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Comparing identification of clinically relevant *Prevotella* species by VITEK MS and MALDI biotyper

NURVER ULGER TOPRAK^{1*}, ALIDA C. M. VELOO²,
EDIT URBAN³, INGRID WYBO⁴, HELENE JEAN-PIERRE⁵,
TREFOR MORRIS⁶, ULRİK STENZ JUSTESEN⁷,
VESNA TRIPKOVIC⁸, SAMO JEVERICA⁹, GUNER SOYLETIR¹,
ELISABETH NAGY³ and ON BEHALF OF THE ESCMID STUDY
GROUP FOR ANAEROBIC INFECTIONS (ESGAI)**

¹Department of Medical Microbiology, School of Medicine, Marmara University, Istanbul, Turkey

²Department of Medical Microbiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

³Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary

⁴Department of Microbiology and Infection Control, Universitair Ziekenhuis Brussel, Brussels, Belgium

⁵Laboratoire de Bactériologie, Hôpital Arnaud de Villeneuve, Centre Hospitalier Régional Universitaire de Montpellier, Montpellier, France

⁶UK Anaerobe Reference Unit, Public Health Wales Microbiology, Cardiff, UK

⁷Department of Clinical Microbiology, Odense University Hospital, Odense, Denmark

⁸Department of Clinical and Molecular Microbiology, University Hospital Center, Zagreb, Croatia

⁹Faculty of Medicine, Institute of Microbiology and Immunology, University of Ljubljana, Ljubljana, Slovenia

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ABSTRACT

In this multicenter study, we aimed to evaluate the performance of MALDI Biotyper and VITEK MS, for identification of *Prevotella* species. Three hundred and fourteen clinical isolates, collected in eight European countries between January 2014 and April 2016, were identified at the collecting sites by MALDI Biotyper (versions 3.0 and 3.1) and then reidentified by VITEK MS (version 3.0) in the central laboratory. 16S rRNA gene sequencing was used as a standard method. According to sequence analysis, the 314 *Prevotella* strains belonged to 19 species. MALDI Biotyper correctly identified 281 (89.5%) isolates to the species level and 33 (10.5%) only at the genus level. VITEK MS correctly identified 253 (80.6%) isolates at the species level and 276 (87.9%) isolates at the genus level. Thirty-three isolates belonging to *P. bergensis*, *P. conceptionensis*, *P. corporis*, *P. histicola*, and *P. nanciensis*, unavailable in the VITEK MS 3.0 database, were resulted in genus level or no identification. Six *Prevotella* strains, belonged to *P. veroralis*, *P. timonensis*, and *P. conceptionensis* not represented in the MALDI Biotyper system database, were misidentified at the genus level. In conclusion, both VITEK MS and MALDI Biotyper provided reliable and rapid identification. However, the permanent extension of the databases is needed.

KEYWORDS

anaerobic bacteria, *Prevotella*, 16S rRNA gene sequencing, mass spectrometry, VITEK MS, MALDI Biotyper

INTRODUCTION

Prevotella species, obligate anaerobic Gram-negative bacilli, are important members of oral, upper respiratory, intestinal, and female genital tract microbiota. However, these organisms

* Corresponding author:
Nurver Ulger Toprak
Department of Medical Microbiology,
School of Medicine, Marmara
University, Basıbuyuk Campus, 34854
Maltepe, Istanbul, Turkey
Phone: +90 533 450 1489;
Fax: +90 216 421 2222
E-mail: nulger@marmara.edu.tr

**Members of the ESGAI participated
in the study: Eva Leitner (Austria),
Suzana C. Stingue, Ame C. Rodloff
(Germany), Joseph Paparaskevas
(Greece), Wafaa Jamal, Vincent O.
Rotimi (Kuwait), Guven Kulekci, and
Hrisi B. Tokman (Turkey).

can also cause opportunistic infections, which may involve any type of oral infection, various types of abscesses, soft tissue, and sterile site infections in the human body. Susceptibility profiles may vary at the species level; consequently, a rapid and an accurate identification of *Prevotella* isolates plays a critical role in successful treatment [1, 2].

The *Prevotella* genus has undergone recent taxonomic changes and the number of validated species has increased to 50 (<http://www.bacterio.net/prevotella.html>). The identification of *Prevotella* isolates classically relies on laborious and time-consuming phenotypic assays [3]. Nucleic acid sequencing methods have been developed for more rapid and reliable identification. However, DNA-based methods for routine identification of clinical isolates are too expensive, technically complex, labor-intensive, and need expert interpretation of the sequences [4]. Due to the various shortcomings of these methods, there is an increasing interest in more accurate identification of anaerobic bacteria including *Prevotella* spp. in routine microbiological laboratories.

Previous studies have confirmed that matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) is an accurate, rapid, and satisfactory method for routine identification of anaerobes in diagnostic laboratories [5–7]. At present, two MALDI-TOF MS systems are commercially available for routine use: the MALDI Biotyper (Bruker Daltonics Inc., Germany) and VITEK MS (bioMérieux Inc., France). Methodology of the two systems is similar, but differences are present in composition of databases and application of software packages for data analyses [8]. Earlier studies evaluating MALDI-TOF MS identification of *Prevotella* spp. mostly used the MALDI Biotyper system [9, 10].

In a multicenter study organized by ESCMID Study Group for Anaerobic Infections (ESGAI), we assessed the performance of the VITEK MS system for the identification of *Prevotella* isolates ($n = 508$) collected in 13 countries for a European antibiotic resistance surveillance. We found that VITEK MS offers a reliable and rapid identification of the most frequently isolated *Prevotella* species [11]. About two third of the isolates were also identified by MALDI Biotyper system in eight participating countries, which made possible to compare the performance of the two MALDI-TOF MS systems. Comparative studies in the field of clinically relevant anaerobic isolates on the applicability of different MS systems are limited. In this study, we describe the identification ability of both MALDI Biotyper and VITEK MS systems for clinically relevant *Prevotella* species using the 16S rRNA gene sequencing as a reference method.

MATERIALS AND METHODS

Bacterial isolates

A total of 314 non-duplicate clinically relevant *Prevotella* isolates were involved in this study. They were a subset of 508 *Prevotella* strains collected for European antibiotic resistance surveillance with the aim to determine the differences in antibiotic resistance among various species [2]. The 314

Prevotella strains, involved in this study, were collected between January 2014 and April 2016 in eight European countries [Belgium ($n = 45$), Croatia ($n = 33$), Denmark ($n = 29$), France ($n = 45$), Great Britain ($n = 41$), Hungary ($n = 47$), Netherlands ($n = 45$), and Slovenia ($n = 29$)], where mass spectrometry-based identification was already used in the routine practice. All centers involved used the MALDI Biotyper system for identification; however, the software and databases were slightly different in the different collecting sites (Table 1). The request was to submit all clinical isolates belonging to genus *Prevotella* identified by the Bruker Biotyper system. *Prevotella* isolates were sent to Turkey, in anaerobic transport medium (Anaerobe systems, Morgan Hill, USA). Upon arrival, the isolates were immediately grown on Brucella agar (Difco, USA) supplemented with 5% sheep blood, hemin, and vitamin K1 at 36 °C for 48 h in an anaerobic chamber (Bactron-I, SHELLAB, USA). Cultures were assessed in terms of purity. Pure and viable strains were stored at –80 °C in 10% skimmed milk until further investigations.

To compare the performance of the two MALDI-TOF MS systems for the identification of *Prevotella* strains, the isolates were reidentified using the VITEK MS (database version 3.0) in the central laboratory in Turkey. Only isolates that gave species-level identification by the sequencing data were further compared for the MS-based identification.

Identification by MALDI Biotyper MS

The isolates were identified according to the manufacturer's guidelines. Briefly, a colony was smeared onto a spot on the MALDI plate. The spots were covered with 1 µl of matrix solution (Bruker Daltonik α -cyano-4-hydroxy-cinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) and allowed to air dry. The plate is placed in the ionization chamber of the Biotyper MS machine. The generated mass spectra were compared against a database of mass spectra by the software, resulting in identification of the organism.

All collecting sites used the same criteria for the identification during the collection of the strains. Species-level identification by the MALDI Biotyper was accepted if the log score was ≥ 2.000 and genus-level identification if the log score was between >1.700 and <2.000 . Only isolates with correct genus-level identification were accepted for the study and were sent to the central laboratory.

Identification by VITEK MS

In case of VITEK MS, according to the manufacturer's guidelines, a single colony was applied onto a spot of target slide as a thin film and allowed to air dry. The spots were covered with 1 µl of matrix solution (VITEK MS α -cyano-4-hydroxycinnamic acid) and allowed to dry at room temperature. Then, the prepared target slide was inserted into the VITEK MS machine (bioMérieux). Microbial identification was performed by generating spectra from the bacterial extracts and comparing them with the reference spectra using the VITEK MS version 3.0 database.



Table I. Number of *Prevotella* strains collected in different countries and the MALDI Biotyper software and database used for their identification at the different collection sites

Collecting sites (no. of isolates)	No. of strains identified on species/genus level by Biotyper ^a	No. of different <i>Prevotella</i> spp. identified by Biotyper at the collection sites	Data about the Biotyper used		
			Software	Database	No. of <i>Prevotella</i> spp./MSPs in the database
Belgium (45)	41/4	14	3.0	5,989	31/77 ^b
Croatia (33)	33/0	6	3.1	5,989	31/77
Denmark (29)	28/1	9	3.1	6,903	31/126
France (45)	40/5	12	3.0	5,627	23/53
Great Britain (41)	41/0	8	3.0	5,989	31/77
Hungary (47)	46/1	9	3.0	5,627	23/53
The Netherlands (45)	45/0	12	3.0	4,613	23/52
Slovenia (29)	29/0	12	3.0	5,627	23/53

Note: MALDI: matrix-assisted laser desorption ionization; MSPs: main spectra profiles.

^aSpecies-level identification was accepted if log score was ≥ 2.000 and genus-level identification was accepted if log score was ≥ 1.700 – < 2.000 .

^bIn this center, an extended database was used according to Wybo et al. [9].

Species-level identification was considered accurate, if confidence level was $\geq 99.9\%$ and for a confidence level ranging between 60% and 99.9% with a single species choice. Low-level confidence score ($< 50\%$) is found in a low discrimination identification consisting of a list of two to four choices for an identification match. These isolates were identified only genus level. The bacterium was considered non-identified, if confidence level value was $< 60\%$ and no match for the composite spectra. These criteria were used for the identification of the isolates in the central laboratory.

Analysis of the MALDI-TOF MS data

MALDI-TOF MS identification data by the two systems obtained in the collecting laboratories and in the central laboratory were compared with the identification results obtained by sequencing and the data were categorized as follows: (1) correct identification at the species level; (2) correct identification at the genus level, including different species within the *Prevotella* genus; (3) misidentification (minor error: different *Prevotella* species; major error: different genus); and (4) no identification.

16S rRNA gene sequencing

A “gold standard” 16S rRNA gene sequencing of all isolates was carried out in the central laboratory in Turkey using the methodology described by Song et al. [12]. Sequences were analyzed using GenBank (www.ncbi.nlm.nih.gov). Identification at species level was accepted in the presence of $\geq 99.0\%$ similarity with the 16S rRNA gene sequence and 97.0%–99.0% similarity at the genus level [13].

RESULTS

All 314 *Prevotella* isolates were identified at the species level with $\geq 99.0\%$ sequence similarity using the 16S RNA gene sequencing. A total of 19 different *Prevotella* species were identified, most of which were *P. bivia* ($n = 74$), followed by *P. buccae* ($n = 41$). *P. nigrescens* and *P. denticola* were represented equally (34 isolates each), but some of the species were present only in very low numbers (≤ 4 isolates), such as *P. buccalis*, *P. conceptionensis*, *P. corporis*, *P. histicola*, *P. salivae*, and *P. veroralis*. Table II shows the distribution of the *Prevotella* spp. identified by the 16S RNA gene sequencing and the identification results obtained by the MALDI Biotyper at the isolation sites applying different databases (Table I) and by the VITEK MS in the central laboratory. Concordant species-level identification between the three identification systems was measured; 16S rRNA gene sequencing, MALDI Biotyper, and VITEK MS gave the same species-level identification in 76.4% ($n = 240$) of the isolates (Table II). There were discrepancies (minor or major errors) compared with the sequencing data for the remaining 74 (23.6%) isolates. Discrepant results are shown in Table III.

Comparing the sequencing data with the identification obtained by the MALDI Biotyper using different databases at the collecting sites, 281 (89.4%) of the 314 isolates were identified correctly at the species level and 33 isolates were only correctly identified at genus level showing different species (minor error) or only being identified as *Prevotella* spp. (Table III). Most of the misidentified organisms belonged to *P. bivia*, *P. buccae*, *P. denticola*, and *P. timonensis*, but many other species were misidentified by showing

Table II. The distribution of *Prevotella* species according to the sequencing data and identification results obtained by MALDI Biotyper and VITEK MS

16S rRNA gene sequencing		MALDI Biotyper used at the collecting sites		VITEK MS used at the central laboratory		Concordant species identification by the two MS systems (n)
<i>Prevotella</i> species	n (%)	Correct species ID (n)	Correct genus ID (n)	Correct species ID (n)	Correct genus ID (n)	
<i>P. baroniae</i>	13 (4.1)	12	13	11	12	11
<i>P. bergensis</i> ^a	12 (3.8)	10	12	0	1	0
<i>P. bivia</i>	74 (23.6)	69	74	74	74	69
<i>P. buccae</i>	41 (13.1)	36	41	38	38	36
<i>P. buccalis</i>	4 (1.3)	3	4	4	4	3
<i>P. conceptionensis</i> ^{a,b}	1 (0.3)	0	1	0	0	0
<i>P. corporis</i> ^a	4 (1.3)	3	4	0	1	0
<i>P. denticola</i>	34 (10.8)	30	34	32	34	30
<i>P. disiens</i>	18 (5.7)	17	18	17	17	17
<i>P. histicola</i> ^a	4 (1.3)	3	4	0	4	0
<i>P. intermedia</i>	9 (2.9)	9	9	7	8	7
<i>P. melaninogenica</i>	27 (8.6)	25	27	25	26	25
<i>P. nanceiensis</i> ^a	12 (3.8)	12	12	0	2	0
<i>P. nigrescens</i>	34 (10.8)	33	34	24	29	24
<i>P. oralis</i>	5 (1.6)	3	5	5	5	3
<i>P. oris</i>	8 (2.5)	8	8	8	8	8
<i>P. salivae</i>	4 (1.3)	3	4	2	4	2
<i>P. timonensis</i>	8 (2.5)	4	8	4	7	4
<i>P. veroralis</i>	2 (0.6)	1	2	2	2	1
Total [n (%)]	314 (100)	281 (89.4)	314 (100)	253 (80.6)	276 (87.9)	240 (76.4)

Note: Concordant species identification by the two MALDI-TOF MS systems is also included. MALDI: matrix-assisted laser desorption ionization; MS: mass spectrometry.

^aNot included in the database of the VITEK MS system during the investigation.

other species as well. The MALDI Biotyper database versions DB 4613 and DB 5627 did not contain *P. conceptionensis*, *P. timonensis*, or *P. veroralis*; therefore, six isolates evaluated by these data sets were only identified at the genus level by the MALDI Biotyper (Table III).

Comparative analysis of the identification obtained by the VITEK MS system and the 16S RNA gene sequencing of the isolates revealed that VITEK MS correctly identified 253 isolates (80.6%) at the species level and 276 (87.9%) at the genus level (Table II) and misidentified 27 isolates with minor error ($n=23$) or major error ($n=4$; Table III). A total of 34 isolates (10.8%) were not identified. Thirty-three isolates from five *Prevotella* species (*P. bergensis*, *P.*

conceptionensis, *P. corporis*, *P. histicola*, and *P. nanceiensis*) were not available in the VITEK MS IVD 3.0 database, and were not identified or misidentified as different *Prevotella* species or different genus (Tables II and III).

DISCUSSION

Recently, in microbiological diagnostic laboratories, there is an increased usage of the MALDI-TOF MS technique for rapid identification of clinically significant bacteria including anaerobes [8]. Various studies indicated that the performance of the MALDI-TOF MS systems is highly

Table III. The discordant results between 16S rRNA gene sequencing and the two MALDI-TOF MS systems for identification of *Prevotella* species

Species identification by 16S rRNA gene sequencing	MALDI Biotyper data at the collection sites				VITEK MS data at the central laboratory			
	Discordant ID vs. 16S rRNA gene sequencing	Misidentified		No identification	Discordant ID vs. 16S rRNA gene sequencing	Misidentified		No identification
		As different spp.	As different <i>Prevotella</i> spp.			As different <i>Prevotella</i> spp.	As different genus	
<i>P. baroniae</i>	1	<i>P. melaninogenica</i> (1)	0	0	2	<i>P. melaninogenica</i> (1)	0	1
<i>P. bergensis</i> ^a	2	<i>P. bivia</i> (1) <i>Prevotella</i> sp. (1)	0	0	12	<i>P. bivia</i> (1)	<i>Porphyromonas asaccharolytica</i> (1)	10
<i>P. bivia</i>	5	<i>P. buccae</i> (2) <i>P. intermedia</i> (1) <i>P. melaninogenica</i> (1) <i>P. nigrescens</i> (1)	0	0	0	0	0	0
<i>P. buccae</i>	5	<i>P. intermedia</i> (1) <i>P. nanceiensis</i> (1) <i>P. nigrescens</i> (1) <i>Prevotella</i> sp. (2)	0	0	3	0	<i>Peptostreptococcus anaerobius</i> (1)	2
<i>P. buccalis</i>	1	<i>Prevotella</i> sp. (1)	0	0	0	0	0	0
<i>P. conceptionensis</i> ^{a,b}	1	<i>Prevotella</i> sp. (1)	0	0	1	0	0	1
<i>P. corporis</i> ^a	1	<i>P. nigrescens</i> (1)	0	0	4	<i>P. intermedia/disiciens</i> ^c (1)	0	3
<i>P. denticola</i>	4	<i>P. bivia</i> (1) <i>P. disiens</i> (1) <i>P. melaninogenica</i> (1) <i>Prevotella</i> sp. (1)	0	0	2	<i>P. intermedia</i> (1) <i>P. melaninogenica</i> (1)	0	0
<i>P. disiens</i>	1	<i>P. melaninogenica</i> (1)	0	0	1	0	0	1
<i>P. histicola</i> ^a	1	<i>P. melaninogenica</i> (1)	0	0	4	<i>P. denticola</i> (2) <i>P. melaninogenica</i> (2)	0	0

(Continued)

Table III. The discordant results between 16S rRNA gene sequencing and the two MALDI-TOF MS systems for identification of *Prevotella* species (Continued)

Species identification by 16S rRNA gene sequencing	MALDI Biotyper data at the collection sites			VITEK MS data at the central laboratory			
	Discordant ID vs. 16S rRNA gene sequencing	Misidentified		Discordant ID vs. 16S rRNA gene sequencing	Misidentified		No identification
		As different <i>Prevotella</i> spp.	As different genus		As different <i>Prevotella</i> spp.	As different genus	
<i>P. intermedia</i>	0	0	0	2	<i>P. disiens</i> (1)	<i>Gamella sanguinis</i> / <i>Streptococcus alactolyticus</i> / <i>Pediococcus parvulus</i> (1)	0
<i>P. melaninogenica</i>	2	<i>P. nanceiensis</i> (1) <i>Prevotella</i> sp. (1)	0	2	<i>Prevotella</i> sp. (1)	0	1
<i>P. nanceiensis</i> ^a	0	0	0	12	<i>P. disiens</i> (1) <i>P. oralis</i> (1)	<i>Coronobacter sakazakii</i> (1)	9
<i>P. nigrescens</i>	1	<i>P. intermedia</i> (1)	0	10	<i>P. intermedia</i> (1) <i>Prevotella</i> spp. ^d (4)	0	5
<i>P. oralis</i>	2	<i>P. intermedia</i> (1) <i>P. melaninogenica</i> (1)	0	0	0	0	0
<i>P. salivae</i>	1	<i>P. histicola</i> (1)	0	2	<i>P. bivia</i> (1) <i>Prevotella</i> sp. (1)	0	0
<i>P. timonensis</i>	4	<i>P. denticola</i> (1) <i>Prevotella</i> sp. (3)	0	4	<i>P. salivae</i> (1) <i>P. buccae</i> (1) <i>Prevotella</i> sp. (1)	0	1
<i>P. veroralis</i>	1	<i>Prevotella</i> sp. (1)	0	0	0	0	0
Total	33	33	0	61	23	4	34

Note: MALDI: matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry.

^aNot included in the database VITEK MS system.

^bNot included in the database of the MALDI Biotyper system.

^c*P. intermedia*/*P. disiens*, with 50.0% identification rates.

^d*Prevotella* spp.: multiple species within the *Prevotella* genus, identification rates ranging from 11% to 33% with a sum of confidence values equal to 100% in the VITEK MS system.

dependent on the expansion of the accompanying databases and the specific group of isolates studied [4–7, 14]. Recent reports show that the MALDI-TOF MS can provide identification for >90% of anaerobes [5, 10], whereas initial studies put this number at <70% [7, 15]. The inclusion of sequenced clinical isolates to the databases of the MALDI-TOF systems has also been suggested [9, 16].

To support this trend, in this multicenter study, we compared the applicability of two MALDI-TOF MS systems for the identification of *Prevotella* species and compared the results with 16S rRNA gene sequencing data. The results showed that the performances of VITEK MS and MALDI Biotyper were highly accurate for species identification (80.6% and 89.4%, respectively) and were even better for genus-level identification (87.9% and 100%, respectively). However, 33 isolates from five different *Prevotella* species, such as *P. bergensis*, *P. conceptionensis*, *P. corporis*, *P. histicola*, and *P. nanciensis*, were not identified or misidentified by the current VITEK MS database version 3.0, which does not cover these *Prevotella* species. Out of the different databases used for the MALDI Biotyper during this study, some species of *Prevotella* (*P. conceptionensis*, *P. veroralis*, and *P. timonensis*) were also unavailable, which also influenced the identification accuracy of the system.

Previous studies on the identification of anaerobic bacteria with MALDI-TOF MS used only a few *Prevotella* isolates representing limited number of species [5, 15, 17–20]. All those earlier studies that focused for the identification of *Prevotella* species used the MALDI Biotyper in comparison with 16S rRNA gene sequencing. The first of these studies was published by Wybo et al. [9] and was conducted in 2012. This study tested 102 clinical isolates belonging to different species of *Prevotella* of which only 62.7% were identified at the species level and 73.5% at the genus level. Accurate species and genus identification went up to 83.3% and 89.2%, respectively, when the commercial database was extended with in-house reference spectra from 23 sequenced *Prevotella* reference strains and 7 clinical isolates. The second study, Gursoy et al. [10] in 2017, used 123 oral *Prevotella* isolates to test the diagnostic accuracy of the MALDI Biotyper. This study yielded 88.6% accuracy at the species level and 100% accuracy at the genus level. The most likely reason for this increase in accuracy in the past 5 years is the expanded database of the MALDI Biotyper covering more *Prevotella* spp. The database used by Wybo et al. [9] contained 20 *Prevotella* species, whereas the database for the 2017 study by Gursoy et al. [10] contained 30. The results of this study (89.2% of species-level identification of 314 isolates), obtained using MALDI Biotyper system with different main spectra profiles of 23–31 *Prevotella* spp. in the databases used in the collecting laboratories, are similar to those ones obtained by Gursoy et al. [10].

The first study evaluating how well the VITEK MS (database version 2.0) identifies anaerobic bacteria on a multicenter level was conducted by Garner et al. [21]. This study tested a total of 652 anaerobic clinical isolates, including 90 isolates from five different *Prevotella* species (*P. bivia*,

P. buccae, *P. denticola*, *P. intermedia*, and *P. melaninogenica*). Of these strains, 91.1% were identified at the species level. In this study, using the VITEK MS database version 3.0, 95.1% of the *Prevotella* strains belonging to the same species were correctly identified at the species level. The overall lower percentage of the species-level identification by the VITEK MS achieved in this study evaluating all the isolates may be due to the much higher number of species represented. The strains used in this study belonged to 19 different species, including species not present in the current VITEK MS database.

There are limited number of studies, which compare the performance of the two commercially available MALDI-TOF MS systems especially for the identification of special anaerobic bacteria such as *Prevotella*. The limitation of conducting this type of research is the relatively low number of *Prevotella* species in the VITEK MS database (16 species at the moment), compared with the MALDI Biotyper databases, which contained reference spectra for 23 species (DB 4613 and DB 5627) and 31 species (DB 5989 and DB 6903) used in this study. However, this study also shows that, besides the number of the species present in the databases, many other factors such as incubation time, sample preparation, or maintenance of the system (which were not tested during this study) may influence the correct species-level identification as well [22].

The strengths of this study are the wide range of clinically relevant *Prevotella* spp. tested and the use of 16S rRNA gene sequencing as the reference method. However, there were some limitations of this study such as the identification of the isolates by the two MALDI-TOF MS systems was not carried out in the same laboratory from the same culture plate simultaneously. Furthermore, the collecting sites used different MALDI Biotyper databases for the original identification of the isolates and we have not collected data whether the sample preparation was the same in all sites where the MALDI Biotyper was used. Several database developments for both systems were carried out, since we closed down this study [16].

In conclusion, both MALDI-TOF MS systems performed well in identifying most clinically important *Prevotella* strains. MALDI-TOF MS is a reliable alternative to 16S rRNA gene sequencing for these species, considered to be the gold standard for identification of bacteria. Moreover, it is easier and more applicable in routine laboratories. However, expanding and optimizing the MS databases to include reference spectra for more species is needed. Controlled sample preparation and careful evaluation of the cut-off values to accept species determination are advisable. Accurate and simple identification of isolates will increase interest in studying anaerobic organisms, enhancing our knowledge of the epidemiology, pathogenicity, and clinical relevance of *Prevotella* species.

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