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Evaluation of the Bruker MALDI Biotyper for Identification of Fastidious Gram-Negative Rods

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Matrix-assisted laser desorption ionization−time of flight mass spectrometry (MALDI-TOF MS) has entered clinical laboratories, facilitating identification of bacteria. Here, we evaluated the MALDI Biotyper (Bruker Daltonics) for the identification of fastidious Gram-negative rods (GNR). Three sample preparation methods, direct colony transfer, direct transfer plus on-target formic acid preparation, and ethanol-formic acid extraction, were analyzed for 151 clinical isolates. Direct colony transfer applied with the manufacturer's interpretation criteria resulted in overall species and genus identification rates of 43.0% and 32.5%, respectively; 23.2% of the isolates were not identified, and two misidentifications (1.3%) were observed. The species identification rates increased to 46.4% and 53.7% for direct transfer plus formic acid preparation and ethanol-formic acid extraction, respectively. In addition, we evaluated score value cutoff alterations. The identification rates hardly increased by reducing the genus cutoff, while reducing the 2.0 species cutoff to 1.9 and to 1.8 increased the identification rates to up to 66.2% without increasing the rate of misidentifications. This study shows that fastidious GNR can reliably be identified using the MALDI Biotyper. However, the identification rates do not reach those of nonfastidious GNR such as the *Enterobacteriaceae*. In addition, two approaches optimizing the identification of fastidious GNR by the MALDI Biotyper were demonstrated: formic acid-based on-target sample treatment and reductions in cutoff scores to increase the species identification rates.

Fastidious Gram-negative rods (GNR) are slow-growing bacteria that belong to different genera, such as *Neisseria*, *Pasteurella*, and the HACEK organisms (*Haemophilus*, *Aggregatibacter*, *Cardiobacterium*, *Eikenella*, and *Kingella*). Many of these species are commensal organisms of the human or animal oral cavity. They are isolated from wound infections, e.g., after a human or animal bite, but are also associated with endocarditis, septicemia, and abscesses, particularly in immunocompromised and elderly patients (1–4).

Traditionally, identification of fastidious GNR in diagnostic laboratories is based on morphological and biochemical criteria. Identification is particularly challenging and time-consuming as these bacteria usually do not grow on standard media and require supplemental nutrients and/or a CO_2-enriched atmosphere for growth (1–3). 16S rRNA gene sequencing is widely used in addition to conventional identification methods (5–7). Matrix-assisted laser desorption ionization−time of flight mass spectrometry (MALDI-TOF MS) has been suggested as an alternative to molecular methods for the identification of fastidious GNR and other bacteria that are difficult to culture (8–10).

Numerous studies have compared MALDI-TOF MS-based bacterial identification with molecular or biochemical identification methods (11–22). Most of these studies included only limited numbers of fastidious GNR or analyzed only a single genus, such as *Haemophilus* spp. (23–26), *Neisseria* spp. (1, 27), or *Pasteurella* spp. (28). Reported identification rates varied between different studies, mainly because different sets of fastidious GNR were investigated, different sample preparation methods were applied, and different MALDI-TOF MS systems with different databases were used.

We evaluated the capability of the MALDI Biotyper (Bruker Daltonics, Bremen, Germany) to identify 151 clinical isolates of fastidious GNR, including 40 species from 15 genera. We used three sample preparation methods (direct colony transfer, direct transfer plus on-target formic acid preparation, and tube-based extraction with ethanol and formic acid) and applied the manufacturer's interpretation criteria. Furthermore, we evaluated the feasibility of altered identification cutoffs for the species and genus level. Based on our data, we propose an optimized combination of sample preparation and cutoff scores to increase the identification rates for fastidious GNR.

MATERIALS AND METHODS

Bacterial isolates and culture conditions. In this study, 151 clinical isolates of fastidious GNR collected at the Institute of Medical Microbiology, University of Zurich, Switzerland, from 1993 to 2012 were included (Table 1). The isolates were characterized using biochemical assays and 16S rRNA gene sequencing, the latter being used as the identification gold standard. When the discriminatory power of the 16S rRNA gene sequence analysis was insufficient, supplemental biochemical tests were used for the final identification (6). Bacteria were cultivated on Columbia sheep blood agar and on chocolate agar containing PolyViteX (bioMérieux, Marcy l'Etoile, France) at 37°C with 7.5% CO_2 for 24 to 48 h.

Biochemical identification. Fastidious GNR isolates were identified by means of biochemical reactions as specified by de Melo Oliveira et al. (6).

16S rRNA gene sequence analysis. The identification of fastidious GNR by partial 16S rRNA gene sequencing was performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI)

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(29), and as described by de Melo Oliveira et al. (6). A 16S rRNA gene fragment, corresponding to *Escherichia coli* positions 10 to 806, was amplified using primers BAK11w (5′-AGTTTGATC[A/C]TGGCTCAG) and BAK2 (5′-GGACTAC[C/T/A]AGGGTATCTAAT) and sequenced with forward primer BAK11w (6). Sequences were analyzed for homology using a GenBank BLAST search and SmartGene IDNS software (SmartGene GmbH, Zug, Switzerland). The identification was done according to the following criteria: (i) species identification when the sequence determined had a similarity score of ≥99% with that of a reference sequence of a classified species and when the sequence divergence between different species was ≥0.5%; (ii) a genus was assigned for similarity scores of <99% to ≥95%; and (iii) a family was assigned for similarity scores of less than 95% (6).

Sample preparation for MALDI-TOF MS. Bacteria were prepared for MALDI-TOF MS as described previously (30). In brief, for the direct colony transfer procedure, colony material from overnight cultures was deposited on a polished steel MSP 96 target (Bruker Daltonics) and overlaid with 1 μl of a saturated α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution (Bruker Daltonics). Direct transfer plus formic acid preparation was done by the addition of 1 μl of a 70% formic acid solution to the bacterial spot prior to overlaying of the sample with the matrix solution. The tube-based extraction was performed by suspending a loopful of bacterial colony material in 300 μl of distilled water and 900 μl of 100% ethanol. The cell suspension was centrifuged at 17,000 × *g* for 2 min, and the pellet was resuspended in 5 to 50 μl of 70% formic acid according to the pellet size. Subsequently, acetonitrile was added in an equal volume. The suspension was centrifuged at 17,000 × *g* for 2 min, and 1 μl of the supernatant was spotted on the MALDI target. After being dried, the spot was overlaid with 1 μl of matrix solution.

MALDI-TOF MS analysis. Mass spectra were acquired and analyzed using a microflex LT mass spectrometer (Bruker Daltonics) in combination with research-use-only (RUO) versions of the MALDI Biotyper software package (version 3.0) and the reference database V.3.1.2.0 (3,995 entries) as previously published (30). Calibration was done by following the manufacturer's instructions and using the manufacturer's recommended bacterial test standard (Bruker Daltonics). Analyses of all strains were done in duplicate from independent preparations.

MALDI-TOF MS data interpretation. Data were interpreted by application of the manufacturer's standard criteria (30). In short, species were assigned for scores of ≥2.0, and genera were assigned for scores of ≥1.7 but <2.0. If scores were lower than 1.7, no identification was assigned. The cutoff scores were altered by reducing the standard 2.0 species cutoff to 1.9, 1.8, and 1.7 and the standard 1.7 genus cutoff to 1.6 and 1.5.

Statistical analysis. Statistical calculations were performed with IBM SPSS, version 20 (SPSS Inc., Chicago, IL). Overall differences between the three MALDI-TOF MS preparation methods and the altered cutoff scores were tested using the Friedman test. In addition, follow-up tests were conducted using the Wilcoxon signed-rank test for pairwise comparison of the three preparation methods and the altered cutoffs. A *P* value of <0.05 was considered statistically significant. A Bonferroni correction was applied at the 0.0166 (0.05/3) and 0.0083 (0.05/6) levels of significance, respectively, across pairwise comparisons.

Nucleotide sequence accession numbers. Partial 16S rRNA gene sequences of all 151 isolates have been deposited in NCBI GenBank by de Melo Oliveira et al. (6) (GU797849, KC866143 to KC866155, KC866157, KC866158, KC866160 to KC866167, KC866170 to KC866178, KC866182 to KC866197, KC866199 to KC866208, KC866210 to KC866224, KC866226, KC866229, KC866232 to KC866237, KC866239 to KC866242, KC866244, KC866245, KC866247 to KC866249, KC866251 to KC866256, KC866259 to KC866268, KC866270, KC866271, KC866274, KC866275, KC866277, KC866281 to KC866283, KC866285 to KC866287, KC866290 to KC866292, KC866294, and KC866299) or were deposited in the course of the present study (KJ557352 to KJ557379).

RESULTS

Identification of fastidious GNR by the MALDI Biotyper. We analyzed 151 clinical fastidious GNR, including 40 species from 15 genera, by the Bruker MALDI Biotyper (Table 1). Using direct colony transfer and the manufacturer's recommended criteria for the identification of species (cutoff of 2.0) and genus (cutoff of 1.7), identification numbers were 65 out of 151 strains (43.0%) at the species level and 49 out of 151 strains (32.5%) at the genus level (Table 1). For 35 of 151 strains (23.2%) no identification was achieved. Incorrect species assignment was seen with two isolates: one *Dysgonomonas capnocytophagoides* isolate was misidentified as *Dysgonomonas gadei*, and one isolate of *Haemophilus* sp. was misidentified as *Haemophilus influenzae* (Table 2). In 5 out of the 49 isolates which were identified only to genus level, more than one species was listed with a score of ≥2.0 in the ranked MALDI-TOF MS results. According to the manufacturer's criteria, this setting indicates inconsistency in species assignment and results in genus assignment only. Such species inconsistency was observed for *Actinobacillus hominis* (scores of ≥2.0 for *Actinobacillus suis*/*Actinobacillus equuli*/*Actinobacillus ureae*), *Bordetella bronchiseptica* (scores of ≥2.0 for *Bordetella bronchiseptica*/*Bordetella parapertussis*), *Neisseria sicca* (scores of ≥2.0 for *Neisseria mucosa*/*Neisseria macacae*), and *Neisseria subflava* (scores of ≥2.0 for *Neisseria perflava*/*Neisseria flavescens*).

Species coverage of the Bruker database. Ten species, accounting for 15 isolates in this study (i.e., *Actinobacillus hominis*, *Dysgonomonas capnocytophagoides*, *Dysgonomonas mossii*, *Leptotrichia trevisanii*, *Neisseria animaloris*, *Neisseria oralis*, *Neisseria shayeganii*, *Pasteurella bettyae*, *Pasteurella stomatis*, and *Psychrobacter pulmonis*), were not included in the Bruker database version (3,995 entries) we used (Table 1). While 11 of these 15 isolates were not identified by MALDI-TOF MS, both *A. hominis* isolates and the *P. stomatis* isolate were assigned to the genus *Actinobacillus* and *Pasteurella*, respectively. The *D. capnocytophagoides* isolate was misidentified as *Dysgonomonas gadei*. The latter discrepancy was also reported by Bizzini et al. (19).

Comparison of different MALDI-TOF MS sample preparation methods. The rate of species identification increased significantly from 43.0% to 46.4% ($Z = -2.646$, $P = 0.008$) and to 53.7% ($Z = -4.491$, $P < 0.0001$), respectively, when direct transfer plus formic acid sample preparation and ethanol-formic acid extraction were used. In parallel, the number of isolates identified only to the genus level decreased from 32.5% to 30.5% and 25.8%, and the rate of no identifications decreased from 23.2% to 21.9% and 19.2%, respectively (Table 3). The rate of misidentification was 1.3% for all three methods. The average MALDI score values for direct colony transfer (1.98 ± 0.29), for direct transfer plus formic acid preparation (1.99 ± 0.28), and for ethanol-formic acid extraction (2.02 ± 0.31) were comparable.

Individual cutoff scores for species and genus identification. The manufacturer recommends cutoff scores for an identification at the species and genus level of 2.0 and 1.7, respectively. In this study, we analyzed the effect of altering the cutoff for species assignment from 2.0 to 1.9, 1.8, and 1.7 and for genus assignment from 1.7 to 1.6 and 1.5 (Table 3).

The reduction in the genus cutoff value did not significantly increase the genus identification rate for any of the three methods [$\chi^2(2,|151) = 3.957$, $P = 0.138$ for direct colony transfer; $\chi^2(2.|151) = 4.333$, $P = 0.115$ for direct transfer plus formic acid

TABLE 1 Identification of 151 fastidious GNR by MALDI-TOF MS by applying direct colony transfer and standard Bruker interpretation criteria (species cutoff of 2.0; genus cutoff of 1.7)

Organism[a]	No. (%) of isolates	No. (%) of MALDI-TOF MS results[b]:				No. of reference spectra[c]
		Species identification	Genus identification	No identification	Misidentification	
Actinobacillus hominis	2		2			0
Aggregatibacter actinomycetemcomitans	2	1	1			5
Aggregatibacter aphrophilus	10	5	3	2		3
Aggregatibacter sp.	3		3			9
Bordetella bronchiseptica	1		1			9
Bordetella petrii	2	2				4
Bordetella trematum	1	1				3
Capnocytophaga canimorsus	3		1	2		1
Capnocytophaga gingivalis	1			1		1
Capnocytophaga sputigena	6	6				2
Capnocytophaga sp.	1		1			10
Cardiobacterium hominis	7		3	4		1
Dysgonomonas capnocytophagoides	1				1	0
Dysgonomonas mossii	1			1		0
Eikenella corrodens	13	10	3			2
Haemophilus influenzae	5	4		1		10
Haemophilus parainfluenzae	5	3	2			5
Haemophilus sp.	1				1	23
Kingella denitrificans	2	1	1			2
Kingella kingae	1	1				4
Leptotrichia trevisanii	1			1		0
Moraxella atlantae	1		1			1
Moraxella canis	1		1			2
Moraxella lacunata	2		1	1		1
Moraxella nonliquefaciens	5	5				3
Moraxella osloensis	10	1	5	4		7
Neisseria animaloris	1			1		0
Neisseria bacilliformis	4			4		1
Neisseria cinerea	2		2			1
Neisseria elongata	9	1	7	1		2
Neisseria oralis	1			1		0
Neisseria shayeganii	2			2		0
Neisseria sicca	1		1			3
Neisseria subflava	2		2			2
Neisseria weaveri	5	5				1
Neisseria zoodegmatis	5	5				1
Neisseria sp.	2		2			42
Oligella urethralis	6	3	3			2
Pasteurella bettyae	4			4		0
Pasteurella canis	1			1		3
Pasteurella dagmatis	1		1			1
Pasteurella multocida	11	11				10
Pasteurella stomatis	1		1			0
Pasteurella sp.	3		1	2		17
Psychrobacter pulmonis	1			1		0
Psychrobacter sp.	1			1		1
Total	151 (100)	65 (43.0)	49 (32.5)	35 (23.2)	2 (1.3)	

[a] Identification by 16S rRNA gene sequence analysis and biochemical methods according to de Melo Oliveira et al. (6).
[b] MALDI-TOF MS identification applying direct transfer plus formic acid preparation, a genus cutoff value of 1.7, and a species cutoff value of 2.0 with Bruker database V.3.1.2.0 (3,995 entries).
[c] Number of reference entries for the corresponding species and genus, respectively, included in Bruker database V.3.1.2.0 (3,995 entries).

preparation; and $\chi^2(2,|151) = 1.867$, $P = 0.393$ for ethanol-formic acid extraction]. However, decreasing the genus cutoff resulted in increasing numbers of genus inconsistencies, i.e., scores of ≥1.6 or ≥1.5 for more than one genus. A genus inconsistency rate of 2% was observed for a genus cutoff of 1.7; however, genus inconsistency increased to up to 15% when a genus cutoff of 1.5 was applied. This increase was mainly caused by *Aggregatibacter* spp. that showed high homology (scores of ≥1.5) to *H. influenzae* reference entries.

The reduced species cutoffs led to significantly higher species

TABLE 2 Overview of misidentifications by MALDI-TOF MS

Organism[a]	No. (%) of isolates	MALDI-TOF MS results[b]			Homology to 16S rRNA reference sequence[c]
		Misidentification as:	Misidentification at cutoff 2.0	Misidentification at cutoff 1.7	
Aggregatibacter sp.	3	*A. aphrophilus*	No	Yes	97.7%–98.3% *A. aphrophilus*
Capnocytophaga sp.	1	*C. sputigena*	No	Yes	98.5% *C. ochracea*
Dysgonomonas capnocytophagoides	1	*D. gadei*	Yes	Yes	100% *D. capnocytophagoides*
Haemophilus sp.	1	*H. influenzae*	Yes	Yes	96.8% *H. haemolyticus*
Neisseria sp.	2	*N. zoodegmatis*	No	Yes	97.8%–97.9% *N. zoodegmatis*
Pasteurella stomatis	1	*P. canis*	No	Yes	99.6% *P. stomatis*
Pasteurella sp.	1	*P. canis*	No	Yes	98.4% *P. stomatis*

[a] Identification by 16S rRNA gene sequence analysis and biochemical methods according to de Melo Oliveira et al. (6).
[b] MALDI-TOF MS identification applying direct transfer plus formic acid preparation, a genus cutoff value of 1.7, and a species cutoff value of 2.0 with Bruker database V.3.1.2.0 (3,995 entries).
[c] Species identification when sequence homology is ≥99% and a sequence divergence with the next homologous species is ≥0.5%; genus assignment when sequence homology is <99% and ≥95%.

identification rates (Table 3). Decreasing the species cutoff from 2.0 to 1.7 resulted in a species identification rate of 64.9% compared to 43.0% for direct colony transfer ($Z = -5.972$, $P < 0.0001$), of 66.9% compared to 46.4% for direct transfer plus formic acid preparation ($Z = -5.684$, $P < 0.0001$), and of 69.5% compared to 53.7% for ethanol-formic acid extraction ($Z = -4.992$, $P < 0.0001$). Concomitantly, the rate of inconsistencies at the species level increased from 4.0% to 6.0%. Species inconsistency was observed in particular for *Neisseria* spp. Misidentifications at the species level increased from 1.3% applying a cutoff of 2.0 to ~6.0% applying a cutoff of 1.7. An increased misidentification rate was observed mainly for those isolates that could be assigned only to the genus level by 16S rRNA gene sequence analysis due to the low sequence homology with reference sequences (Table 2). These isolates yielded erroneous species assignments with MALDI-TOF MS.

DISCUSSION

Recent studies have evaluated the identification of fastidious GNR using the Vitek MS system (bioMérieux, Marcy l'Etoile, France) and show reliable identification of HACEK organisms and other fastidious Gram-negative bacteria (31, 32). The aim of this study was to evaluate the capability of the MALDI Biotyper system (Bruker Daltonics) to correctly identify fastidious GNR. We analyzed the feasibility of various sample preparation methods and of altered MALDI-TOF MS interpretation criteria for improving reliable identification rates, in particular the alteration of score value cutoffs.

Sample preparation. If fastidious GNR bacteria were pretreated with formic acid either on-target or using the tube-based extraction method, the rate of species identification increased compared to the identification by direct colony transfer without formic acid. Our observations parallel those of previous reports that demonstrated improved identification rates when formic acid was used in the process of sample preparation for MALDI-TOF MS analysis of bacteria (30, 33, 34). The highest identification rates were yielded by applying ethanol-formic acid extraction, which is the manufacturer-recommended gold standard and which is the standard method for generating reference spectra.

TABLE 3 Identification of 151 fastidious GNR by MALDI-TOF MS: comparison of three sample preparation methods and different score value cutoffs

MALDI-TOF MS method	No. (%) of isolates identified using a species score value cutoff of:				No. (%) of isolates identified using a genus score value cutoff of:		
	2.0	1.9	1.8	1.7	1.7	1.6	1.5
Direct colony transfer							
Species identification	65 (43.0)	85 (56.3)	92 (60.9)	98 (64.9)			
Genus identification	49 (32.5)	29 (19.2)	22 (14.6)	8 (5.3)	116 (76.8)	125 (82.8)	115 (76.2)
No identification	35 (23.2)	35 (23.2)	35 (23.2)	35 (23.2)	35 (23.3)	26 (17.2)	36 (23.8)
Misidentification	2 (1.3)	2 (1.3)	2 (1.3)	10 (6.6)	0 (0)	0 (0)	0 (0)
Direct transfer plus formic acid preparation							
Species identification	70 (46.4)	86 (57.0)	95 (62.9)	101 (66.9)			
Genus identification	46 (30.5)	29 (19.2)	17 (11.3)	8 (5.3)	118 (78.1)	128 (84.8)	119 (78.8)
No identification	33 (21.9)	33 (21.9)	33 (21.9)	33 (21.9)	33 (21.9)	23 (15.2)	32 (21.2)
Misidentification	2 (1.3)	3 (2.0)	6 (4.0)	9 (6.0)	0 (0)	0 (0)	0 (0)
Ethanol-formic acid extraction							
Species identification	81 (53.7)	89 (58.9)	100 (66.2)	105 (69.5)			
Genus identification	39 (25.8)	27 (17.9)	16 (10.6)	9 (6.0)	122 (80.8)	124 (82.1)	128 (84.8)
No identification	29 (19.2)	29 (19.2)	29 (19.2)	29 (19.2)	29 (19.2)	27 (17.9)	23 (15.2)
Misidentification	2 (1.3)	6 (4.0)	6 (4.0)	8 (5.3)	0 (0)	0 (0)	0 (0)

Ethanol-formic acid extraction, however, comprises a considerable number of manual preparation steps and is, thus, time-consuming and laborious (35). Use of the direct transfer plus formic acid preparation, on the other hand, hardly increases the total time to the result compared to that for direct colony transfer (30). Therefore, the direct transfer plus formic acid preparation better meets the requirements of high-throughput clinical laboratories.

Altered MALDI Biotyper data interpretation criteria. No significant increase in overall genus assignments was observed when the genus cutoff was reduced. In contrast, the rate of species identification was significantly improved for all sample preparation methods tested by a reduction of the species cutoff value from 2.0 to 1.7. However, reducing the cutoff to 1.7 led to a significantly higher number of misidentifications (up to 6.6% instead of 1.3%). Similar observations were made previously by Bizzini et al., who reported that a species cutoff of 1.7 led to a higher identification rate for difficult-to-identify bacteria but that this increase was accompanied by additional misidentifications (19). Species cutoffs of 1.9 and 1.8 increased the identification rates less than a cutoff of 1.7. However, the misidentification rates were significantly lower. Thus, species cutoffs of 1.9 or 1.8 may offer an optimal balance between increased correct identifications and limited misidentifications. This finding is supported by other studies that suggest lower species cutoffs (mostly 1.9) for the identification of bacteria in general (34).

Coverage of the reference database. While this study was ongoing, a new version of the Bruker database was released (v.4.0.0.1, 5,627 entries). The rate of species identification increased from 53.7% to 64.2% when the new database was used with the standard interpretation criteria and ethanol-formic acid extraction (data not shown). Applying a species identification cutoff of 1.8 instead of 2.0 led to an additional increase in the identification rate to 70.2%. The increase was due to the addition of new species to the database, such as *Pasteurella bettyae* and *Leptotrichia trevisanii*, and additional reference entries for existing species, such as *Capnocytophaga gingivalis*, *Moraxella osloensis*, *Neisseria bacilliformis*, and *Oligella urethralis*. These data indicate that the low identification rates for fastidious GNR do not reflect a technical issue, but are rather the result of poor database coverage and that further expansion of the database is needed.

Comparison of MALDI Biotyper-based identification and conventional identification algorithms. Traditionally the workflow of our diagnostic laboratory for the identification of fastidious GNR comprises both phenotypic and molecular methods (6). Initially, the identification of putative fastidious GNR isolates is attempted by investigating phenotypic and biochemical characteristics. For isolates which cannot be identified by phenotypic and biochemical methods, 16S rRNA gene sequencing is performed. In a previous study, we reported rates of 40%, 13%, and 47% for species identification, genus identification, and no identification, respectively, of fastidious GNR by phenotypic methods (6). The use of MALDI-TOF MS identification in the clinical laboratory will, therefore, reduce the number of additional 16S rRNA gene sequence analyses and lead to less cost, time, and effort for identification of fastidious GNR. We suggest an integrated approach combining MALDI-TOF MS and 16S rRNA gene sequencing: MALDI-TOF MS is used as the primary identification method. If no identification is achieved, 16S rRNA gene analysis is done as the secondary identification method. To optimize the species identification rate, direct transfer plus on-target formic acid sample preparation in combination with a species cutoff of 1.8 is proposed.

In conclusion, we demonstrated that the Bruker MALDI Biotyper is a reliable tool for the identification of fastidious GNR, although the identification rates were not as high as those reported for Gram-positive cocci or nonfastidious Gram-negative bacteria. The number of reliable species identifications can be increased by the following three measures: direct transfer plus on-target formic acid sample pretreatment, alteration of the species cutoff score value from 2.0 to 1.8, and amelioration of the database with additional spectra.

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