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SPECIAL ARTICLE

Comparison of two MALDI-TOF MS systems for the identification of clinically relevant anaerobic bacteria in Argentina

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KEYWORDS
MALDI-TOF MS systems;
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Abstract The aim of this study was to compare the performance of two MALDI-TOF MS systems in the identification of clinically relevant strict anaerobic bacteria. The 16S rRNA gene sequencing was the gold standard method when discrepancies or inconsistencies were observed between platforms. A total of 333 isolates were recovered from clinical samples of different centers in Buenos Aires City between 2016 and 2021. The isolates were identified in duplicate using two MALDI-TOF MS systems, BD Bruker Biotyper (Bruker Daltonics, Bremen, Germany) and Vitek MS (bioMèrieux, Marcy-l'Etoile, France). Using the Vitek MS system, the identification of anaerobic isolates yielded the following percentages: 65.5% (n: 218) at the species or species–complex level, 71.2% (n: 237) at the genus level, 29.4% (n: 98) with no identification and 5.1% (n: 17) with misidentification. Using the Bruker Biotyper system, the identification rates were as follows: 85.3% (n: 284) at the species or species–complex level, 89.7% (n: 299) at the genus level, 14.1% (n: 47) with no identification and 0.6% (n: 2) with misidentification.

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Differences in the performance of both methods were statistically significant (p-values <0.0001). In conclusion, MALDI-TOF MS systems speed up microbial identification and are particularly effective for slow-growing microorganisms, such as anaerobic bacteria, which are difficult to identify by traditional methods. In this study, the Bruker system showed greater accuracy than the Vitek system. In order to be truly effective, it is essential to update the databases of both systems by increasing the number of each main spectrum profile within the platforms.

PALABRAS CLAVE
Sistemas MALDI-TOF MS;
Identificación;
Bacterias anaerobias estrictas

Comparación de dos sistemas MALDI-TOF MS en la identificación de bacterias anaerobias de relevancia clínica en Argentina

Resumen El objetivo de este estudio fue comparar el desempeño de dos sistemas MALDI-TOF MS en la identificación de bacterias anaerobias estrictas de interés clínico. La secuenciación del gen 16S ARNr fue el método de referencia utilizado cuando se observaron discrepancias o inconsistencias entre plataformas. Se recuperaron 333 aislados de muestras clínicas de diferentes centros de la Ciudad Autónoma de Buenos Aires entre 2016 y 2021. Los aislados se identificaron por duplicado mediante dos sistemas MALDI-TOF MS: el BD Bruker Biotyper (Bruker Daltonics, Bremen, Alemania) y el Vitek MS (bioMèrieux, Marcy-l'Etoile, Francia). A través del sistema Vitek MS, los mismos fueron identificados a nivel de especie o complejo de especies en un 65,5% (n: 218) y de género en un 71,2% (n: 237), mientras que no se identificaron en un 29,4% (n: 98) y fue incorrecta en el 5,1% (n: 17). Mediante el sistema Bruker Biotyper, dichos valores fueron del 85,3% (n: 284), del 89,7% (n: 299), del 14,1% (n: 47) y del 0,6% (n: 2), respectivamente. La diferencia entre ambos métodos fue estadísticamente significativa ($p < 0,0001$). En conclusión, los sistemas MALDI-TOF MS aceleran la identificación microbiana. Son especialmente útiles para los microorganismos de crecimiento lento, como las bacterias anaerobias, que son difíciles de identificar con los métodos tradicionales. El sistema Bruker demostró ser más preciso que el Vitek MS. Para que estos métodos sean realmente efectivos es fundamental actualizar las bases de datos de ambos sistemas e incrementar el número de espectros de referencia dentro de las plataformas.

Introduction

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a fast and accurate tool for the routine identification of microorganisms, being particularly effective for anaerobic bacteria, since their classical phenotypic identification requires long-term cultivation (at least 48 h) and a substantial quantity of inoculum[4,5,36,57,58]. On the other hand, identification using MALDI-TOF MS requires low inoculum and takes only a few minutes. In this sense, this technology brought a new light to anaerobic microbiology and has been an important turning point[4,5,17,36,57,58].

Numerous studies have described the efficacy of identifying anaerobic bacteria by MALDI-TOF MS, but most of them performed the identification using only one instrument (VITEK MS or Bruker Biotyper)[2,38,55,82], and took into account the most common microorganisms that are routinely identified in clinical laboratories. Several authors concluded that there is a need to optimize and constantly update the existing MALDI-TOF MS databases[8,26,75].

In Argentina, the commercially available MALDI-TOF MS systems used for the identification of microorganisms isolated from clinical specimens are MALDI-TOF Biotyper (Bruker Daltonics, Bremen, Germany), and VITEK MS (bioMèrieux, Marcy l'Etoile, France). Scarce literature compared the efficiency of both platforms in the identification of anaerobic bacteria[33,34,39,52].

The aim of this study was to compare the performances of two MALDI-TOF MS systems in the identification of clinical strict anaerobic isolates, using the sequence analysis of the 16S ribosomal RNA (16S rRNA) gene as the gold standard method when discrepancies or inconsistencies were observed between these two mass spectrometry methods.

Materials and methods

Bacterial isolates

A total of 333 isolates were recovered from clinical samples collected from six different centers in Buenos Aires City between 2016 and 2021 (Table 1). Gram staining of the

Table 1 Distribution of sources and isolates from six centers.

Site or specimen	No. of isolates[a]						
	IL[b]	HG[c]	HM[d]	HC[e]	HA[f]	CE[g]	Total
Head and neck		13	7		4	2	26
Dental, oral cavity					6	6	12
Lower respiratory tract	2	9	5	2	2		20
Upper respiratory tract		10				2	12
Gastro-intestinal tract	8	9	3	3	18	21	62
Obstetric/gynecological			2		7	2	11
Urinary tract	2			2			4
Skin and soft tissues	13	13	16	9	14	9	74
Bone and joint		7	2	2		4	15
Blood	18		12	31	6	6	73
Stool	1	7	3	4			15
Others		1	3	5			9
Total	44	69	53	58	57	52	333

[a] Number of isolates available from each center.
Centers:
[b] Instituto Lanari.
[c] Hospital Garrahan.
[d] Hospital Muñiz.
[e] Hospital de Clínicas.
[f] Hospital Alemán.
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recovered anaerobic isolates was performed, and an oxygen tolerance test was conducted to ensure culture purity. Identification was carried out by phenotypic methods as previously described[53]. Using phenotypic methods in several isolates we achieved genus level identification every time[45]. Isolates were stored at −70 °C in trypticase soy broth with 20% glycerol. Frozen isolates were then sub-cultured on Brucella blood agar and incubated for 48 h under an anaerobic atmosphere before MALDI-TOF MS identification.

MALDI-TOF MS identification and interpretation

The isolates were identified in duplicate using the two MALDI-TOF MS systems. Bacterial isolates were identified by the direct colony on-plate extraction method with the Bruker Biotyper system, using the MALDI Biotyper software 3.1 (library version 10.0 containing 9607 main spectrum profile [MSP] entries), as previously described[69].

The cut-off score used for identification using Bruker Biotyper was ≥1.5 for the genus level, and ≥1.7 for the species level. Otherwise, the identification was considered unreliable. A minimum difference of 10% between the top score and the next closest score was required for the bacterial isolate to be considered a different species, based on the interpretative criteria of the National Network for Microbiological Identification by Mass Spectrometry (RENAEM) (http://www.anlis.gov.ar/renaem/)[32,56].

Identification was carried out using the Vitek MS system with the platform v3.2 knowledge base for clinical use. All procedures followed the manufacturer's instructions. Values between 60.0 and 99.9% indicated reliable species discrimination.

To compare system performance, MALDI-TOF MS results were classified into four categories: 1 – correct identification of species or species–complex; 2 – correct identification of genus; 3 – no identification, and 4 – misidentification. The species whose names were updated in recent years and assigned by MALDI-TOF MS with their previous name were considered correct identification. Rare species were defined as such when fewer than 10 articles related to the subject were retrieved from the PubMed database (https://pubmed.ncbi.nlm.nih.gov/)[36].

Genetic identification

The sequence analysis of 16S rRNA gene was used as a reference method when: (1) isolates could not be identified by any MALDI-TOF MS, (2) isolate identifications showed discrepancies between both systems either because they were different or one of the platforms yielded a ''no identification'' result and (3) identification was unreliable, that is, when the percentage/score of identification fell below the cut-off established by any system[34].

Genomic DNA was extracted using a commercial preparation kit (ADN PuriPrep-B, Inbio Highway), and the 16S rRNA gene was amplified using the specific primers 63f (5′-CAGGCCTAACACATGCAAGTC-3′) and 1387r (5′-GGGCGGWGTGTACAAGGC-3′). Amplicons were sequenced at external facilities (Macrogen, South Korea). Sequences obtained were analyzed using the Blastn online tool at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST) and the VECTOR NTI 11.5 program.

To achieve the identification at species level the sequences should exhibit an identity of >99% with full

gene coverage (>80%) with 16S rRNA gene of reference sequences based on guideline MM18-A of the Clinical and Laboratory Standards Institute (CLSI)[12]. However, for *Bacteroides thetaiotaomicron/Bacteroides faecis*, *Bacteroides ovatus/Bacteroides xylanisolvens*, *Fusobacterium nucleatum/Fusobacterium naviforme*, *Porphyromonas asaccharolytica/Porphyromonas uenonis* and *Peptoniphilus harei/Peptoniphilus indolicus*, the identification was considered correct as a complex, since they could not be differentiated by 16S rRNA sequencing[14].

Statistical analysis

Confidence interval, proportions and differences between platforms were calculated by the proportion methods using Statistix 10.0. Statistical significance was assigned to *p*-values <0.05.

Results and discussion

Table 2 summarizes the identification of the 333 clinical anaerobic isolates, with 141 isolates corresponding to gram negative bacilli (GNB),110 isolates to gram positive bacilli (GPB), 75 isolates to gram positive cocci (GPC), and 7 isolates to gram negative cocci (GNC) distributed among 58 different genera and 139 species, many of which are recognized as causes of human infections.

In this study, the identification at the species or species–complex level comprised 85.3% (n: 284/333) (CI_{95}: 81.3–98.2) of the isolates using the Bruker System, but Vitek MS achieved less efficacy, given that only 65.5% (n: 218/333) (CI_{95}: 60.2–70.7) of the isolates could be identified (*p*-value <0.0001) (Table 2). Previous studies reported that correct identification at the species level using the Bruker Biotyper platform ranged from 70.8 to 95.7%, while the Vitek MS platform reached 82.2–91.2% of the isolates[2,26,38,45,57,65,75].

- Identification of gram negative bacilli (GNB) isolates

The performances in the identifications are shown in Tables 2 and 3. Of the GNB, 89.4% (126/141) and 74.5% (105/141) were identified at the genus level using the Bruker Biotyper system and the Vitek MS System, respectively (*p*-value: 0.002).

The GNB belonged to the following genera: *Bacteroides*, *Porphyromonas*, *Prevotella*, *Alloprevotella*, *Parabacteroides*, *Alistipes*, *Odoribacter*, *Gabonibacter*, *Fusobacterium*, *Campylobacter*, *Dialister*, *Bilophila*, *Desulfovibrio*, *Pyramidobacter*, *Eikenella*, *Tidjanibacter* and *Casaltella*, which were distributed into the eight different classes and orders and *Fenollaria* (S1).

At the species level, 83.7% (118/141) of GNB were identified by Bruker Biotyper, while Vitek MS identified 70.2% (99/141) (*p*-value: 0.011). The proportion of unidentified GNB was 14.9% (21/141) and 25.5% (36/141) by Bruker Biotyper and Vitek MS, respectively (*p*-value: 0.038). Both systems misidentified these bacteria in 1.4% by Bruker Biotyper, and in 4.3% by Vitek MS (*p*-value: 0.282). GNB not reliably identified mainly corresponded to the *Porphyromonas* genus followed by the *Prevotella* genus.

- Class *Bacteroidia*. Order *Bacteroidales*

Regarding *Bacteroides* spp., Vitek MS system identified 34/37 isolates at the genus level and showed difficulties in the discrimination of 8 isolates at the species level (Tables 2 and 3). For the following species: *Bacteroides coprocola*, *Bacteroides salyersiae*, *Bacteroides nordii*, and *Bacteroides clarus*, the identification failed since they are not included in the database.

Bruker Biotyper identified all *Bacteroides* isolates at the genus level, but one (L5) was not identified at the species level since the score difference between the two species was less than 10% (Table 4), and another isolate was misidentified at the species level.

The isolates that were not identified or that showed discrepancies were further analyzed by 16S rRNA gene sequencing (Table 4). In 7 isolates (L5, G47, G60, C22, HA66, CEM30 and CEM33) 16S rRNA sequencing and Bruker Biotyper identification were concordant. Inconsistency was observed in the identification of G58 isolate that was identified as *Bacteroides koorensis* by its 16S rRNA sequence, but Bruker Biotyper assigned it as *Bacteroides ovatus*.

The analysis of the 16S rRNA gene sequence was useful to correct the discrepancies in 8 out of 10 *Bacteroides* isolates; however, in 2 isolates, it was observed that there was >99% sequence identity for *B. ovatus* and *B. koorensis*. In 2017 Shin et al. described a new species, *B. koorensis*, from isolates recovered in human feces, which displayed relatedness with *B. ovatus* and *B. xylanisolvens*[64].

It is important to highlight that an additional note from the Vitek MS database (V3.2.0) revealed that *B. ovatus* is grouped with a diagonal line with *B. xylanisolvens*; however, *B. xylanisolvens* is not included in the Bruker Biotyper database yet. Therefore, an alternative could be to inform the complex *B. ovatus/B. xylanisolvens* when *B. ovatus* is present in clinical environments. *B. ovatus/B. xylanisolvens* showed a close phylogenetic relationship and they evidence biochemical similarity[15]. Undoubtedly, to enable a more accurate identification, the databases should be expanded.

Nevertheless, the identification accuracy of MALDI-TOF MS for *Bacteroides* species was studied and proved to be superior to biochemical testing[15,46].

Bacteroides is one of the most common and well-known genera that contains numerous species that can be found in the human gut microbiome. Other related genus such as *Parabacteroides*, *Alistipes* and *Odoribacter* belonging to the same *Bacteroidales* order, are relatively new and can also be relevant in some infectious diseases[31,35,49,59]. Two isolates of *Alistipes* genus (*Alistipes indistinctus* and *Alistipes onderdonkii*) were correctly identified only by Bruker Biotyper. This identification was confirmed by 16S rRNA gene sequencing (Table 4).

Alistipes genus includes 10 species validly published (https://lpsn.dsmz.de/search?word=alistipes). Currently, 5 species are represented in the Bruker Biotyper database (*Alistipes finegoldii*, *A. onderdonkii*, *Alistipes shahii*, *A. indistinctus* and *Alistipes putredinis*) and only one species is represented in the Vitek MS database (*A. finegoldii*). Parker et al. mentioned that the identification in clinical samples is often underestimated and that it would be necessary to update databases[49].

Table 2 Identification of 333 anaerobic bacteria by the Vitek MS and Bruker Biotyper systems.

Microorganism (n)	No. of isolates identified by system							
	Vitek MS				Bruker Biotyper			
	Species/ complex level	Genus level	Not identified*	Misidentified**	Species/ complex level	Genus level	Not identified*	Misidentified**
Gram negative bacilli (GNB)								
Bacteroides (37)	29	34	3	5	35	37	1	1
B. fragilis (9)	9	9	0	0	9	9	0	0
B. thetaiotaomicron/B. faecis (8)	8	8	0	0	8	8	0	0
B. ovatus/B. xylanisolvens (5)	5	5	0	0	5	5	0	0
B. vulgatus[a] (3)	3	3	0	0	3	3	0	0
B. nordii (3)	0	1	2	1	3	3	0	0
B. uniformis (2)	2	2	0	0	2	2	0	0
B. pyogenes (1)	1	1	0	0	1	1	0	0
B. clarus (1)	0	1	0	1	0	1	1	0
B. massiliensis[a] (1)	0	1	0	1	1	1	0	0
B. stercoris (1)	1	1	0	0	1	1	0	0
B. coprocola[a] (1)	0	1	0	1	1	1	0	0
B. salyersiae (1)	0	1	0	1	1	1	0	0
B. koorensis (1)	0	0	1	0	0	1	0	1
Prevotella/Alloprevotella (29)	20	20	9	0	23	24	6	0
P. buccae (4)	4	4	0	0	4	4	0	0
P. baroniae (4)	4	4	0	0	4	4	0	0
P. nigrescens (3)	3	3	0	0	3	3	0	0
P. bivia (5)	3	3	2	0	4	4	1	0
P. timonensis (3)	3	3	0	0	3	3	0	0
P. bergensis (1)	1	1	0	0	1	1	0	0
P. nanciensis (1)	1	1	0	0	1	1	0	0
P. denticola (1)	1	1	0	0	1	1	0	0
P. heparinolytica (1)	0	0	1	0	1	1	0	0
P. maculosa (1)	0	0	1	0	1	1	0	0
P. ihumii (2)	0	0	2	0	0	1	2	0
P. oris (1)	0	0	1	0	0	0	1	0
Prevotella sp. (1)	0	0	1	0	0	0	1	0
Alloprevotella rava (1)	0	0	1	0	0	0	1	0
Porphyromonas (22)	12	12	10	0	16	16	6	0
P. asaccharolytica/P. uenonis (16)	11	11	5	0	13	13	3	0
P. endodontalis (1)	0	0	1	0	1	1	0	0
P. somerae (1)	0	0	1	0	1	1	0	0

Table 2 (*Continued*)

Microorganism (n)	No. of isolates identified by system							
	Vitek MS				Bruker Biotyper			
	Species/ complex level	Genus level	Not identified*	Misidentified**	Species/ complex level	Genus level	Not identified*	Misidentified**
P. bennonis (1)	0	0	1	0	0	0	1	0
P. gingivalis (1)	1	1	0	0	1	1	0	0
Porphyromonas spp. (2)	0	0	2	0	0	0	2	0
Fusobacterium (16)	14	15	1	1	13	16	3	0
F. nucleatum (7)	6	6	1	0	6	7	1	0
F. mortiferum (3)	3	3	0	0	3	3	0	0
F. gonidiaformans (2)	2	2	0	0	1	2	1	0
F. necrophorum (2)	2	2	0	0	2	2	0	0
F. varium (1)	1	1	0	0	1	1	0	0
F. canifelinum (1)	0	1	0	1	0	1	1	0
Campylobacter (11)	11	11	0	0	11	11	0	0
C. ureolyticus (7)	7	7	0	0	7	7	0	0
C. rectus (3)	3	3	0	0	3	3	0	0
C. fetus (1)	1	1	0	0	1	1	0	0
Parabacteroides (6)	4	4	2	0	4	5	1	1
P. distasonis (3)	3	3	0	0	3	3	0	0
P. merdae (1)	1	1	0	0	1	1	0	0
P. chongii (1)	0	0	1	0	0	1	0	1
P. faecis (1)	0	0	1	0	0	0	1	0
Dialister (5)	5	5	0	0	5	5	0	0
D. micraerophilus (2)	2	2	0	0	2	2	0	0
D. pneumosintes (3)	3	3	0	0	3	3	0	0
Odoribacter (2)	2	2	0	0	2	2	0	0
O. splanchnicus (2)	2	2	0	0	2	2	0	0
Alistipes (2)	0	0	2	0	2	2	0	0
A. indistinctus (1)	0	0	1	0	1	1	0	0
A. onderdonkii (1)	0	0	1	0	1	1	0	0
Bilophila (2)	1	1	1	0	1	2	1	0
B. wadsworthia (2)	1	1	1	0	1	2	1	0
Desulfovibrio (1)	1	1	0	0	1	1	0	0
D. desulfuricans (1)	1	1	0	0	1	1	0	0

Table 2 (*Continued*)

Microorganism (n)	No. of isolates identified by system							
	Vitek MS				Bruker Biotyper			
	Species/ complex level	Genus level	Not identified*	Misidentified**	Species/ complex level	Genus level	Not identified*	Misidentified**
Rare species								
Pyramidobacter (2)	0	0	2	0	2	2	0	0
P. piscolens (2)	0	0	2	0	2	2	0	0
Gabonibacter (1)	0	0	1	0	1	1	0	0
G. massiliensis (1)	0	0	1	0	1	1	0	0
Eikenella (1)	0	0	1	0	0	0	1	0
E. longinqua (1)	0	0	1	0	0	0	1	0
Tidjanibacter (1)	0	0	1	0	0	0	1	0
T. massiliensis (1)	0	0	1	0	0	0	1	0
Casaltella (1)	0	0	1	0	0	0	1	0
C. massiliensis (1)	0	0	1	0	0	0	1	0
Fenollaria (2)	0	0	2	0	2	2	0	0
F. massiliensis (2)	0	0	2	0	2	2	0	0
Total GNB (141)	**99**	**105**	**36**	**6**	**118**	**126**	**21**	**2**
Gram positive bacilli								
Clostridium and related genera (46)	37	39	7	2	40	43	6	0
C. sporogenes (4)	4	4	0	0	3	3	1	0
C. septicum (3)	3	3	0	0	3	3	0	0
C. perfringens (2)	2	2	0	0	2	2	0	0
C. paraputrificum (2)	2	2	0	0	2	2	0	0
C. ramnosum (1)	1	1	0	0	1	1	0	0
C. baratii (1)	1	1	0	0	1	1	0	0
C. innocuum (1)	1	1	0	0	1	1	0	0
C. fallax (1)	1	1	0	0	1	1	0	0
C. tunisiense (1)	0	0	1	0	1	1	0	0
C. bifermentans (1)	1	1	0	0	1	1	0	0
C. hydrogeniformans (1)	0	0	1	0	0	0	1	0
C. argentinense[b] (1)	0	0	1	0	1	1	0	0
C. symbiosum (1)	0	0	1	0	1	1	0	0
Clostridium sp. (1)	0	1	0	1	0	1	1	0

Table 2 (*Continued*)

Microorganism (n)	No. of isolates identified by system							
	Vitek MS				Bruker Biotyper			
	Species/ complex level	Genus level	Not identified*	Misidentified**	Species/ complex level	Genus level	Not identified*	Misidentified**
Clostridioides (15)								
C. difficile (15)	15	15	0	0	15	15	0	0
Paeniclostridium (5)								
P. sordelli (5)	5	5	0	0	5	5	0	0
Enterocloster (3)								
E. bolteae (2)	1	2	0	1	1	2	1	0
E. aldenensis (1)	0	0	1	0	1	1	0	0
Lacrimispora (2)								
L. celerecrescens (1)	0	0	1	0	0	1	1	0
L. amygdalina (1)	0	0	1	0	0	0	1	0
Cutibacterium (13)	13	13	0	0	13	13	0	0
Cutibacterium acnes (8)	8	8	0	0	8	8	0	0
Cutibacterium avidum (4)	4	4	0	0	4	4	0	0
Cutibacterium granulosum (1)	1	1	0	0	1	1	0	0
Eggerthella (8)	5	5	3	0	6	6	2	0
E. lenta (8)	5	5	3	0	6	6	2	0
Paraeggerthella (1)	0	0	1	0	0	0	1	0
P. hongkongensis (1)	0	0	1	0	0	0	1	0
Atopobium (2)	0	0	2	0	1	1	1	0
A. minutum (2)	0	0	2	0	1	1	1	0
Lancefieldella (5)	5	5	0	0	5	5	0	0
L. rimae (2)	2	2	0	0	2	2	0	0
L. parvula (3)	3	3	0	0	3	3	0	0
Fannyhessea (1)	0	0	1	0	1	1	0	0
F. vaginae (1)	0	0	1	0	1	1	0	0
Bifidobacterium (5)	0	3	5	0	5	5	0	0
B. dentium (2)	0	2	2	0	2	2	0	0
B. breve (2)	0	1	2	0	2	2	0	0
B. scardovii (1)	0	0	1	0	1	1	0	0

Table 2 (*Continued*)

Microorganism (n)	No. of isolates identified by system							
	Vitek MS				Bruker Biotyper			
	Species/ complex level	Genus level	Not identified*	Misidentified**	Species/ complex level	Genus level	Not identified*	Misidentified**
Gram positive bacilli (GPB)								
Slackia (5)	3	3	2	0	5	5	0	0
S. exigua (5)	3	3	2	0	5	5	0	0
Solobacterium (4)	0	0	4	0	4	4	0	0
S. moorei (4)	0	0	4	0	4	4	0	0
Actinomyces/Actinotignum (4)	4	4	0	0	4	4	0	0
Actinotignum schaalii (1)	1	1	0	0	1	1	0	0
Actinomyces urogenitalis (1)	1	1	0	0	1	1	0	0
Actinomyces turicensis[c] (1)	1	1	0	0	1	1	0	0
Actinomyces odontolyticus[c] (1)	1	1	0	0	1	1	0	0
Eggerthia (3)	3	3	0	0	3	3	0	0
E. catenaformis (3)	3	3	0	0	3	3	0	0
Eubacterium (2)	2	2	0	0	1	1	1	0
E. limosum/E. callanderi (2)	2	2	0	0	1	1	1	0
Tissierella (2)	0	0	2	0	2	2	0	0
T. praeacuta (2)	0	0	2	0	2	2	0	0
Moryella (2)	0	0	2	0	2	2	0	0
M. indoligenes (2)	0	0	2	0	2	2	0	0
Propionimicrobium (1)	0	0	1	0	1	1	0	0
P. lymphophilum (1)	0	0	1	0	1	1	0	0
Olsenella (1)	0	0	1	0	1	1	0	0
O. uli(1)	0	0	1	0	1	1	0	0
Robinsoniella (1)	1	1	0	0	1	1	0	0
R. peoriensis (1)	1	1	0	0	1	1	0	0
Terrisporobacter (1)	1	1	0	0	1	1	0	0
T. glycolicus (1)	1	1	0	0	1	1	0	0
Filifactor (1)	0	0	1	0	1	1	0	0
F. alocis (1)	0	0	1	0	1	1	0	0

Table 2 (*Continued*)

Microorganism (n)	No. of isolates identified by system							
	Vitek MS				Bruker Biotyper			
	Species/ complex level	Genus level	Not identified*	Misidentified**	Species/ complex level	Genus level	Not identified*	Misidentified**
Collinsella (1)	1	1	0	0	1	1	0	0
C. aerofaciens (1)	1	1	0	0	1	1	0	0
Criibacterium (1)	0	0	1	0	0	0	1	0
C. bergeronii (1)	0	0	1	0	0	0	1	0
Total GPB (110)	**75**	**80**	**33**	**2**	**98**	**101**	**12**	**0**
Gram positive cocci (GPC)								
Peptoniphilus (18)	5	13	5	8	17	17	1	0
P. indolicus/P. harei (10)	2	10	0	8	10	10	0	0
P. lacrimalis (3)	3	3	0	0	3	3	0	0
P. duerdenii (1)	0	0	1	0	1	1	0	0
P. tyrreliae (2)	0	0	2	0	2	2	0	0
P. lacydonensis (1)	0	0	1	0	1	1	0	0
P. nemausensis (1)	0	0	1	0	0	0	1	0
Anaerococcus (17)	6	6	10	1	11	13	6	0
A. octavius (3)	0	0	3	0	3	3	0	0
A. vaginalis (2)	2	2	0	0	1	2	1	0
A. prevotii (2)	2	2	0	0	1	2	1	0
A. murdochii (2)	0	0	2	0	2	2	0	0
A. tetradius (2)	2	2	0	0	2	2	0	0
A. nagyae (1)	0	0	1	0	1	1	0	0
A. hydrogenalis (1)	0	0	1	0	1	1	0	0
A. provencensis (1)	0	0	1	0	0	0	1	0
A. urinomassiliensis (1)	0	0	1	0	0	0	1	0
A. mediterraneensis (1)	0	0	1	0	0	0	1	0
A. jeddahensis (1)	0	0	0	1	0	0	1	0
Finegoldia (17)	17	17	0	0	16	17	1	0
F. magna (17)	17	17	0	0	16	17	1	0
Peptostreptococcus (8)	5	5	3	0	6	6	2	0
P. anaerobius (5)	5	5	0	0	5	5	0	0
P. stomatis (3)	0	0	3	0	1	1	2	0

Table 2 (*Continued*)

Microorganism (n)	No. of isolates identified by system							
	Vitek MS				Bruker Biotyper			
	Species/ complex level	Genus level	Not identified*	Misidentified**	Species/ complex level	Genus level	Not identified*	Misidentified**
Parvimonas (7)	6	6	1	0	6	7	1	0
P. micra (7)	6	6	1	0	6	7	1	0
Staphylococcus (2)	1	1	1	0	2	2	0	0
S. saccharolyticus (2)	1	1	1	0	2	2	0	0
Murdochiella (2)	0	0	2	0	1	1	1	0
M. asaccharolytica (1)	0	0	1	0	1	1	0	0
Murdochiella sp. (1)	0	0	1	0	0	0	1	0
Fastidiosipila (2)	0	0	2	0	2	2	0	0
F. sanguinis (2)	0	0	2	0	2	2	0	0
Ruminococcus (1)	1	1	0	0	1	1	0	0
R. gnavus (1)	1	1	0	0	1	1	0	0
Lagierella (1)	0	0	1	0	0	0	1	0
L. massiliensis (1)	0	0	1	0	0	0	1	0
Total GPC (75)	**41**	**49**	**25**	**9**	**62**	**66**	**13**	**0**
Gram negative cocci (GNC)								
Acidaminococcus (3)	0	0	3	0	2	2	1	0
A. intestini (3)	0	0	3	0	2	2	1	0
Veillonella (3)	3	3	0	0	3	3	0	0
V. atypica (2)	2	2	0	0	2	2	0	0
V. parvula (1)	1	1	0	0	1	1	0	0
Negativicoccus (1)	0	0	1	0	1	1	0	0
N. succinicivorans (1)	0	0	1	0	1	1	0	0
Total GNC (7)	**3**	**3**	**4**	**0**	**6**	**6**	**1**	**0**
Total microorganisms (333)	**218 (65.5%)[d]**	**237 (71.2%)**	**98 (29.4%)**	**17 (5.1%)**	**284 (85.3%)**	**299 (89.7%)**	**47 (14.1%)**	**2 (0.6%)**

[a] Current nomenclature: *Phocaeicola vulgatus*, *Phocaeicola massiliensis* and *Phocaeicola coprocola*.
[b] *C. argentinense* is synonymous with *C. subterminale*.
[c] Current nomenclature: *Schaalia turicensis* and *Schaalia odontolytica*.
[d] In parentheses, percentage of each identification level.
* Not identified at the species level.
** Misidentified at the species level.

Table 3 Overall picture of the identification of the species included in this work and their presence in databases.

Microorganism	Spectra included in		Identified, misidentified, or not identified at the species level by	
	Vitek system	Bruker Biotyper system	Vitek system	Bruker Biotyper system
Gram negative bacilli (GNB)				
Bacteroides				
B. fragilis	x	x	Identified[b]	Identified
B. thetaiotaomicron/B. faecis	x	x	Identified	Identified
B. ovatus/B. xylanisolvens	x	x[a]	Identified	Identified
*B. vulgatus**	x	x	Identified	Identified
B. nordii	–	x	Not Identified/misidentified[c]	Identified
B. uniformis	x	x	Identified	Identified
B. pyogenes	x	x	Identified	Identified
B. clarus	–	x	Misidentified[d]	Identified
*B. massiliensis**	–	x	Misidentified	Identified
B. stercoris	x	x	Identified	Identified
*B. coprocola**	–	x	Misidentified	Identified
B. salyersiae	–	x	Misidentified	Identified
B. koorensis	–	–	Not identified[e]	Misidentified
Prevotella/Alloprevotella				
P. buccae	x	x	Identified	Identified
P. baroniae	x	x	Identified	Identified
P. nigrescens	x	x	Identified	Identified
P. bivia	x	x	Identified/Not identified[f]	Identified/Not identified
P. timonensis	x	x	Identified	Identified
P. bergensis	x	x	Identified	Identified
P. nanciensis	x	x	Identified	Identified
P. denticola	x	x	Identified	Identified
P. heparinolytica	–	x	Not identified	Identified
P. maculosa	–	x	Not identified	Identified
P. ihumii	–	–	Not identified	Not identified
P. oris	x	x	Not identified	Not identified
Alloprevotella rava	–	x	Not identified	Not identified
Porphyromonas				
P. asaccharolytica/P. uenonis	x	x	Identified/Not identified	Identified/Not identified
P. endodontalis	–	x	Not identified	Identified
P. somerae	–	x	Not identified	Identified
P. bennonis	–	x	Not identified	Not identified
P. gingivalis	x	x	Identified	Identified

Table 3 (*Continued*)

Microorganism	Spectra included in		Identified, misidentified, or not identified at the species level by	
	Vitek system	Bruker Biotyper system	Vitek system	Bruker Biotyper system
Fusobacterium				
F. nucleatum	x	x	Identified/Not identified	Identified/Not identified
F. mortiferum	x	x	Identified	Identified
F. gonidiaformans	x	x	Identified	Identified/Not identified
F. necrophorum	x	x	Identified	Identified
F. varium	x	x	Identified	Identified
F. canifelinum	–	x	Misidentified	Not identified
Campylobacter				
C. ureolyticus	x	x	Identified	Identified
C. rectus	x	x	Identified	Identified
C. fetus	x	x	Identified	Identified
Parabacteroides				
P. distasonis	x	x	Identified	Identified
P. merdae	x	x	Identified	Identified
P. chongii	–	–	Not identified	Misidentified
P. faecis	–	–	Not identified	Not identified
Dialister				
D. micraerophilus	x	x	Identified	Identified
D. pneumosintes	x	x	Identified	Identified
Odoribacter				
O. splanchnicus	x	x	Identified	Identified
Alistipes				
A. indistinctus	–	x	Not identified	Identified
A. onderdonkii	–	x	Not identified	Identified
Bilophila				
B. wadsworthia	x	x	Identified/Not identified	Identified/Not identified
Desulfovibrio				
D. desulfuricans	x	x	Identified	Identified
Rare species				
Pyramidobacter				
P. piscolens	–	x	Not identified	Identified
Gabonibacter				
G. massiliensis	–	x	Not identified	Identified

Table 3 (*Continued*)

Microorganism	Spectra included in		Identified, misidentified, or not identified at the species level by	
	Vitek system	Bruker Biotyper system	Vitek system	Bruker Biotyper system
Eikenella				
E. longinqua	–	–	Not identified	Not identified
Tidjanibacter				
T. massiliensis	–	–	Not identified	Not identified
Casaltella				
C. massiliensis	–	–	Not identified	Not identified
Fenollaria				
F. massiliensis	–	x	Not identified	Identified
Gram positive bacilli (GPB)				
Clostridium				
C. sporogenes	x	x	Identified	Identified/Not identified
C. septicum	x	x	Identified	Identified
C. perfringens	x	x	Identified	Identified
C. paraputrificum	x	x	Identified	Identified
C. ramnosum	x	x	Identified	Identified
C. baratii	x	x	Identified	Identified
C. innocuum	x	x	Identified	Identified
C. fallax	x	x	Identified	Identified
C. tunisiense	–	x	Not identified	Identified
C. bifermentans	x	x	Identified	Identified
C. hydrogeniformans	–	–	Not identified	Not identified
*C. argentinense***	–	x	Not identified	Identified
C. symbiosum	–	x	Not identified	Identified
Clostridioides				
C. difficile	x	x	Identified	Identified
Paeniclostridium				
P. sordelli	x	x	Identified	Identified
Enterocloster				
E. bolteae	x	x	Identified/Misidentified[g]	Identified/Not identified
E. aldenensis	–	x	Not identified	Identified
Lacrimispora				
L. celerecrescens	–	x	Not identified	Not identified
L. amygdalina	–	–	Not identified	Not identified

Table 3 (*Continued*)

Microorganism	Spectra included in		Identified, misidentified, or not identified at the species level by	
	Vitek system	Bruker Biotyper system	Vitek system	Bruker Biotyper system
Cutibacterium				
Cutibacterium acnes	x	x	Identified	Identified
Cutibacterium avidum	x	x	Identified	Identified
Cutibacterium granulosum	x	x	Identified	Identified
Eggerthella				
E. lenta	x	x	Identified/Not identified	Identified/Not identified
Paraeggerthella				
P. hongkongensis	–	–	Not identified	Not identified
Atopobium				
A. minutum	–	x	Not identified	Identified/Not identified
Lancefieldella				
L. rimae	x	x	Identified	Identified
L. parvula	x	x	Identified	Identified
Fannyhessea				
F. vaginae	–	x	Not identified	Identified
Bifidobacterium				
B. dentium	x	x	Not identified	Identified
B. breve	x	x	Not identified	Identified
B. scardovii	x	x	Not identified	Identified
Slackia				
S. exigua	x	x	Identified/Not identified	Identified
Solobacterium				
S. moorei	–	x	Not identified	Identified
Actinomyces/Actinotignum				
Actinotignum schaalii	x	x	Identified	Identified
Actinomyces turicensis[***]	x	x	Identified	Identified
Actinomyces odontolyticus[***]	x	x	Identified	Identified
Actinomyces urogenitalis	x	x	Identified	Identified
Eggerthia				
E. catenaformis	x	x	Identified	Identified
Eubacterium				
E. limosum/E. callanderi	x	x	Identified	Identified/Not identified

Table 3 (*Continued*)

Microorganism	Spectra included in		Identified, misidentified, or not identified at the species level by	
	Vitek system	Bruker Biotyper system	Vitek system	Bruker Biotyper system
Tissierella				
T. praeacuta	–	x	Not identified	Identified
Moryella				
M. indoligenes	–	x	Not identified	Identified
Olsenella				
O. uli	–	x	Not identified	Identified
Robinsoniella				
R. peoriensis	x	x	Identified	Identified
Terrisporobacter				
T. glycolicus	x	x	Identified	Identified
Filifactor				
F. alocis	–	x	Not identified	Identified
Collinsella				
C. aerofaciens	x	x	Identified	Identified
Criibacterium				
C. bergeronii	–	–	Not identified	Not identified
Gram positive cocci (GPC)				
Peptoniphilus				
P. indolicus/P. harei	x	x	Identified/Misidentified	Identified
P. lacrimalis	x	x	Identified	Identified
P. duerdenii	–	x	Not identified	Identified
P. tyrreliae	–	x	Not identified	Identified
P. lacydonensis	–	x	Not identified	Identified
P. nemausensis	–	–	Not identified	Not identified
Anaerococcus				
A. octavius	–	x	Not identified	Identified
A. vaginalis	x	x	Identified	Identified/Not identified
A. prevotii	x	x	Identified	Identified/Not identified
A. murdochii	–	x	Not identified	Identified
A. tetradius	x	x	Identified	Identified
A. nagyae	–	x	Not identified	Identified
A. hydrogenalis	–	x	Not identified	Identified
A. provencensis	–	–	Not identified	Not identified
A. urinomassiliensis	–	–	Not identified	Not identified
A. mediterraneensis	–	–	Not identified	Not identified
A. jeddahensis	–	–	Misidentified	Not identified

Table 3 (*Continued*)

Microorganism	Spectra included in		Identified, misidentified, or not identified at the species level by	
	Vitek system	Bruker Biotyper system	Vitek system	Bruker Biotyper system
Finegoldia				
F. magna	x	x	Identified	Identified/Not identified
Peptostreptococcus				
P. anaerobius	x	x	Identified	Identified
P. stomatis	–	x	Not identified	Identified/Not identified
Parvimonas				
P. micra	x	x	Identified/Not identified	Identified/Not identified
Staphylococcus				
S. saccharolyticus	x	x	Identified/Not identified	Identified
Murdochiella				
M. asaccharolytica	–	x	Not identified	Identified
Fastidiosipila				
F. sanguinis	–	X	Not identified	Identified
Ruminococcus				
R. gnavus	x	X	Identified	Identified
Lagierella				
L. massiliensis	–	–	Not identified	Not identified
Gram negative cocci (GNC)				
Acidaminococcus				
A. intestini	–	X	Not identified	Identified/Not identified
Veillonella				
V. atypica	x	X	Identified	Identified
V. parvula	x	X	Identified	Identified
Negativicoccus				
N. succinicivorans	–	X	Not identified	Identified

Notes: x, spectra included in system; –, spectra not included in system.
[a] Only *B. ovatus*.
[b] Identified: total isolates studied.
[c] Not identified/misidentified: some of the isolates studied/some of the isolates studied.
[d] Misidentified: total isolates studied.
[e] Not identified: total isolates studied.
[f] Identified/not identified: some of the isolates studied/some of the isolates studied.
[g] Identified/misidentified: some of the isolates studied/some of the isolates studied.
[*] Current nomenclature: *Phocaeicola vulgatus*, *Phocaeicola massiliensis* and *Phocaeicola coprocola*.
[**] *C. argentinense* is synonymous with *C. subterminale*.
[***] Current nomenclature: *Schaalia turicensis* and *Schaalia odontolytica*.

Table 4 Comparison between identifications provided by both MALDI-TOF MS systems and the reference method for the isolates with discrepancies, or with the same identification but low scores.

Isolate/number	Vitek MS ID 99%	Bruker Biotyper ID (score)	16S rRNA sequencing
Gram negative bacilli (GNB)			
Bacteroides (n: 10)			
L5	*B. stercoris*	*B. clarus* (2.34), *B. stercoris* (2.23)	*B. clarus*
L16	*B. dorei/B. vulgatus*	*B. massiliensis* (2.39)	*B. massiliensis*
G12	*B. ovatus/B. xylanisolvens*	*B. ovatus* (1.97)	*B. ovatus/B. koorensis*
G47	*B. ovatus/B. xylanisolvens*	*B. ovatus* (2.06)	*B. ovatus/B. koorensis*
G58	No identification	*B. ovatus* (1.80)	*B. koorensis*
G60	No identification	*B. nordii* (1.96)	*B. nordii*
C22	*B. dorei*	*B. salyersiae* (2.27)	*B. salyersiae*
HA66	*B. dorei/B. uniformis*	*B. coprocola* (2.12)	*B. coprocola*
CEM30	No identification	*B. nordii* (1.92)	*B. nordii*
CEM33	*B. fragilis*	*B. nordii* (1.93)	*B. nordii*
Prevotella (n: 9)			
G31	No identification	*P. heparinolytica* (2.44)	*P. heparinolytica*
A35	No identification	*P. maculosa* (2.03)	*P. maculosa*
A16	No identification	*P. bivia* (2.25)	*P. bivia*
A21	No identification	No identification	*P. bivia*
A33	No identification	No identification	*P. ihumii*
CEM28	No identification	No identification	*P. oris*
G75	No identification	No identification	*Prevotella* sp. DNF 00663
A58	No identification	No identification	*Alloprevotella rava*
M60	No identification	*Prevotella* sp. (2.00)	*P. ihumii*
Porphyromonas (n: 12)			
L13	*P. asaccharolytica/P. uenonis*	No identification	*P. asaccharolytica*
L19	No identification	No identification	*P. asaccharolytica*
L50	*P. asaccharolytica/P. uenonis*	No identification	*P. asaccharolytica*
A61	No identification	*P. asaccharolytica/P. uenonis* (2.01)	*P. asaccharolytica*
A62	No identification	*P. asaccharolytica/P. uenonis* (2.12)	*P. asaccharolytica*
CEM24	No identification	No identification	*P. bennonis*
A68	No identification	*P. asaccharolytica/P. uenonis* (1.88)	*P. asaccharolytica*
A69	No identification	*P. asaccharolytica/P. uenonis* (1.83)	*P. asaccharolytica*
CEM25	No identification	No identification	*Porphyromonas* sp. oral taxon 275
A34	No identification	No identification	*Porphyromonadaceae* sp.
A24	No identification	*P. endodontalis* (2.11)	*P. endodontalis*
C27	No identification	*P. somerae* (1.96)	*P. somerae*
Fusobacterium (n: 4)			
L46	*F. nucleatum*	*F. nucleatum* (1.53)	*F. nucleatum*
A8	No identification	*F. nucleatum* (1.86)	*F. nucleatum*
L37	*F. gonidiaformans*	*F. gonidiaformans* (1.59)	*F. gonidiaformans*
L36	*F. periodonticum*	*F. canifelinum* (1.53)	*F. canifelinum*
Alistipes (n: 2)			
CEM 20	No identification	*A. indistinctus* (2.18)	*A. indistinctus*
CEM 22	No identification	*A. onderdonkii* (2.08)	*A. onderdonkii*
Parabacteroides (n: 2)			
L24	No identification	No identification	*P. faecis*
Gram negative bacilli (GNB)			
Bilophila (n: 2)			
CEM 10	No identification	*B. wadsworthia* (1.84)	*B. wadsworthia*
L42	*B. wadsworthia*	*B. wadsworthia* (1.51)	*B. wadsworthia*
Pyramidobacter (n: 2)			
CEM 29	No identification	*P. piscolens* (2.33)	*P. piscolens*
CEM44	No identification	*P. piscolens* (1.91)	*P. piscolens*

Table 4 (*Continued*)

Isolate/number	Vitek MS ID 99%	Bruker Biotyper ID (score)	16S rRNA sequencing
Gabonibacter (n: 1)			
L43	No identification	*G. massiliensis* (2.14)	*G. massiliensis*
Eikenella (n: 1)			
A34	No identification	No identification	*Eikenella longinqua*
Tidjanibacter (n: 1)			
CEM 35	No identification	No identification	*T. massiliensis*
Casaltella (n: 1)			
G41	No identification	No identification	*C. massiliensis*
Fenollaria (n: 2)			
L31	No identification	*F. massiliensis* (1.93)	*F. massiliensis*
M59	No identification	*F. massiliensis* (1.97)	*F. massiliensis*
Gram positive bacilli (GPB)			
Clostridium (n: 10)			
G71	No identification	*Clostridium tunisiense* (2.00)	*Clostridium tunisiense*
M50	*Clostridium sporogenes*	No identification	*Clostridium sporogenes*
M55	No identification	*Clostridium subterminale* (2.12)	*Clostridium argentinense*
G73	No identification	No identification	*Clostridium hydrogeniformans*
L9	No identification	*Clostridium symbiosum* (2.20)	*Clostridium symbiosum*
C9	*Clostridium sporogenes*	*Clostridium* sp. *110324* (1.90)	*Clostridium moniliforme/C. sardiniense*
L10	*Clostridium clostridioforme*	*C. clostridioforme* (1.89)/*C. bolteae* (1.80)	*Enterocloster bolteae*
L51	No identification	*C. aldenense* (2.27)	*Enterocloster aldenensis*
G69	No identification	*C. celerecrescens* (1.99)/*C. sphenoides* (1.85)	*Lacrimispora celerecrescens*
CEM21	No identification	No identification	*Lacrimispora amygdalina*
Eggerthella (n: 3)			
G8	No identification	No identification	*E. lenta*
L33	No identification	*Eggerthella lenta* (2.13)	*E. lenta*
CEM18	No identification	*Eggerthella lenta* (1.89)	*E. lenta*
Paraeggerthella (n: 1)			
G35	No identification	No identification	*P. hongkongensis*
Gram positive bacilli (GPB)			
Atopobium/Fannyhessea (n: 3)			
G53	No identification	No identification	*Atopobium minutum*
CEM 41	No identification	*Atopobium minutum* (2.22)	*Atopobium minutum*
CEM 16	No identification	*Atopobium vaginae* (1.72)	*Fannyhessea vaginae*
Bifidobacterium (n: 5)			
L2	No identification	*B. scardovii* (1.97)	*B. scardovii*
C48	No identification	*B. breve* (1.98)	*B. breve*
CEM 13	*Bifidobacterium* sp.	*B. breve* (2.06)	*B. breve*
CEM 14	*Bifidobacterium* sp.	*B. dentium* (2.18)	*B. dentium*
G66	*Bifidobacterium* sp.	*B. dentium* (1.76)	*B. dentium*
Slackia (n: 2)			
L14	No identification	*S. exigua* (2.07)	*S. exigua*
CEM 11	No identification	*S. exigua* (2.24)	*S. exigua*
Solobacterium (n: 4)			
CEM 4	No identification	*S. moorei* (2.39)	*S. moorei*
CEM 5	No identification	*S. moorei* (2.00)	*S. moorei*
CEM 6	No identification	*S. moorei* (2.18)	*S. moorei*
CEM 7	No identification	*S. moorei* (1.80)	*S. moorei*
Eubacterium (n: 1)			
C37	*E. callanderi*	No identification	*E. limosum/callanderi*

Table 4 (*Continued*)

Isolate/number	Vitek MS ID 99%	Bruker Biotyper ID (score)	16S rRNA sequencing
Tissierella (n: 2)			
C11	No identification	*T. praeacuta* (2.12)	*T. praeacuta*
G72	No identification	*T. praeacuta* (2.08)	*T. praeacuta*
Moryella (n: 2)			
CEM 27	No identification	*M. indoligenes* (2.12)	*M. indoligenes*
CEM 45	No identification	*M. indoligenes* (1.91)	*M. indoligenes*
Propionimicrobium (n: 1)			
G70	No identification	*P. lymphophilum* (1.95)	*P. lymphophilum*
Olsenella (n: 1)			
G21	No identification	*O. uli* (1.85)	*O. uli*
Filifactor (n: 1)			
M51	No identification	*F. alocis* (1.87)	*F. alocis*
Criibacterium (n: 1)			
G64	No identification	No identification	*Criibacterium bergeronii*
Gram positive cocci (GPC)			
Peptoniphilus (n: 16)			
A20	No identification	*P. duerdenii* (2.36)	*P. duerdenii*
A38	*P. asaccharolyticus*	*P. indolicus* (1.96)/*P.harei* (1.82)	*P. harei*
A40	*P. asaccharolyticus*	*P. indolicus* (2.30)/*P.harei* (2.14)	*P. harei*
C56	*P. asaccharolyticus*	*P. indolicus* (2.14)/*P.harei* (2.15)	*P. harei*
G11	No identification	*P. tyrrelliae* (2.34)/*P. senegalensis* (2.30)	*P. tyrrelliae*
G17	*P. asaccharolyticus*	*P. harei* (2.24)/*P. indolicus* (2.23)	*P. harei*
G37	No identification	No identification	*P. nemausensis*
G40	No identification	*P. tyrrelliae* (2.22)/*P. indolicus* (2.17)	*P. tyrrelliae*
G42	*P. asaccharolyticus*	*P. indolicus* (2.37)/*P. harei* (2.17)	*P. harei*
G43	*P. asaccharolyticus*	*P. indolicus* (2.02)/*P. harei* (1.85)	*P. harei*
G49	*P. asaccharolyticus*	*P. indolicus* (2.13)/*P. harei* (2.06)	*P. harei*
G50	No identification	*P. rhinitidis* (1.96)	*P. lacydonensis*
G51	*P. asaccharolyticus*	*P. indolicus* (2.32)/*P. harei* (2.28)	*P. harei*
Anaerococcus (n: 11)			
G10	No identification	*A. octavius* (1.74)	*A. octavius*
G38	No identification	*A. murdochii* (2.27)	*A. murdochii*
A54	No identification	*A. murdochii* (1.83)	*A. murdochii*
G28	No identification	No identification	*A. mediterraneensis*
G44	No identification	*A. octavius* (2.02)	*A. octavius*
G49	No identification	*A. nagyae* (2.09)	*A. nagyae*
A63	No identification	No identification	*A. urinomassiliensis*
A64	No identification	No identification	*A. provencensis*
C55	*A. vaginalis*	No identification	*A. jeddahensis*
G55	No identification	*A. octavius* (2.12)	*A. octavius*
G56	No identification	*A. hydrogenalis* (1.91)	*A. hydrogenalis*
Finegoldia (n: 1)			
C54	*F. magna*	*F. magna* (1.68)	*F. magna*
Peptostreptococcus (n: 3)			
G5	No identification	No identification	*P. stomatis*
G15	No identification	No identification	*P. stomatis*
G24	No identification	*P. stomatis* (1.90)	*P. stomatis*
Parvimonas (n: 1)			
M48	No identification	*Parvimonas micra* (1.63)	*Parvimonas* sp.
Staphylococcus (n: 1)			
C62	No identification	*S. saccharolyticus* (1.94)	*S. saccharolyticus*

Table 4 (*Continued*)

Isolate/number	Vitek MS ID 99%	Bruker Biotyper ID (score)	16S rRNA sequencing
Murdochiella (n: 2)			
G30	No identification	No identification	*Murdochiella vaginalis*
C61	No identification	*M. asaccharolytica* (2.10)	*M. asaccharolytica*
Lagierella (n: 1)			
G46	No identification	No identification	*L. massiliensis*
Gram positive cocci (GPC)			
Fastidiosipila (n: 2)			
CEM36	No identification	*F. sanguinis* (1.98)	*F. sanguinis*
CEM37	No identification	*F. sanguinis* (1.89)	*F. sanguinis*
Gram negative cocci (GNC)			
Acidaminicoccus (n: 3)			
CEM1	No identification	*A. intestini* (2.36)	*A. intestini*
CEM2	No identification	*A. intestini* (2.38)	*A. intestini*
L45	No identification	*No identification*	*A. intestini*
Negativicoccus (n: 1)			
CEM38	No identification	*N. succinivorans* (1.71)	*N. succinivorans*

Odoribacter genus includes two species validly published in human samples, *Odoribacter laneus* and *Odoribacter splanchnicus*. The two isolates of *O. splanchnicus* included in this work were correctly identified by both MALDI-TOF MS systems (Tables 2 and 3)[31].

With regard to *Parabacteroides* spp., 4/6 isolates included in this study were identified at the species level by both MALDI-TOF MS systems. The remaining two unidentified isolates (L24 and C57) corresponded to *Parabacteroides faecis* and *Parabacteroides chongii* as per their 16S rRNA sequence. None of these species are included in the MALDI-TOF MS databases. *P. faecis* was described in 2015 as a new species isolated from human feces and closest related (96% closest similarity) to *Parabacteroides gordonii* based on 16S rRNA sequence analysis[58]. *P. chongii* is a recently described species and the 16S rRNA gene sequence is closely related to *P. faecis* (97.3% identity), *P. gordonii* (96.6% identity), and *P. goldsteinii* (95.7% identity)[35].

Both MALDI-TOF MS systems presented troublesome identification when isolates of *Prevotella* spp. and *Porphyromonas* spp. were analyzed. The reason could be their pigmented nature that hindered the quality of spectra obtained, and the inadequate number of spectra of the represented species[13,54,81].

Accurate identification of *Prevotella* isolates plays a critical role in the success of the treatments, especially since the antibiotic susceptibility profile differs between species[73]. In 2012, Wybo et al. studied 102 clinical *Prevotella* isolates and only 63% were identified at the species level[80]. In a subsequent study, the expansion of the commercial database increased the correct identification of the species reaching 89%[27]. In the present study, the Vitek MS system identified 20/28 isolates (71.4%), and Bruker Biotyper 23/28 isolates (82.1%) (Table 2). The species identified by both systems were: *Prevotella buccae*, *Prevotella baroniae*, *Prevotella nigrescens*, *Prevotella timonensis*, *Prevotella nanciensis*, *Prevotella denticola* and *Prevotella bergensis*.

Nine isolates were sequenced due to discrepancies or lack of identification between the MALDI-TOF MS systems (Table 4). Three isolates were identified by Bruker Biotyper and confirmed by 16S rRNA sequencing as *Prevotella heparinolytica*, *Prevotella maculosa* and *Prevotella bivia*. Among the isolates that could not be identified by any of the MALDI-TOF MS systems not even at the genus level, one isolate corresponded to *Prevotella ihumii*, and another one to *Alloprevotella rava*, which are currently missing or there is only one MSP in the databases. Anani et al. recently described the species *P. ihumii*, a bacterium isolated from a stool specimen of a healthy woman whose reference MSP was imported into their own database (http://www.mediterranee-infection.com/article.php?larub=280&titer=urms-database)[3]. Only Bruker Biotyper identified the M60 isolate at the genus level, which corresponded to *P. ihumii* (Table 4).

However, some species that are well-represented in the database such as *Prevotella oris* and *P. bivia* were not identified.

One isolate could neither be identified by the MALDI-TOF MS sytems nor by 16S rRNA secquencing, and only displayed identity with *Prevotella* sp. DNF 00663 (Accession Number KF280297.1). More studies should be performed to reach the species level, as it could correspond to a new species.

In the identification of *Porphyromonas* spp., the performance of the MALDI-TOF MS systems was different. Using the Vitek MS system, only 12 out of 22 isolates were identified. Nowadays, the database includes only three species out of 25 listed in the List of Prokaryotic names with Standing in Nomenclature (LPSN). According to Vitek MS, 12/22 isolates studied, belonged to *Porphyromonas gingivalis* and *Porphyromonas asaccharolytica/Porphyromonas uenonis*, but further analysis evidenced that they were distributed among six different species. The analysis of the 16S rRNA sequences for five isolates from this complex (L19, A61, A62, A68 y A69) showed the highest identity with *P. asaccharolytica*

(Table 4). Moreover, the analysis of the 16S rRNA sequences was needed to identify 3/22 isolates that corresponded to *Porphyromonas endodontalis*, *Porphyromonas somerae* and *Porphyromonas bennonis*. Of these, *P. endodontalis* and *P. somerae* were correctly identified using the Bruker Biotyper system. Although *P. bennonis* is represented in the Bruker Biotyper database, the isolate CEM24 was not identified. It should be noted that there is only one MSP of this species. Seng et al. previously reported that poor bacterial identification is mostly due to an insufficient number of spectra in the database[63]. However, for other several species, low database sampling does not interfere with a good identification level[36]. Isolates L13, L19 and L50 corresponding to *P. asaccharolytica* by 16S rRNA sequences displayed a very low score (<1.5) for *P. asaccharolytica* in the top ten identification using the Bruker Biotyper system (Table 3). On the other hand, isolates CEM25 and A34 that were not identified by the MALDI-TOF MS systems corresponded to unidentifiable taxa by their 16S rRNA sequence analysis, being suggestive of a new species; however, more studies are necessary to classify these isolates.

The literature reported that the identification of *Porphyromonas* spp. by MALDI-TOF MS exhibited problems at both the species and genus levels. Vega-Castaño et al. showed that none of the 10 isolates of *P. asaccharolytica* studied could be identified by MALDI-TOF MS, although a different database version was used (version 2.0)[72]. In another study of Rodríguez-Sánchez et al., none of the two isolates (*P. somerae* and *P. asacharolytica*/*P. uenonis*) could be identified by MALDI-TOF MS[57]. In a recent analysis, Alcalá et al. included 14 isolates and only *P. somerae* and *P. gingivalis* could reach the identification at the species level by MALDI-TOF MS[2]. The difference in the identification performance by both authors could be explained by the number of MSP entries in the database (Rodríguez-Sánchez et al., 5627 MSP entries vs Alcalá et al., 9234 MSP entries).

Among the rare species detected in this order, one isolate was identified as *Gabonibacter massiliensis* and the other corresponded to *Tidjanibacter massiliensis*.

G. massiliensis was correctly identified by the Bruker Biotyper system and confirmed by the 16S rRNA analysis. However, both MALDI-TOF MS failed to identify *T. massiliensis* and only the 16S rRNA analysis allowed it. According to LPSN, the *Tidjanibacter* genus belongs to the *Rikenellaceae* family and contains only one species published in 2017 from the human colon, which was phylogenetically closest to *Alistipes putredinis* (divergence of >5%)[43]. MALDI-TOF MS MSP of *T. massiliensis* is available online (http://www. mediterranee infection.com/article.php?laref=256&titre=urmsdatabase); however, it was not included in the Vitek and Bruker Biotyper databases.

- Class *Fusobacteriia*. Order *Fusobacteriales*

Identification at the species level was achieved in 14/16 and 13/16 isolates using Vitek MS and Bruker Biotyper, respectively. One out of two isolates, L36, which could not be identified at the species level by Vitek MS corresponded to *Fusobacterium canifelinum*, a species not included in its database (Tables 2 and 3). Interestingly, the L36 isolate showed the top ten identification score with *F. canifelinum* using Bruker Biotyper; however, the species level could not be assigned since the score was <1.7. The other two isolates did not achieve the proper score for species level identification using Bruker Biotyper: *Fusobacterium nucleatum*, score 1.53, and *Fusobacterium gonidiaformans*, score 1.59. However, these species were confirmed by the 16S rRNA analysis (Table 4).

The difficulty of identifying *Fusobacterium* spp. by MALDI-TOF MS has been previously reported. On the other hand, for one of the most common species *F. nucleatum*, the 16S rRNA sequences showed divergences between 0.6% and 1.9%, defining it as a highly heterogeneous species[2,23,63].

- Class *Epsilonproteobacteria*. Order *Camkpylobacterales* and
- Class *Negativicutes*. Order *Veillonellales*

The identification of *Campylobacter* spp. (n: 11) and *Dialister* spp. (n: 5) was correctly achieved by both MALDI-TOF MS systems.

- Class *Deltaproteobacteria*. Order *Desulfovibrionales*

Two isolates corresponded to the genus *Bilophila* using 16S rRNA sequencing, which were correctly identified at the genus level using the Bruker Biotyper system, but only one at the species level, as the score was <1.7. Using the Vitek MS system, one isolate was assigned as *Bilophila wadsworthia*, but the remaining one was not identified. In a recent study, Alcalá et al. also reported reliable identification for this genus by MALDI-TOF MS[2]. One isolate recognized as *Desulfovibrio desulfuricans* was correctly identified by both systems.

- Rare species

Among GNB, only the Bruker Biotyper system correctly identified isolates that corresponded to *Pyramidobacter piscolens* (n: 2) which belongs to class *Synergistia* and order *Synergistales*[19], as well as *Fenollaria massiliensis* (n: 2) isolates from class *Clostridia* and order *Eubacteriales*. Boiten et al. have pointed out the importance of adding more spectra of less common species such as *F. massiliensis* to the MALDI-TOF MS databases to gain insight into their clinical relevance[10].

Two isolates of GNB have only been identified by the 16S rRNA analysis. One isolate corresponded to class *Betaproteobacteria* and order *Neisseriales* and has been identified as *Eikenella longinqua*. Until 2020, the genus *Eikenella* contained a single species, *Eikenella corrodens*, which belongs to the HACEK (*Haemophilus*, *Aggregatibacter*, *Cardiobacterium*, *Eikenella* and *Kingella*) group considered as miscellaneous or fastidious gram negative facultative anaerobic bacteria. Recently, the emendation of this genus included three new species of strict anaerobic GNB: *E. longinqua*, *Eikenella halliae* and *Eikenella exigua*[9].

The other one was identified as *Casaltella massiliensis*. This species was mentioned by La Scola et al. in an infected lipectomy as a small gram negative bacillus, indole positive, whose sequence was introduced in Genbank in 2017 (Accession Number HM587320)[36]. Although, *C. massiliensis* is not validated nor included in LPSN.

- Identification of gram positive bacilli (GPB) isolates

The GPB corresponded to five classes and eight orders. Out of 110 GPB isolates, 75 (68.2%) and 80 (72.7%) were identified at the species and genus levels, respectively, using the Vitek MS system. Using the Bruker Biotyper system, high performances were observed, as 98 (89.1%) and 101 (91.8%) of GBP were identified at the species and genus levels, respectively. The differences in performances for species and genus, were statistically significant for both systems, with respective *p*-values of 0.0003 and 0.0004.

Thirty-three isolates (30%) were not identified at the species level and two isolates (1.8%) were misidentified using the Vitek MS system. Meanwhile, 12 isolates (10.9%) were not identified and there were no misidentified isolates using the Bruker Biotyper system (Tables 2 and 3). In GPB identification, the main trouble was related to the lack of spectra in the databases, which was more evident using the Vitek MS system.

- Class *Clostridia*. Order *Eubacteriales*.
- Family *Clostridiaceae*

With regard to *Clostridium* spp., four isolates were not identified using the Vitek MS system that corresponded to *Clostridium tunisiense*, *Clostridium symbiosum*, *Clostridium argentinense*, and *Clostridium hydrogeniformans*, which have not been included in the database. Isolate M55 identified as *C. argentinense* corresponded to *Clostridium subterminale* by the Bruker Biotyper system. Suen et al. described this species as the first toxigenic strain isolated from Argentinian soil, and they mentioned it as a genetically homogeneous group of strains previously identified as *C. subterminale*[67]. Therefore, identification using the Bruker Biotyper system was considered correct to the species level for this isolate. On the other hand, the Bruker Biotyper system failed to identify *C. hydrogeniformans*, since its database does not include it. Additionally, this system failed in the identification of one isolate of *Clostridium sporogenes* despite being part of the database. *C. hydrogeniformans* as well as *C. tunisiense* were described from chlorinated solvent-contaminated groundwater and olive mill wastewater, respectively, but they were not described in human samples[11,68].

Both systems were efficient in the identification of *Clostridioides difficile*, the most common nosocomial pathogen in antibiotic-related diarrhea into health care facilities[16].

Furthermore, the five *Paeniclostridium sordellii* (former *Clostridium sordellii*) isolates were correctly identified by the two platforms.

The data obtained from the whole genome sequencing entailed taxonomic changes in the genus *Clostridium*, which was reclassified into two separate clades. One clade which includes *Clostridium clostridioforme*, *Clostridium aldenense* and *Clostridium bolteae*, which was reclassified as *Enterocloster* gen. nov., and another clade that comprises *Clostridium sphenoides*, *Clostridium amygdalinum* and *Clostridium celerecrescens*, which was reclassified as *Lacrimispora* gen. nov.[28]. Isolate G69, identified by 16S rRNA sequencing as *Lacrimispora celerecrescens* could not be identified by the Vitek MS system, since it is not included in the database. Instead, the Bruker Biotyper system identified it as *C. celerecrescens*/*C. sphenoides*. A unique identification could not be assigned because the difference in scores between them did not exceed 10%. These two species display the closest relationship between them (98.1% identity). In a recent case of chronic osteomyelitis by *C. sphenoides*, several discrepancies in identification using MALDI-TOF MS and 16S rRNA sequencing were observed. Whole genome sequencing was necessary to solve it[51]. In summary, we suggest considering these two species as a complex when MALDI-TOF MS is used as the identification method.

Neither the Vitek MS nor Bruker Biotyper databases included the species *Lacrimispora amygdalina* (or *C. amygdalinum*); therefore, they failed in the identification at the species or genus levels.

With respect to species that were isolated in small numbers in this study, one isolate of *Robinsoniella peoriensis* was correctly identified by both MALDI-TOF MS, since this species is included in both databases. Only a few cases were published in which *R. peoriensis* was identified as the cause of an infection. Schrottner et al. described the detection of *R. peoriensis* in multiple bone samples of a trauma patient. The bacterium could only be identified using 16S rRNA sequencing, since the results by MALDI-TOF MS system gave a score below 1.7, which could not be considered as secure identification at the species level. They used the Bruker Biotyper database which contains 7854 reference spectra (version 8.0), which is an older version than the one that was used in the present study[62].

Veloo et al. recently validated the use of the Bruker MALDI-TOF MS database, by using a large set of anaerobic strains isolated from human clinical specimens where 4 isolates of *C. aerofaciens* were identified with score $\geq$2.0[75]. In contrast to our study, Lee et al. could not identify the only one isolate of *C. aerofaciens* using the Vitek MS system, since they used an older version[38].

Two isolates of *Eubacterium* spp. were included in this study and were both identified by the Vitek MS system, but only one was identified by the Bruker Biotyper system. When 16S rRNA sequencing was performed, in the C37 isolate (Table 4) we observed that both species, *Eubacterium callanderi* and *Eubacterium limosum*, could not be differentiated using this gene sequence, as they share more than 99% identity. Therefore, in this case, the correct identification might be considered as *E. callanderi*/*E. limosum* complex. Li et al. reported that the identification accuracy of MALDI-TOF MS was 57% for *Eubacterium* spp.[40].

The genera *Moryella* and *Filifactor* are only included in the Bruker Biotyper database, therefore, the species *Moryella indoligenes* (n: 2 isolates) and *Filifactor alocis* (n: 1) were only identified using this system and later confirmed by 16S rRNA gene sequencing.

An isolate that could not be identified by either of the two MALDI-TOF MS systems, was identified by 16S rRNA gene sequencing as *Criibacterium bergeronii*. This novel species was recently described in 2021 from a vaginal sample of a woman with bacterial vaginosis, and has not been included in these databases yet[42].

- Class *Actinomycetes*.
- Order *Propionibacteriales*

All the isolates (n: 13) that belong to *Cutibacterium* spp., formerly *Propionibacterium* spp., were correctly identified by both MALDI-TOF MS systems. Traditionally, the species within this genus were grouped as either classical or cutaneous propionibacteria. This group that used to comprise the species *Propionibacterium*. *Propionibacterium acnes*, *Propionibacterium avidum* and *Propionibacterium granulosum* were accommodated into the genus *Cutibacterium* gen. nov. by Scholz and Killian[61]. Peel et al., highlighted the ability of MALDI-TOF MS to quickly identify this kind of gram positive bacilli, meaning an important tool to assess their clinical significance[50].

Only one isolate from a patient with a urinary tract infection was identified as *Propionimicrobium lymphophylum* by the Bruker Biotyper system and confirmed with 16S rRNA sequencing; however, it could not be identified by Vitek MS, as it is not present into the database. This genus was created to accommodate *P. lymphophylum*, a species that is a rarely encountered anaerobic gram positive non-spore forming rod that might be an emergent uropathogen[66,78].

- Order *Bifidobacteriales*

Regarding the order *Bifidobacteriales*, *Bifidobacterium*, is one of the genera that includes the largest number of species, either facultative or strict anaerobic. All isolates, *Bifidobacterium dentium* (n: 2), *Bifidobacterium breve* (n: 2) and *Bifidobacterium scardovii* (n: 1), were correctly identified at the species level by the Bruker Biotyper system. However, when using Vitek MS, they could only be identified at the genus level as *Bifidobacterium* spp. Moreover, the Vitek MS database manual mentions that for the species *Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, *B. breve*, *B. dentium*, and *Bifidobacterium longum*, the system will identify them as *Bifidobacterium* spp. Only one *B. scardovii* isolate could not be identified, although this species is included in the Vitek MS database.

- Order *Actinomycetales*

The order *Actinomycetales* does not include strict anaerobic species, but since they are fastidious and slow growing species in the aerobic atmosphere, they were included in our study. *Actinomyces* (n: 3) and *Actinotignum* (n: 1) isolates were correctly identified by both MALDI-TOF MS systems.

Barberis et al. demonstrated high efficacy in the identification at the species level in these bacteria using Bruker Biotyper MALDI-TOF MS[6].

- Class *Coriobacteriia*.
- Order *Eggerthellales*

Eight isolates of *Eggerthella lenta* (first described in 1935 as *Eubacterium lentum* by Arnold Eggerth) were included[20]. This bacterium was characterized in more detail through genetic analysis in 1999, placing it in its distinct genus[76]. Three isolates could not be identified by the Vitek MS system, and two isolates could not be identified by the Bruker Biotyper system, although this species is included in both databases. Conversely, Alcalá et al. recently reported, in a four-year experience in MALDI-TOF MS identification of anaerobic bacteria, one of the largest studies about *E. lenta* isolates (n: 71), and most of them (n: 66) were correctly identified at the species level[2].

The complete genomic sequence of a relatively new closely related species, *Paraeggerthella hongkongensis*, was published in 2009, but it was not included in the MALDI-TOF MS systems[79]. Thus, the only isolate included in our study was not identified. This species was also involved in bacteremia cases, similar to *E. lenta*[37]. Five isolates of *Slackia exigua* were included and were correctly identified at the species level by Bruker Biotyper, only three of them were identified at the species level using the Vitek MS system. Li et al. observed that the identification accuracy of MALDI-TOF MS against the *Slackia* genus was 83%[40].

- Order *Coriobacteriales*

Eight isolates of *Atopobium* spp. were included in this study. The two isolates that were identified as *Atopobium minutum* by 16S rRNA gene sequencing were not identified by the Vitek MS system, as this species is not included in the database, and only one isolate was correctly identified at the species level by the Bruker Biotyper system. The five isolates of *Atopobium rimae* (n: 2) and *Atopobium parvulum* (n: 3) agreed in the identification by both systems. On the other hand, *Atopobium vaginae* (n: 1) was correctly identified only by the Bruker Biotyper system even though this species is included in Vitek MS system database. Recently Nouioui et al. reported changes based on the genome taxonomic classification that placed the species *A. rimae* and *A. parvulum* into the genus *Lancefieldella*, and *A. vaginae* into the genus *Fannyhessea* being the correct names *Lancefieldella rimae*, *Lancefieldella parvula and Fannyhessea vaginae*, respectively[47].

With regard to species that were isolated in small numbers in this study, one isolate of *Collinsella aerofaciens* was correctly identified by both MALDI-TOF MS systems, as this species is included in both databases.

The *Olsenella* genus is only included in the Bruker Biotyper database; therefore, *Olsenella uli* (n: 1) included in this study, was only identified at the species level using this system and then confirmed by 16S rRNA sequencing.

- Class *Erysipelotrichia*. Order *Erysipelothricales*

Isolates from the genera *Eggerthia* and *Solobacterium* were analyzed. We observed that three isolates of *Eggerthia catenaformis*, were correctly identified by both MALDI-TOF MS systems, as these species are included in both databases. *E. catenaformis* (formerly known as *Lactobacillus catenaformis*) is a member of the human fecal microbiota, rarely associated with human infections and reassigned to the *Eggerthia* genus by Salvetti et al. in 2011[60]. Foronda et al. reported the second case of bacteremia due to this organism, which could be identified using MALDI-TOF MS[25].

Four isolates of *Solobacterium moorei*, the only species included in the genus, were evaluated. As we observed with other anaerobes, *S. moorei* is an example of the differences that exist between currently available databases, as it is absent from the Vitek MS database. Alauzet et al. carried out a retrospective analysis of 27 cases of infection involv-

ing *S. moorei* that had to be identified by 16S rRNA gene sequencing because they used the Vitek MS system[1].

○ Class *Tissierellia*. Order *Tissierellales*

The *Tissierella* genus is included in the Bruker Biotyper database, but not in that of the Vitek MS. Therefore, the two *Tissierella praeacuta* isolates included in this study were only identified at the species level using the Bruker Biotyper system, and then confirmed by 16S rRNA sequencing.

Veloo et al. validated the Bruker database optimized for anaerobic bacteria, including strains of species less commonly encountered in human infections. They observed that the addition of more spectra optimized the database and improved identification with higher confidence[75]. Undoubtedly, Vitek MS should expand its database to include less frequently isolated species as this would allow to gain insight into the clinical relevance of these less common anaerobic bacteria.

- Identification of gram positive cocci (GPC) isolates

Most of the GPC belong to the class *Clostridia*, order *Eubacteriales* with the genera *Peptoniphilus* spp., *Anaerococcus* spp., *Finegoldia* sp., *Peptostreptococcus* spp., *Parvimonas* sp., *Murdochiella* spp., *Fastidiosipila* sp., *Ruminococcus* sp., and *Lagierella* sp. Additionally, two isolates of class *Bacteria*, order *Caryophanales* were included. Eighty-eight percent (66/75) and 82.7% (62/75); 65.3% (49/75) and 54.7% (41/75) of the GPC were identified at the genus level and species level using the Bruker Biotyper system and the Vitek MS system, respectively. The differences in the performances of both systems for genus and species, were statistically significant, with respective *p*-values of 0.002 and 0.0004.

○ Class *Clostridia*. Order *Eubacteriales*.
○ Family *Peptoniphilaceae*

In a recent study conducted by our group, the identification of 18 isolates of *Peptoniphilus* spp. was analyzed and it was demonstrated that the performance of the Bruker Biotyper system outperformed the Vitek MS system for this genus[7]. These results were included in this study (Tables 2 and 4).

Finegoldia is a genus represented by only one species, *Finegoldia magna* (https://lpsn.dsmz.de/genus/finegoldia). It is part of the human normal microbiota; however, it is considered one of the most pathogenic species among anaerobic gram positive cocci, as it displays a variety of virulence factors[41]. All the isolates included in this study (n: 17) were correctly identified at the species level using the Vitek MS system. Bruker Biotyper identified 16 isolates at the species level, as one isolate gave a score <1.7. Alcalá et al. in an extensive study of the identification of anaerobes by MALDI-TOF MS (Bruker) that included 299 isolates of *Finegoldia magna*, showed that most of the isolates (n: 290) were identified with scores ≥2.0, 95 isolates with scores 1.99–1.7, and 13 isolates with scores 1.6–1.69[2]. Therefore, we consider that not only the MALDI-TOF MS is a great tool for the identification of this species, but also lower scores than those recommended by the manufacturer could be considered when the top ten assigns *F. magna*.

Regarding *Anaerococcus* spp., we observed that it was one of the CGP that presented more difficulties in the identification at the species level. At present, nine species are represented in the Bruker Biotyper database (version 10.0) (*Anaerococcus degeneri*, *Anaerococcus hydrogenalis*, *Anaerococcus lactolyticus*, *Anaerococcus murdochii*, *Anaerococcus nagyae*, *Anaerococcus octavius*, *Anaerococcus prevotii*, *Anaerococcus tetradius*, *Anaerococcus vaginalis*), and only three species are represented in the Vitek MS database (*A. prevotii*, *A. tetradius* and *A. vaginalis*). Consequently, all isolates included in this study that belong to the species *A. prevotii*, *A. tetradius* and *A. vaginalis* were correctly identified by the Vitek MS system. However, the other isolates included in the study that belong to species not included in the Vitek MS database (n: 10) were not identified or misidentified. One isolate identified by 16S rRNA gene sequencing as *Anaerococcus jeddhahensis* was misidentified as *A. vaginalis*. Conversely, using the Bruker Biotyper system, six isolates could not be identified, but none were misidentified. These isolates were identified by 16S rRNA gene sequencing as *Anaerococcus urinomassiliensis*, *A. jeddahensis*, *Anaerococcus mediterraneensis*, *Anaerococcus provencensis*[18,44,48]. None of these recently described species have been included in other studies evaluating the performance of MALDI-TOF MS[2]. This highlights the importance of including new species in the database to accurately assess their clinical impact on human infections.

After the reclassification of the species of *Peptostreptococcus* into other several genera, the remaining members were *Peptostreptococcus anaerobius*[21,74] and then, *Peptostreptococcus stomatis*, both isolated from human samples. These two species are included in the Bruker Biotyper database but only one species, *P. anaerobius*, is included in the Vitek MS database. As expected, all *P. anaerobius* isolates included in this study (n: 5) were correctly identified by both MALDI-TOF MS systems, but none of the *P. stomatis* isolates (n: 3) could be identified using the Vitek MS system. One of three *P. stomatis* was identified only at the genus level using the Bruker system, as the score obtained was <1.7. In the study by Alcalá et al., the only species included was *P. anaerobius* and only 2/36 isolates showed a score <1.7[2].

It is known that only one species is included in the genus *Parvimonas*. *Parvimonas micra* has a long-standing presence in nomenclature and its role in human health and disease has been studied to some extent[70]. Recently a new species, *Parvimonas parva* was described. All but one of the isolates included in this study (n: 7) were correctly identified at the species level for both MALDI-TOF MS systems. Alcalá et al. showed, in one of the largest number of isolates (n: 255) included in the evaluation of MALDI-TOF MS performance, that more than 99% were correctly identified at the species level[2].

Two isolates of *Murdochiella* spp. were also included in this study. One of them was identified as *Murdochiella asaccharolytica* by the Bruker Biotyper system and confirmed by 16S rRNA sequencing. It was not identified by the Vitek MS system, as it is not present in its database. The other isolate was not identified by any MALDI-TOF MS system. Its 16S rRNA

analysis corresponded to *Murdochiella vaginalis* (Accession Number LT576397.1).

Fastidiosipila sanguinis, the only species of the genus *Fastidiosipila*, is currently not included in the MALDI-TOF database for the Vitek MS system, but has just been included in the Bruker Biotyper database revision no. 10. For the two isolates included in this study, identification using 16S rRNA sequencing resulted in species identification with more than 99.9%. So far, in the literature, the few published cases that involved *F. sanguinis* infection reported the same results[22,29].

For species of which only one strain was encountered, we found one isolate of *Ruminococcus gnavus*. This species is represented in both databases and therefore was correctly identified by both MALDI-TOF MS systems. Fontanals et al. were able to identify *R. gnavus* using the Bruker Biotyper system with a score >2.0 from blood culture[24].

Hansen et al. initially identified two *R. gnavus* bloodstream infections with scores of 1.675 and 1.723 using the Bruker Biotyper software v.3.0 and later obtained scores of 2.181 and 2.124 using software v.3.1. This underlines the importance of keeping databases up to date[30].

Conversely, one isolate of *Lagierella massiliensis* was only identified by 16S rRNA sequencing, as it is not represented in the databases. The MALDI-TOF MS MSP of *L. massiliensis* is only available at http://www.mediterraneeinfection.com/article.php?laref=256&titreurms-database[71].

○ Class *Bacilli*. Order *Caryophanales*

Staphylococcus saccharolyticus is a rarely encountered coagulase-negative cocci and is the only strictly anaerobic species within the genus *Staphylococcus*[77]. The two isolates included in this study were correctly identified at the species level using the Bruker Biotyper system; however, although this species is also included in the Vitek database, only one of the isolates was identified by Vitek MS.

- Identification of gram negative cocci (GNC) isolates

The GNC corresponded to class *Negativicutes* and the orders *Acidaminococcales* and *Veillonellales*, which include the most frequently described genera in the literature.

Regarding the order *Veillonellales*, two isolates of *Veillonella atypica* and one isolate of *Veillonella parvula* were correctly identified at the species level by both MALDI-TOF MS systems. Accordingly, Alcalá et al. showed that 99.5% of the GNC included in their study, mostly *Veillonella* species, were correctly identified at the species level[2]. However, Veloo et al. have pointed out that several species of *Veillonella*, such as *Veillonella dispar*, *V. parvula*, *Veillonella denticariosi* and *Veillonela rogosae*, are difficult to separate by MALDI-TOF MS and 16S rRNA gene sequencing; therefore, any of these four species should be named as *Veillonella* spp.[75]. Further research into the identification of *Veillonella* using MALDI-TOF MS is necessary.

Negativicoccus spp. could not be identified by Vitek MS, as these species are not included in the database. The only included isolate of *Negativicoccus succinivorans* was correctly identified by Bruker Biotyper.

With regard to the order *Acidaminococcales*, neither of the *Acidaminococcus* spp. could be identified by Vitek MS, since they are not included into its database. Two out of three *Acidaminococcus intestini* isolates were correctly identified at species level by Bruker Biotyper, and all of them were confirmed by 16S rRNA gene sequencing.

Conclusions

MALDI-TOF MS systems speed up microbial identification and are especially effective for slow-growing microorganisms, such as anaerobic bacteria, which are difficult to identify by traditional methods.

When assessing GNBs, isolates of *Bacteroides* spp., the most frequent GNB found in clinical laboratories, were correctly identified by both systems. However, the GNBs that were not reliably identified mainly corresponded to the *Porphyromonas* genus, followed by the *Prevotella* genus. The species most frequently isolated within GPBs, *Clostridium*/*Clostridioides*, were correctly identified by both systems. However, Bruker showed advantages in specific species within this genus, as they were absent in the Vitek MS database. Concerning CGPs, *Anaerococcus* spp. was the genus that presented more difficulties in the identification at the species level for both systems. Misidentification observed in *Peptoniphilus* spp. by the Vitek MS system was mainly attributed to its failure to identify *P. harei*.

In summary, the Bruker system was more accurate than the Vitek system. In order to be truly effective, it is essential to update the databases of both systems by increasing the number of each MSP within their platforms.

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Conflict of interest

None declared.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ram.2023.12.001.

References

1. Alauzet C, Aujoulat F, Lozniewski A, Ben Brahim S, Domenjod C, Enault C, Lavigne JP, Marchandin H. A new look at the genus *Solobacterium*: a retrospective analysis of twenty-seven cases of infection involving *S. moorei* and a review of sequence databases and the literature. Microorganisms. 2021;9:1229.
2. Alcalá L, Marín M, Ruiz A, Quiroga L, Zamora-Cintas M, Fernández-Chico MA, Muñoz P, Rodríguez-Sánchez B. Identifying anaerobic bacteria using MALDI-TOF mass spectrometry: a four-year experience. Front Cell Infect Microbiol. 2021;11:521014.
3. Anani H, Guilhot E, Andrieu C, Fontanini A, Raoult D, Fournier PE. *Prevotella ihumii* sp. nov., a new bacterium isolated from a stool specimen of a healthy woman. New Microbes New Infect. 2019;32:100607.

4. Bachli P, Baars S, Simmler A, Zbinden R, Schulthess B. Impact of MALDI-TOF MS identification on anaerobic species and genus diversity in routine diagnostics. Anaerobe. 2022;75:102554.
5. Barba MJ, Fernández A, Oviaño M, Fernández B, Velasco D, Bou G. Evaluation of MALDI-TOF mass spectrometry for identification of anaerobic bacteria. Anaerobe. 2014;30:126–8.
6. Barberis C, Almuzara M, Join-Lambert O, Ramirez MS, Famiglietti A, Vay C. Comparison of the Bruker MALDI-TOF mass spectrometry system and conventional phenotypic methods for identification of Gram-positive rods. PLoS One. 2014;9:e106303.
7. Barberis C, Litterio M, Venuta ME, Maldonado ML, Abel S, Fernández-Canigia L, Vaustat D, Azula N, Castello L, Legaria MC, Pereyra A, Rossetti A, Predari SC, Rollet R, Cejas D. The dilemma of identifying *Peptoniphilus* species by using two MALDI-TOF MS systems. Anaerobe. 2022;73:102500.
8. Barreau M, Pagnier I, La Scola B. Improving the identification of anaerobes in the clinical microbiology laboratory through MALDI-TOF mass spectrometry. Anaerobe. 2013;22:123–5.
9. Bernard KA, Burdz T, Wiebe D, Bernier AM. Description of *Eikenella halliae* sp. nov. and *Eikenella longinqua* sp. nov., derived from human clinical materials, emendation of *Eikenella exigua* Stormo et al., 2019 and emendation of the genus *Eikenella* to include species which are strict anaerobes. Int J Syst Evol Microbiol. 2020;70:3167–78.
10. Boiten KE, Jean-Pierre H, Veloo ACM. Assessing the clinical relevance of *Fenollaria massiliensis* in human infections, using MALDI-TOF MS. Anaerobe. 2018;54:240–5.
11. Bowman KS, Dupre RE, Rainey FA, Moe WM. *Clostridium hydrogeniformans* sp. nov. and *Clostridium cavendishii* sp. nov., hydrogen-producing bacteria from chlorinated solvent-contaminated groundwater. Int J Syst Evol Microbiol. 2010;60:358–63.
12. Clinical and Laboratory Standard Institute. Interpretative criteria for identification of bacteria and fungi by DNA target sequencing. Approved standards MM18-A. 1st ed. Wayne, PA: EE.UU.; 2008.
13. Coltella L, Mancinelli L, Onori M, Lucignano B, Menichella D, Sorge R, Raponi M, Mancini R, Russo C. Advancement in the routine identification of anaerobic bacteria by MALDI-TOF mass spectrometry. Eur J Clin Microbiol Infect Dis. 2013;32:1183–92.
14. Conrads G, Claros MC, Citron DM, Tyrrell KL, Merriam V, Goldstein EJC. 16S-23S rDNA internal transcribed spacer sequences for analysis of the phylogenetic relationships among species of the genus *Fusobacterium*. Int J Syst Evol Microbiol. 2002;52:493–9.
15. Culebras E, Rodríguez-Avial I, Betriu C, Gómez M, Picazo JJ. Rapid identification of clinical isolates of *Bacteroides* species by matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry. Anaerobe. 2012;18:163–5.
16. Davies KA, Longshaw CM, Davis GL, Bouza E, Barbut F, Barna Z, Delmee M, Fitzpatrick F, Ivanova K, Kuijper E, Macovei IS, Mentula S, Mastrantonio P, von Muller L, Oleastro M, Petinaki E, Pituch H, Noren T, Novakova E, Nyc O, Rupnik M, Schmid D, Wilcox MH. Underdiagnosis of *Clostridium difficile* across Europe: the european, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea (EUCLID). Lancet Infect Dis. 2014;14:1208–19.
17. Di Conza JA. Aplicaciones de la espectrometría de masas MALDI-TOF MS en la microbiología clínica. Rev Argent Microbiol. 2022;54:163–5.
18. Diop K, Bretelle F, Fournier P-E, Fenollar F. *Anaerococcus mediterraneensis* sp. nov., a new species isolated from human female genital tract. New Microbes New Infect. 2017;17:75–6.
19. Downes J, Vartoukian SR, Dewhirst FE, Izard J, Chen T, Yu WH, Sutcliffe IC, Wade WG. *Pyramidobacter piscolens* gen. nov., sp. nov., a member of the phylum *Synergistetes* isolated from the human oral cavity. Int J Syst Evol Microbiol. 2009;59:972–80.
20. Eggerth AH. The gram-positive non-spore-bearing anaerobic bacilli of human feces. J Bacteriol. 1935;30:277–99.
21. Ezaki T, Kawamura Y, Li N, Li ZY, Zhao L, Shu S. Proposal of the genera *Anaerococcus* gen. nov., *Peptoniphilus* gen. nov. and *Gallicola* gen. nov. for members of the genus *Peptostreptococcus*. Int J Syst Evol Microbiol. 2001;51:1521–8.
22. Falsen E, Collins MD, Welinder-Olsson C, Song Y, Finegold SM, Lawson PA. *Fastidiosipila sanguinis* gen. nov., sp. nov., a new gram-positive, coccus-shaped organism from human blood. Int J Syst Evol Microbiol. 2005;55:853–8.
23. Fedorko DP, Stock F, Murray PR, Drake SK. Identification of clinical isolates of anaerobic bacteria using matrix-assisted laser desorption ionization-time of flight mass spectrometry. Eur J Clin Microbiol Infect Dis. 2012;31:2257–62.
24. Fontanals D, Larruzea A, Sanfeliu I. Direct identification of *Ruminococcus gnavus* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) on a positive anaerobic blood culture bottle. Anaerobe. 2018;54:264–6.
25. Foronda C, Calatrava E, Casanovas I, Martin-Hita L, Navarro-Mari JM, Cobo F. *Eggerthia catenaformis* bacteremia in a patient with an odontogenic abscess. Anaerobe. 2019;57:115–6.
26. Garner O, Mochon A, Branda J, Burnham C, Bythrow M, Ferraro M, Ginocchio C, Jennemann R, Manji R. Multi-centre evaluation of mass spectrometric identification of anaerobic bacteria using the VITEK MS system. Clin Microbiol Infect. 2013;20:335–9.
27. Gursoy M, Harju I, Matomaki J, Bryk A, Kononen E. Performance of MALDI-TOF MS for identification of oral *Prevotella* species. Anaerobe. 2017;47:89–93.
28. Haas KN, Blanchard JL. Reclassification of the *Clostridium clostridioforme* and *Clostridium sphenoides* clades as *Enterocloster* gen. nov. and *Lacrimispora* gen. nov., including reclassification of 15 taxa. Int J Syst Evol Microbiol. 2020;70:23–34.
29. Hansen K, Løfberg SV, Nielsen DK, Kobberø H, Justesen US. Bacteremia with *Moryella indologenes* and *Fastidiosipila sanguinis*: a case report. Access Microbiol. 2020;2, acmi000108.
30. Hansen SG, Skov MN, Justesen US. Two cases of *Ruminococcus gnavus* bacteremia associated with diverticulitis. J Clin Microbiol. 2013;51:1334–6.
31. Hiippala K, Barreto G, Burrello C, Diaz-Basabe A, Suutarinen M, Kainulainen V, Bowers JR, Lemmer D, Engelthaler DM, Eklund KK, Facciotti F, Satokari R. Novel *Odoribacter splanchnicus* strain and its outer membrane vesicles exert immunoregulatory effects in vitro. Front Microbiol. 2020;11:575455.
32. Hsu YM, Burnham CA. MALDI-TOF MS identification of anaerobic bacteria: assessment of pre-analytical variables and specimen preparation techniques. Diagn Microbiol Infect Dis. 2014;79:144–8.
33. Jamal WY, Shahin M, Rotimi VO. Comparison of two matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry methods and API 20AN for identification of clinically relevant anaerobic bacteria. J Med Microbiol. 2013;62:540–4.
34. Justesen US, Holm A, Knudsen E, Andersen LB, Jensen TG, Kemp M, Skov MN, Gahrn-Hansen B, Moller JK. Species identification of clinical isolates of anaerobic bacteria: a comparison of two matrix-assisted laser desorption ionization-time of flight mass spectrometry systems. J Clin Microbiol. 2011;49:4314–8.
35. Kim H, Im WT, Kim M, Kim D, Seo YH, Yong D, Jeong SH, Lee K. *Parabacteroides chongii* sp. nov., isolated from blood of a patient with peritonitis. J Microbiol. 2018;56:722–6.
36. La Scola B, Fournier PE, Raoult D. Burden of emerging anaerobes in the MALDI-TOF and 16S rRNA gene sequencing era. Anaerobe. 2011;17:106–12.

37. Lau SK, Woo PC, Woo GK, Fung AM, Wong MK, Chan KM, Tam DM, Yuen KY. *Eggerthella hongkongensis* sp. nov. and *Eggerthella sinensis* sp. nov., two novel *Eggerthella* species, account for half of the cases of *Eggerthella* bacteremia. Diagn Microbiol Infect Dis. 2004;49:255–63.
38. Lee W, Kim M, Yong D, Jeong SH, Lee K, Chong Y. Evaluation of VITEK mass spectrometry (MS), a matrix-assisted laser desorption ionization time-of-flight MS system for identification of anaerobic bacteria. Ann Lab Med. 2015;35:69–75.
39. Lévesque S, Dufresne PJ, Soualhine H, Domingo MC, Bekal S, Lefebvre B, Tremblay C. A side by side comparison of Bruker Biotyper and VITEK MS: utility of MALDI-TOF MS technology for microorganism identification in a Public Health Reference Laboratory. PLoS One. 2015;10:1–21.
40. Li Y, Shan M, Zhu Z, Mao X, Yan M, Chen Y, Zhu Q, Li H, Gu B. Application of MALDI-TOF MS to rapid identification of anaerobic bacteria. BMC Infect Dis. 2019;19:941.
41. Litterio Burki M, Rollet R. Cocos anaerobios. Cocos anaerobios gram positivos y negativos Parte IIIb. In: Lopardo H, Predari SC, Vay CA, editors. Manual de Microbiologia Clínica, vol. I. Bacterias de importancia clínica. AAM; 2016. p. 6–41.
42. Maheux AF, Boudreau DK, Abed JY, Berube E, Brodeur S, Bernard KA, Hashimi A, Ducrey E, Guay EF, Raymond F, Corbeil J, Domingo MC, Roy PH, Boissinot M, Tocheva EI, Omar RF. *Criibacterium bergeronii* gen. nov., sp. nov., a new member of the family *Peptostreptococcaceae*, isolated from human clinical samples. Int J Syst Evol Microbiol. 2019;71:004691.
43. Mailhe M, Ricaboni D, Benezech A, Lagier JC, Fournier PE, Raoult D. *Tidjanibacter massiliensis* gen. nov., sp. nov., a new bacterial species isolated from human colon. New Microbes New Infect. 2017;17:21–2.
44. Morand A, Tall ML, Kuete Yimagou E, Ngom II, Lo CI, Cornu F, Tsimaratos M, Lagier JC, Levasseur A, Raoult D, Fournier PE. *Anaerococcus urinimassiliensis* sp. nov., a new bacterium isolated from human urine. Sci Rep. 2021;11:2684.
45. Nagy E, Becker S, Kostrzewa M, Barta N, Urban E. The value of MALDI-TOF MS for the identification of clinically relevant anaerobic bacteria in routine laboratories. J Med Microbiol. 2012;61:1393–400.
46. Nagy E, Maier T, Urban E, Terhes G, Kostrzewa M, Nord CE, Hedberg M, Könönen E, Dubreuil L, Dosa E, Kalenic S, Piérard D, Degener J, Wildeboer-Veloo A, Chmelarova E, Mazzariol A, Gürler N, Güner S, Papaparakevas J, Villa J. Species identification of clinical isolates of *Bacteroides* by matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry. Clin Microbiol Infect. 2009;15:796–802.
47. Nouioui I, Carro L, García-López M, Meier-Kolthoff JP, Woyke T, Kyrpides NC, Pukall R, Klenk HP, Goodfellow M, Goker M. Genome-based taxonomic classification of the phylum Actinobacteria. Front Microbiol. 2018;9:2007.
48. Pagnier I, Croce O, Robert C, Raoult D, La Scola B. Non-contiguous finished genome sequence and description of *Anaerococcus provenciensis* sp. nov. Stand Genomic Sci. 2014;9:1198–210.
49. Parker BJ, Wearsch PA, Veloo ACM, Rodríguez-Palacios A. The genus *Alistipes*: gut bacteria with emerging implications to inflammation, cancer, and mental health. Front Immunol. 2020;11:906.
50. Peel TN, Cole NC, Dylla BL, Patel R. Matrix-assisted laser desorption ionization time of flight mass spectrometry and diagnostic testing for prosthetic joint infection in the clinical microbiology laboratory. Diagn Microbiol Infect Dis. 2015;81:163–8.
51. Perkins MJ, Snesrud E, McGann P, Duplessis CA. *Clostridium sphenoides* chronic osteomyelitis diagnosed via matrix-assisted laser desorption ionization time of flight mass spectrometry, conflicting with 16S rRNA sequencing but confirmed by whole genome sequencing. Mil Med. 2017;182:1669–72.
52. Porte L, García P, Braun S, Ulloa MT, Lafourcade M, Montana A, Miranda C, Acosta-Jamett G, Weitzel T. Head-to-head comparison of Microflex LT and Vitek MS systems for routine identification of microorganisms by MALDI-TOF mass spectrometry in Chile. PLoS One. 2017;12:e0177929.
53. Predari SC. Microorganismos anaerobiois. Parte III. In: Lopardo H, Predari SC, Vay CA, editors. Manual de Microbiología Clinica, vol. I. Bacterias de importancia clínica. AAM; 2016.
54. Rahi P, Prakash O, Shouche YS. Matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry (MALDI-TOF MS) based microbial identifications: challenges and scopes for microbial ecologists. Front Microbiol. 2016;7:1359.
55. Ramos LS, Rodloff AC. Identification of *Clostridium* species using the VITEK MS. Anaerobe. 2018;54:217–23.
56. Rocca MF, Almuzara M, Barberis C, Vay C, Vines P, Prieto M. Presentation of the national network for microbiological identification by mass spectrometry website guide for the interpretation of MALDI-TOF MS results. Rev Argent Microbiol. 2020;52:83–4.
57. Rodríguez-Sánchez B, Alcalá L, Marín M, Ruiz A, Alonso E, Bouza E. Evaluation of MALDI-TOF MS (matrix-assisted laser desorption-ionization time-of-flight mass spectrometry) for routine identification of anaerobic bacteria. Anaerobe. 2016;42:101–7.
58. Shannon S, Kronemann D, Patel R, Schuetz AN. Routine use of MALDI-TOF MS for anaerobic bacterial identification in clinical microbiology. Anaerobe. 2018;54:191–6.
59. Sakamoto M, Tanaka Y, Benno Y, Ohkuma M. *Parabacteroides faecis* sp. nov., isolated from human faeces. Int J Syst Evol Microbiol. 2015;65:1342–6.
60. Salvetti E, Felis GE, Dellaglio F, Castioni A, Torriani S, Lawson PA. Reclassification of *Lactobacillus catenaformis* (Eggerth 1935) Moore and Holdeman 1970 and *Lactobacillus vitulinus* Sharpe et al., 1973 as *Eggerthia catenaformis* gen. nov., comb. nov. and *Kandleria vitulina* gen. nov., comb. nov., respectively. Int J Syst Evol Microbiol. 2011;61:2520–4.
61. Scholz CFP, Kilian M. The natural history of cutaneous propionibacteria, and reclassification of selected species within the genus *Propionibacterium* to the proposed novel genera *Acidipropionibacterium* gen. nov., *Cutibacterium* gen. nov. and *Pseudopropionibacterium* gen. nov. Int J Syst Evol Microbiol. 2016;66:4422–32.
62. Schrottner P, Hartwich K, Bunk B, Schober I, Helbig S, Rudolph WW, Gunzer F. Detection of *Robinsoniella peoriensis* in multiple bone samples of a trauma patient. Anaerobe. 2019;59:14–8.
63. Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, Raoult D. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis. 2009;49:543–51.
64. Shin Y, Park SJ, Paek J, Kim JS, Rhee MS, Kim H, Kook JK, Chang YH. *Bacteroides koreensis* sp. nov. and *Bacteroides kribbi* sp. nov., two new members of the genus *Bacteroides*. Int J Syst Evol Microbiol. 2017;67:4352–7.
65. Schmitt BH, Cunningham SA, Dailey AL, Gustafson DR, Patel R. Identification of anaerobic bacteria by Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometry with on-plate formic acid preparation. J Clin Microbiol. 2013;51:782–6.
66. Stackebrandt E, Schumann P, Schaal KP, Weiss N. *Propionimicrobium* gen. nov., a new genus to accomodate *Propionibacterium lymphophilum* (Torrey 1916) Johnson and Cummins 1972 1057AL as *Propionimicrobium lymphophilum* comb. nov. Int J Syst Evol Microbiol. 2002;52:1925–7.
67. Suen JC, Hathegaw CL, Steigerwalt AG, Brenner DJ. *Clostridium argentinense* sp. nov.: a genetically homogeneous group composed of all strains of *Clostridium botulinum* toxin type G and some nontoxigenic strains previously identified as *Clostridium*

subterminale or *Clostridium hastiforme*. Int J Syst Bacteriol. 1988;38:375–81.
68. Thabet OB, Fardeau ML, Joulian C, Thomas P, Hamdi M, Garcia JL, Ollivier B. *Clostridium tunisiense* sp. nov., a new proteolytic, sulfur-reducing bacterium isolated from an olive mill wastewater contaminated by phosphogypse. Anaerobe. 2004;10:185–90.
69. Theel ES, Schmitt BH, Hall L, Cunningham SA, Walchak RC, Patel R, Wengenack NL. Formic acid-based direct, on-plate testing of yeast and *Corynebacterium* species by Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2012;50:3093–5.
70. Tindall BJ, Euzeby JP. Proposal of *Parvimonas* gen. nov. and *Quatrionicoccus* gen. nov. as replacements for the illegitimate, prokaryotic, generic names *Micromonas* Murdoch and Shah 2000, and *Quadricoccus* Maszenan et al., 2002, respectively. Int J Syst Evol Microbiol. 2006;56:2711–3.
71. Traore SI, Khelaifia S, Dubourg G, Sokhna C, Raoult D, Fournier PE. ''*Lagierella massiliensis*'' a new bacterium detected in human feces. New Microbes New Infect. 2016;14:53–5.
72. Vega-Castaño S, Ferreira L, González-Ávila M, Sánchez-Juanes F, García-García MI, García-Sánchez JE, González-Buitrago JM, Muñoz-Bellido JL. Reliability of MALDI-TOF mass spectrometry in the identification of anaerobic bacteria. Enferm Infecc Microbiol Clin. 2012;30:597–601.
73. Veloo AC, Boiten KE, Wekema-Mulder GJ, Rurenga P, Singadji ZM, Scoop GG, van Winkelhoff AJ. Antibiotic susceptibility profiles of *Prevotella* species in The Netherlands. Int J Antimicrob Agents. 2015;45:554–6.
74. Veloo AC, Welling GW, Degener JE. Mistaken identity of *Peptoniphilus asaccharolyticus*. J Clin Microbiol. 2011;49:1189.
75. Veloo ACM, Jean-Pierre H, Justesen US, Morris T, Urban E, Wybo I, Kostrzewa M, Friedrich AW. Validation of MALDI-TOF MS Biotyper database optimized for anaerobic bacteria: the ENRIA project. Anaerobe. 2018;54:224–30.
76. Wade WG, Downes J, Dymock D, Hiom SJ, Weightman AJ, Dewhirst FE, Paster BJ, Tzellas N, Coleman B. The family *Coriobacteriaceae*: reclassification of *Eubacterium exiguum* (Poco et al., 1996) and *Peptostreptococcus heliotrinreducens* (Lanigan 1976) as *Slackia exigua* gen. nov., comb. nov. and *Slackia heliotrinireducens* gen. nov., comb. nov., and *Eubacterium lentum* (Prevot 1938) as *Eggerthella lenta* gen. nov., comb. nov. Int J Syst Bacteriol. 1999;49:595–600.
77. Wang P, Liu Y, Xu Y, Xu Z. *Staphylococcus saccharolyticus* infection: case series with a PRISMA-compliant systemic review. Medicine. 2020;99:e20686.
78. Williams GD. Two cases of urinary tract infection caused by *Propionimicrobium lymphophilum*. J Clin Microbiol. 2015;53:3077–80.
79. Wurdemann D, Tindall BJ, Pukall R, Lunsdorf H, Strompl C, Namuth T, Nahrstedt H, Wos-Oxley M, Ott S, Schreiber S, Timmis KN, Oxley AP. *Gordonibacter pamelaeae* gen. nov., sp. nov., a new member of the *Coriobacteriaceae* isolated from a patient with Crohn's disease, and reclassification of *Eggerthella hongkongensis* Lau et al., 2006 as *Paraeggerthella hongkongensis* gen. nov., comb. nov. Int J Syst Evol Microbiol. 2009;59:1405–15.
80. Wybo I, Soetens O, De Bel A, Echahidi F, Vancutsem E, Vandoorslaer K, Pierard D. Species identification of clinical *Prevotella* isolates by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2012;50:1415–8.
81. Zamoras-Cintas M, Marín M, Quiroga L, Martínez A, Fernández-Chico MA, Bouza E, Rodríguez-Sánchez B. Identification of *Porphyromonas* isolates from clinical origin using MALDI-TOF mass spectrometry. Anaerobe. 2018;54:197–200.
82. Zárate MS, Romano V, Nievas J, Smayevsky J. Utility of MALDI-TOF MS for the identification of anaerobic bacteria. Rev Argent Microbiol. 2014;46:98–102.