* (*n*=11), and *A. hydrophila/caviae* (*n*=1)

[c] Number (percentage of total tested)

[d] Correct identification to genus level with either two or three species listed, including the correct species (*n*=47) or without the correct species (*n*=1); or single identification correct to genus level, but incorrect to species level (*n*=3)

[e] Total identifications correct to genus level only (*n*=51) or genus and species levels (*n*=290)

[f] Single identification correct to genus level, but incorrect to species level (*n*=3). Results are also included in the correct to genus level only category

[g] Total with incorrect identification to genus and species levels (*n*=1) or single identification correct to genus level, but incorrect to species species level (*n*=3)

[h] Total with no identification owing to either mixed genera (*n*=6) or "no ID" (*n*=23)

correct to genus level for 97.1 % of the isolates, with identification to species level in 64 % for *A. hydrophilia/caviae* and 40 % for *A. sobria*. All *P. multocida* were identified correctly to species level.

Quality control

In total, for all studies included in the clinical trial, the *P. aeruginosa* control was tested 189 times across the five clinical

Table 3 Non-*Enterobacteriaceae* Gram-negative bacilli incorrectly identified by VITEK MS to species or genus level

Number	Organism	VITEK MS identification(s) incorrect at species level[c]
2	*Aeromonas caviae*[a]	*Aeromonas sobria*
1	*Pseudomonas putida*[a]	*Pseudomonas viridiflava*
1	*Acinetobacter junii*[b]	*Acinetobacter haemolyticus*
1	*Aeromonas sobria*[b]	*Aeromonas hydrophila/caviae*
		VITEK MS Identification(s) incorrect at genus level
1	*Stenotrophomonas maltophilia*[a]	*Ochrobactrum anthropi*
1	*Alcaligenes faecalis* ssp. *faecalis*[b]	*Staphylococcus aureus*
1	*Rhizobium radiobacter*[b]	*Obesumbacterium proteus*
1	*Rhizobium radiobacter*[b]	*Achromobacter denitrificans* and *Achromobacter xylosoxidans*

[a] Isolates not used to develop the knowledge base

[b] Isolates used to develop the knowledge base

[c] Specimen was considered incorrect to species level when only one ID was given, correct to genus level and incorrect to species level

trial sites and was correctly identified in 186 out of 189 (98.4 %, 95 % CI 95.4; 99.7 %) VITEK MS v2.0 runs. The three quality control tests not reported as *P. aeruginosa* were reported as "no ID" owing to bad spectra and not because of an incorrect identification.

Discussion

Correct identification of NEGNB clinical isolates to genus level and species level was comparable for all isolates (n = 588) tested in this study (91.8 % and 77.8 % respectively) compared with those isolates (n = 371) not used to develop the knowledge base (91.9 % and 78.2 % respectively). Additionally, 7.5 % of the total tested were reported as "no ID" or mixed genera compared with 7.8 % of the isolates not used to develop the knowledge base. These data demonstrate that the testing of strains used to develop the knowledge base did not bias the overall performance of the VITEK MS when all isolates were included in the analysis.

Overall, the results of this study were comparable to those of other studies that evaluated MALDI-TOF MS performance for identification of NEGNB. However, a true comparison is difficult because of major differences in the types and total numbers of each species tested in the different published studies. In addition, MALDI-TOF MS databases are expanding at a rapid rate, limiting the value of comparisons of the current results with those of older studies.

Bizzini et al., using the Microflex LT instrument (Bruker Daltonics, GmbH, Leipzig, Germany), demonstrated that MALDI-TOF MS could identify 90.8 % of the 141 isolates representing seven common NEGNB species to either species level or genus level [30]. The VITEK MS v2.0 tested 192 isolates representing the same seven species and correctly identified to either species level or genus level 181 out of 192 (94.3 %) of the study isolates. A study by van Veen et al. tested 88 NEGNB (10 genera comprising 17 species) using MicroFlex LT [49]. Although the variety of strains tested was different, the overall correct identification to genus level were similar with the MicroFlex LT (94.3 %) and VITEK MS v2.0 (91.8 %). Similar results were demonstrated in a study by Martiny et al., which compared Microflex LT, VITEK MS RUO (Axima Assurance-Saramis database, bioMérieux) and VITEK MS v1.0 (pre-commercialized database) for identifying 71 NEGNB, including *A. xylosoxidans*, *A. baumannii*, *P. aeruginosa*, *Pseudomonas fluorescens* and *S. maltophilia* [44]. Microflex LT, VITEK MS RUO, and VITEK MS v1.0, correctly identified to species level and/or genus level: 97.2 %, 88.7 %, and 94.4 % of the isolates respectively. In our study we tested similar NEGNB (n = 206) and the VITEK MS v2.0 correctly identified 93.7 % to species and/or genus level. Dubois et al. tested 192 NEGNB representing 12 genera and 21 species using VITEK MS v1.1 [36]. Overall, 94.8 % of NEGNB were identified to species level (86.2 %) or genus level (8.5 %). Included in their study were NEGNB isolates (n = 88) representing similar genera (n = 10) and species (n = 15) that were tested by VITEK MS v2.0 (n = 357). Overall, the performance of the VITEK MS v1.1 (94.4 %) and VITEK MS v2.0 (91.9 %) for the identification of this group of NEGNB to genus and/or species level was comparable. Differences between VITEK MS v1.2 and v2.0 include more taxa (161 additional species) and respective changes to the bin matrix for additions of new spectra or deletions of certain lower quality spectra after review and clean-up.

The performance of MALDI-TOF MS is of special interest for the identification of NEGNB commonly isolated from CF patients [12, 33, 34, 39, 42, 43, 48]. A study by Marko et al. compared BioTyper (Bruker) and VITEK MS RUO (Saramis database) [43] for the identification of non-fermenting Gram-negative bacilli from CF patients. Included in their study were 174 isolates representing eight genera (*Achromobacter* spp., *A. xyloxidans*, *B. multivorans*, *B. bronchioseptica*, *C. indologenes*, *E. meningioseptica*, *P. aeruginosa*, and *S. maltophilia*) tested in our study. For these 174 isolates the Biotyper and VITEK MS RUO identified to species level 79.9 % and 87.4 % of the isolates respectively, and 19.5 % and 6.9 % respectively, to genus level for an overall agreement of 99.4 % for Biotyper and 94.3 % for VITEK MS RUO. VITEK MS RUO identified to family level 7 *Achromobacter* spp. isolates, increasing the overall agreement to 98.3 %. For the same genera and species, the VITEK MS v2.0 identified 149 out of 204 of the strains (73.0 %) to species level and 40 out of 204 (19.6 %) to genus level for overall agreement of 92.6 %. The

lower species level identification by VITEK MS v2.0 was due to strain bias as a greater percentage (20.1 %) of the total number of strains tested in this study versus 8.6 % in the Marko study were *Achromobacter* spp., which is commonly found to be difficult to speciate using MS systems [30, 34, 36, 43, 44].

Bcc species-level identification is recommended for treatment and infection control purposes [9, 10, 12, 13]. A study by Lambiase et al. tested 57 Bcc isolates using the Biotyper system. All isolates were correctly identified to the species level [42]. Additionally, Degand et al. tested 52 Bcc strains representing seven species with the Bruker Autoflex System, and after modifications to their original database they were able to detect 96 % of the Bcc to species level [33]. Overall, VITEK MS v2.0 identified 95 % of Bcc isolates to species/complex level and 67.5 % to species level. Our study was limited since we did not test additional members of the Bcc, including *B. vietnamiensis* and *B. cenocepacia*. In addition to, but not part of this data set, we tested *Burkholderia gladioli* strains, which were identified to species level. Further studies are necessary to assess the true performance of the VITEK MS v2.0 with this complex group of organisms.

Only 3 (7.9 %) *Vibrio* spp. did not yield an identification, 89.5 % were correct to species level and 1 *V. parahaemolyticus* was correct to genus level. Our data are consistent with two studies that demonstrated that MALDI-TOF MS can accurately identify *Vibrio* spp. and differentiate from other *Aeromonadaceae* [35, 41].

The rate of incorrect VITEK MS identifications was low at both the genus level (0.7 %) and species level (0.9 %). Incorrect identification could have a significant clinical impact, especially for resistant bacteria (e.g., *Acinetobacter* spp.) or for bacteria with limited therapeutic choices (e.g., *Stenotrophomonas* spp.) [5, 6, 11]. All VITEK MS results for *Acinetobacter* spp. were correct to genus level, only one isolate was incorrect to species level, and one result for *S. maltophilia* was incorrect to genus level. Our data were consistent with a study using the MicroFlex LT (Bruker) that correctly identified 98.3 % of the *Acinetobacter* spp. to species level [60]. Additionally, a study by Dubois et al. using the VITEK MS RUO system identified 93.3 % of the *Acinetobacter* spp. to genus level, 60 % to species level and all isolates of *S. maltophilia* to species level [36].

For the four tests with an incorrect identification at genus level, a Gram stain would have proved useful for one isolate (*A. faecalis* subsp. *faecalis* called *S. aureus*), while an oxidase test would have been helpful for one isolate (*S. maltophilia* called *O. anthropi*). The remaining two tests incorrectly identified one *R. radiobacter* isolate as *A. denitrificans* or *A. xylosoxidans*, and one *R. radiobacter* as *O. proteus*. Confirmation of the identity of these isolates would require more extensive biochemical testing or molecular identification. Considering the rarity of these isolates, it is reasonable that clinical laboratories would retest such isolates, particularly if an isolate feature (e.g., morphology) did not match the MS identification. This study protocol did not permit repeat testing if an acceptable identification was generated. Repeat testing may have yielded the correct result. Four of the five incorrect results at the species level most likely would not have affected clinical care as antibiotic susceptibility profiles are similar.

Studies using the same sets of organisms are required to make an accurate comparison between the different MS systems and the different VITEK MS knowledge bases. The improved knowledge base of VITEK MS v2.0 may enhance the performance for NEGNB identification compared with VITEK MS RUO (Saramis database).

Time studies from our laboratory (data not shown) have demonstrated that MALDI-TOF testing, compared with traditional microbiology methods, is easy to perform as VITEK MS v2.0 does not require any pre-processing of NEGNB prior to inoculation of the target slide, and rapid (2 min per sample for set up and 56±4 min for 48 identifications). Importantly, studies have demonstrated the clinical benefit and financial value of using MALDI-TOF MS in the clinical laboratory [38, 53, 61–64]. Tan et al. demonstrated that MALDI-TOF MS can provide organism identification up to 30 h faster than conventional methods, therefore reducing reagent and labor costs, while providing important information within a time frame that can have an impact on patient care [64].

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