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Species Identification of Clinical Isolates of Anaerobic Bacteria: a Comparison of Two Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Systems▽

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We compared two matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) systems (Shimadzu/SARAMIS and Bruker) on a collection of consecutive clinically important anaerobic bacteria ($n = 290$). The Bruker system had more correct identifications to the species level (67.2% versus 49.0%), but also more incorrect identifications (7.9% versus 1.4%). The system databases need to be optimized to increase identification levels. However, MALDI-TOF MS in its present version seems to be a fast and inexpensive method for identification of most clinically important anaerobic bacteria.

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is a fast and inexpensive technology for identification of bacteria. The technique has in a short time been widely adopted and is integrated into many clinical microbiology laboratories. Several papers have reported the advantages and performance of MALDI-TOF MS versus conventional systems (1, 14–16). Although MALDI-TOF MS has revolutionized bacterial identification, there are still some limitations (e.g., the viridans group streptococci), and moreover, there are groups of bacteria which have not been evaluated on a larger scale or in a clinical setting (2). This is partly the case for the anaerobic bacteria. The *Bacteroides fragilis* group has been evaluated by Nagy et al. as part of an antibiotic resistance surveillance study (13). Veloo et al. have evaluated the Gram-positive anaerobic cocci and also compared two MALDI-TOF MS systems on a collection ($n = 79$) of clinically relevant anaerobic bacteria (17, 18). The existing data from these studies were recently reviewed by Veloo et al., the application of MALDI-TOF MS in routine identification of anaerobic bacteria was discussed, and it was concluded that the existing databases need optimizing for routine identification of anaerobic bacteria (19). However, databases are continuously updated, and MALDI-TOF MS represents a fast and inexpensive technology for species identification. Species identification of anaerobic bacteria from serious infections (e.g., blood cultures) is important because information about virulence, potential resistance to certain antimicrobial agents, and primary site of infection can be obtained (5). MALDI-TOF MS can be applied as a first-line identification system without delaying the final identification result. To our knowledge, an evaluation of MALDI-TOF MS in a clinical setting with consecutive clinical isolates, regardless of whether the species included are present in the system databases, has not been performed before.

To evaluate the performance of the Bruker system (Bruker Daltonik, Bremen, Germany) and the Shimadzu/SARAMIS system (Shimadzu Corporation, Kyoto, Japan; and AnagnosTec GmbH, Potsdam-Golm, Germany), a head-to-head comparison of the two systems with 16S rRNA gene sequencing was performed on a collection of consecutive clinically important anaerobic bacteria.

(The preliminary results from this study were presented at the 21st European Congress of Clinical Microbiology and Infectious Diseases, Milan, Italy, 7 to 10 May 2011 [9].)

Odense University Hospital is a 1,300-bed tertiary referral hospital in the Region of Southern Denmark. The hospital department of clinical microbiology offers service to a surrounding area of approximately ½ million inhabitants. Since November 2007, 16S rRNA gene sequencing (MicroSeq 500 system; Perkin-Elmer, Applied Biosystems Division, Foster City, CA) has been applied to pure culture material (originating from blood cultures) from solid media as soon as anaerobic bacteria, including *Lactobacillus* spp. and *Actinomyces* spp., were suspected (8). From November 2008, this strategy has also included pure culture material from other sterile body sites (e.g., the central nervous system, pleura, bones, and joints) and suspected *Fusobacterium* spp. and *Clostridium* spp. from any body site. All isolates identified by 16S rRNA gene sequencing were stored at −80°C.

Identification by 16S rRNA gene sequencing was chosen as the "gold standard" in this study, as it has been shown to be an excellent method for the identification of anaerobic bacteria (8). All isolates identified to the species level with a ≥99% match by 16S rRNA gene sequencing, according to CLSI guideline MM18-A (6), from the period November 2007 to

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October 2010, were included in the study (only one isolate of the same species per patient). Consensus sequences were initially compared with the MicroSeq ID 2.0 500-bp library. If there were no matches at the species level, the sequences were compared with the EzTaxon server, followed by the NCBI BLAST search engine (4). Thus, a total of 278 consecutive clinical isolates, representing a 3-year period, were included in this study. In addition, five United Kingdom National External Quality Assessment Service (NEQAS) and seven American Type Culture Collection (ATCC)/National Collection of Type Cultures (NCTC) isolates were included. The 290 isolates represented 80 species from 27 genera. The isolates were mainly from blood cultures (74.8%), tissue (7.2%), sterile body fluids (6.2%) and pus samples (4.5%). The included ATCC/NCTC isolates were *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, *Bacteroides vulgatus* ATCC 29327, *Clostridium difficile* ATCC 700057, *Clostridium perfringens* ATCC 13124, *Fusobacterium necrophorum* ATCC 25286, and *Peptostreptococcus anaerobius* NCTC 11460. The isolates from NEQAS were *Bacteroides fragilis*, *Clostridium perfringens*, *Fusobacterium periodonticum*, *Peptostreptococcus anaerobius*, and *Propionibacterium acnes* (all confirmed with 16S rRNA gene sequencing).

The isolates were cultured on an anaerobe agar, a modified chocolate agar containing hemin and supplemented with vitamin K and cysteine as the reducing agent (Statens Serum Institut Diagnostica, Copenhagen, Denmark), in batches of approximately 40 (10). Subsequently, the isolates were subcultured on two anaerobe agars for 48 h. One of the anaerobe agars was transported for 45 min in an anaerobic environment to the Department of Clinical Microbiology at Vejle Hospital for analysis with the Bruker system. The other was used for analysis at Odense University Hospital with the Shimadzu/SARAMIS system. One dedicated laboratory technician operated the systems at each hospital.

The systems were operated as described recently in detail by Cherkaoui et al. (3). The colonies were picked from the anaerobe agar and inoculated onto a ground steel MALDI target plate. The Bruker system (comprising a Microflex MALDI-TOF mass spectrometer with Flex Control 3.0 software and the MALDI BioTyper DB Update_V3.1.1.0) was operated with a matrix (1 μl) consisting of a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile–2.5% trifluoroacetic acid (Bruker α-cyano) (Bruker Daltonik, Bremen, Germany). The Shimadzu/SARAMIS system (comprising an Axima Assurance mass spectrometer system [Shimadzu Corporation], with the Shimadzu Biotech Launchpad software program and the SARAMIS database application with the November 2010 update) was operated with two matrices delivered from AnagnosTec, a solution of α-cyano-4-hydroxycinnamic acid in acetonitrile, ethanol, water, and trifluoroacetic acid (Shimadzu/SARAMIS α-cyano) at 1 μl, and a solution of 2,5-dihydroxybenzoic acid in acetonitrile, ethanol, water, and trifluoroacetic acid (DHB) at 0.7 μl. Accordingly, the study compared three different system-matrix combinations. All isolates were run in duplicate. If both scores from the first run were <2.0 with the Bruker system or <80% with the Shimadzu/SARAMIS system, a second run in duplicate was immediately performed with the system-matrix combinations that were below the cutoff score. If the scores were still below the cutoff, the inoculated material was pretreated with 1 μl of a 70% formic acid solution on the target plate and air dried at room temperature before the matrix was applied, and a third run in duplicate was immediately performed. The highest of all the scores was considered the final result, and scores below the cutoff were considered invalid results with the conclusion "no identification". A full extraction protocol was not applied, since the study aimed at keeping hands-on time acceptable for use in a routine clinical microbiology laboratory. Both systems were calibrated immediately prior to analysis with a bacterial test standard according to the manufacturer's instructions. *Escherichia coli* ATCC 8739 was included in duplicate as quality control in each run with both systems and matrices.

Results are presented as number and percentage with the correct species and genus or family-only identification (i.e., results above the specified scores). Also the number and percentage of incorrect identifications, as well as no (invalid) identification (i.e., species included in the system database and species not included in the system database) are presented.

Identification results of the 290 isolates, representing 80 species from 27 genera, included in the study, are presented in Tables 1, 2, and 3. *Bacteroides* (36.9%), *Clostridium* (22.8%), *Fusobacterium* (8.6%), *Propionibacterium* (7.6%), *Lactobacillus* (5.2%), *Actinomyces* (3.1%), and *Veillonella* (2.8%) were the most prominent genera. The Shimadzu/SARAMIS system identified 63.2% (67/106) and 56.1% (37/66) of the *Bacteroides* spp. and *Clostridium* spp. to the species level, whereas the Bruker system identified 84.9% (90/106) and 93.9% (62/66), respectively (Table 1). Depending on the matrix and system, there was a variable number of isolates that were not identified, although the species were included in the databases of the two systems (from 18.3% to 29.3%). There was no uniform pattern, but the metronidazole-resistant Gram-positive rods (*Actinomyces*, *Lactobacillus*, and *Propionibacterium*) were difficult to identify for both systems (Table 1).

The isolates that were not identified and which were not included in the system databases were mainly very rare species or recently described species: e.g., *Robinsoniella peoriensis*, *Solobacterium moorei*, or *Turicibacter sanguinis* (Table 2). With our test collection of anaerobic bacteria, the Bruker system database had the lowest number of species that were not included in the database: 14 versus 31 for the Shimadzu/SARAMIS system.

The higher number of incorrect identifications with the Bruker system mainly consisted of minor errors with the correct genus, but the wrong species: e.g., *Bacteroides dorei* (n = 8) identified as *Bacteroides vulgatus*, *Bacteroides xylanisolvens* (n = 2) identified as *Bacteroides ovatus*, and different *Veillonella* spp. (n = 6) all identified as *Veillonella parvula* (Table 3).

Table 4 summarizes the performance characteristics of the two systems. Of the 176 isolates identified to the species, genus, or family-only level with the Shimadzu/SARAMIS system and the DHB matrix, 90.4% were identified in the first run, another 6.6% were identified in the second run, and the last 3.0% were identified after pretreatment with formic acid. With the α-cyano matrix, the number of identifications was 180, of which 85% were identified in the first run, another 8.9% in the second run, and 6.1% after pretreatment with formic acid. For the Bruker system, the number of identifications was 218, of which 83.5% were identified in the first run, another 9.2% in the

TABLE 1. Species and number of isolates that were identified to the species level or to the genus or family level only or which were not identified

Species (*n*)	No. of isolates identified to species level/genus or family level (no. not identified) by[a]:		
	Shimadzu/SARAMIS		Bruker α-cyano
	DHB	α-Cyano	
Actinomyces europaeus (1)	0/0 (1)	0/0 (1)	0/0 (1)
Actinomyces funkeii (1)			0/0 (1)
Actinomyces graevenitzii (1)	0/0 (1)	0/0 (1)	0/0 (1)
Actinomyces meyeri (3)	0/0 (3)	0/0 (3)	0/0 (3)
Actinomyces odontolyticus (1)	0/0 (1)	0/0 (1)	0/0 (1)
Actinomyces radingae (1)			1/0 (0)
Actinomyces turicensis (1)	0/0 (1)	0/0 (1)	0/0 (1)
Bacteroides caccae (3)	3/0 (0)	3/0 (0)	3/0 (0)
Bacteroides dorei (8)	0/8 (0)	0/8 (0)	
Bacteroides fragilis (60)	47/13 (0)	52/8 (0)	60/0 (0)
Bacteroides nordii (1)			1/0 (0)
Bacteroides ovatus (2)	0/1 (1)	0/2 (0)	2/0 (0)
Bacteroides pyogenes/suis (4)			0/0 (1)[b]
Bacteroides thetaiotaomicron (16)	12/0 (4)	8/0 (8)	16/0 (0)
Bacteroides uniformis (4)	4/0 (0)	4/0 (0)	4/0 (0)
Bacteroides ureolyticus (1)	0/0 (1)	0/0 (1)	0/0 (1)
Bacteroides vulgatus (4)	0/4 (0)	0/4 (0)	4/0 (0)
Bacteroides xylanisolvens (2)	0/2 (0)	0/2 (0)	
Bifidobacterium breve (2)	0/0 (2)	0/0 (2)	0/0 (2)
Bifidobacterium scardovii (2)	0/1 (1)	0/1 (1)	
Bilophila wadsworthia (1)	1/0 (0)	1/0 (0)	
Blautia coccoides (1)			1/0 (0)
Clostridium butyricum (2)	0/0 (2)	0/0 (2)	1/0 (1)
Clostridium cadaveris (1)	0/0 (1)	0/0 (1)	1/0 (0)
Clostridium citroniae (1)			1/0 (0)
Clostridium clostridioforme (2)			2/0 (0)
Clostridium difficile (1)	0/0 (1)	0/0 (1)	1/0 (0)
Clostridium hathewayi (3)			3/0 (0)
Clostridium innocuum (1)			1/0 (0)
Clostridium paraputrificum (5)	0/0 (5)	0/0 (5)	5/0 (0)
Clostridium perfringens (22)	22/0 (0)	22/0 (0)	22/0 (0)
Clostridium ramosum (11)	6/0 (5)	11/0 (0)	8/0 (3)
Clostridium scindens (1)			1/0 (0)
Clostridium septicum (7)	0/0 (7)	1/0 (6)	7/0 (0)
Clostridium sordelli (4)	0/0 (4)	0/0 (4)	4/0 (0)
Clostridium sporogenes (2)	0/0 (2)	0/0 (2)	2/0 (0)
Clostridium tertium (3)	3/0 (0)	3/0 (0)	3/0 (0)
Eggerthella lenta (1)	0/0 (1)	0/0 (1)	1/0 (0)
Finegoldia magna (1)	1/0 (0)	1/0 (0)	1/0 (0)
Fusobacterium naviforme (1)			1/0 (0)
Fusobacterium necrophorum (15)	11/0 (4)	14/0 (1)	15/0 (0)
Fusobacterium nucleatum (7)	0/0 (6)[b]	0/0 (6)[b]	0/0 (7)
Flavonifractor plautii (1)			1/0 (0)
Gemella morbillorum (1)	0/0 (1)	0/0 (1)	0/0 (1)
Lactobacillus catenaformis (1)			1/0 (0)
Lactobacillus fermentum (1)	0/0 (1)	0/0 (1)	0/0 (1)
Lactobacillus paracasei (1)	0/1 (0)	0/1 (0)	1/0 (0)
Lactobacillus rhamnosus (9)	2/0 (7)	5/0 (4)	3/0 (6)
Lactobacillus sakei (1)			1/0 (0)
Lactobacillus salivarius (2)	0/0 (2)	0/0 (2)	2/0 (0)
Parabacteroides distasonis (2)	2/0 (0)	2/0 (0)	2/0 (0)
Parabacteroides merdae (1)	1/0 (0)	0/1 (0)	
Parvimonas micra (1)	0/0 (1)	1/0 (0)	1/0 (0)
Peptoniphilus indolicus (1)	0/0 (1)	0/0 (1)	
Peptostreptococcus anaerobius (3)	3/0 (0)	3/0 (0)	3/0 (0)
Porphyromonas uenonis (1)		0/1 (0)	
Prevotella baroniae (1)	1/0 (0)	1/0 (0)	1/0 (0)
Prevotella bivia (1)	1/0 (0)	0/0 (1)	1/0 (0)
Prevotella buccae (2)	0/0 (2)	0/0 (2)	1/0 (1)
Prevotella nanceiensis (1)			0/0 (1)
Propionibacterium acnes (21)	7/0 (14)	10/0 (10)[b]	3/0 (18)
Propionibacterium avidum (1)			0/0 (1)
Staphylococcus saccharolyticus (1)			0/0 (1)
Tissierella praeacura (1)			1/0 (0)
Veillonella atypica (1)	0/0 (1)	0/0 (1)	1/0 (0)
Veillonella dispar (2)	0/2 (0)	0/2 (0)	
Veillonella rodentium (4)	0/3 (1)	0/4 (0)	

[a] DHB, 2,5-dihydroxybenzoic acid in acetonitrile, ethanol, water, and trifluoroacetic acid; Shimadzu/SARAMIS α-cyano, α-cyano-4-hydroxycinnamic acid in acetonitrile, ethanol, water, and trifluoroacetic acid; Bruker α-cyano, α-cyano-4-hydroxycinnamic acid in 50% acetonitrile–2.5% trifluoroacetic acid.

[b] A number of isolates were incorrectly identified (Table 3).

TABLE 2. Species and number of isolates that were not identified and not included in the database of the systems

Species (*n*)	No. of isolates not identified by[a]:		
	Shimadzu/SARAMIS		Bruker α-cyano (*n* = 19)
	DHB (*n* = 39)	α-Cyano (*n* = 39)	
Actinomyces funkeii (1)	1	1	
Actinomyces radingae (1)	1	1	
Bacteroides denticanoris (1)	1	1	1
Bacteroides faecis (1)	1	1	
Bacteroides nordii (1)	1	1	
Bacteroides pyogenes/suis (4)	4	4	
Bifidobacterium infantis (1)	1	1	
Bifidobacterium scardovii (2)			2
Bilophila wadsworthia (1)			1
Blautia coccoides (1)	1	1	
Clostridium citroniae (1)	1	1	
Clostridium clostridioforme (2)	2	2	
Clostridium hathewayi (3)	3	3	
Clostridium innocuum (1)	1	1	
Clostridium scindens (1)	1	1	
Desulfovibrio desulfuricans (1)	1	1	1
Dialister pneumosintes (1)	1	1	1
Flavonifractor plautii (1)	1	1	
Fusobacterium gonidiaformans (1)	1		1
Fusobacterium naviforme (1)	1	1	
Fusobacterium periodonticum (1)	1	1	1
Lactobacillus catenaformis (1)	1	1	
Lactobacillus sakei (1)	1	1	
Leptotrichia trevisanii (1)	1	1	1
Parabacteroides merdae (1)			1
Porphyromonas uenonis (1)			1
Prevotella nanceiensis (1)	1	1	
Prevotella timonensis (1)		1	1
Propionibacterium avidum (1)	1	1	
Robinsoniella peoriensis (2)	2	2	2
Solobacterium moorei (4)	3	3	4
Staphylococcus saccharolyticus (1)	1	1	
Tissierella praeacura (1)	1	1	
Turicibacter sanguinis (1)	1	1	1
Veillonella rogosae (1)	1	1	

[a] DHB, 2,5-dihydroxybenzoic acid in acetonitrile, ethanol, water, and trifluoroacetic acid; Shimadzu/SARAMIS α-cyano, α-cyano-4-hydroxycinnamic acid in acetonitrile, ethanol, water, and trifluoroacetic acid; Bruker α-cyano, α-cyano-4-hydroxycinnamic acid in 50% acetonitrile–2.5% trifluoroacetic acid.

second run, and 7.3% after pretreatment with formic acid. For both systems and matrices, *Clostridium ramosum* was the only species which frequently required pretreatment with formic acid for identification. The overall performance of the Shimadzu/SARAMIS system was better with the α-cyano matrix than with the DHB matrix.

The performance of the MALDI-TOF MS systems for species identification is faster and less expensive than those of the commercial phenotypic systems like the Rapid ID 32A or Vitek 2 ANC card (bioMérieux, Marcy l'Etoile, France) (7, 11). Although a higher number of species can be identified with 16S rRNA gene sequencing, it is resource saving to use the MALDI-TOF MS system as a first-line identification system and to reserve gene sequencing for isolates that cannot be identified to the species level by MALDI-TOF MS (8). It also seems to be worthwhile to try for a second run in duplicate, as this in our laboratories adds another 6.6 to 9.2% to the identification total of isolates. Furthermore, 3.0 to 7.3% of the isolates were identified after pretreatment with 70% formic acid. It is not clear whether this was an effect of the pretreatment or just a result of yet another run, but the fact that *C. ramosum* consistently needed formic acid to be identified indicates that this could be used to improve the identification of some species.

TABLE 3. Species and number of isolates that were incorrectly identified

Species	Species (no. of isolates) incorrectly identified by[a]:		
	Shimadzu/SARAMIS		Bruker α-cyano (n = 23)
	DHB (n = 4)	α-Cyano (n = 4)	
Bacteroides dorei			*Bacteroides vulgatus* (8)
Bacteroides faecis			*Bacteroides thetaiotaomicron*
Bacteroides pyogenes/suis			*Bacteroides tectus* (3)
Bacteroides xylanisolvens			*Bacteroides ovatus* (2)
Bifidobacterium infantis			*Bifidobacterium longum*
Fusobacterium gonidiaformans		*Fusobacterium necrophorum*	
Fusobacterium nucleatum	*Parvimonas micra*	*Parvimonas micra*	
Peptoniphilus indolicus			*Peptoniphilus harei*
Porphyromonas uenonis	*Bacteroides fragilis*		
Prevotella timonensis	*Prevotella bivia*		
Propionibacterium acnes		*Trichophyton interdigitale*	
Solobacterium moorei	*Parvimonas micra*	*Parvimonas micra*	
Veillonella dispar			*Veillonella parvula* (2)
Veillonella rodentium			*Veillonella parvula* (4)
Veillonella rogosae			*Veillonella atypica*

[a] DHB, 2,5-dihydroxybenzoic acid in acetonitrile, ethanol, water, and trifluoroacetic acid; Shimadzu/SARAMIS α-cyano, α-cyano-4-hydroxycinnamic acid in acetonitrile, ethanol, water, and trifluoroacetic acid; Bruker α-cyano, α-cyano-4-hydroxycinnamic acid in 50% acetonitrile–2.5% trifluoroacetic acid.

More isolates could be identified to the species level with the Bruker system: 67.2% versus 43.8 to 49.0%. This is in contrast to a recent paper by Veloo et al., where the corresponding numbers were 61% with the Shimadzu/SARAMIS system and 51% with the Bruker system (18). However, that study included a selection of 79 nonconsecutive clinical isolates, representing 47 species of anaerobic bacteria, of which 24 (30.4%) were Gram-positive anaerobic cocci compared to 2.8% in the present study. In another paper by Veloo et al., the construction and validation of a Shimadzu/SARAMIS system database with focus on the Gram-positive anaerobic cocci are described, which could explain the difference in levels of performance between the two systems (17). The work with the Gram-positive anaerobic cocci is a good example of how databases can be optimized in collaboration with clinical laboratories (17).

TABLE 4. Summary performance characteristics of the Shimadzu/SARAMIS and Bruker MALDI-TOF MS systems

Parameter	No. with result/total (%) by[a]:		
	Shimadzu/SARAMIS		Bruker α-cyano
	DHB	α-Cyano	
Identification to:			
Species level	127/290 (43.8)	142/290 (49.0)	195/290 (67.2)
Genus or family level only	35/290 (12.1)	34/290 (11.7)	0/290 (0.0)
Incorrect species identification	4/290 (1.4)	4/290 (1.4)	23/290 (7.9)
No. of identifications by:			
Species included in system database	85/290 (29.3)	71/290 (24.5)	53/290 (18.3)
Species not included in system database	39/290 (13.4)	39/290 (13.4)	19/290 (6.5)

[a] DHB, 2,5-dihydroxybenzoic acid in acetonitrile, ethanol, water, and trifluoroacetic acid; Shimadzu/SARAMIS α-cyano, α-cyano-4-hydroxycinnamic acid in acetonitrile, ethanol, water, and trifluoroacetic acid; Bruker α-cyano, α-cyano-4-hydroxycinnamic acid in 50% acetonitrile–2.5% trifluoroacetic acid.

The Shimadzu/SARAMIS system uses the option of reporting results to the genus or family level frequently. The Shimadzu/SARAMIS system could not differentiate between *B. vulgatus* and *B. dorei*, *B. xylanisolvens* and *B. ovatus*, or the different *Veillonella* spp. and reported the isolates as *B. vulgatus/dorei* (three spectra), *B. xylanisolvens/ovatus* (one spectrum), or just *Veillonella* sp. (two spectra), respectively. The same species were incorrectly identified by the Bruker system (correct genus, but incorrect species) as *B. dorei* and *B. xylanisolvens* were not included in the Bruker system database. This for the most part explains the larger number of misidentifications by the Bruker system (7.9% versus 1.4%) and also the large number of isolates that were only identified to the genus level by the Shimadzu/SARAMIS system (≈12%). The described problems might be solved by optimizing the databases with more spectra. However; if the problem is that the species are too closely related and the spectra are indistinguishable, the more cautious approach by the Shimadzu/SARAMIS system could easily be adopted by the Bruker system. Still, as most of the incorrect identifications were within the correct genus, 80.6% (25/31), the clinical significance is questionable in these cases.

The relatively large number of isolates that could not be identified, although the species were included in the system databases, mainly belonged to the metronidazole-resistant Gram-positive rods. There may be a species diversity problem (it is known that *Propionibacterium acnes* can be divided into several subtypes), and this might again be solved by database optimization (12). It is also possible that the Gram-positive cell wall in these species inhibits protein release from the bacteria. There are several published pretreatment extraction protocols that might enhance protein release and possibly help in solving this problem (1, 16, 18).

Had the cutoff been set lower at 1.7 with the Bruker system, as in the study by Cherkaoui et al. (3), 235/290 (81.0%) would

have been correctly identified to the species level, and 30/290 (10.3%) would have been incorrectly identified. Out of 21 *P. acnes* isolates, only 3 were identified with a cutoff at 2.0, while all but one was identified with a cutoff at 1.7. With the 1.7 cutoff, the additional incorrect identifications included two *Fusobacterium* spp., *F. gonidiaformans* and *F. nucleatum*, which were identified as *F. necrophorum* and *F. johnsonii*, respectively. This illustrates that the appropriate cutoff may vary between different genera and species. Had the cutoff been set at 70% with the Shimadzu/SARAMIS system, the same as in the study by Cherkaoui et al. (3), 132/290 (45.5%) would have been correctly identified to the species level with the DHB matrix and 148/290 (51.0%) with the α-cyano matrix. No further isolates would have been incorrectly identified. The additional isolates that were identified were mainly *P. acnes* and *Lactobacillus rhamnosus*.

As already emphasized, the system databases need to be optimized with more spectra for certain genera and species, and the very rare species or recently described species need to be included in the databases to increase identification levels. However, MALDI-TOF MS in its present version seems to be a fast and inexpensive method for identification of most clinically important anaerobic bacteria to the species level.

In summary; the Bruker system had more correct identifications to the species level, but also more incorrect identifications. Second and third runs of isolates add considerably to the identification total for both systems. The isolates that were included in the system database but not identified mainly belonged to the metronidazole-resistant Gram-positive rods. Finally; pretreatment with 70% formic acid enhanced identification of *C. ramosum*.

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