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Application de la spectrométrie de masse MALDI-TOF en microbiologie clinique

Pour obtenir le grade de Doctorat d'Aix-Marseille Université
Spécialité Maladies Transmissibles et Pathologies Tropicales

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Sommaire

Avant-propos

Le format de présentation de cette thèse correspond à une recommandation de la spécialité Maladies Infectieuses et Microbiologie, à l'intérieur du Master des Sciences de la Vie et de la Santé qui dépend de l'Ecole Doctorale des Sciences de la Vie de Marseille.

Le candidat est amené à respecter des règles qui lui sont imposées et qui comportent un format de thèse utilisé dans le Nord de l'Europe et qui permet un meilleur rangement que les thèses traditionnelles. Par ailleurs, la partie introduction et bibliographie est remplacée par une revue envoyée dans un journal afin de permettre une évaluation extérieure de la qualité de la revue et de permettre à l'étudiant de commencer le plus tôt possible une bibliographie exhaustive sur le domaine de cette thèse. Par ailleurs, la thèse est présentée sur article publié, accepté ou soumis associé d'un bref commentaire donnant le sens général du travail. Cette forme de présentation a paru plus en adéquation avec les exigences de la compétition internationale et permet de se concentrer sur des travaux qui bénéficieront d'une diffusion internationale.

Professeur Didier RAOULT

Résumé

L’objectif de cette thèse est d’appliquer la méthode d’identification bactérienne par spectrométrie de masse MALDI-TOF (Matrix-assisted laser desorption/ionization time-of-flight) pour une utilisation en routine dans un laboratoire de microbiologie clinique.

Dans un premier temps et de manière prospective, nous avons évalué la performance et le coût-efficacité de l'identification bactérienne de routine par MALDI-TOF par rapport aux techniques conventionnelles d'identification phénotypique dans un laboratoire de microbiologie clinique. Durant la période des 16 semaines d’étude, nous avons comparé la performance de la technique par MALDI-TOF aux techniques conventionnelles d'identification phénotypique comprenant la coloration de Gram (Aerospray Wiescor; Elitech), l’identification des bactéries anaérobies par des galeries API ANA (BioMérieux, France) et l’identification automatique par le système Vitek 2 (BioMérieux, France). En cas de résultats discordants entre la technique de MALDI-TOF et les techniques conventionnelles, l’identification était réalisée par biologie moléculaire par séquençage des gènes 16S ribosomal RNA ou des gènes rpoB.

Lors de cette première étude, nous avons montré que la technique de MALDI-TOF est un moyen efficace et rentable pour l’identification rapide des bactéries dans la pratique quotidienne au sein de notre laboratoire de microbiologie clinique. Cette technique de MALDI-TOF peut être utilisée en première intention dans l'identification bactérienne avant la coloration de Gram ou d’autres techniques

d’identifications phénotypiques basées sur les caractéristiques chimiques des bactéries.

Dans un deuxième temps, nous avons évalué rétrospectivement la performance et le coût-efficacité de l’utilisation exclusive de MALDI-TOF en diagnostic bactériologique de routine en comparaison avec les techniques conventionnelles d'identification phénotypique. En analysant les données des 11 dernières années, nous avons montré que la technique de MALDI-TOF est efficace et tout à fait adaptée pour l'identification d’espèce bactérienne en routine. La performance de MALDI-TOF dans l’identification d’un grand nombre d'espèces bactériennes conduit de nombreux laboratoires cliniques à abandonner les techniques d’identification phénotypique dites traditionnelles. Nous avons également prouvé que MALDI-TOF est un outil puissant pour identifier les espèces bactériennes rarement impliquées dans les maladies infectieuses humaines. Cette technique (MALDI-TOF) pourrait être une alternative aux méthodes moléculaires dans le laboratoire clinique.

Mots clés : la spectrométrie de masse MALDI-TOF, MicroFlex, AutoFlex, Vitek, API, 16S rRNA, rpoB, biologie moléculaire, laboratoire de microbiologie clinique, bactéries, espèce rarement pathogène, Archaea, virus, champignons.

Abstract

The objective of this thesis is to apply the method of bacterial identification by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) in daily practice in a routine clinical microbiological laboratory. Firstly, we prospectively evaluated the performance and the cost-effective of bacterial identification by MALDI-TOF in comparison with conventional phenotypic identification methods.

During a 16-week study, we compared the performance of MALDI-TOF with conventional techniques of identification including Gram staining (Aerospray Wiescor; Elitech), API ANA identification strip for anaerobes (BioMérieux, France) and automated identification using the Vitek 2 (BioMérieux, France). The unmatched identifications between MALDI-TOF and conventional phenotypic identification methods were resolved by 16S ribosomal RNA and rpoB gene sequence–based molecular identification. In this study, we showed that MALDI-TOF was an effective tool and less expensive for the rapid identification of bacterial species from direct colony in clinical microbiology laboratory. MALDI-TOF can be used in first intention for identification before Gram staining or other phenotypic identification techniques based on physicochemical properties of bacteria.

Secondly, we retrospectively evaluated the performance and the cost-effectiveness of the exclusive use of MALDI-TOF in bacteriological diagnosis in comparison with conventional phenotypic identification. 11-year retrospective analysis of data showed that MALDI-TOF was efficient and completely adapted for the routine identification

of bacterial species. The performance of MALDI-TOF to identify a large number of bacterial species leads many clinical laboratories to give up traditional phenotypic identifications. We also showed that MALDI-TOF had capacity to identify bacterial species that were rarely involved in human diseases. This technique (MALDI-TOF) could be an alternative to molecular methods in the clinical laboratory.

Keywords: MALDI-TOF mass spectrometry, MicroFlex, AutoFlex, Vitek, API, 16S rRNA, *rpoB*, molecular methods, clinical laboratory, bacterial rare species in human pathogen, bacteria, Archaea, virus, fungi.

Introduction

L'identification rapide et précise des espèces bactériennes est une étape cruciale dans la prise en charge des patients dans la spécialité des maladies infectieuses pour aboutir à une antibiothérapie précoce et appropriée. L'identification des bactéries en routine dans des laboratoires de microbiologie clinique est basée principalement sur les tests phénotypiques en analysant la morphologie après la coloration de Gram, la caractéristique de culture et de croissance et la caratéristique biochimique (1). Bien que certains de ces essais soient réalisés en quelques minutes, l'identification complète est par habitude réalisée en plusieurs heures dans le meilleur des cas ou en plusieurs jours pour les organismes fastidieux.

L’identification par biologie moléculaire, surtout le séquençage du gène 16s rRNA a augmenté de manière considérable le nombre de bactéries nouvellement décrites dans la pathologie humaine (2-9). L'identification moléculaire des bactéries est une des techniques les plus efficaces mais demeure chère et inadaptée pour l'usage en routine dans un laboratoire de microbiologie clinique.

L’identification des bactéries, se basant sur des spectres peptidiques obtenus par la spectrométrie de masse MALDI-TOF (Matrix-assisted laser desorption/ionization time-of-flight), a été utilisé dans la recherche fondamentale depuis plus de 30 ans (10-17). Cette technique d'identification a été utilisée pour identifier les microorganismes au niveau de l’espèce (18-65), des sous-espèces et des clones épidémiques (66-83) dans les laboratoires de microbiologie clinique pendant les 10

dernières années. Certaine résistances antibiotiques (53, 84-95) et quelques toxines bactériennes (96, 97) pourraient être détectées par MALDI-TOF.

Après une revue de la littérature sur l'application de la spectrométrie de masse MALDI-TOF en microbiologie clinique (chapitre 1), l'objectif de notre travail a été d'évaluer de manière prospective la performance, le coût et l'efficacité de MALDI-TOF dans l'identification bactérienne de routine comparativement aux techniques conventionelles (la coloration de Gram, l'identification des bactéries anaérobies par des galeries API ANA et l'identification semi-automatique par le système Vitek 2) dans un laboaratoire de microbiologie clinique (chapitre 2).

Nous avons ensuite évalué de manière rétrospective, l'identification bactérienne en routine, en analysant nos données de 11 années, afin d'évaluer la performance, le coût et l'efficacité de MALDI-TOF en usage de routine par rapport aux techniques d'identification phénotypique conventionelle et aux techniques d'identification moléculaire (chapitre 3).

Chapitre 1 : Revue de la litterature
« MALDI-TOF mass spectrometry applications in clinical microbiology. »

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RESUME DE PUBLICATION N°1 « Revue : L'application de spectrométrie de masse MALDI-TOF (Matrix-assisted laser desorption/ionization time-of-flight) en microbiologie clinique. »

La méthode d'identification bactérienne par spectrométrie de masse MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight) a été adaptée avec succès pour l'identification de routine des microorganismes dans les laboratoires de microbiologie clinique au cours des dix dernières années. Cette technique révolutionnaire permet de faire le diagnostic plus facilement et plus rapidement des agents pathogènes humains, avec une fiabilité et une rentabilité incontestable, comparativement aux méthodes d'identification phénotypique conventionnelle et d'identification moléculaire. Dans cette revue de la littérature, nous allons décrire en détail l'utilisation de cet outil « MALDI-TOF » dans le diagnostic clinique de routine, y compris l'identification des espèces, des sous-espèces, des clones, des toxines et des types de résistance aux antibiotiques des bactéries. Nous discuterons également l'application de MALDI-TOF dans l'identification des Archaea, Eucaryotes, et de virus. Nous parlerons également de l'identification des bactéries directement sur les colonies bactériennes, sur les prélèvements cliniques, et les échantillons environnementaux.

MALDI-TOF-mass spectrometry applications in clinical microbiology

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MALDI-TOF-mass spectrometry (MS) has been successfully adapted for the routine identification of microorganisms in clinical microbiology laboratories in the past 10 years. This revolutionary technique allows for easier and faster diagnosis of human pathogens than conventional phenotypic and molecular identification methods, with unquestionable reliability and cost-effectiveness. This article will review the application of MALDI-TOF-MS tools in routine clinical diagnosis, including the identification of bacteria at the species, subspecies, strain and lineage levels, and the identification of bacterial toxins and antibiotic-resistance type. We will also discuss the application of MALDI-TOF-MS tools in the identification of Archaea, eukaryotes and viruses. Pathogenic identification from colony-cultured, blood-cultured, urine and environmental samples is also reviewed.

The identification of microorganisms by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is a technical revolution, which is increasingly used in microbiology laboratories. For more than 30 years, it has been shown that bacteria could be identified based on their proteins. However, the high cost of the apparatus and the absence of specific reagents have limited the development of this technology for economic reasons. In practice, the expense of using MALDI-TOF-MS for identification now lies in the acquisition of a machine that costs between €100,000 and 200,000, reagents that have almost negligible costs, and the use of a databank that can be increased as needed. The clinical use of MALDI-TOF-MS for bacterial isolates, or biological samples, was demonstrated in recent studies for the first time with high efficacy [1,2]. By testing colonies, it only takes a few minutes to obtain a precise identification, which makes identification of microorganisms at the species level, as well as the subspecies and strain levels possible, allowing the detection of epidemic lineages. In addition, antibiotic resistances and bacterial toxins might be detected. Databanks have quickly increased in size to identify not only bacteria and fungi, but also viruses and animals. MALDI-TOF-MS is a revolutionary approach for the identification of living organisms, which will change the strategies for identification in the next 15 years. It will replace many phenotypic and genetic identification methods, owing to its low cost and outstanding performance.

Three MALDI-TOF mass spectrometers, the MALDI BioTyper™ (Bruker Daltonics), SARAMIS™ (Shimadzu & Anagnostec), and the MALDI micro MX™ (Waters Corporation), have entered the market of bacterial identification tools. Bacterial species can be identified by two algorithms: a manual identification using the previously created mass spectra databank, or automated identification using commercial software packages with their own databases, such as BioTyper, SARAMIS, and MicrobeLynx™ (Bruker Daltonics, Shimadzu & Anagnostec and Waters Corporation, respectively). Cluster analysis with dendrograms using characteristic mass fingerprints allows for bacterial identification and classification at the species, subspecies, strain and lineage levels, in some cases. This article will focus on the application of MALDI-TOF-MS diagnosis to clinical microbiology, including the identification of bacteria at the various levels, and the identification of bacterial virulence factors, antibiotic susceptibility, Archaea, eukaryotes and viruses. We will also discuss the application of MALDI-TOF-MS tools in bacterial identification, from colonies, as well as direct samples such as from blood culture, urine and the environment (Table 1).

Review
Future Microbiology

Keywords
■ Archaea ■ bacteria ■ fungi ■ human ■ MALDI-TOF ■ mass spectrometry ■ microorganism ■ virus

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Table 1. Microorganism identification by matrix-assisted laser desorption ionization time-of-flight-mass spectrometry and its application in clinical microbiology laboratories.

Species level	Study (year)	Ref.
Gram-negative bacteria		
Aeromonas spp.	Donohue *et al.* (2006); Donohue *et al.* (2007); Dieckmann *et al.* (2009)	[10–12]
Campylobacter spp.	Winkler *et al.* (1999); Fagerquist *et al.* (2005); Mandrell *et al.* (2005); Kolinska *et al.* (2008); Alispahic *et al.* (2010)	[13–17]
Haemophilus spp.	Haag *et al.* (1998)	[18]
Helicobacter spp.	Nilsson (1999); Winkler *et al.* (1999); Ilina *et al.* (2010)	[13,19,20]
Neisseria spp.	Ilina *et al.* (2009)	[21]
Vibrio spp.	Dieckmann *et al.* (2009); Hazen *et al.* (2009)	[12,22]
Yersinia spp.	Mazzeo *et al.* (2006); unpublished data	[23]
Pseudomonas spp.	Teramoto *et al.* (2007)	[24]
Other nonfermenting Gram-negative bacteria	Degand *et al.* (2008); Mellmann *et al.* (2008); Vanlaere *et al.* (2008); Mellmann *et al.* (2009)	[25–28]
Fastidious bacteria		
Bartonella spp.	Fournier *et al.* (2009)	[29]
Coxiella burnetii	Shaw *et al.* (2004); Pierce *et al.* (2007)	[30,31]
Legionella spp.	Fujinami *et al.* (2010); Moliner *et al.* (2010)	[32,33]
Gram-positive bacteria		
Arthrobacter spp.	Vargha *et al.* (2006)	[34]
Lactobacillus	Sun *et al.* (2006)	[35]
Listeria spp.	Barbuddhe *et al.* (2008)	[36]
Staphylococcus spp.	Carbonnelle *et al.* (2007); Dupont *et al.* (2009); Rajakaruna *et al.* (2009); Dubois *et al.* (2010); Spanu *et al.* (2010)	[37–41]
Streptococcus spp.	Rupf *et al.* (2005); Friedrichs *et al.* (2007); Eigner *et al.* (2009); Seng *et al.* (2009); Blondiaux *et al.* (2010); van Veen *et al.* (2010)	[1,42–46]
Corynebacterium pseudodiphtheriticum	Bittar *et al.* (in press)	[47]
Mycobacteria	Claydon *et al.* (1996); Hettick *et al.* (2004); Hettick *et al.* (2006); Pignone *et al.* (2006)	[4,48–50]
Anaerobic bacteria	Shah *et al.* (2002); Grosse-Herrenthey *et al.* (2008); Stingu *et al.* (2008); Nagy *et al.* (2009)	[51–54]
Planctomycetes and environmental microorganisms	Fastner *et al.* (2001); Cayrou *et al.* (in press)	[55,56]
Subspecies or strains levels		
Salmonella spp.	Lynn *et al.* (1999); Leuschner *et al.* (2004); Dieckmann *et al.* (2008).	[57–59]
Escherichia spp.	Ochoa *et al.* (2005); Mazzeo *et al.* (2006).	[23,60]
Streptococcus spp.	Kumar *et al.* (2004); Moura *et al.* (2008); Williamson *et al.* (2008); Lartigue *et al.* (2009)	[61–64]
Bacillus spp.	Krishnamurthy *et al.* (1996); Ryzhov *et al.* (2000); Ryzhov *et al.* (2000); Elhanany *et al.* (2001); Demirev *et al.* (2008); Lasch *et al.* (2009)	[65–70]
Francisella tularensis	Seibold *et al.* (2010)	[71]
Rhodococcus erythropolis	Teramoto *et al.* (2009)	[72]
Bacterial toxin	Bernardo *et al.* (2002); Bittar *et al.* (2009)	[73,74]
Antibiotic-resistance study	Edwards-Jones *et al.* (2000); Bernardo *et al.* (2002); Du *et al.* (2002); Walker *et al.* (2002); Jackson *et al.* (2005); Majcherczyk *et al.* (2006); Camara *et al.* (2007); Russell *et al.* (2007); Marinach *et al.* (2009); Rajakaruna *et al.* (2009)	[39,75–83]
Identification of Archaea by MALDI-TOF-MS	Krader and Emerson (2004) Unpublished data	[84]

MS: Mass spectrometry.

Table 1. Microorganism identification by matrix-assisted laser desorption ionization time-of-flight-mass spectrometry and its application in clinical microbiology laboratories (cont.).

Species level	Study (year)	Ref.
Identification of eukaryotes by MALDI-TOF-MS		
Identification of fungi	Li *et al.* (2000); Welham *et al.* (2000); Amiri-Eliasi and Fenselau (2001); Valentine *et al.* (2002); Moura *et al.* (2003); Chen and Chen (2005); Neuhof *et al.* (2007); Neuhof *et al.* (2007); Degenkolb *et al.* (2008); Hettick *et al.* (2008); Hettick *et al.* (2008); Qian *et al.* (2008); Dong *et al.* (2009); Kemptner *et al.* (2009); Marinach-Patrice *et al.* (2009); Marklein *et al.* (2009); Pfohler *et al.* (2009); Santos *et al.* (2009); Sulc *et al.* (2009); Ferroni *et al.* (2010); Marinach-Patrice *et al.* (2010)	[85–105]
Identification of protists	Marks *et al.* (2004); Papadopoulos *et al.* (2004); Agranoff *et al.* (2005); Dea-Ayuela *et al.* (2006); Makioka *et al.* (2007); Sharma *et al.* (2007); Liu *et al.* (2009)	[106–112]
Identification of multicellular organisms	Zhang *et al.* (2006); Karger *et al.* (2010)	[113,114]
Identification of viruses by MALDI-TOF-MS	Lopaticki *et al.* (1998); Kim *et al.* (2001); Yao *et al.* (2002); Ilina *et al.* (2005); Colquhoun *et al.* (2006); Luan *et al.* (2009); Michael *et al.* (2009), La Scola *et al.* (2010)	[115–122]
MS application in clinical microbiology		
Bacteria identification by MALDI-TOF-MS in clinical laboratories	Valentine *et al.* (2005); Wunschel *et al.* (2005); Liu *et al.* (2007); Mellmann *et al.* (2008); Mellmann *et al.* (2009); Szabados *et al.* (2010)	[7,25,26,123–125]
Routine bacterial identifications by MALDI-TOF-MS	Anzai *et al.* (2000); Eigner *et al.* (2009); Seng *et al.* (2009); Bizzini *et al.* (2010); Blondiaux *et al.* (2010); Cherkaoui *et al.* (2010); van Veen *et al.* (2010)	[1,44–46,126–128]
Microbial identification by MALDI-TOF-MS without colony cultures		
Bloodstream samples	La Scola and Raoult (2009); Christner *et al.* (2010); Ferroni *et al.* (2010); Marinach-Patrice *et al.* (2010); Stevenson *et al.* (2010); Szabados *et al.* (2010)	[2,103,104,129–131]
Urine samples	Ferreira *et al.* (2010)	[132]
Environmental samples	Ochoa and Harrington (2005); Sun, Teramoto *et al.* (2006); Parisi *et al.* (2008)	[35,60,133]

MS: Mass spectrometry.

MALDI-TOF-MS tools for the identification of bacteria

Apart from protein extraction from whole-cell bacteria lysates prepared with chemical treatments, direct bacterial analysis is usually adopted for bacterial identification by MALDI-TOF-MS. Protein profiles can be obtained from a single colony of bacteria directly deposited on the MALDI-TOF target plate and overlaid with matrix solution (i.e., a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoracetic acid, after air-drying at room temperature for 5 min) (Figure 1) [1].

Identification of bacteria at the species level

In previous decades, MALDI-TOF-MS has been used in basic research to classify bacteria at the genus and species levels in a few isolates of some Gram-negative and Gram-positive bacteria [3–9]. In this section, we will discuss some species of bacteria that were identified recently, with strains that were subsequently added into specific mass spectra reference databases.

Gram-negative bacteria

Aeromonas spp.

Donohue *et al.* used MALDI-TOF-MS to identify 32 strains of 17 *Aeromonas* spp. at the species and strain levels using the variability of 17–25 mass peaks from the mass spectra fingerprint of each strain [10]. The accuracy of MALDI-TOF-MS identification of *Aeromonas* spp. was reconfirmed by a follow-up study, which used databases of 45 reference stains of 17 *Aeromonas* spp. to blindly identify 52 *Aeromonas* strains from drinking water samples. Compared to biochemical methods used as a positive control, MALDI-TOF-MS identified 82.7% of 52 environmental strains [11]. *Aeromonas* spp. identification using MALDI-TOF-MS was also reported by Dieckmann *et al.* in the study of *Vibrio* spp. (see later) [12].

Campylobacter spp.

A preliminary study of *Campylobacter* spp. identification using a high-mass range of protein (10–12 kDa) was reported in the same study of *Helicobacter pylori* by Winkler *et al.* [13].

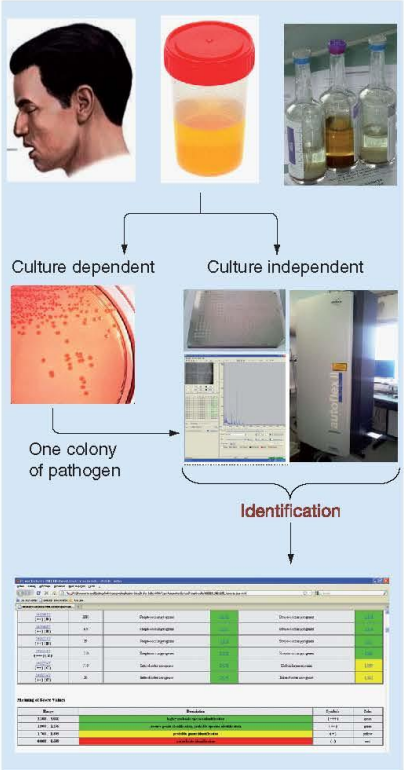

Figure 1. Rapid identification of clinical pathogens using matrix-assisted laser desorption ionization time-of-flight-mass spectrometry tools.

The identification of four *Campylobacter* spp. using specific biomarkers predicted from the DNA-binding protein HU was reported by Fagerquist *et al.* [14]. Based on the concept of species identification using a few species-specific mass peaks, Mandrell *et al.* correctly identified 139 *Campylobacter* strains and 75 clinical isolates at the species level [15]. *Campylobacter* spp. could be also identified using their mass spectra fingerprints. Databases of mass spectra fingerprints of three *Campylobacter* spp. have been used to identify all 42 *Campylobacter* isolates at the species level, and cluster analysis of these strains revealed two subspecies of *Campylobacter jejuni*, *Campylobacter jejuni* spp. *jejuni* and *Campylobacter jejuni* spp. *doylei* [16]. The reproducibility of this approach was recently confirmed by Alispahic *et al.*, who demonstrated the advantage of this approach over a PCR restriction fragment-length polymorphism (PCR-RFLP) assay based on the hippuricase-encoding gene [17].

Haemophilus *spp.*

Using MALDI-TOF-MS, Haag *et al.* successfully identified *Haemophilus* spp. implicated as human pathogens, including *Haemophilus influenza*, *Haemophilus parainfluenzae*, *Haemophilus aphrophilus*, and *Haemophilus ducreyi*. MALDI-TOF-MS was also used to identify strain-specific biomarkers of *H. ducreyi* isolated from different patients with chancroid [18].

Helicobacter *spp.*

Nilsson *et al.* first reported that *H. pylori* spp. could be identified by their high mass range (6588–18,480 *m/z*) using cell lysates and protein extraction [19]. In the same year, Winkler *et al.* distinguished *H. pylori* from *Helicobacter mustelae* using a direct analysis of bacterial colonies stored in a 50% methanol–water solution [13]. The species identification of *H. pylori* was performed using mass profiles of two reference strains (266695 and J99) available in the BioTyper database. Nine and eight clinical isolates were identified at the species and genus level, respectively [20].

Neisseria *spp.*

Two pathogenic *Neisseria* spp., including 29 strains of *Neisseria meningitidis*, 13 strains of *Neisseria gonorrhoeae* and 15 nonpathogenic *Neisseria* strains, were successfully identified at the species level using MALDI-TOF-MS. *Neisseria* spp. were correctly identified by analysis of the main spectra from 57 strains using BioTyper software. All strains were correctly classified into three clusters: *N. meningitidis N. gonorrhoeae* and nonpathogenic *Neisseria.* However, the subspecies and serotype levels could not be identified because of low intraspecies variability of the *N. meningitidis* and *N. gonorrhoeae* mass spectra [21].

Vibrio *spp.*

Recently, MALDI-TOF-MS was used to identify the species of 83 *Vibrio* and *Aeromonas* isolates using a classification model created from putative species-specific mass peaks, obtained from the comparison of ribosomal protein candidates predicted from genomic sequences of seven *Vibrio* and one *Aeromonas* spp. to their MALDI-TOF mass peaks. The dendrogram of mass spectra profiles was similar to the phylogenetic tree based on the *rpoB* gene sequence [12]. Using the same methods, Hazen *et al.* used a database of seven *Vibrio* spp. to correctly identify 20 strains of clinical and environmental *Vibrio* spp. at the species

level. The phyloproteomic characterization of *Vibrio* strains based on mass spectra fingerprinting allowed clustering of *Vibrio* strains by their species and subspecies, and by their geographic location relative to pandemic *Vibrio parahaemolyticus* clones [22].

Yersinia *spp.*

Successful species identification of four *Yersinia* spp. by MALDI-TOF-MS was reported by Mazzeo *et al.* using five environmental *Yersinia* strains [23]. A recent study reported the mass spectrometric identification of *Yersinia* spp., and successfully identified two *Yersinia pestis* isolates and 11 *Yersinia enterocolitica* isolates. An updated BioTyper database was initially built from 40 *Yersinia* strains, representative of 12 species, including 13 *Y. pestis* strains. All *Yersinia* strains were correctly identified at the species level, and MALDI-TOF-MS was advantageous for *Y. pestis* identification in artificially infected talc [Ayyadurai *et al.*, Unpublished Data].

Pseudomonas *spp.*

The phylogenetic classification of *Pseudomonas putida* by MALDI-TOF-MS was established from 43 putative species and strain-specific biomarker ions obtained by the comparison of specific biomarkers. These biomarkers were predicted from genomic sequences of MALDI-TOF mass lists from cell lysates with purified ribosomal proteins of *P. putida* strain KT2440. Cluster analysis that used these biomarkers allowed the identification of 16 *P. putida* isolates at the strain level, and dendrograms were comparable to phylogenetic data based on DNA gyrase subunit-B gene sequences. Interestingly, diversity of mass profiles has been observed in biovars A and B of *P. putida* strains, which have been correctly classified by phyloproteomic techniques [24]. Further study of several strains of *P. putida* and other related species, particularly *Pseudomonas aeruginosa*, are needed.

Other nonfermenting Gram-negative bacteria (except *Pseudomonas* spp.)

An updated database was initially created from 248 strains of 37 genera of human pathogenic nonfermenting bacteria using BioTyper software. Compared to 16S rRNA gene sequencing, 82.5% of blind-coded clinical nonfermenting isolates were correctly identified by MALDI-TOF-MS at the species level [25]. This approach has been further evaluated in eight clinical laboratories from different countries using 60 blind-coded nonfermenting bacteria, with a 98.75% accuracy rate [26]. Along similar lines, Degand *et al.* used a MALDI-TOF-MS database created from 58 reference strains to identify 512 clinical nonfermenting Gram-negative bacilli from cystic fibrosis patients, and 47 reference strains. All *P. aeruginosa*, *Stenotrophomonas maltophilia* and *Alcaligenes xylosoxidans* strains were correctly identified at the species level. After adding four *Ralstonia*, five *Cupriavidus* and 21 *Burkholderia cepacia* complex strains to the database, MALDI-TOF-MS was used to identify 98% of *B. cepacia* complex and *Ralstonia* isolates at the species level [27].

Recently, 75 clinical and environmental isolates of nine *B. cepacia* complex species were analyzed using two data-analysis algorithms, SARAMIS and BioNumerics™ software (Applied MathsNV), which identified 65 and 69 out of 75 isolates, respectively. Interestingly, the cluster analysis with the dendrogram correctly classified *B. cepacia* complex and non-*B. cepacia* complex isolates at group and species levels [28].

Fastidious bacteria

Bartonella *spp.*

Fournier *et al.* recently reported rapid and cost-effective *Bartonella* spp. identification using automated MALDI-TOF-MS. The BioTyper database was updated by adding 20 strains of 17 *Bartonella* spp., and was used to blindly identify 36 out of 39 *Bartonella* isolates at the species level (score ≥2) and one *Bartonella clarridgeiae* isolate at the genus level (score = 1.88). Two *Bartonella bovis* strains were not identified (score <1.7) owing to the intraspecies diversity of *B. bovis* and *B. clarridgeiae*, and only one strain of each species was present in the database. MALDI-TOF-MS analysis has also identified specific biomarkers of three *Bartonella vinsonii* subspecies [29].

Coxiella burnetii

Analysis by MALDI-TOF-MS of *Coxiella burnetii* phase I strains previously treated with 2×10^6 rad of γ-irradiation identified 16 reproducible mass peaks, including three peaks of proteins identified as ScvA protein, heat-shock inducible protein, and Orf 109, corresponding to peaks of 3612, 10,504 and 11,130 *m/z* [30]. In a later study, five *C. burnetii* phase I strains (Nine Mile phase I, KAV, PAV, Henzerling and Ohio) and two phase II strains (Australian QD and M44) were analyzed by MALDI-TOF-MS. The phase- and strain-specific biomarker ions

were identified in low-mass ranges (1000–6000 *m/z*) using visual peak selection, completed by partial least squares-discriminant analysis (PLS-DA) [31].

Legionella *spp.*

Legionella spp. identification using automated MALDI-TOF-MS was recently performed using 59 strains of 21 species. After adding the mass spectra of 13 misidentified strains into the updated BioTyper database, 90% of 237 clinical and environmental strains representative of 19 *Legionella* spp. were identified at the species level. MALDI-TOF-MS has been used to identify all eight *Legionella* spp. most frequently involved in human disease, including *Legionella pneumophila*. Further mass spectral analysis was not successful in identifying *Legionella* strains at the serotype group level [32]. A parallel study of *Legionella* spp. identification by MALDI-TOF-MS confirmed the accuracy of this method to identify *Legionella* spp. at the species level [33]. Mass spectra analysis with dendrograms classified 13 *L. pneumophila* isolates from two different hot-water springs into two separate clusters; the difference between these clusters is likely related to the location of sampling, excluding two isolates.

Gram-positive bacteria

Arthrobacter *spp.*

Vargha *et al.* successfully classified 16 *Arthrobacter* isolates at the strain level using MALDI-TOF mass spectra from different cell preparation methods, matrices and solvents, cell concentrations, growth stages and culture media. Dendrograms from cluster analysis of mass spectra profiles were correlated with the phylogenetic tree based on 16S rRNA gene sequences [34].

Lactobacillus *spp.*

Species identification of *Lactobacillus plantarum* by MALDI-TOF-MS was reported by Sun *et al.* Mass spectra from intact cells, cell lysates and ribosomal protein extracts were compared with 53 subunits of ribosomal proteins assigned from the genomic sequence of *L. plantarum* WCFS1. Finally, 34 proteins were identified as specific biomarkers of *L. plantarum*, and 30 of these proteins were observed in mass spectra from cell lysates. These 34 biomarkers were used to correctly identify *L. plantarum* KT using the industrial starter culture, and yogurt fermented by *L. plantarum*. All mass peaks obtained from *L. plantarum* 8826 were found in the industrial starter culture, and 18 peaks were found in the yogurt culture [35].

Listeria *spp.*

Listeria spp. databases were created from eight reference strains of six species using BioTyper software and used to correctly identify 138 *Listeria* strains at the species level, including 86 *Listeria monocytogenes*, nine *Listeria innocua*, 20 *Listeria ivanovii*, 15 *Listeria seeligeri*, four *Listeria welshimeri* and four *Listeria grayi* isolates. Further mass spectra profile analyses of different *L. monocytogenes* serotypes showed a higher sensitivity compared with conventional pulsed-field gel electrophoresis in identifying the lineage level of epidemical clones [36].

Staphylococcus *spp.*

Staphylococcus spp. were identified by MALDI-TOF-MS using between three and 14 species-specific mass peaks obtained from 23 reference *Micrococcaceae* strains, including *Staphylococcus aureus* and *Micrococcus luteus*, and 22 strains of coagulase-negative *Staphylococcus* (CoNS). Using this reference database, 196 *Micrococcaceae* isolates, including 68 *S. aureus*, 127 CoNS and one *M. luteus* isolate, were correctly identified at the species and subspecies levels, but the accuracy was lost with changes in culture conditions and duration [37]. Recently, Dubois *et al.* blindly identified 98.3% of 152 *Staphylococcus* isolates using the BioTyper database compared with PCR sequencing of *sodA*. Dendrograms of mass profiles clustered clinical *Staphylococcus epidermidis* isolates apart from environmental isolates, and were more identical to the phylogenetic tree based on the 16s rRNA sequence than the tree based on the *sodA* sequence [38]. Using the same procedure, Rajakaruna *et al.* identified 97% of 134 *S. aureus* strains at the species levels by MALDI-TOF-MS, using the MicrobeLynx™ database [39]. MALDI-TOF-MS was also used to identify 93.2% of 234 clinical isolates of CoNS at the species level using the BioTyper database; this ratio was increased to 97.4% by adding three missing CoNS species to the database [40]. More recently, Spanu *et al.* identified 100% of *S. aureus* and 99.1% of CoNS isolates from 450 bloodstream-infection staphylococcal isolates using MALDI-TOF-MS and the BioTyper database, compared with PCR sequencing of *rpoB*. Only one isolate each of *S. epidermidis*, *Staphylococcus warneri*, and *Staphylococcus pettenkoferi* was not identified [41].

Streptococcus *spp.*

A primary study of MALDI-TOF-MS identification of viridians *Streptococci* spp. was reported by Friedrichs *et al.* A MALDI-TOF mass spectra database created from ten reference strains was used to identify 99 clinical strains at the species level. Compared to conventional methods of identification, species-specific PCR and 16s rRNA sequencing, MALDI-TOF correctly identified 71 *Streptococci mitis*, 23 *Streptococci anginosus* and five *Streptococci salivarius* isolates at the species level, using low-mass ranges [42].

The species identification of mutans *Streptococci* using MALDI-TOF-MS was reported by Rupf *et al.* A reference database was first built from five mutans group *Streptococcus* spp. (*Streptococcus criceti*, *Streptococcus downei*, *Streptococcus mutans*, *Streptococci ratti* and *Streptococcus sobrinus*) and four non-mutans group *Streptococcus* spp. (*Streptococcus oralis*, *S. mitis*, *S. salivarius* and *Streptococcus sanguinis*). Using this database, MALDI-TOF-MS identified all 159 *S. mutans*, 16 *S. sobrinus* and two *S. anginosus* isolates from 177 isolates previously identified by conventional methods as mutans *Streptococcus*. This result was confirmed by PCR sequencing of 16S rRNA and species-specific PCR gene sequencing of *S. mutans* and *S. sobrinus*. Authors have observed much diversity in the mass spectra profiles of 14 *S. mutans* strains and three *S. sobrinus* strains that were used to identify isolates at the subspecies level [43]. However, *Streptococcus* subspecies identification using MALDI-TOF-MS requires reliable molecular taxonomy identification to establish comparative groups.

The MALDI-TOF-MS technique was used in one of our studies to identify *Streptococcus* at the species level in 65.4% of 81 clinical isolates of 16 *Streptococcus* spp. [1]. The same accuracy rate of mass spectrometric identification of *Streptococcus* spp. was reported by Blondiaux *et al.* [44]. Higher rates of identification of this species of 77 and 93.7% were reported by van Veen *et al.* and Eigner *et al.*, respectively [45,46]. However, only 16S rRNA gene sequencing was used to confirmed the *Streptococcus* spp. in those studies, whereas both 16S rRNA and *rpoB* gene sequencing were used in our study. Thus far, the identification of *Streptococcus* spp. remains a challenge for MALDI-TOF-MS identification tools, especially in closely related species, such as *S. pneumoniae*, *S. mitis* and *S. parasanguinis*.

Corynebacterium pseudodiphtheriticum

The MALDI-TOF-MS identification of *C. pseudodiphtheriticum* isolates from cystic fibrosis patients was recently reported in our laboratory. Compared to *rpoB* gene sequencing, MALDI-TOF-MS perfectly identified all 18 *C. pseudodiphtheriticum* isolates at the species level. Cluster analysis of *C. pseudodiphtheriticum* mass fingerprints was generally in agreement with the phylogenetic tree of the partial *rpoB* gene sequence (Bittar *et al.* in press) [47].

Mycobacteria

Many attempts to identify mycobacteria by MALDI-TOF-MS have been reported. Claydon *et al.* identified some mass peaks from a *Mycobacterium smegmatis* strain [4]. Later, MALDI-TOF-MS, combined with 'canonical discriminant analysis', was used by Hettick *et al.* to differentiate six species of *Mycobacterium* (*Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium kansasii*, *Mycobacterium intracellulare*, *Mycobacterium fortuitum* and *Mycobacterium avium*). The authors reported that mass spectra profiles of mycobacteria from cell extracts and intact cells were similar, and that strains incubated in an acetonitrile–trifluoroacetic acid solution can be used to inactivate mycobacteria [48]. Pignon *et al.* identified mycobacteria species using MALDI-TOF-MS fingerprints of 37 strains of 13 *Mycobacterium* spp. with lower mass ranges (500–2000 Da) [49]. The species identification was confirmed by the second study reported by Hettick *et al.*, in which 16 strains of four *Mycobacterium* spp. (*M. tuberculosis*, *M. avium*, *M. intracellulare* and *M. kansasii*) were correctly identified at the species level. Random forest analysis of mass spectra has facilitated the accurate classification of all 16 strains into *M. tuberculosis* or nontuberculosis groups. Although the error rate of the strain classification model was acceptable, only four strains of each *Mycobacterium* spp. were used in this study, which did not allow extension to clinical diagnosis [50]. Many species- and genus-specific mass peaks have been identified in reported studies, but no study has succeeded in identifying mycobacteria using the ribosomal protein biomarkers that are mostly used in routine MALDI-TOF-MS identification of bacteria species.

Anaerobic bacteria

The genera of *Bacteroides*, *Prevotella*, *Porphyromonas*, *Fusobacteria*, *Treponema*, *Peptostreptococcus* and *Clostridium* were identified by their low-mass profiles (500–3000 *m/z*). Preliminary data indicated that higher mass ranges (500–20,000 *m/z*) were used to identify species of anaerobic bacteria [51]. Recently,

Stingu *et al.* used a MALDI-TOF-MS database created from nine reference strains and ten clinical isolates of anaerobic bacteria to identify 92.3% of 65 clinical anaerobic isolates compared with biochemical identification. Five unidentified or misidentified strains were not in the database. The dendrogram constructed from mass profiles classified 39 *Prevotella* strains into two separate species, *P. intermedia* and *P. nigrescens* [52]. MALDI-TOF-MS and the BioTyper database were used to identify species of *Bacteroides* in 89.1% of 277 clinical strains compared with conventional methods. This rate was increased to 95.6% (265 out of 277) after adding four out of seven unidentified strains of *Parabacteroides distasonis* into the database, and confirming 11 out of 23 discrepancies by 16S rRNA gene sequencing [53]. A variety of clostridial species could be identified by MALDI-TOF-MS using the updated BioTyper database created from 64 reference strains of 31 *Clostridium* spp. The dendrogram constructed from the mass profiles of all 64 *Clostridium* strains was similar to the phylogenetic tree based on 16S rRNA gene sequences [54].

Identification of *Planctomycetes* & environmental microorganisms

Various MALDI-TOF-MS tools have mainly been used to investigate human pathogenic microorganisms, but some studies have focused on environmental microorganism identification, such as the species identification of *Mycrocystis* spp. (cyanobacteria), which was first reported by Fastner *et al.* [55]. This technique has also allowed us to identify seven isolates of *Planctomycetes* spp. at the species level. The cluster analysis of mass fingerprinting was strongly correlated to the phylogenetic tree based on the 16S rRNA gene sequence of *Planctomycetes* spp. (Cayrou *et al.*, in press) [56].

Identification of bacteria at the subspecies or strain levels

Species identification by MALDI-TOF-MS has been advantageous for subtyping some closely related bacterial species (i.e., for subspecies, serovar, lineage or strain level characterization of *Salmonella* spp., *Escherichia* spp., *Streptococcus* spp., *Bacillus* spp., *Francisella tularensis* and *Rhodococcus erythropolis*).

Salmonella spp.

For *Salmonella* spp., Lynn *et al.* identified genus- and species-specific biomarkers from the MALDI-TOF-MS analysis of two *Salmonella* subspecies, Typhimurium and Dublin [57]. Later, Leuschner *et al.* identified consensus peaks specific for *Salmonella* serovars by mass spectra analysis of 22 strains, including six serovars of *S. enterica* subspecies *enterica*. In that study, the authors were unable to identify seven *S.* Typhimurium isolates using the species-specific biomarkers previously described by Lynn *et al.* [58]. Recently, the identification of *Salmonella* isolates on the species, subspecies and serovar levels was reported by Dieckmann *et al.*, who designated presumed genus-, species- and subspecies-identifying biomarker ions based on available genome sequence data, before classifying 126 *Salmonella* isolates. To identify *Salmonella* isolates at the subspecies level, high-mass ranges (10,000–40,000 *m/z*) were needed, which were obtained by adding sinapinic acid to the matrix. In total, 57 genus-identifying biomarker ions of *Salmonella* spp., 27 species-identifying biomarker ions of *S. bongori*, and 14 species-identifying biomarker ions of *S. enterica* were identified. The dendrogram based on cluster analysis of biomarker profiles was shown to correctly classify *Salmonella* at the subspecies level [59].

Escherichia spp.

In the process of species identification of 29 environmental strains of *Escherichia* spp., including 25 *Escherichia coli* and four other *Escherichia* spp., MALDI-TOF-MS identified one serotype-specific biomarker ion in enterohemorragic *E. coli* O157:H7 strains at 9740 *m/z*. None of the *E. coli* O157:H7 strains presented mass peaks at 9060 *m/z* [23]. Discrimination of the enterohemorragic *E. coli* O157:H7 serotype has been reported using immunomagnetic isolation before MALDI-TOF mass spectrometric analysis. A unique strain of *E. coli* O157:H7 was used, and correctly discriminated from three other *E. coli* strains [60].

Streptococcus spp.

During a MALDI-TOF mass spectrometric study of *Streptococcus agalactiae*, all 110 isolates were correctly identified by automated data analysis using BioTyper software. Interestingly, MALDI-TOF-MS identified 109 out of 110 strains at the serotype level with scores of at least 2.3, after adding the main spectra of five *Streptococcus agalactiae* strains with sequence types ST1, ST10, ST17, ST19 and ST23 into the database [61]. Williamson *et al.* used MALDI-TOF-MS to successfully classify 13 *S. pneumoniae* isolates that cause conjunctivitis outbreaks

in the USA. The authors analyzed 45 isolates, including 13 nontypeable strains, seven strains of heptavalent vaccine, one nonencapsulated isolate R6, one encapsulated isolate TIGR4, four isolates from the bloodstream and cerebrospinal fluid, 15 other *Streptococcus* spp., and three strains from other genera. All *S. pneumoniae* conjunctivitis-outbreak isolates have unique common peaks at 2944 *m/z*. Cluster analysis of mass spectra indicated that their mass spectra profiles shared 70–86% similarity, compared with only 45% similarity with *S. pneumoniae* vaccine strains [62].

For the identification of β-hemolytic streptococci groups, Kumar *et al.* used the group-specific biomarker ions for 54 isolates of group A streptococci (12 pyoderma isolates, 21 throat isolates and 21 carrier isolates), 13 isolates of group C streptococci and three isolates of group G streptococci. Only visual peak analysis and low-mass ranges (400–3000 *m/z*) were used to differentiate β-hemolytic streptococci groups [63]. Differentiation between invasive and noninvasive *S. pyogenes* isolates using MALDI-TOF fingerprinting was reported by Moura *et al.*, and the *S. pyogenes* isolates included nine invasive isolates from necrotizing fasciitis cases, four noninvasive isolates from throat and skin infections and one reference strain. Mass spectra analysis and cluster analysis were able to identify *S. pyogenes* at the species level, and classify strains into invasive or noninvasive groups [64].

Bacillus spp.

Identification of different species-specific biomarkers from *Bacillus anthracis* spores and vegetative forms have been reported in many studies [65–69]. The use of MALDI-TOF-MS to identify *B. anthracis* was recently reported by Lasch *et al.* Mass spectra of 102 *B. anthracis* isolates, 121 *B. cereus* isolates and 151 other *Bacillus* and related genera isolates, were analyzed first. Mass spectra analysis by gel view and unsupervised hierarchical cluster analysis permitted the classification of *Bacillus* strains into two groups (*cereus* and non-*cereus*), and *B. anthracis* were correctly classified into two different clusters of six subgroups of the *B. cereus* group. Classification models using artificial neural networks created from 296 mass spectra were used to blindly identify 100% of *B. anthracis*, 94.5% of *B. cereus* and 92.9% of *Bacillus* non-*cereus* strains [70]. The complexity of these data-analysis methods prevents their application in clinical laboratories for routine diagnoses.

Francisella tularensis

Species and subspecies of *Francisella tularensis*, the agent of tularemia, have been successfully identified by MALDI-TOF-MS using the BioTyper database, previously updated by adding five representative strains of *Francisella* spp. All 40 *F. tularensis* and five *Francisella philomiragia* isolates were correctly identified at the species and subspecies level, compared with molecular identifications. The phyloproteomic classification constructed from mass spectra fingerprints correctly classified all *Francisella* strains into five species and three different subclusters, which represented the three *F. tularensis* subspecies [71].

Rhodococcus erythropolis

Rhodococcus strains have been successfully identified by their ribosomal protein fingerprints using MALDI-TOF-MS. First, comparison of mass spectra from cell lysates and the extracted ribosomal proteins of reference strain R17, identified 30 reference mass peaks. Second, mass spectra from cell lysates of 21 *Rhodococcus* strains, including antibiotic-producer and antibiotic-nonproducer groups were compared with those of reference strain R17. In total, 20 mass peaks were commonly identified in all *Rhodococcus* strains, and ten inhomogeneous mass peaks were sufficient for the identification of *Rhodococcus* isolates at the strain level. Cluster analysis of all mass spectra compared with phylogenetic analysis based on 16S rRNA and *gyrB* gene sequences correctly classified all strains and groups [72].

Bacterial toxin identification by MALDI-TOF-MS

S. aureus exotoxin identification by MALDI-TOF-MS has been reported. However, previous protein extraction from sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was needed to identity this exotoxin [73]. Recently, Bittar *et al.* successfully identified Panton–Valentine leukocidin (PVL)-positive *Staphylococcus* using MALDI-TOF-MS. One PVL-positive group-specific biomarker ion, with a mass peak at 4448 *m/z*, was identified by comparing the mass spectra of 24 PVL-positive *S. aureus* strains with 57 PVL-negative *S. aureus* strains using ClinProTools™ software (Bruker Daltonics, Germany). The classification model created from this specific biomarker ion was used to blindly identify 34 *S. aureus* isolates, including two PVL-positive strains. The sensitivity, specificity, predictive positive value and predictive negative value were determined to be 100, 90.6, 40 and 100%, respectively [74].

Antibiotic-resistance study by MALDI-TOF-MS

The use of MALDI-TOF-MS to differentiate methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) was first reported by Edwards-Jones *et al.* The intact-cell mass spectra of seven reference strains of MSSA, seven clinical isolates of MRSA, and six strains of CoNs were compared, in a mass range of 500–10,000 *m/z*. Group-specific biomarker ions were identified in the lower mass range of 500–2000 *m/z*, which allowed the authors to correctly differentiate MRSA from MSSA strains [75]. This result was confirmed by two follow-up studies that revealed the reproducibility of MRSA detection by MALDI-TOF-MS in different laboratories. However, this approach has dramatic losses in accuracy after media cultures are changed [76,77]. In accordance with previous studies, Du *et al.* identified only 74% of 76 *S. aureus* strains, and MRSA was correctly distinguished from MSSA using cluster analysis with a dendrogram. Discrepancies between MALDI-TOF and molecular detection of *mecA* were observed in seven *S. aureus* identified as MRSA by MALDI-TOF-MS that were negative for *mecA* [78]. It should be noted that all previous studies used low-mass ranges to differentiate MRSA from MSSA. Bernardo *et al.* published contradictory results, showing that MALDI-TOF-MS was not able to differentiate MRSA from MSSA using a higher mass range (2000–7000 *m/z*) [79].

Similarly, Rajakaruna *et al.* were unable to identify consistent mass ions that could distinguish MRSA strains from other *S. aureus* using the same technique reported by Edwards-Jones *et al.* in a set of well-characterized *S. aureus* strains, including reference and clinical strains [39]. Rapid tentative identification of MRSA susceptible and resistant to teicoplanin using MALDI-TOF-MS was recently reported by Majcherczyk *et al.* The cluster analysis with dendrograms constructed from low-mass ranges (<3500 *m/z*) of four MRSA strains correctly distinguished MRSA teicoplanin-susceptible strains from MRSA teicoplanin-resistant strains [80].

Differentiation between wild-type and ampicillin-resistant *E. coli* using MALDI-TOF-MS was reported by Camara *et al.* Mass spectra of different *E. coli* strains, including one wild-type *E. coli* strain K12 MG1655, one *E. coli* strain transformed with a β-lactamase-producing plasmid (MG1655/pUC19), and one MG1655/pUC19 strain grown in LB medium with 100 µg/ml of ampicillin, were used in this study. One specific mass peak at 29,000 *m/z* was identified in the MG1655/pUC19 strain grown with and without ampicillin, and the identity of this mass peak was confirmed by SDS-PAGE. To identify ampicillin-resistant *E. coli*, the authors proposed optimizing MALDI-TOF-MS using unconventional bacterial identification to detect a high-mass range (~29,000 *m/z*) [81]. The reported specific biomarker for ampicillin-resistant *E. coli* was recently reinforced by the identification of an *E. coli* plasmid inserted at 28,907 *m/z* of the MALDI-TOF mass spectra [82]. Additionally, Camara *et al.* have shown a great diversity of mass spectra fingerprints in their database between wild-type and β-lactamase-producing *E. coli* strains, at a mass range of 6409–17,529 *m/z*. This variability in mass spectra fingerprints could be used to distinguish ampicillin-resistant *E. coli* from wild-type *E. coli* strains, without needing to resort to the specific peak.

In our preliminary results, MALDI-TOF-MS was used to identify *Klebsiella pneumoniae* producing extended-spectrum β-lactamase, with sensitivity and specificity evaluated at 72 and 92%, respectively, compared with molecular detection methods [Raoult *et al.*, Unpublished Data]. Another approach, using MALDI-TOF-MS to test the drug susceptibility of *Candida albicans* strains, was recently reported by Marinach *et al.* In their preliminary results, the mass spectra profiles of *C. albicans* fluconazole-resistant strains changed after exposure to 2 µg/ml, which was different from the affect observed for fluconazole minimum inhibitory concentration (MIC), which was 8 µg/ml. Statistical calculations showed that minimal profile change concentrations were highly correlated with the MICs of fluconazole for 17 *C. albicans* strains [83].

Identification of Archaea by MALDI-TOF-MS

Krader *et al.* reported the identification of 28 Archaea and 42 extremophilic bacteria using a MALDI-TOF mass spectrum range of 500–2000 Da [84]. Based on our results of environmental microorganism identification using MALDI-TOF-MS, we are able to identify Archaea strains at the species level using routine MALDI-TOF-MS tools (2000–20,000 *m/z*).

Identification of eukaryotes by MALDI-TOF-MS

Identification of fungi

In past 10 years, MALDI-TOF-MS tools for microorganism identification were successfully adapted for the identification of fungi. An early

study of fungal identification using the MALDI-TOF-MS approach was published in 2000 by Welham *et al.* Three fungal species (*Penicillium* spp., *Scytalidium dimidiatum* and *Trichophyton rubrum*) were distinguished from each other by their mass profiles [85]. Recently, many studies have focused on the application of the MALDI-TOF-MS technique to characterize more strains of diverse fungal species and groups, and create mass spectra databases for further identification. Several species of *Penicillium* were identified by Chen *et al.* and Hettick *et al.*, at species and genus levels, using MALDI-TOF-MS analysis of intact fungal spores, or short chemical extract ions [86,87].

Different mass peak profiles of aflatoxigenic and non-aflatoxigenic strains of *Aspergillus* spp. were mentioned by Li *et al.* [88]. However, intact-spore MALDI-TOF mass spectra did not correctly classify these four closely related *Aspergillus* spp. MALDI-TOF-MS tools were then applied to identify *Aspergillus* spp. at the species level. This technique was extended to identify *Aspergillus* strains, with an accuracy rate at 95% for *Aspergillus flavus* and 100% for *Aspergillus fumigatus* [89,90]. The challenge of identifying *Aspergillus niger* strains, which were classified in the *Aspergillus chevalieri* cluster, has been noted [89]. The application of *Aspergillus* spp. identification by automated MALDI-TOF-MS in clinical laboratories requires reliable reference databases from several strains of different species of *Aspergillus* spp. (~180 species).

The identification of *Fusarium* spp. by MALDI-TOF-MS was reported in many studies. Marinach-Patrice *et al.* updated the BioTyper database by adding 13 strains of five *Fusarium* spp., and accurately identified 89.7% of 49 clinical *Fusarium* isolates at the species level, compared with molecular methods. Only one *Fusarium pseudonygamai* isolate was misidentified, and four unidentified *Fusarium* isolates had no reference in the database [91]. MALDI-TOF-MS has been also applied to identify the species of *Fusarium* spores [92,93]. Other fungal species have been successfully identified, such as human *Microsporidia* spp., *Candida* spp., *Cryptococcus* spp., *Saccharomyces* spp., *Trichosporon* spp., *Geotrichum* spp., *Pichia* spp., *Blastoschizomyces* spp., *Trichoderma* spp., *Rhizopus oryzae*, *Trichoderma reesei*, *Phanerochaete chrysosporium* and *Saccharomyces cerevisiae* [94–102].

Recently, many studies reported the identification of fungal species using MALDI-TOF-MS analysis of direct samples, such as positive blood culture specimens from patients with candidemia, or nail samples from patients with onychomycosis [103–105].

Identification of protists

Some reported studies have used proteomic approaches on protozoa but, to our knowledge, no study has identified protozoans using whole-cell MALDI-TOF-MS tools [106–112].

Identification of multicellular organisms

Zang *et al.* successfully identified three mammalian cell lines (the K562 human myelomonocytic cell line, the BHK21 fibroblast cell line derived from baby hamster kidney cells, and the GM15226 human lymphoblast cell line) using MALDI-TOF-MS [113]. Recently, MALDI-TOF-MS was adapted to identify 66 cell line samples of 34 species of Eukarya, from insects to primates [114].

Identification of viruses by MALDI-TOF-MS

To date, only a few reports have been published on the identification of viruses using MALDI-TOF-MS. Some studies used MALDI-TOF-MS to identify viral fusion protein, which indicated the virulence of the viral strains (i.e., Newcastle disease virus), viral glycoproteins (i.e., Sindbis virus) and capsid proteins (i.e., Norovirus) [115–118]. Most studies used MALDI-TOF-MS to identify specific restriction fragments of viral genomes by their molecular weights, or to determine a specific gene mutation (i.e., hepatitis B virus, hepatitis C virus or avian influenza virus) [119–121].

Recently, La Scola *et al.* used MALDI-TOF-MS identification tools on 19 types of purified *Acanthamoeba*-growing giant viruses. The authors identified 13–110 reproducible mass peaks, which allowed the correct identification of *Acanthamoeba*-growing giant viruses, and a cluster analysis confirmed that their positions correlated with the phylogenetic tree based on the *PoIB* gene sequence [122]. Other viruses, such as cowpox virus and human herpes simplex virus (HSV)-1, have also been identified using MALDI-TOF-MS tools in our laboratory [Raoult *et al.*, Unpublished Data].

MS application in clinical microbiology

Bacteria identification by MALDI-TOF-MS in clinical laboratories

In recent years, several mass spectra fingerprints of a large number of human pathogenic bacteria species were added to separate reference mass spectra databases, as described previously. Soon thereafter, MALDI-TOF-MS was reported as an accurate and

reproducible method for bacterial identification after intra- and inter-laboratory evaluation with different culture conditions, sample preparation methods, MALDI-TOF instruments and diverse types of matrices. MALDI-TOF-MS bacterial identification is possible using conserved protein signatures, despite differences in the culture media and conditions [7,25,26,123,124]. The accuracy rate of MALDI-TOF-MS in identifying bacteria at the species or strain level was evaluated using a species comparison to an updated species-specific database (as described previously). Recently, MALDI-TOF-MS tools, and the commercial BioTyper database, were used in a retrospective study to identify 100% of 602 *S. aureus* (363 MSSA and 243 MRSA) and 412 CoNs isolates, compared with molecular identification methods [125].

Routine bacterial identification by MALDI-TOF-MS

In this section, we will focus on some retrospective and prospective studies that evaluated the performance of automated MALDI-TOF-MS tools in the routine identification of bacterial isolates in the clinical laboratory. We reported a prospective study of an automated MALDI-TOF-MS application in routine bacterial identification in a clinical microbiology laboratory [1]. In total, 1660 bacteria isolates were identified by MALDI-TOF-MS, using an automated comparison to the BioTyper database (updated in June 2008), and conventional phenotypic identification. Discrepancies were checked later by 16S rRNA and *rpoB* gene sequencing. Overall, the correct identification rate was 84.1% at the species level, and 95.4% at the genus level when the direct analysis of bacteria without additional protein extraction was conducted. Frequently misidentified bacteria included 11 *S. pneumoniae*, seven *Stenotrophomonas maltophilia*, and all five *S. sonnei* isolates, identified by MALDI-TOF-MS as *S. parasanguinis*, *P. hibiscicola*, and *E. coli*, respectively. The difficulty in *S. pneumoniae* identification was caused by poor mass-peak signals, which usually could not be distinguished from the noise produced by the medium culture (e.g. 5% sheep blood and chocolate agar). According to the recent taxonomy of *Pseudomonas* spp. based on the 16S rRNA sequence, *P. hibiscicola* and *P. beteli* are heterotypic synonyms of *S. maltophilia* [126]. Therefore, all of the reference spectra listed under *P. hibiscicola* or *P. beteli* in the BioTyper database were changed to *S. maltophilia*. Further mass spectrometric study of *Shigella* spp. is needed to identify closely related species-specific biomarkers to improve identification of this species. Most unidentified species (e.g., *Propionibacterium acnes* and other anaerobic bacteria) were absent from the database. Figures 2 & 3 show the identification of bacteria by their different raw spectra and the comparison of phylogenic tree based on 16S rRNA sequences and MALDI-TOF-MS dendrogram of clinical isolates of bacteria.

A retrospective study of 1116 previously collected clinical isolates, with 108 additional reference strains, was reported recently [46]. Bacterial identification from the colonies by MALDI-TOF-MS using the BioTyper database was compared with manual and automated conventional identification methods (i.e., API, Vitek 2®). All discrepancies were clarified by 16S rRNA gene sequencing. Overall, the correct species-identification rates were determined to be 93.5% for reference strains and 95.2% for clinical isolates. At the species level, MALDI-TOF-MS identified 100, 99.5, 95.5, 93.7 and 79.7% of enterococci, staphylococci, Enterobacteriaceae, streptococci and nonfermenting Gram-negative bacilli, respectively. All species of *Staphylococcus* isolates were correctly identified, except for one *S. haemolyticus* isolate, which was misidentified as *S. epidermidis* by MALDI-TOF-MS. The great difficulty in clearly identifying *Streptococcus* spp. in this study could be explained by the inability of 16S rRNA sequencing to correctly identify *Streptococcus* isolates at the species level. Most of the microorganisms misidentified by MALDI-TOF-MS were usually from closely related strains (i.e., two *Shigella sonnei* reference strains incorrectly identified as *E. coli*) [46]. The same difficulty in identifying species of *Streptococcus* spp. and *Shigella* spp. was also reported by Blondiaux *et al.* [44].

The reliability of MALDI-TOF-MS identification of microorganisms was also evaluated by van Veen *et al.* in a retrospective study, followed by another prospective study [45]. In the retrospective study, the correct rate of MALDI-TOF-MS identification at the species level was 85.6% of 327 isolates, including 19 yeast isolates. For the prospective study, MALDI-TOF-MS correctly identified the species in 92% of 980 clinical isolates, with 61 yeast isolates. By group, the performance of MALDI-TOF-MS identification was determined to be 97.7, 94.3, 92, 85.2, 84.8 and 84% for Enterobacteriaceae, Gram-positive cocci in clusters, nonfermenting Gram-negative rods, yeast, Gram-positive cocci in chains and miscellaneous bacteria, respectively. All hemolytic streptococci were properly identified. However, only 86.4, 77.8 and 9.5% of *S. pneumoniae*, *S. milleri* and *S. viridans* were identified, respectively.

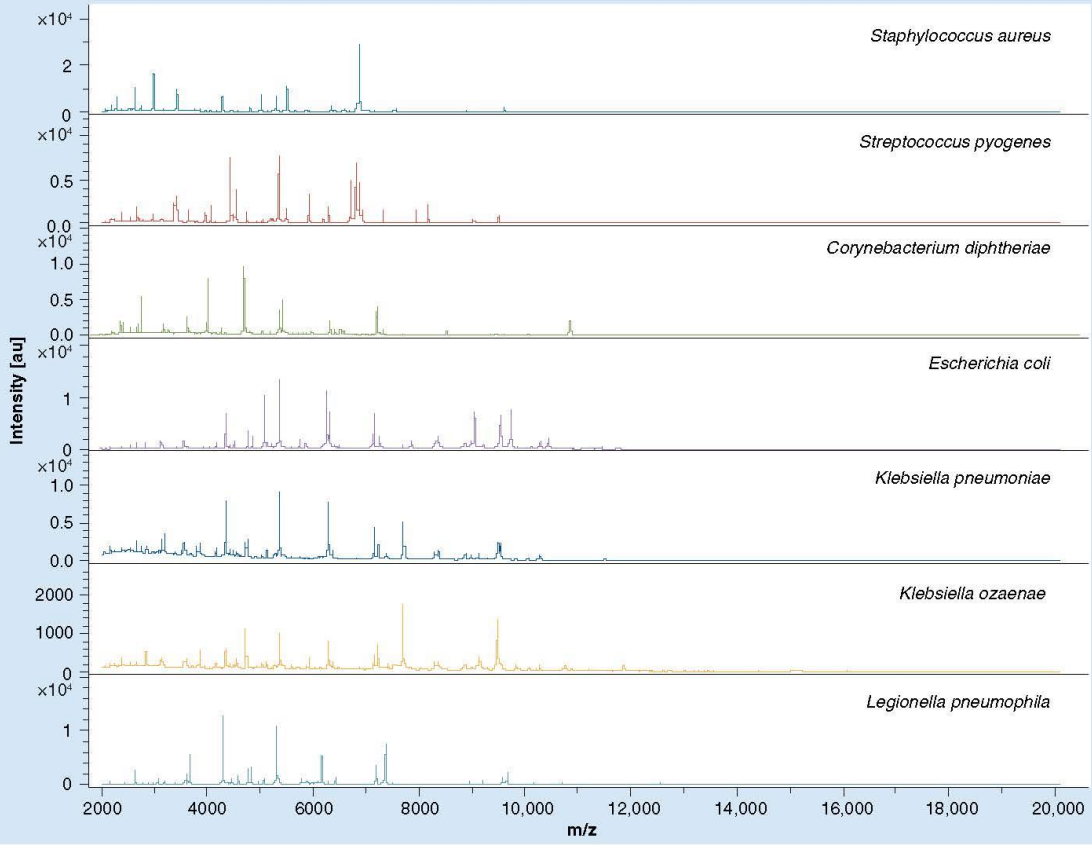

Figure 2. Alignment of raw spectra of seven bacteria, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium diphtheriae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella ozaenae* and *Legionella pneumophila*.

Another study of routine identification of bacteria and yeast using MALDI-TOF-MS was published by Bizzini *et al.* Compared to conventional methods, MALDI-TOF-MS correctly identified 95.1% of 1278 isolates at the species level that had log scores of at least 2.0, and 88.6% of 1371 tested clinical isolates. Direct bacteria analysis identified bacteria with scores of at least 2 in only 70.3% of all studied isolates; identification of an additional 22.9% was achieved by additional protein extraction. No other molecular identification was used to clarify discrepancies. Other authors reported the same difficulty noted in our study regarding the correct identification of *Shigella* spp. and *P. acnes* strains [127]. Low reproducibility of Gram-negative mass spectra grown on MacConkey agar could be explained by the presence of crystal violet-inhibited mass peak signals.

Cherkavoui *et al.* used two systems of MALDI-TOF-MS in the routine identification of bacterial species in the clinical laboratory, with comparison to conventional identification. Two mass spectrometers from Bruker and Shimadzu, with two reference databases (BioTyper and SARAMIS), were used to identify 720 clinical isolates, with putative species identification in 99.1 and 88.8% of isolates, respectively [128]. Discrepancies and identified isolates were resolved by 16S rRNA sequencing. Accuracy rate for the identification of *Streptococcus* spp. and anaerobic Gram-negative bacteria was low (i.e., only 57.1 and 71.4% of streptococci for Bruker and Shimadzu mass spectrometers, respectively) [128].

The accuracy rate of automated MALDI-TOF-MS in routine bacterial identification at the species level in clinical microbiology laboratories was 84.1–99.1% using commercial

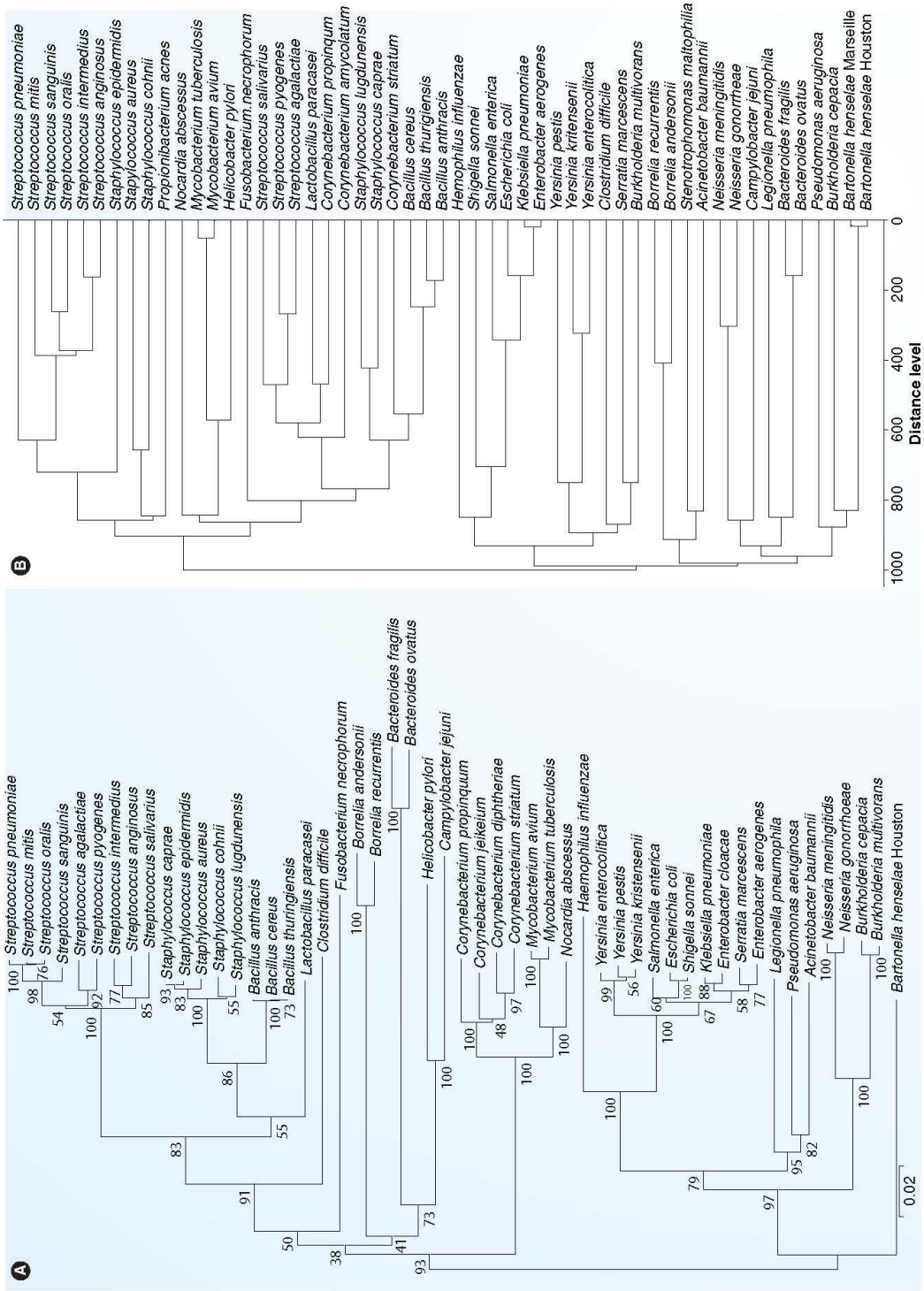

Figure 3. Comparison of phylogenic tree based on (A) 16S rRNA sequences and (B) matrix-assisted laser desorption ionization time-of-flight mass spectrometry dendrogram of clinical isolates of bacteria.

reference databases (BioTyper or SARAMIS). Three out of four studies on the clinical evaluation of MALDI-TOF-MS identification of microorganisms used molecular methods to resolve discrepancies. Some species that were misidentified (anaerobic bacteria, CoNS and *P. acnes*) would be resolved by completion of the database. More studies of *Streptococcus* spp. are urgently needed to improve the accuracy, speed and cost–effectiveness of MALDI-TOF-MS in medical routine microorganism identification.

Microbial identification by MALDI-TOF-MS without colony cultures

Bloodstream samples

In the past 10 years, accurate and rapid MALDI-TOF-MS tools for bacterial identification from direct colony analysis were successfully adapted for the direct identification of bacteria in blood-culture bottles. We will focus on some studies of MALDI-TOF-MS performance in identifying microorganisms in clinical laboratories based on diagnosis from positive blood-culture broths.

La Scola *et al.* reported the successful identification of bacteria directly from positive blood-culture bottles. Among 599 positive blood-culture bottles, 584 were correctly identified by routine MALDI-TOF-MS and phenotypic identification of bacteria colonies, and 562 positive blood-culture bottles for one bacterial species were analyzed by MALDI-TOF-MS using two different protocols. The primary 322 positive blood-culture bottles were tested using trifluoroacetic acid (TFA); this protocol identified only 59% of bacteria at the species level compared with routine MALDI-TOF-MS identification. The second protocol using formic acid (FA) improved bacterial species identification to 67%. Gram-positive bacteria were correctly identified less frequently than Gram-negative bacteria (i.e., 37 and 67% of Gram-positive bacteria vs 94 and 88% of Gram-negative bacteria were identified using protocol 1 and protocol 2, respectively) [2].

The MALDI-TOF mass spectra of blood-culture bottles artificially infected by 12 strains of six different *Candida* spp. were used to create reference databases, which allowed the correct identification of 36 additional *Candida* strains and one *C. albicans* isolate from a patient admitted with candidemia [103]. Further studies on other fungal species are needed to update the MALDI-TOF-MS database for routine application in clinical microbiological diagnosis.

Rapid identification of bacteria from blood-culture broths using MALDI-TOF-MS was also reported by Stevenson *et al.* [129] Among 170 samples identified at the species level from 212 positive blood-culture broths, only 138 samples were correctly identified at the species level, 24 samples were identified at the genus level, and eight were misidentified. Overall, the accuracy rate of MALDI-TOF-MS tools for direct bacterial identification from blood-culture bottles was 65 and 76% at the species and genus levels, respectively. The authors mentioned that the minimal concentration of bacteria needed to obtain excellent spectra was less than 1×10^6 colony-forming units, and nine out of ten positive blood-culture broths infected with mixed microbial species had mass spectra scores of more than 7. The same difficulty was reported in a study by La Scola *et al.*, in which eight *S. mitis* strains were misidentified as *S. pneumoniae* [2].

Direct identification of bacteria and yeast from positive blood-culture broths was recently reported by Ferroni *et al.* In this study, 312 negative blood-culture flasks, artificially infected by adding 1×10^4 cells of 292 bacterial strains and 20 *Candida* strains were used, as well as 388 positive blood-culture flasks from patients, and 46 other fluid samples grown in blood culture broth. The accuracy rates of MALDI-TOF-MS in identifying microorganisms at the species level were 89, 91 and 96% for artificial-infected blood cultures, clinical blood cultures and fluid samples in blood-culture broth, respectively. The authors mentioned the difficulty in identifying closely related species, owing to the low quality of mass spectra from the direct blood-culture broth analysis. Interestingly, the Slidex pneumo-kit™ (bioMérieux, France) has been used on supernatants of blood-culture broths to identify 98% of cases of *S. pneumoniae* previously ambiguously identified as *S. pneumoniae* or *S. mitis*. Of 15 blood cultures from patients containing mixed bacteria, the pathogens present in the mixture was detected at the same score or only one was detected [104].

To optimize the performance of bacterial identification from positive blood-culture bottles, Christner *et al.* used spiking experiments to determine the effects of bacterial concentration and duration of culture on the performance of MALDI-TOF-MS identification. Preincubation and prolonged blood culture incubation had negative effects on species identification by MALDI-TOF-MS, and required higher concentrations of bacteria. The optimal bacterial concentration was at least 1×10^8 colony-forming units per ml, and bacterial concentrations ranged from 2×10^7 to 7×10^9 colony-forming units per ml in positive blood-culture bottles. Among 277 monospecies-positive blood-culture

bottles, the accuracy rate of species identification was 95%, with log scores of at least 1.9. Other positive blood-culture bottles showed mixed microbial, yeast-positive and no signal in 16, eight and three cases, respectively. Of 15 misidentified species, two *S. thermophilus* isolates and one *S. pettenkoferi* isolate were absent from the database, and the 12 other misidentified isolates were resolved by MALDI-TOF-MS analysis of bacterial colonies. Higher identification rates in anaerobic blood-culture bottles were only observed for *S. aureus* isolates. Misidentified species included *Streptococcus* spp. (13 isolates) and CoNS (five isolates) [130].

Szabados *et al.* reported that the sensitivity of MALDI-TOF-MS to identify bacteria in blood-culture bottles in the BacT/ALLERT™M incubator was lower than that of the BACTEC system, with sensitivities of 28 and 76%, respectively. Using an optimized extraction protocol, only 36% of 268 positive blood-culture bottles in the BacT/ALLERT™ incubator were identified at the species level, and 64% were identified at the genus level [131].

Urine samples

Using the same approach of identifying bacteria in positive blood cultures, Ferreira *et al.* successfully adapted MALDI-TOF-MS tools to identify bacteria directly from urine samples. For this, 260 urine samples identified as positive for bacterial infection by flow cytometry (UF-1000i; bioMérieux, France) were applied to the collection and identification of bacteria by MALDI-TOF-MS, in parallel with conventional identification methods. This technique allowed the identification of 91.8% of positive urine cultures infected with only one bacterium species, and two species were identified at the genus level. All 20 urine samples identified as false positive by flow cytometry were negative by MALDI-TOF-MS. This technique only requires 5–6 min more than routine bacterial identification using MALDI-TOF-MS, and could be applied and re-evaluated in the routine diagnosis of urinary tract infections in clinical laboratories [132].

Environmental samples

The identification of bacteria directly from environmental samples using MALDI-TOF-MS (i.e., the detection of enterohemorragic *E. coli* serotype O157:H7 directly from spiked bovine meat [60,133]), requires an additional step of bacterial enrichment that lasts 8–20 h. In a study of MALDI-TOF-MS identification of *Lactobacillus plantarum*, Sun *et al.* successfully identified *L. plantarum* from cell lysates obtained directly from 1 g of yogurt after short enrichment cultivation [35].

Comparison of MALDI-TOF-MS & alternative identification methods in bacteria identification

Compared to the routine phenotypic identification methods, such as semiautomated Gram staining (Aerospray Wescor®; Elitech), catalase and oxidase determination, automated identification by Vitek® (BioMérieux) and the Api Anaérobie Bio Mérieux® identification strip for anaerobes (BioMérieux), we have evaluated the performance of MALDI-TOF-MS to identify 84.1 and 11.3% of 1660 clinical isolates at species and genus levels, respectively [1]. Similarly, Eigner *et al.* retrospectively identified 95.2% of 1116 clinical isolates. Van Veen *et al.* have identified 79.9 and 17.3% of 980 clinical isolates at the species and genus levels, respectively [45]. Bizzini *et al.* have correctly identified 95.1% of 1278 isolates at the species level that had log scores of at least 2.0, and 88.6% of 1371 tested clinical isolates [127]. Cherkaoui *et al.* have correctly identified 93.6 and 88.3% of 720 clinical isolates at the species level using Bruker Daltonic and Shimadzu systems, respectively [128].

The cost of bacterial identification by MALDI-TOF-MS was evaluated at €1.43 compared with €4.60–8.23 in the conventional identification in our study [1]. This estimation has been supported by two other prospective studies [127,128]. The time needed for bacterial identification from a direct colony was 6–8.5 min versus 5–48 h for conventional identification [1]. Only three prospective studies of routine bacterial identification by MALDI-TOF-MS have used molecular-identification methods to check discrepancies between MALDI-TOF-MS and conventional identification [1,46,128].

Aside from the commercial spectra databank of BioTyper (Bruker Daltonics), SARAMIS and MicrobeLynx, other species-specific databases have improved the performance of MALDI-TOF-MS in routine bacterial identification (i.e., *Bartonella* spp. and *Legionella* spp.)

Future perspective

The technique of MALDI-TOF-MS has existed for a long time, but its place in clinical microbiology laboratories has only been identified recently. The first publication demonstrating its use in routine diagnosis was reported in 2009 [1]. The diagnostic industry in this field has shown rapid growth, and it is probable that many laboratories will have an apparatus for biotyping microbes

within 2–3 years. It appears that the identification of bacteria is simple, and that biotyping by MALDI-TOF-MS should completely replace phenotypic detection in the next 10 years. The currently important issues are the recognition of antibiotic resistance, which is still incompletely defined, and the automation of collection and detection for blood cultures (also for urine samples). In addition, the role of MALDI-TOF-MS in the identification of viruses or eukaryotes remains to be defined. Last, we believe that MALDI-TOF-MS will play a considerable role in the evaluation of whole collections of microorganisms and cells, while quickly making possible the evaluation of reliable identification. Using two collections of bacteria, *Bartonella* spp. and *Legionella* spp., we have shown that errors in identification can occur. The capacity of MALDI-TOF-MS to identify cell lines will simplify the management of microorganism collections. Finally, if MALDI-TOF-MS becomes economically feasible on a widespread basis, it is likely that this technology will improve interpretation significantly, and will make MS essential for every clinical microbiology laboratory.

Financial & competing interests disclosure

Public hospital system of Marseille (Assistance Publique – Hôpitaux de Marseille) has two patents pending on virus identification by MALDI-TOF-MS with Bernard La Scola and Didier Raoult as co-inventors, and eukaryote identification by MALDI-TOF-MS with Michel Drancourt and Didier Raoult as co-inventors. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

MALDI-TOF-mass spectrometry tools for bacterial identification

- matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has been used in basic research to identify bacteria by their respective proteins for more than 30 years.
- In the last decade, this technique of identification was successfully adapted to identify clinical and environmental microorganisms in clinical microbiology laboratories.
- MALDI-TOF-MS was used to identify bacteria and Archaea, not only at the species level, but also at the subspecies and strain levels, allowing the detection of epidemic lineages.
- Some antibiotic resistances, and well as some bacterial toxins, might be detected using MALDI-TOF-MS.

Identification of eukaryotes by MALDI-TOF-MS

- The databanks have quickly increased in size to identify, not only bacteria, but also fungi and animals.
- Fungal species that have been identified correctly by MALDI-TOF-MS include *Penicillium* spp., *Scytalidium dimidiatum*, *Trichophyton rubrum*, *Aspergillus* spp., *Fusarium* spp., human *Microsporidia* spp., *Candida* spp., *Cryptococcus* spp., *Saccharomyces* spp., *Trichosporon* spp., *Geotrichum* spp., *Pichia* spp., *Blastoschizomyces* spp., *Trichoderma* spp., *Rhizopus oryzae*, *Trichoderma reesei*, *Phanerochaete chrysosporium* and *Saccharomyces cerevisiae*.
- To date, whole-cell MALDI-TOF-MS is not able to identify any protozoan species.
- MALDI-TOF-MS was used to successfully identify 66 cell line samples of 34 species of Eukarya, from insects to primates, and three other mammalian cell lines (the K562 human myelomonocytic cell line, the BHK21 fibroblast cell line derived from baby hamster kidney cells and the GM15226 human lymphoblast cell line).

Identification of viruses by MALDI-TOF-MS

- MALDI-TOF-MS was used to identify the viral fusion protein, which indicated the virulence of the Newcastle disease virus, the viral glycoprotein of Sindbis virus and the capsid protein of Norovirus.
- Most studies used MALDI-TOF-MS to identify specific restriction fragments of viral genomes by their molecular weights, or to determine specific gene mutations of hepatitis B virus, hepatitis C virus and avian influenza virus.
- MALDI-TOF-MS was also used to identify 19 *Acanthamoeba*-growing giant viruses, the cowpox virus and the human herpes virus.

MS application in clinical microbiology

- MALDI-TOF-MS was recently applied to the routine diagnosis of clinical microbes, not only from direct colonies of pathogens, but also from blood-culture bottles, urine and environmental samples.

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CONCLUSION & PERSPECTIVE DE CHAPITRE 1 « Revue: L'application de spectrométrie de masse MALDI-TOF (Matrix-assisted laser desorption/ionization time-of-flight) en microbiologie clinique. »

La technique de MALDI-TOF MS existe depuis longtemps et a commencé à trouver sa place dans les laboratoires de microbiologie clinique que très récemment. La première publication montrant son utilisation dans le diagnostic de routine a été rapportée en 2009 (55). L'industrie du diagnostic est exceptionnellement avancée dans ce domaine et il est probable que de nombreux laboratoires aient dans deux ou trois ans un appareil afin de pouvoir biotyper la microbiologie. Actuellement, il s'avère que l'identification des bactéries par MALDI-TOF est facile et que dans les prochaines années, le biotypage par MALDI-TOF sera amené à remplacer l'ensemble des identifications phénotypiques. Les questions importantes restant à définir sont la reconnaissance du type de résistance aux antibiotiques et l'automatisation dans la détection dans l'échantillonnage clinique (par exemple dans les urines, les prélèvements profonds et dans les flacons d'hémoculture). En outre, la place de MALDI-TOF dans l'identification des virus ou des Eucaryotes reste à définir. Enfin, nous pensons que MALDI-TOF jouera un rôle considérable dans l'évaluation des collections de micro-organismes et des cellules en permettant d'obtenir rapidement et de manière fiable l'identification du microorganisme. Le MALDI-TOF nous permettrait d'éviter des erreurs d'identification comme cela peut se produire dans les deux espèces de bactéries, *Bartonella* sp. et *Legionella* sp. La capacité de MALDI-TOF à identifier les lignées cellulaires permettra de simplifier la gestion des collections. En conclusion, si MALDI-TOF-MS devient un véritable enjeu

économique, nous espérons que cette technologie d'identification s'améliorera de manière très significative et rendra la spectrométrie de masse essentielle à tout laboratoire de microbiologie clinique.

Chapitre 2 : « Ongoing revolution in bacteriology: routine identification of bacteria by MALDI-TOF-mass spectrometry. »

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RESUME DE PUBLICATION N° 2 : « Ongoing revolution in bacteriology: routine identification of bacteria by MALDI-TOF-mass spectrometry.»

Contexte :

La méthode d'identification bactérienne par spectrométrie de masse MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight) a montré être une méthode précise d'identification de bactéries sélectionnées et de bactéries dans des situations cliniques données.

Cette technique n'a pas été évaluée pour une utilisation en routine en microbiologie clinique.

Méthodes :

Nous avons analysé de manière prospective l'identification de routine des bactéries par le MALDI-TOF MS en comparaison avec l'identification phénotypique conventionnelle indépendamment du phylum ou de l'origine du prélèvement. Les divergences entre les deux techniques ont été tranchées par une identification par biologie moléculaire avec un séquençage du gène 16S ribosomal RNA ou du gène de *rpoB*. Nous avons utilisé un spectromètre de masse Autoflex II (Brüker, Daltonik) pour analyser des colonies bactériennes à partir de 4 dépôts d'isolat directement sur une plaque de MALDI-TOF. Des spectres peptidiques ont été comparés à la base de données de Bruker BioTyper, la version 2,0, et le score d'identification a été noté. Le délai et les coûts d'identification ont été mesurés.

Résultats :

Parmi les 1660 isolats bactériens analysés, 95,4% ont été correctement identifiés par MALDI-TOF MS; 84,1% ont été identifiés au niveau de l'espèce, et 11,3% ont été identifiés au niveau du genre. Dans la plupart des cas, l'absence d'identification (2,8% des isolats) et l'identification incorrecte (1,7% des isolats) étaient dues aux entrées inexactes de base de données. L'identification par MALDI-TOF MS a été significativement corrélée avec la présence de 10 spectres de référence dans la base de données (p=0,01). Le temps moyen requis pour l'identification par MALDI-TOF MS pour un isolat était de 6 minutes pour un coût de 22% à 32% moins cher de celui des méthodes d'identification phénotypique conventionnelle.

Conclusions :

La spectrométrie de masse (MALDI-TOF MS) est une méthode rentable et précise pour l'identification en routine d'isolats bactériens en moins d'une heure en utilisant une base de données comportant des spectres de référence ≥ 10 par espèce bactérienne et un score d'identification ≥1,9 (Bruker, Daltonik). Cette Technique de MALDI-TOF MS pourrait remplacer dans un avenir très proche la coloration de Gram et l'identification par caractère biochimique des bactéries en laboratoire de microbiologie clinique.

MAJOR ARTICLE

Ongoing Revolution in Bacteriology: Routine Identification of Bacteria by Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

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(See the editorial commentary by Nassif on pages 552–3)

Background. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry accurately identifies both selected bacteria and bacteria in select clinical situations. It has not been evaluated for routine use in the clinic.

Methods. We prospectively analyzed routine MALDI-TOF mass spectrometry identification in parallel with conventional phenotypic identification of bacteria regardless of phylum or source of isolation. Discrepancies were resolved by 16S ribosomal RNA and *rpo*B gene sequence–based molecular identification. Colonies (4 spots per isolate directly deposited on the MALDI-TOF plate) were analyzed using an Autoflex II Bruker Daltonik mass spectrometer. Peptidic spectra were compared with the Bruker BioTyper database, version 2.0, and the identification score was noted. Delays and costs of identification were measured.

Results. Of 1660 bacterial isolates analyzed, 95.4% were correctly identified by MALDI-TOF mass spectrometry; 84.1% were identified at the species level, and 11.3% were identified at the genus level. In most cases, absence of identification (2.8% of isolates) and erroneous identification (1.7% of isolates) were due to improper database entries. Accurate MALDI-TOF mass spectrometry identification was significantly correlated with having 10 reference spectra in the database ($P = .01$). The mean time required for MALDI-TOF mass spectrometry identification of 1 isolate was 6 minutes for an estimated 22%–32% cost of current methods of identification.

Conclusions. MALDI-TOF mass spectrometry is a cost-effective, accurate method for routine identification of bacterial isolates in <1 h using a database comprising ⩾10 reference spectra per bacterial species and a ⩾1.9 identification score (Brucker system). It may replace Gram staining and biochemical identification in the near future.

Bacterial identification is routinely based on phenotypic tests, including Gram staining, culture and growth characteristics, and biochemical pattern [1]. Although some of these tests are performed within minutes, complete identification is routinely achieved within hours in the best cases or days for fastidious organisms. Such conventional, time-consuming procedures hamper proper treatment of patients with respect to antibiotic and supportive treatments. Rapid and accurate identification of routinely encountered bacterial species is therefore warranted to improve the care of patients with infectious diseases.

Bacterial identification based on peptidic spectra obtained by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry was proposed >30 years ago [2–4]. It has only recently been used as a rapid, inexpensive, and accurate method for

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identifying isolates that belong to certain bacterial phyla (Figure 1). It has also proved useful for identifying bacteria isolated in selected clinical situations, such as cystic fibrosis [5]. However, previous studies did not evaluate the effectiveness of MALDI-TOF mass spectrometry identification for routine use in the clinics, because they included bacterial isolates gathered from past collections and grown in conditions selected for the study [6] or incorporated isolates subcultured in selected growth conditions prior to MALDI-TOF mass spectrometry analysis [7].

We evaluated the performance and cost-effectiveness of MALDI-TOF mass spectrometry for the routine identification of bacteria, regardless of their phylogeny and relation to any specific clinical situation.

MATERIALS AND METHODS

Bacterial isolates. All isolates recovered from blood, cerebrospinal fluid, pus, biopsy, respiratory tract, wound, and stool specimens were prospectively included over a 16-week period. The isolates were recovered after aerobic, microaerophilic, and anaerobic incubation of clinical specimens on 5% sheep-blood, chocolate, Mueller-Hinton, trypticase soy, and MacConkey agar media (bioMérieux). After semi-automated Gram staining (Aerospray Wiescor; Elitech) and determination of catalase and oxidase activities, isolates were inoculated into the appropriate Vitek identification strip using the Vitek 2 apparatus (bioMérieux) or API ANA identification strip for anaerobes (bioMérieux). In parallel, 1 single colony was directly deposited on a MALDI-TOF MTP 384 target plate (Bruker Daltonik GmbH), and 4 such deposits were made for each isolate. The preparation was overlaid with 2 μL of matrix solution (saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile, and 2.5% tri-fluoracetic-acid). A total of 15 isolates (4×15 spots) were deposited per plate, and the matrix-sample was crystallized by air-drying at room temperature for 5 minutes.

Mass spectrometry. Measurements were performed with an Autoflex II mass spectrometer (Bruker Daltonik) equipped with a 337-nm nitrogen laser. Spectra were recorded in the positive linear mode (delay, 170 ns; ion source 1 voltage, 20 kV; ion source 2 voltage, 18.5 kV; lens voltage, 7 kV; mass range, 2–20 kDa). Each spectrum was obtained after 675 shots in automatic mode at a variable laser power, and the acquisition time ranged from 30 to 60 s per spot. Data were automatically acquired using AutoXecute acquisition control software. The 2 first raw spectra obtained for each isolate were imported into BioTyper software, version 2.0 (Bruker Daltonik GmbH), and were analyzed by standard pattern matching (with default parameter settings) against the spectra of 2881 species used as

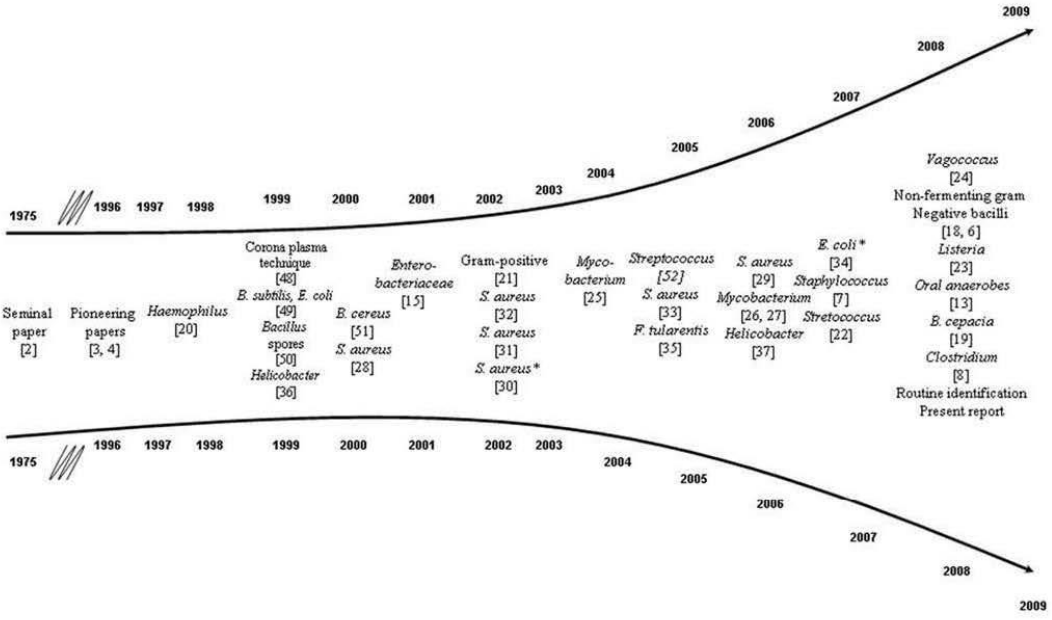

Figure 1. Increasing number of publications related to matrix-assisted laser desorption ionization time-of-flight mass spectrometry applications in medical microbiology. Applications include the identification of isolates, the identification of specific antibiotic-resistance profile, and typing of isolates.

Table 1. Concordance between Conventional Routine Identification (Vitek; bioMérieux) and Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry Identification (Brucker Mass Spectrometer and Database Complemented with Local Database)

	Routine phenotypic identification, no. of isolates				
MALDI-TOF identification	Species identification	Genus identification	No identification	Misidentification	Total
Species identification	1392	0	4	1	1397
Genus identification	185	0	2	2	189
No identification	18	0	26	2	46
Misidentification	27	0	0	1	28
Total	1622	0	32	6	1660

reference database in the BioTyper database (these spectra are an integrated part of the BioTyper software version, as updated in June 2008). When both spots yielded score ≥1.9, the analysis stopped. When 1 or both spots yielded score <1.9, the MALDI-TOF mass spectrometry read the 2 other spots. The method of identification included the m/z from 3 to 15 kDa. For each spectrum, no more than 100 peaks were taken into account and compared with peaks in the database. The 15 bacterial species exhibiting the most similar peptidic pattern with the isolate were ranked by their identification score.

Criteria for identification of isolates. Accurate identification of isolates using the Vitek system was confirmed when the index T was ≥0.25; identification using the API system was confirmed when the percentage of identification was ≥90% and the index T was ≥0.25. As for MALDI-TOF analysis, we used modified score values proposed by the manufacturer: (1) a score ≥1.9 indicated species identification, (2) a score of 1.7–1.9 indicated genus identification, and (3) a score <1.7 indicated no identification. An isolate was considered correctly identified by MALDI-TOF mass spectrometry if ≥2 of 4 spectra had a score ≥1.9 for species identification or ≥1.7 for genus identification. For isolates discrepantly identified by routine phenotype analysis and MALDI-TOF mass spectrometry, we performed partial 16S ribosomal RNA (rRNA) or *rpo*B gene sequencing, as described elsewhere [8–10]. An isolate was correctly identified when its almost full-length 16S rRNA gene sequence yielded ≥98.7% sequence similarity with the closest bacterial species sequence in GenBank [11]; it was correctly identified when its partial *rpo*B gene sequence yielded ≥97% sequence similarity with the closest bacterial species sequence in GenBank or a local database [10, 12].

MALDI-TOF delay and cost analysis. We defined MALDI-TOF mass spectrometry identification delay as the delay between the deposit of bacteria on the MALDI-TOF plate by the technician and the end of the informatics interpretation of spectra (ie, identification ready to be transmitted to the clinician). This delay was randomly measured in 10 nonconsecutive days. Costs of identification were measured by adding the cost of specific consumables, the cost for salary of personals, and the provisions for 5-year depreciation of the respective apparatus (Gram staining apparatus, microscope, identification apparatus, and mass spectrometer) on the basis of 20,000 isolates analyzed per year.

Statistical analyses. For bacterial species under study comprising ≥5 isolates tested by MALDI-TOF mass spectrometry, we tested the correlation between the precision of MALDI-TOF mass spectrometry identification (>85% of isolates identified at the species level—that is, with a MALDI-TOF mass spectrometry identification score ≥1.9) and the number of reference spectra for that bacterial species in the BioTyper database using a Mantel-Haenszel test.

RESULTS

Concordant MALDI-TOF mass spectrometry identification. Of 1660 isolates prospectively analyzed over a 16-week period, 260 isolates (15.7%) did not yield an accurate identification after reading of 2 spots because 1 or both spots were either empty or too small to allow any analysis (Table 1). For these 260 isolates, a peptidic profile was then gathered after reading the 2 further spots. Of 1660 isolates (including 45 genera and 109 species, with 1–347 isolates per species), 1586 (95.5%) yielded identical identifications by current methods of identification and MALDI-TOF mass spectrometry. Of these isolates, 1397 (84.1%) yielded the same species identification by MALDI-TOF mass spectrometry and routine tests, and 189 (11.3%) yielded the same genus identification by MALDI-TOF mass spectrometry and routine tests. Isolates identified at the genus level comprised 2 (100%) of 2 *Actinomyces* species, 2 (6.7%) of 30 *Bacteroides* species, 1 (7.1%) of 14 *Citrobacter* species, 7 (46.7%) of 15 *Corynebacterium* species, 1 (1.4%) of 72 *Enterobacter* species, 13 (15.5%) of 84 *Enterococcus* species, 2 (1%) of 206 *Escherichia coli*, 1 (20%) of 5 *Fusobacterium* species, 2 (28.6%) of 7 *Haemophilus* species, 1 (50%) of 2 *Kingella kingae*, 2 (1.9%) of 104 *Klebsiella* species, 1 (50%) of 2 *Lactobacillus* species, 2 (66.7%) of 3 *Micrococcus luteus* iso-

Table 2. Discrepancies and Errors in Routine Phenotypic Tests and Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry Identification

Final identification	No. of isolates			
	MALDI-TOF identification		Current methods of identification	
	No identification	Misidentification	No identification	Misidentification
Actinomyces naeslundii (*n* = 1)	0	0	0	1[a]
Anaerococcus vaginalis (*n* = 3)	3	0	3	0
Anaerotruncus colihominis (*n* = 1)	1	0	1	0
Atopobium rimae (*n* = 2)	2	0	2	0
Bacteroides fragilis (*n* = 10)	0	0	1	0
Bacteroides ureolyticus (*n* = 1)	1	0	1	0
Bilophila wadsworthia (*n* = 2)	2	0	2	0
Clostridium hatherium (*n* = 1)	1	0	1	0
Clostridium perfringens (*n* = 4)	0	0	1	0
Clostridium symbosium (*n* = 1)	1	0	0	0
Corynebacterium pseudodiphtheriticum (*n* = 2)	0	0	1	0
Eggerthella lenta (*n* = 1)	1	0	0	0
Enterobacter aerogenes (*n* = 23)	0	1[b]	0	0
Enterobacter cloacae (*n* = 39)	0	1[c]	0	0
Escherichia coli (*n* = 206)	0	0	0	0
Finegoldia magna (*n* = 5)	5	0	0	0
Fusobacterium nucleatum (*n* = 4)	3	0	0	0
Lactobacillus zeae (*n* = 1)	0	0	0	1[d]
Parabacteroides distasonis (*n* = 1)	1	0	1	0
Peptoniphilus harei (*n* = 1)	1	0	1	0
Peptoniphilus lacrimalis (*n* = 1)	1	0	1	0
Peptostreptococcus anaerobius (*n* = 1)	0	0	1	0
Peptostreptococcus micros (*n* = 5)	5	0	5	0
Peptostreptococcus vaginalis (*n* = 1)	1	0	1	0
Porphyromonas asacharolytica (*n* = 1)	1	0	1	0
Prevotella bivia (*n* = 2)	0	0	1	0
Prevotella buccae (*n* = 2)	0	0	2	0
Prevotella denticola (*n* = 1)	1	0	1	0
Prevotella intermedia (*n* = 3)	3	0	2	0
Prevotella loescheii (*n* = 1)	1	0	1	0
Propionibacterium acnes (*n* = 58)	8	0	0	0
Shigella sonnei (*n* = 5)	0	5[e]	0	0
Staphylococcus epidermidis (*n* = 272)	1	0	0	2[f]
Staphylococcus saccharolyticus (*n* = 1)	1	0	0	0
Stenotrophomonas maltophilia (*n* = 10)	0	7[g]	0	0
Streptococcus infantis (*n* = 1)	0	1[h]	0	1[i]
Streptococcus sanguinis (*n* = 4)	0	0	0	1[j]
Total (*n* = 678)	45	15	32	6

[a] *Streptococcus mitis.*
[b] *Citrobacter freundii.*
[c] *Klebsiella oxytoca.*
[d] Group G *Corynobacterium* species.
[e] *Escherichia coli.*
[f] *Propionibacterium* species for one and *Staphylococcus lugdunensis* for the other.
[g] *Pseudomonas hibiscicola.*
[h] *Streptococcus parasanguinis.*
[i] *Aerococcus viridans.*
[j] *Gemella morbilorum.*

lates, 27 (45%) of 60 *Propionibacterium* species, 2 (2.4%) of 82 *Pseudomonas aeruginosa* isolates, 23 (6.6%) of 347 *Staphylococcus aureus* isolates, 86 (22.3%) of 385 coagulase-negative *Staphylococcus* species, and 14 (17.3%) of 81 *Streptococcus* species.

Lack of identification and erroneous MALDI-TOF mass spectrometry identification. Forty-six isolates (2.8%) were not identified by MALDI-TOF mass spectrometry (Table 2). These isolates included 8 (13.8%) of 58 *Propionibacterium acnes* isolates, 5 (100%) of 5 *Peptostreptococcus micros* isolates, 5

(100%) of 5 *Finegoldia maga* isolates, 3 (75%) of 4 *Fusobacterium nucleatum* isolates, 3 (100%) of 3 *Anaerococcus vaginalis* isolates, 3 (100%) of 3 *Prevotella intermedia* isolates, 2 (100%) of 2 *Atopobium rimae* isolates, 2 (100%) of 2 *Bilophila wadsworthia* isolates, and 1 isolate for each of 15 additional species (Table 2). An additional 28 isolates (1.7%) were erroneously identified by MALDI-TOF mass spectrometry even though they had scores ⩾1.9. These isolates included 11 (45.8%) of 24 *Streptococcus pneumoniae* isolates (identified as *Streptococcus parasanguinis*), 7 (70%) of 10 *Stenotrophomonas maltophilia* isolates (identified as *Pseudomonas hibiscicola*), 5 (100%) of 5 *Shigella sonnei* isolates (identified as *E. coli*), 1 (4.3%) of 23 *Enterobacter aerogenes* isolates (identified as *Citrobacter freundii*), 1 (2.6%) of 39 *Enterobacter cloacae* isolates (identified as *Klebsiella oxytoca*), 1 (1.1%) of 90 *Klebsiella pneumoniae* isolates (identified as *E. coli*), 1 *Lactobacillus casei* isolate (identified as *Lactobacillus rhamnosus*), and 1 *Streptococcus infantis* isolate (identified as *S. parasanguinis*) (Table 2). When the spectra of the aforementioned isolates were added to the Bruker database, further identification was accurate.

Phenotype erroneous identifications. The current methods of identification failed for 32 isolates (1.9%), which were all anaerobes (Table 2). Phenotypic identification was erroneous for 28 isolates (1.7%). One isolate phenotypically identified as *Streptococcus mitis* was identified as *Actinomyces* species by MALDI-TOF mass spectrometry and was confirmed to be *Actinomyces naeslundii* by 16S rRNA gene sequencing. One isolate phenotypically identified as *Aerococcus viridans* was identified as *S. parasanguinis* by MALDI-TOF mass spectrometry and as *S. infantis* by partial *rpo*B gene sequencing. One isolate phenotypically identified as *Gemella morbilorum* was identified as *Streptococcus* species by MALDI-TOF mass spectrometry and was confirmed to be *Streptococcus sanguinis* by partial *rpo*B gene sequencing. One *Corynebacterium* group G isolate was identified as *Lactobacillus* species by MALDI-TOF mass spectrometry and was confirmed to be *Lactobacillus zeae* by 16S rRNA gene sequencing. One isolate phenotypically identified as *Staphylococcus epidermidis* was identified as *Propionibacterium* species by MALDI-TOF mass spectrometry and as *S. epidermidis* by *rpo*B sequencing.

MALDI-TOF mass spectrometry identification performances. For bacterial species comprising ⩾5 isolates under study, the fact that ⩾85% of isolates were identified to the species level by MALDI-TOF mass spectrometry analysis was borderline correlated with the fact that the reference database for that species comprised >5 reference spectra ($P = .45$). Accurate MALDI-TOF mass spectrometry identification was significantly correlated with the fact that the reference database for those species included ⩾10 reference spectra ($P = .01$).

Comparative delay and cost of MALDI-TOF mass spectrometry identification. The delay for MALDI-TOF mass spectrometry identification (15 isolates; 4 spots per isolate) was 90 minutes, including 25 minutes for plate preparation, 15 minutes for plate loading, and 50 minutes for plate reading and spectra interpretation, for a mean delay of 6 minutes per isolate (Figure 2). Furthermore, use of only 2 spots per isolate resulted in a delay of identification of 55 minutes for 15 colonies and a mean delay of 3.5 minutes per isolate. Because our protocol includes a 5-minute matrix drying step regardless of the number of isolates, the minimum delay for MALDI-TOF mass spectrometry identification of 1 isolate would be 8.5 minutes, including 7 minutes for colony and matrix deposition and drying, a 0.5-minute spectra acquisition, and 1 minute for informatics interpretation and identification of spectra. The cost for 1 MALDI-TOF mass spectrometry identification as tabulated in this laboratory is presented in Table 3.

DISCUSSION

We tested a large collection of bacteria by mass spectrometry for the first time in a routine laboratory. The proof-of-concept that mass spectrometry could identify crude bacteria was established >30 years ago [2], but the pioneering works were published in nonmedical, specialized mass spectrometry journals [2, 4, 5]. Such studies dealt with anaerobic bacteria from the oral flora [13]; clostridia [8]; Enterobacteriaceae [14], including *E. coli* [15, 16], *Yersinia enterocolitica* [16], and *Erwinia* species [17]; nonfermenting bacteria [18], such as *Burkholderia cepacia* complex [19]; *Haemophilus* species [20]; various gram-positive cocci [21], including *Staphylococcus* species [7], viridans *Streptococcus* species [22], *Listeria* species [23], and *Vagococcus fluvialis* [24]; and *Mycobacterium* species [25–27]. MALDI-TOF mass spectrometry was also used to discriminate antibiotic resistance within minutes (Table 2); for example, methicillin-resistant *S. aureus* was identified [28–33] because the spectra of methicillin-resistant and methicillin-susceptible *S. aureus* organisms differed in the mass range of m/z 500–3500 Da [29, 30], and spectral profiles were accurately clustered into 2 separate groups (ie, methicillin-resistant and methicillin-susceptible *S. aureus*) [30]. Camara et al [34] demonstrated the usefulness of MALDI-TOF mass spectrometry for rapid discrimination of ampicillin-resistant *E. coli* organisms displaying an m/z 29,000 peak that has been confirmed to be a β-lactamase. Antibiotic resistance–associated specific peak detection depended on the type of culture medium, instruments, and experimental protocols [32, 33], suggesting that local databases should be built for accurate detection of resistance profiles. MALDI-TOF mass spectrometry further discriminated bacteria at the subspecies level (*Francisella tularensis* [35] and *Bartonella* subspecies; P. E. Fournier, unpublished data), at the serotype level (*Salmonella* species), and at the strain level (*Helicobacter pylori* [36, 37], *Haemophilus influenzae* [38] and *Bartonella henselae*; P. E. Fournier, unpublished data). Also, MALDI-TOF

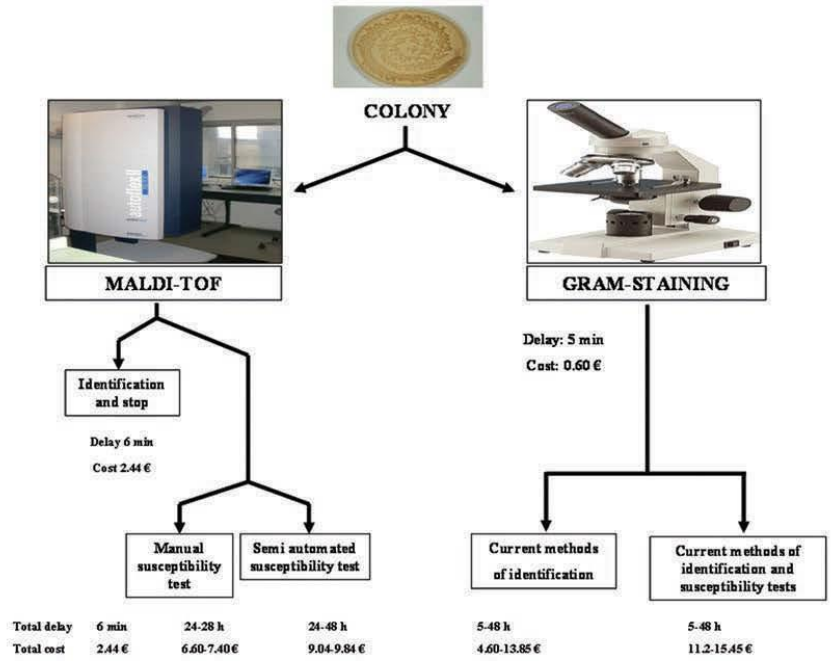

Figure 2. Work flow and delay for matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry identification of bacteria in this study.

mass spectrometry analyses proved to be effective for the identification of bacterial isolates generated from specimens collected in selected clinical situations (eg, respiratory tract specimens obtained from patients with cystic fibrosis) [6]. Bacterial isolates *(E. coli)* tested using the same reagents in different laboratories with different mass spectrometers have also yielded reproducible, identifying spectra [39].

We observed that 95.4% of isolates were identified by MALDI-TOF mass spectrometry at the species and genus levels. With the exception of *F. nucleatum,* the lack of MALDI-TOF mass spectrometry identification was observed almost only for non-*Clostridium* anaerobes, which had no reference in the Bruker database. In fact, when based on accurate databases, MALDI-TOF mass spectrometry will be of particular interest for the identification of anaerobes. As illustrated in this report, these fastidious organisms are poorly identified by current phenotypic methods, which lack specificity and result in ambiguous or even erroneous identification. The availability of easy and rapid MALDI-TOF mass spectrometry identification of anaerobes may encourage microbiologists to further isolate and culture this group of pathogens, the presence of which is often underestimated in situations such as orthopedic prosthesis infections [40] or brain abscess [41]. Likewise, the misidentification of all *S. sonnei* organisms as *E. coli* was due to an absence in the database. This was also the case for almost one-half of *S. pneumoniae* isolates that were misidentified as *S. parasanguinis* (a closely related species within the mitis group of *Streptococcus* species [42]), because the database included only 3 *S. pneumoniae* and 2 *S. parasanguinis* reference spectra. The incorporation of additional *S. pneumoniae* spectra solved this problem. Likewise, 7 *S. maltophilia* isolates were misidentified as *P. hibiscicola* by MALDI-TOF mass spectrometry. We hypothesized that this discordance resulted from a trivial mislabeling of bacterial species in the Bruker database. Indeed, *P. hibiscicola* is an invalid name for a nonfermenting gram-negative rod that was demonstrated to be *S. maltophilia* [43–45]. Addition of correct spectra in the database solved these problems. Approximately 16% of isolates were identified only at the genus level by MALDI-TOF mass spectrometry analysis; an example of this identification was provided by *P. acnes,* for which only 1 spectrum (DSM 1897 strain) was included in the Bruker database. We hypothesized that this unique spectrum may not be representative of the true diversity of *P. acnes* profiles, and the inclusion of additional *P. acnes* spectra in the

Table 3. Delays, Costs, and Level of Training for Isolate Identification Methods

Method	Delay, minutes	Cost, €[a]	Level of training
Manual			
Gram staining	6	0.6	Medium to high
API system identification (bioMérieux)	1080–2880	4.6–6.0	Medium
Antibiotic susceptibility test	1080–2880	6.6–7.4	Medium
Phoenix system identification and susceptibility test (BD Diagnostics)	300–1200	12.65	Medium
Vitek system (bioMérieux)			
Identification	300–480	5.9–8.23	Medium
Identification and susceptibility test	300–480	10.38–12.71	
MALDI-TOF	6–8.5	1.43	Low to medium

NOTE. MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight.

[a] Costs have been tabulated based on December 2008 price list of the providers in France

database resulted in a 100% correct identification (data not shown). The same remark held true for *Bacillus cereus*, for which the Bruker database also included only 1 reference spectrum. We further observed that the statistical significance of the correlation between precision in MALDI-TOF mass spectrometry identification and the number of reference spectra increased from $\geqslant$5 reference spectra to $\geqslant$10 reference spectra in the database, further indicating that a complete and representative database is, unsurprisingly, a critical requirement for the accurate identification of isolates by MALDI-TOF mass spectrometry [46].

This large, prospective study included >1600 isolates, representative of >100 bacterial species, which were analyzed regardless of the source of isolation and bacterial phylum. We used a very simple experimental protocol that involved directly depositing bacterial colonies onto the MALDI-TOF mass spectrometry plate, regardless of the agar-based medium, without any subculture or colony preparation. The direct protocol used in this study mostly suppressed manipulations of organisms and enabled their identification with little delay. The very basic procedure that we used contrasts with some studies in which identification has been performed after subculture onto selective medium [27] or extensive manipulation of colonies [13, 27, 45] after inactivation of the organisms [8, 18]. Studies that also used direct analysis of bacterial colonies found a delay for identification of less than 10 minutes due to the <1-minute delay for spectrum acquisition [4, 45]. Use of such a simple protocol helped to train technicians in $\leqslant$1 hour. In our laboratory, bacteria are typically deposited onto MALDI-TOF mass spectrometry plates at 7:00–7:30 AM, and all identifications are available for the clinician at 9:30 AM. Moreover, on-going improvement in the quality of spotting allowed decreasing the number of spot from 4 to 2 per isolate without alteration of the performances. In our institution, this timing greatly contributes to the clinical management of patients, because most medical decisions, including adaptation of antibiotic regimens, ordering of additional tests, and the prevention of nosocomial infections, are made before 1 PM. We calculated that MALDI-TOF mass spectrometry identification costs 22%–32% of the cost of conventional phenotypic identification. We did not observe any discrepancies between MALDI-TOF mass spectrometry and Gram staining, suggesting that MALDI-TOF mass spectrometry could be used as a first-line technique without prior Gram staining. We propose that Gram staining could be used only for isolates exhibiting a MALDI-TOF mass spectrometry score $\leqslant$1.9 and for both unusual isolates and isolates obtained from unusual clinical sites.

The data prospectively gathered in the present study demonstrated that MALDI-TOF mass spectrometry identification is an efficient, cost-effective method for the rapid and routine identification of bacterial isolates in the clinical microbiology laboratory. It can be used as the first-line method of identification, before Gram staining and any biochemical profiling, when using a database that includes $\geqslant$10 reference spectra per bacterial species and an identification score $\leqslant$1.9. The cost of analysis will decrease as bench-top instruments are used more often. The potential for a identification at the serotype or strain level, and antibiotic resistance profiling within minutes make MALDI-TOF mass spectrometry an on-going revolution in the clinical microbiology laboratory. It will significantly change business models as the diagnostic industry may develop new models to sell, and the cost of reagents will be very low.

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Potential conflicts of interest. All authors: no conflicts.

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Chapitre 3 : « Identification of rare pathogenic bacteria in a clinical microbiology laboratory: impact of MALDI-TOF mass spectrometry.»

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RESUME DE PUBLICATION N° 3 « Identification of rare pathogenic bacteria in a clinical microbiology laboratory: impact of MALDI-TOF mass spectrometry.»

Contexte :

Pendant les cinq dernières années, MALDI-TOF est devenu un puissant outil pour l'identification courante des microorganismes dans beaucoup de laboratoires cliniques.

Méthodes :

Nous avons analysé notre expérience sur 11 ans dans l'identification courante des isolats cliniques (40 mois d'utilisation de MALDI-TOF MS et 91 mois d'identification phénotypique conventionnelle.

Résultats :

Parmi les 286 842 isolats identifiés, 284 899 isolats de 459 espèces ont été identifiés. Parmi les autres 1 951 isolats mal identifiés, 670 ont été confirmés par une deuxième identification phénotypique et 1 273 isolats de 339 espèces bactériennes ont nécessité une identification par la technique de biologie moléculaire. MALDI-TOF MS a annuellement identifié 112 espèces, c.-à-d., 36 espèces/10 000 isolats, comparés à 44 espèces, c.-à-d., 19 espèces/10 000 isolats par l'identification phénotypique conventionnelle. Seulement 50 isolats ont exigé des identifications phénotypiques dans un deuxième temps au cours de la période d'identification par MALDI-TOF (c.-à-d., 4,5 identification/10 000 isolats) comparée à 620 identifications au cours de la période d'identification phénotypique conventionnelle (c.-à-d., 35.2/10,000 isolats). Nous avons identifié 128 espèces bactériennes rarement rapportées en tant qu'agents

pathogènes humains, y compris 48 utilisant les techniques phénotypiques (22 utilisant l'identification phénotypique conventionnelle et 37 utilisant MALDI-TOF MS). 75 espèces rares ont été identifiées par des méthodes moléculaires. MALDI-TOF a réduit le temps requis pour l'identification d'un isolat de 55 fois par rapport à l'identification phénotypique conventionnelle, et de 169 fois par rapport à l'identification moléculaire. MALDI-TOF MS réduit le coût par 5 fois comparativement à l'identification phénotypique conventionnelle et 169 fois par rapport à l'identification moléculaire.

Conclusions :

MALDI-TOF est non seulement un puissant outil pour l'identification bactérienne courante mais également pour l'identification des espèces bactériennes rarement impliquées dans les maladies infectieuses humaines. La capacité d'identification rapide des espèces bactériennes rarement décrites en tant qu'agent pathogène dans des prélèvements cliniques donnés, nous aidera à étudier la charge clinique résultant de l'émergence de ces espèces en tant qu'agents pathogènes humains. Le MALDI-TOF pourra être considéré comme une alternative aux méthodes moléculaires dans les laboratoires cliniques.

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Identification of Rare Pathogenic Bacteria in a Clinical Microbiology Laboratory: Impact of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

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AQ: A **During the past 5 years, matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) has become a powerful tool for routine identification in many clinical laboratories. We analyzed our 11-year experience in routine identification of clinical isolates (40 months using MALDI-TOF MS and 91 months using conventional phenotypic identification [CPI]). Among the 286,842 clonal isolates, 284,899 isolates of 459 species were identified. The remaining 1,951 isolates were misidentified and required confirmation using a second phenotypic identification for 670 isolates and using a molecular technique for 1,273 isolates of 339 species. MALDI-TOF MS annually identified 112 species, i.e., 36 species/10,000 isolates, compared to 44 species, i.e., 19 species/10,000 isolates, for CPI. Only 50 isolates required second phenotypic identifications during the MALDI-TOF MS period (i.e., 4.5 reidentifications/10,000 isolates) compared with 620 isolates during the CPI period (i.e., 35.2/10,000 isolates). We identified 128 bacterial species rarely reported as human pathogens, including 48 using phenotypic techniques (22 using CPI and 37 using MALDI-TOF MS). Another 75 rare species were identified using molecular methods. MALDI-TOF MS reduced the time required for identification by 55-fold and 169-fold and the cost by 5-fold and 96-fold compared with CPI and gene sequencing, respectively. MALDI-TOF MS was a powerful tool not only for routine bacterial identification but also for identification of rare bacterial species implicated in human infectious diseases. The ability to rapidly identify bacterial species rarely described as pathogens in specific clinical specimens will help us to study the clinical burden resulting from the emergence of these species as human pathogens, and MALDI-TOF MS may be considered an alternative to molecular methods in clinical laboratories.**

Early and accurate microbial identification is a critical requisite for early, adequate antibiotic treatment. The number of newly described bacteria has risen impressively during the past few decades (1, 2). Notably, the identification of new pathogens in clinical microbiology has been spectacularly improved during previous decades by the use of molecular identification, especially 16S rRNA gene sequencing (3–8). Molecular identification is one of the most useful techniques but remains expensive and requires a workload that is not adapted for routine use. Moreover, clinical definitions of some species do not match those used for 16S rRNA identification, such as the mismatched definitions used for streptococci (9–11).

Bacterial identification directly from colonies and samples using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) has been described as a revolutionary tool perfectly adapted to the clinical microbiology laboratory (12, 13). MALDI-TOF MS has been used to identify bacterial species and subspecies (14, 15), and in some outbreaks, MALDI-TOF MS has been reported to be able to identify the lineages of strains (16–18). Recently, MALDI-TOF MS has also been used to detect clinical pathogens previously misidentified or ambiguously identified (19–24). Detection of antimicrobial resistance using MALDI-TOF MS has been reported for *Staphylococcus aureus* (25–32), *Acinetobacter baumannii* (26), *Escherichia coli*, and other members of the family *Enterobacteriaceae* (33–35). Several new bacterial species emerging as human pathogens have been identified using MALDI-TOF MS (36–45).

In the present study, we examined data from a large collection of clinical isolates routinely identified during the last 11 years in our laboratory to evaluate the performance of MALDI-TOF MS for routine bacterial identification compared with conventional phenotypic identification (CPI). Particularly, we evaluated the capacity of MALDI-TOF MS to identify bacterial species that were rarely reported as human pathogens compared with conventional phenotypic and molecular identifications.

MATERIALS AND METHODS

Specimen collection. Clinical isolates were recovered from blood samples, cerebrospinal fluid samples, wounds, exudate samples, abscesses, respiratory tract samples, genitourinary samples, bone-joint infection samples, digestive samples, stools, and other clinical samples from 1 January 2002 through 31 December 2012, excluding December 2002 (data not available). In September 2008, an anaerobic laboratory with anaerobic chamber, preincubation of agar plates in strictly anaerobic condition, and a team of dedicated technicians was created with the opening of another laboratory at the University Hospital North in Marseille, France (600 beds) in our 4,000-bed university hospital. AQ: B

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TABLE 1 Summary of 11 years of bacterial identification in our laboratory[a]

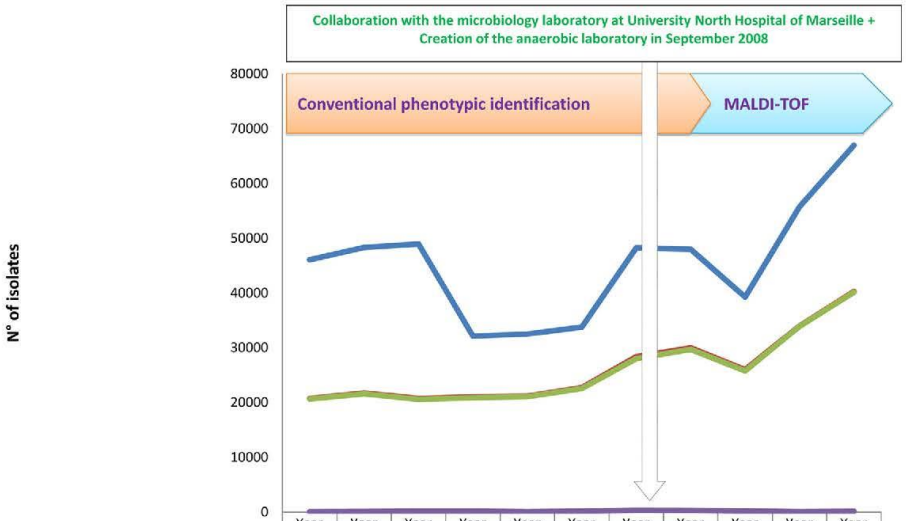
Identification technique (study period [day-mo-yr])	Study period (no. of months)	Total no. of analyses	No. of clonal isolates	No. of isolates identified by 1st PID	No. of species identified by 1st PID	No. of bacterial species identified/year	No. of isolates confirmed by 2nd PID	No. of isolates identified by molecular identification	No. of isolates misidentified by 1st PID	% misidentified
CPI period (1-Jan-02 to 30-Aug-09)	91	322,291	175,999	174,636	336	44	620	743	1,363	0.77
MALDI-TOF MS period (1-Sep-09 to 30-Dec-12)	40	177,888	110,843	110,263	382	112	50	530	580	0.52
AutoFlex II (1-Sep-09 to 30-Nov-10)	15	52,695	34,839	34,497	264	211	32	310	342	0.98
MicroFlex (1-Dec-10 to 31-Dec-12)	25	125,193	76,004	75,766	340	163	18	220	238	0.31
Total	131	500,179	286,842	284,899	459	42	670	1,273	1,951	0.68

[a] We identified 459 bacterial species among 284,899 clinical isolates during nearly 11 years. We identified 112 species per year using MALDI-TOF MS compared with 44 identified using conventional phenotypic identification (CPI) (Gram staining, API, Vitek 2 system identification). PID, phenotypic identification.

Bacterial identification. All isolates were identified after aerobic, microaerophilic, and anaerobic incubation of clinical specimens on 5% sheep blood, chocolate, Mueller-Hinton, Trypticase soy, and MacConkey agar plates (bioMérieux).

(i) Conventional phenotypic identification period. In CPI, we used semiautomated Gram staining (Aerospray Wiescor; Elitech), determined catalase and oxidase activities, and used the Vitek 2 system (bioMérieux), with 330 microorganism strains as references or the API 20A identification strip for anaerobes (bioMérieux) to identify bacterial species from 1 January 2002 to 30 August 2009. Correct identification of an isolate using the Vitek 2 system was confirmed when the T index was ≥0.25; identification using the API system was confirmed when the percentage of identification was ≥90%, and the T index was ≥ 0.25 (46). We reidentified organisms by Gram staining rather than by using the Vitek 2 system. API identification strips included API 20A, API Coryne, API Campy, API 20E, API 20NE, API Strep, API Staph, API NH, and API Listeria strips (bioMérieux) as the second phenotypic identification in the CPI period to identify uncertainly identified isolates at the species level.

(ii) MALDI-TOF MS identification period. ***(a) MALDI-TOF MS analysis.*** We used MALDI-TOF MS as a routine bacterial identification tool to categorize bacterial species from direct colonies, and the procedure was performed as previously described (12). We used a MALDI-TOF MS AutoFlex II system (Brüker Daltonik) for the first part of the MALDI-TOF MS identification period, from 1 September 2009 to 30 November 2010 and a MicroFlex LT mass spectrometer (Brüker Daltonik) for the second part of the MALDI-TOF MS identification period, from 1 December 2010 to 31 December 2012.



	Year 2002	Year 2003	Year 2004	Year 2005	Year 2006	Year 2007	Year 2008	Year 2009	Year 2010	Year 2011	Year 2012
All analysis	46079	48343	48978	32119	32516	33808	48272	48022	39292	55761	66989
Clonal isolates	20737	21725	20754	21048	21179	22717	28368	29978	25983	34024	40329
Isolates well-identified by PID*	20655	21598	20577	20874	21083	22544	28013	29674	25757	33942	40182
Total of misidentified isolates by PID*	82	127	177	174	96	173	355	304	226	82	147

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AQ: L FIG 1 Time course of the numbers of total analyses, clonal isolates analyzed, and clonal isolates identified and misidentified using phenotypic identification (PID*) and MALDI-TOF MS during 11 years of routine identification in our clinical laboratory.

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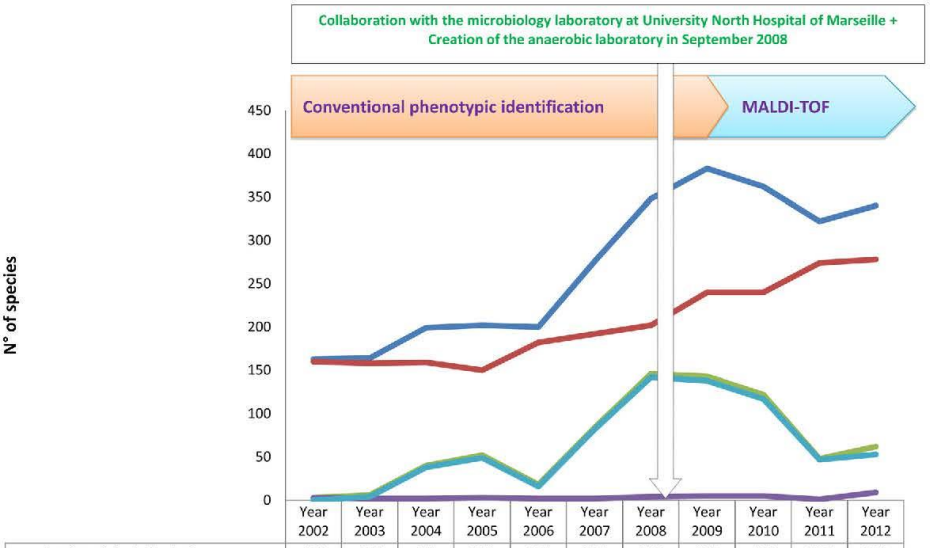

	Year 2002	Year 2003	Year 2004	Year 2005	Year 2006	Year 2007	Year 2008	Year 2009	Year 2010	Year 2011	Year 2012
Species of single bacterium	163	164	199	202	200	276	348	383	362	322	340
Species well-identified by PID*	160	158	159	150	182	192	202	240	240	274	278
Total of misidentified species by PID*	3	6	40	52	18	84	146	143	122	48	62
Misidentified species, confirmed by another PID*	3	2	2	3	2	2	4	5	5	1	9
Misidentified species, confirmed by molecular ID**	0	4	38	49	16	82	142	138	117	47	53

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FIG 2 Time course of the numbers of species of clonal bacteria identified, species identified using an initial phenotypic identification (PID*), total species misidentified, species confirmed by another PID*, and species confirmed by molecular identification (molecular ID**) over 11 years of routine identification in our clinical laboratory.

(b) MALDI-TOF mass spectrum database. The Brüker database updated with a laboratory collection of spectra from clinical isolates identified by 16S rRNA gene sequencing was used from 1 September 2009 to 31 December 2012. For each organism updated, a consensus spectrum was obtained by using the Biotyper MSP (mean spectrum projection) creation standard method from a total of 12 spots made for each isolate, and the manipulation was repeated in two independent runs. The Fisher exact test was used to evaluate the reproducibility. We determined the sensitivity of MALDI-TOF MS by identification of 10 colonies of the same bacterial species in another independent run. Our MALDI-TOF mass spectrum database has 6,213 reference microorganism strain spectra, and we updated the primary Brüker database containing 3,993 microorganism spectra (3,670 of bacteria, 7 of *Archaea*, and 316 of *Eukaryota*) with laboratory bacterial spectra including spectra from well-typed bacterial strains and other human-pathogenic bacteria identified by using a molecular technique.

(c) MALDI-TOF MS identification. Bacterial species were directly identified from one bacterial colony; each colony was covered with 2 ml of matrix solution (saturated α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) without other supplements and extracted as previously described (12). We used MALDI Biotyper 3.0 software to compare the first 100 peaks of each spectrum to our MALDI-TOF mass spectrum database previously updated as described below. An isolate was considered correctly identified at the species level by using MALDI-TOF MS if 2 spectra had scores of ≥1.9. Uncertainly identified isolates at the species level (scores of <1.9) were identified with certainty by MALDI-TOF MS analysis of 2 additional spectra. A second run of MALDI-TOF MS identification with 4 spectra was done for unsatisfied species identification in the MALDI-TOF MS period.

(iii) Molecular identification. Isolates misidentified by the second CPI or MALDI-TOF MS analyses were identified with certainty using molecular identification using 16S rRNA or *rpoB* gene sequencing as described elsewhere (4, 12, 47, 48). An isolate was correctly identified when (i) its 16S rRNA gene sequence yielded ≥98.7% identity with the sequence of the most closely related bacterial species in GenBank (49) or (ii) when its *rpoB* gene sequence yielded ≥97% identity with the sequence of the most closely related bacterial species in GenBank or a local database (12, 48).

Database analysis. Our database included bacterial identification results and their associated clinical information; 500,174 identifications of clinical isolates were performed during the study period. All results were extracted into Microsoft Excel files for further analysis. Duplicate analyses were eliminated by retaining only a single bacterial identification per sample. We also excluded all samples for which there were phenotypic or molecular identifications of fungi, environmental isolates, *Mycobacterium*, and other intra- and extralaboratory strains that were not of human origin.

Meaning of rare species. Rare species were defined as bacterial species with ≤10 reports designating them as human pathogens retrieved from the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/). The possibility of inaccurate classifications as rare species due to taxonomy changes was checked using the National Center for Biotechnology Information (NCBI) taxonomy database (http://www.ncbi.nlm.nih.gov/guide/taxonomy/).

Time, cost, and training requirement evaluation of a MALDI-TOF MS identification technique. We evaluated the time required for the MALDI-TOF mass spectrometry identification as the period between the deposit of a bacterial colony on the MALDI-TOF MS plate by a technician

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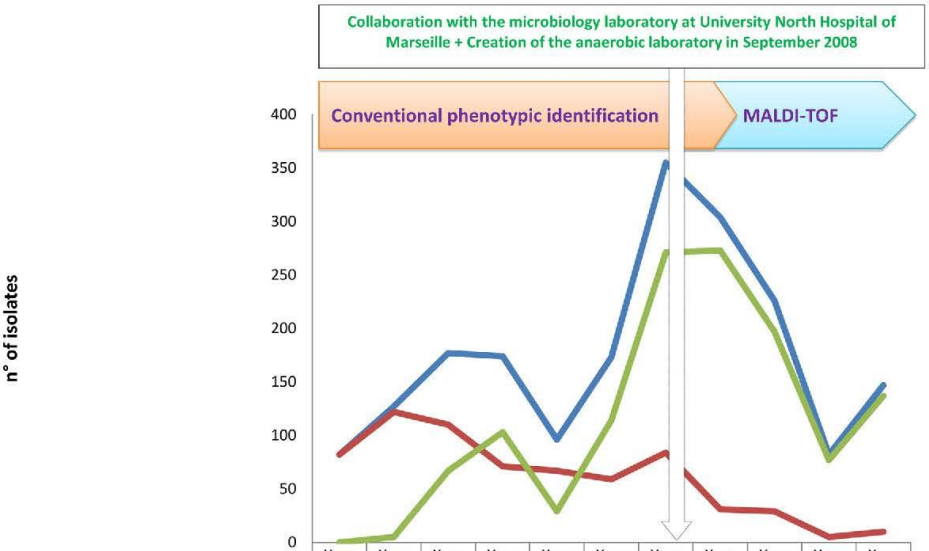


	Year 2002	Year 2003	Year 2004	Year 2005	Year 2006	Year 2007	Year 2008	Year 2009	Year 2010	Year 2011	Year 2012
Total of misidentified isolates by PID*	82	127	177	174	96	173	355	304	226	82	147
Misidentified isolates, confirmed by another PID*	82	122	110	71	67	59	84	31	29	5	10
Misidentified isolates, confirmed by molecular ID**	0	5	67	103	29	114	271	273	197	77	137

COLOR

FIG 3 Time course of the numbers of total isolates misidentified using phenotypic identification (PID*), isolates confirmed by a second PID* and isolates confirmed by molecular identification (ID**) over 11 years of routine identification in our clinical laboratory.

and the completion of the informatics interpretation of the resulting spectra (i.e., identification ready to be transmitted to a clinician). The costs of identification were evaluated by adding the costs of matrix reagents, plates, positive controls, and technician salary, with provisions for 5-year depreciations of the apparatuses used (Gram staining apparatus, microscope, identification apparatus, and mass spectrometer) on the basis of ≈67,000 isolates analyzed per year (the number of samples analyzed in 2012 in our laboratory).

Statistical analysis. Data analyses were performed using IBM SPSS Statistics software version 20.0. Proportions were compared using the chi-squared or Fisher's exact two-tailed tests. A P value of <0.05 was considered statistically significant.

RESULTS

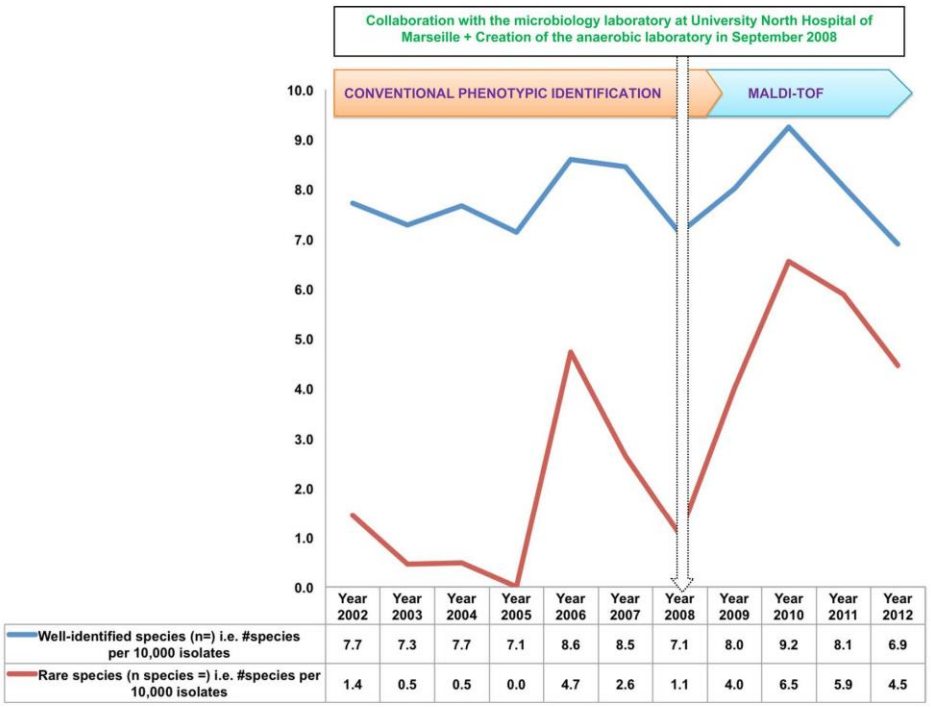
Over 11 years, we performed 500,179 bacterial identifications in our laboratory (Table 1). We grew our capacity for identification between 2002 and 2012, increasing the number of analyses from 46,079 per year to 66,989 per year, by creating an anaerobic laboratory and joining with another microbiology laboratory located at Marseille University North Hospital in September 2008 (Fig. 1). The implementation of a new tool for identification (MALDI-TOF MS) has spectacularly improved our capacity to identify more clinical isolates and more human-pathogenic bacteria. We identified 160 bacterial species during 2002 and 278 species during 2012 (Fig. 2).

Among 286,842 clonal isolates identified, phenotypic identification methods (CPI or MALDI-TOF MS) correctly identified 284,899 isolates including 459 species of 134 genera and 6 phyla. Another 1,951 isolates were misidentified and required identification by another phenotypic or molecular method (Table 1 and Fig. 3).

CPI identified 174,636 isolates, including 336 species of 120 genera and 6 phyla, over the 91 months from 1 January 2002 through 30 August 2009, whereas MALDI-TOF MS identified 110,263 isolates classified in 382 species of 114 genera and 6 phyla over the 40 months from 1 September 2009 through 31 December 2012. Thus, MALDI-TOF MS yearly identified 32,430 isolates of 112 species, i.e., 36 species/10,000 isolates, compared with 22,692 isolates of 44 species, i.e., 19 species per 10,000 isolates, for CIP ($P < 0.0001$) (Table 1 and Fig. 4).

Among the 459 bacterial species identified during 2002 to 2012, 76 species (17%) were identified using only CPI over a 91-month period, 124 species (27%) were identified using only MALDI-TOF MS during a 40-month period (see Table S1 and Table S2 in the supplemental material), and 258 species (56%) were identified using both methods.

In the group of bacterial species identified only by CPI, 15 (20%) of the 76 isolates were absent from our MALDI-TOF mass spectrum database. In the phylum *Actinobacteria*, 16 species of 11 genera were identified using only CPI, and 3 species were absent from our MALDI-TOF MS database. In the phylum *Bacteroidetes*, 5 species of 3 genera were identified using CPI exclusively, and 1 species was absent from the MALDI-TOF MS database. In the



	Year 2002	Year 2003	Year 2004	Year 2005	Year 2006	Year 2007	Year 2008	Year 2009	Year 2010	Year 2011	Year 2012
Well-identified species (n=) i.e. #species per 10,000 isolates	7.7	7.3	7.7	7.1	8.6	8.5	7.1	8.0	9.2	8.1	6.9
Rare species (n species =) i.e. #species per 10,000 isolates	1.4	0.5	0.5	0.0	4.7	2.6	1.1	4.0	6.5	5.9	4.5

C O L O R

FIG 4 Biodiversity of rare species identified in the routine identification of all clinical isolates tested (identified plus misidentified) during the last 11 years.

phylum *Firmicutes*, 19 species of 10 genera were identified using only CPI, and 3 were missing from the MALDI-TOF MS database. In the phylum *Fusobacteria*, 3 species of 2 genera were identified using only CPI, and 1 was missing from the MALDI-TOF MS database. In the phylum *Proteobacteria*, 33 species of 22 genera were identified using CPI exclusively, and 7 were missing from the MALDI-TOF MS database (see Table S1 in the supplemental material).

In the group of bacterial species identified only by MALDI-TOF MS, 21 (17%) of the 124 isolates were present in the Vitek 2 database, whereas 103 (83%) were not (see Table S2 in the supplemental material). In the phylum *Actinobacteria*, 21 species of 12 genera were identified using only MALDI-TOF MS and were lacking in the Vitek 2 database. In the phylum *Bacteroidetes*, 10 species of 7 genera were identified by using MALDI-TOF MS exclusively, and 9 species were absent from the Vitek 2 database. In the phylum *Firmicutes*, 54 species of 18 genera were identified using only MALDI-TOF MS, and 41 were missing from the Vitek 2 database. In the phylum *Fusobacteria*, *Fusobacterium periodonticum* was identified using only MALDI-TOF MS and was missing from the Vitek 2 database. In the phylum *Proteobacteria*, 38 species of 20 genera were identified using MALDI-TOF MS exclusively, and 31 were missing from the Vitek 2 database. No species in the phylum *Tenericutes* was identified by using MALDI-TOF MS exclusively (see Table S2 in the supplemental material).

During the study period, 1,951 isolates were misidentified and required confirmation by another round of phenotypic identification for 670 isolates of 21 species (see Table S3 in the supplemental material) and by molecular identification for 1,273 isolates of 339 species (see Table S4 in the supplemental material). Among 339 species that required confirmation by molecular identification, 63 species were absent from the initial Brüker database, which contained 3,993 bacterial spectra, and only 24 were missing from our updated MALDI-TOF mass spectrum database (6,213 bacterial spectra). Among 24 bacterial species of 46 isolates missed from our MALDI-TOF MS database, 16 species of 32 isolates were identified by a molecular method in the CPI period, and 11 species of 14 isolates were identified by a molecular method in the MALDI-TOF MS period. Despite their presence in our MALDI-TOF database, 315 other species had to be examined by molecular identification; this included 228 species of 711 isolates and 196 species of 516 isolates in the CPI period and the MALDI-TOF MS period, respectively. AQ: E

We identified 40 species of 1,506 anaerobic organisms before MALDI-TOF MS by using the API 20A system (bioMérieux), and we identified 103 species of 1,564 anaerobic organisms at the species level using MALDI-TOF MS identification.

During the CPI period, 1,363 isolates (0.77%) were misidentified; the 1,363 isolates included 620 isolates reidentified using a second CPI as described below (i.e., 35.2 per 10,000 isolates) and 743 confirmed using a molecular technique (i.e., 42 per 10,000 isolates). During the MALDI-TOF MS period, 580 isolates (0.52%) were misidentified; the 580 isolates included 50 isolates reidentified using a second run of identification by MALDI-TOF

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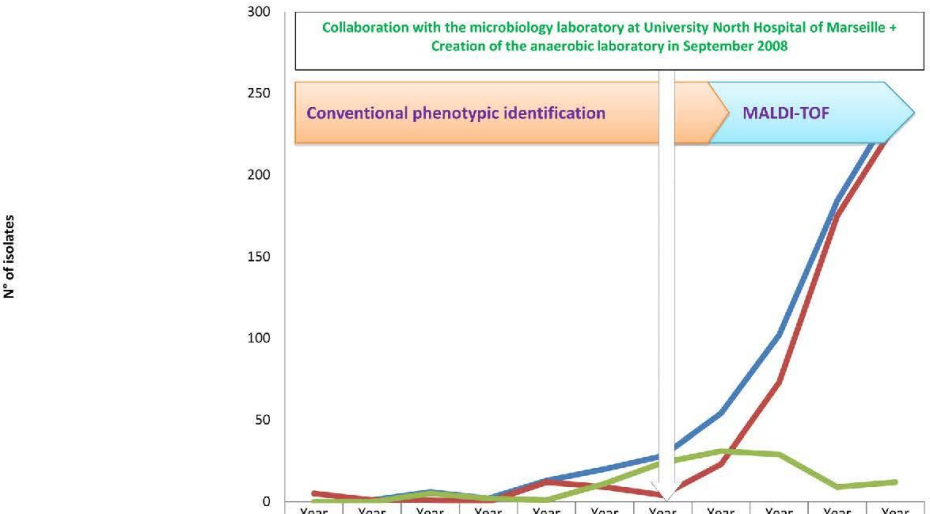

	Year 2002	Year 2003	Year 2004	Year 2005	Year 2006	Year 2007	Year 2008	Year 2009	Year 2010	Year 2011	Year 2012
Isolate of rare species	5	1	6	2	13	20	28	54	102	184	243
Isolate of rare species by PID	5	1	1	0	12	9	4	23	73	175	231
Isolate of rare species by molecular ID	0	0	5	2	1	11	24	31	29	9	12

COLOR

FIG 5 Time course of the numbers of isolates of 128 rare species, 48 of which were identified using phenotypic identification (PID), and 75 of which were identified using molecular identification (ID).

MS, i.e., 4.5 species per 10,000 isolates, and 530 isolates confirmed using a molecular technique, i.e., 47 species per 10,000 isolates (Table 1 and Fig. 3).

The molecular identification requirements were similar during the CPI and MALDI-TOF MS periods at 42 and 47 molecular identifications/10,000 isolates, respectively. However, a decreasing trend was observed during the final 2 years, with 47 and 53 during 2011 and 2012, respectively, compared with 142 molecular identifications in 2008 (Fig. 2 and Fig. 3).

During 11 years of routine identification, we identified 123 rare species of bacteria that were reported to be human pathogens fewer than or equal to 10 times in the literature (PubMed database). Among these species, 48 were identified by phenotypic identification. Another 75 species were confirmed by molecular identification. In addition, CPI identified only 22 rare species during 91 months, and MALDI-TOF MS identified 37 such rare species during 40 months (Fig. 5, Fig. 6, and Fig. 7). Among 196 species of 516 isolates that were not satisfactorily identified in the MALDI-TOF MS period, 365 (71%) isolates represented 10 genera, including *Streptococcus*, *Corynebacterium*, *Pseudomonas*, *Acinetobacter*, *Actinomyces*, *Staphylococcus*, *Bacillus*, *Enterobacter*, *Enterococcus*, and *Nocardia*, that frequently required molecular identification (Fig. 8).

Identification of 11 of the 48 rare species identified using phenotypic methods was performed using only CPI, and 26 other rare species were identified using only MALDI-TOF MS (Table 2). In the phylum *Actinobacteria*, 18 rare species were identified, including 9 exclusively identified using MALDI-TOF MS, 5 using CPI, and 4 species using both techniques. In the phylum *Bacteroidetes*, 6 rare species were identified; the 6 species included 2 exclusively identified using MALDI-TOF MS, 1 using CPI, and 3 using both techniques. In the phylum *Firmicutes*, 12 rare species were identified, including 7 exclusively identified using MALDI-TOF MS, 2 using CPI, and 3 using both techniques. In the phylum *Fusobacteria*, 2 rare species were totally identified using CPI. In the phylum *Proteobacteria*, 10 rare species were identified, including 8 exclusively identified using MALDI-TOF MS, 1 using CPI, and 1 using both techniques (Table 2).

Looking in detail at the group of 48 rare species identified using phenotypic methods, 4 of these were identified more than 10 times in our laboratory during the last 11 years, including 12 isolates of *Actinomyces europaeus*, 20 isolates of *Actinomyces radingae*, 31 isolates of *Pandoraea pulmonicola*, 95 isolates of *Peptoniphilus harei*, and 272 isolates of *Enterobacter kobei* (Table 2).

The rare species identified using phenotypic methods were mostly recovered from bloodstream and urinary traction infections (see Table S5 in the supplemental material). *Enterobacter kobei* was the most frequently identified among the 48 rare species (see Table S5 in the supplemental material). In the following analysis, using MALDI-TOF MS, we identified two bacterial species, *Brevibacterium ravenspurgense* and *Corynebacterium fastidiosum*, that had never been reported as human pathogens in PubMed (Table 2).

Moreover, molecular techniques identified 75 rare species among 124 isolates including 23 that were identified as rare species using phenotypic identification methods (Table 3). In all, 57 of the 75 rare species identified using molecular techniques were absent from the Brüker database and 18 were absent from our MALDI-TOF database. Among 57 bacterial rare species identified by molecular methods which spectrum present in our MALDI-TOF

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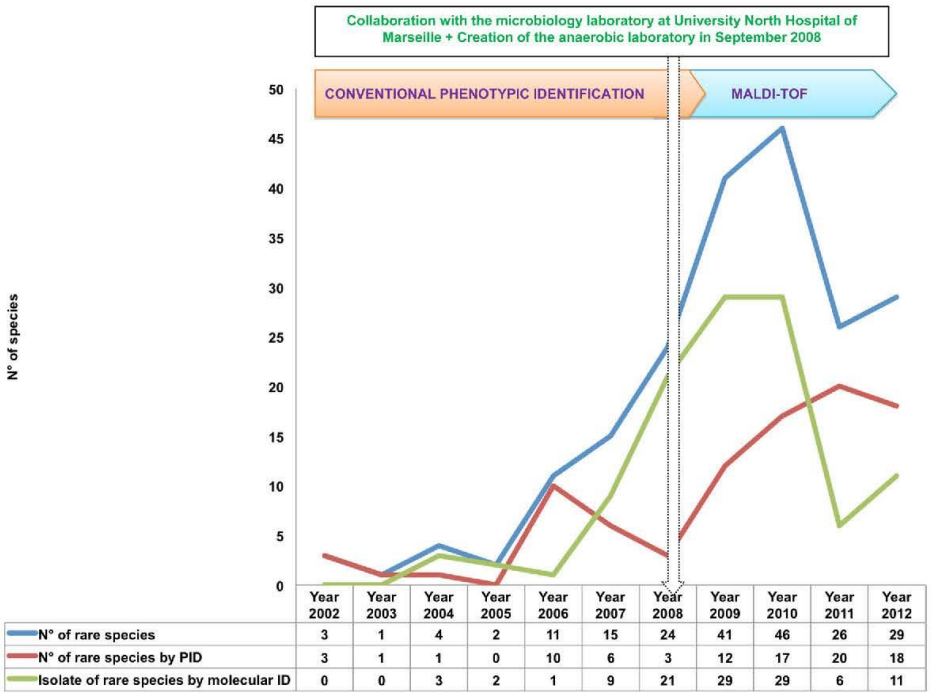

	Year 2002	Year 2003	Year 2004	Year 2005	Year 2006	Year 2007	Year 2008	Year 2009	Year 2010	Year 2011	Year 2012
N° of rare species	3	1	4	2	11	15	24	41	46	26	29
N° of rare species by PID	3	1	1	0	10	6	3	12	17	20	18
Isolate of rare species by molecular ID	0	0	3	2	1	9	21	29	29	6	11

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FIG 6 Time course for the numbers of species identified among 128 rare species, 48 of which were identified using phenotypic identification (PID) and 75 of which were identified using molecular identification (ID).

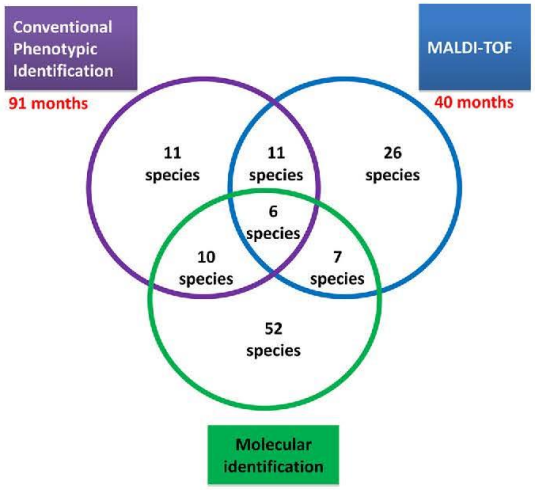

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FIG 7 Of 48 rare species identified using phenotypic techniques, MALDI-TOF MS identified 37 rare species and conventional phenotypic identification identified 22 rare species in 40 and 91 months of study, respectively. Seventy-five rare species were identified using molecular techniques.

database, 39 species were recently created during the study. Fourteen of 18 rare species exclusively identified in the CPI period were recently created. Twenty-five of 39 rare species identified in the MALDI-TOF MS period were recently created in our database. Other 14 rare species that were present in the database but that needed molecular identification in the MALDI-TOF MS period were *Actinomyces europaeus* (2 isolates), *Corynebacterium argentoratense* (2 isolates), *Corynebacterium confusum* (1), *Corynebacterium coyleae* (4 isolates), *Corynebacterium imitans* (1 isolate), *Corynebacterium kroppenstedtii* (1 isolate), *Corynebacterium mucifaciens* (3 isolates), *Corynebacterium riegelii* (1 isolate), *Corynebacterium ureicelerivorans* (1 isolate), *Microbacterium aurum* (1 isolate), *Streptococcus criceti* (3 isolates), *Streptococcus peroris* (1 isolate), *Enterobacter kobei* (3 isolates), and *Pandoraea pulmonicola* (3 isolates).

The time required for identification of one clinical isolate using MALDI-TOF MS was 6 to 8 min 30 s for the AutoFlex II system (Brüker Daltonik) and 1 min 46 s for the MicroFlex LT mass spectrometer (Brüker Daltonik). The cost of identification of one clinical isolate using MALDI-TOF MS was 1.43 euros for the AutoFlex II system (Brüker Daltonik) and 1.35 euros for the MicroFlex LT mass spectrometer (Brüker Daltonik) (Table 4). In comparison, the time required for identification for one clinical isolate using 16S rRNA or *rpoB* sequencing was 24 h. In addition, the cost of bacterial isolate identification using gene sequencing was 137.70 euros.

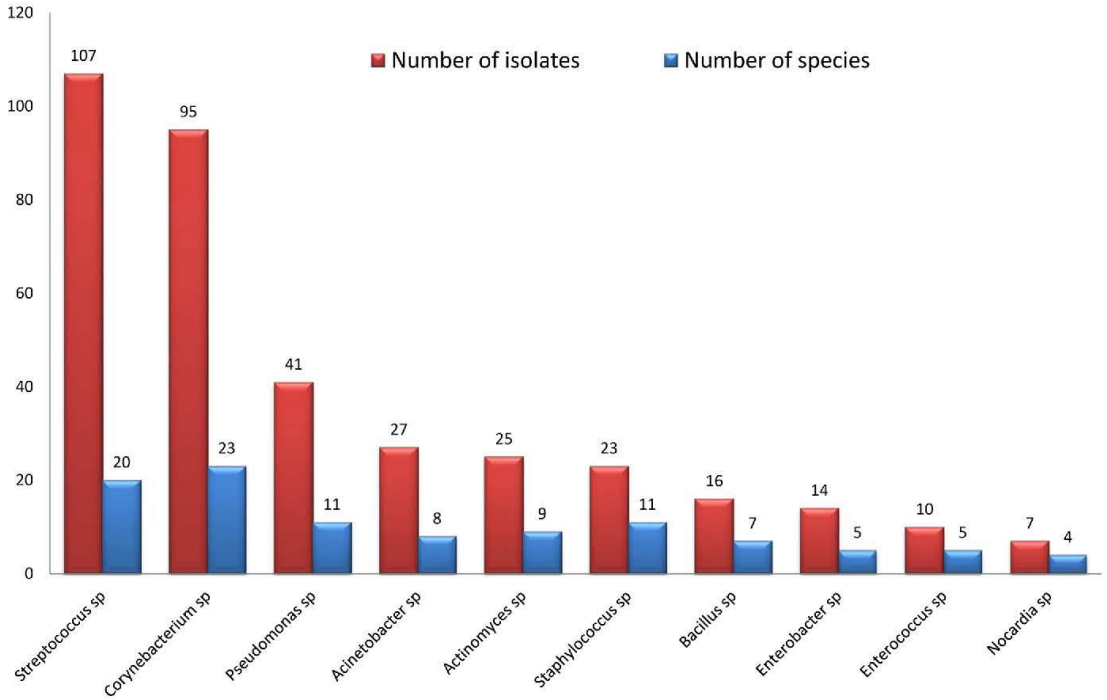

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FIG 8 Ten genera of 365 (71%) isolates that frequently required molecular identification among 196 species of 516 isolates identified unsatisfactorily in the MALDI-TOF period.

DISCUSSION

During the last 11 years, our clinical laboratory has seen an increased ability to analyze bacteriological samples due to several reasons: first, the establishment of another laboratory at the University Hospital North in Marseille, France, and second, the creation of an anaerobic laboratory in September 2008. By optimizing the new tool of MALDI-TOF mass spectrometry for routine identification, we were able to increase our yearly analysis capacity from 46,079 analyses in 2002 to 66,989 in 2012.

In 2008, we evaluated the performance of MALDI-TOF MS to identify 1,660 clinical isolates in a 16-week period by comparing it with routine phenotypic identification methods, such as semiautomated Gram staining (Aerospray Wescor; Elitech), catalase and oxidase assays and automated identifications using the Vitek 2 and API 20A systems (bioMérieux). Since then, more than 300 scientific publications have confirmed that MALDI-TOF MS can be adapted to achieve performances similar to the routine identification methods used in clinical laboratories (14, 50–53). Many clinical laboratories have, like us, adopted bacterial identification using MALDI-TOF MS for biotyping microbes to replace all of the traditional phenotypic methods used for routine diagnoses directly from colony or clinical samples (13, 45, 54–58).

Recently, MALDI-TOF MS was used in culturomics studies to identify 32 new bacterial species and another 177 bacterial species that had never been reported to occur in the human gut microbiota that may explain the involvement of microorganisms in human diseases such as obesity (59, 60). MALDI-TOF MS has been used to identify 233 of 349 bacterial species from 4 stool samples by direct identification from 36,500 colonies. MALDI-TOF MS has also identified 116 unknown bacterial species with the score < 1.9 that was needed to identify by 16S rRNA gene sequencing. Seventy-one of 116 (61%) bacterial species were previously absent in our MALDI-TOF database. Among 45 (39%) species present in our MALDI-TOF database, 24 (20%) have only 1 reference spectrum, and only one species of 18 serovars of *A. pitii* have more than 10 spectra in the database (59–61). We used an incremental database with each spectra identified by 16S rRNA gene sequencing from the first three stool samples that allowed us to use the culturomics study of Dubourg et al. (61) for the fourth stool sample; in the study of Dubourg et al., only 4 of 4,000 bacterial colonies needed molecular identification (61). AQ: H AQ: I

The capacity of MALDI-TOF MS to identify an unknown bacterial species before molecular identification has been previously observed by Bizzini et al. (62) and confirmed after updating the MALDI-TOF database. Among 410 bacterial strains that were not satisfactorily identified by the Vitek 2 and API systems (bioMérieux), 62% of them were concordantly identified by MALDI-TOF MS and 16S rRNA gene sequencing. Failure to identify 85 other bacterial species was due to the absence of spectra of 78 species in the MALDI-TOF database (62).

The 196 species (516 isolates) that were not identified included 57 rare bacterial species present in the MALDI-TOF database that needed molecular identification in the MALDI-TOF period can be attributed to two causes. The first cause is the absence of reference

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TABLE 2 Species of clinical isolates that were identified by phenotypic identification as species that had been rarely reported as human pathogens[a]

Phylum	Genus	Bacterial rare species identified by PID	No. of isolates	Identification method(s)	No. of isolates identified by CPI	No. of isolates identified by MALDI-TOF MS	No. of reports in PubMed
Actinobacteria	*Actinobaculum*	*Actinobaculum massiliense*	1	MALDI-TOF MS	0	1	4
	Actinomadura	*Actinomadura cremea*	1	CPI	1	0	6
	Actinomyces	*Actinomyces europaeus*	12	CPI and MALDI-TOF MS	3	9	9
		Actinomyces radicidentis	3	MALDI-TOF MS	0	3	4
		Actinomyces radingae	20	CPI and MALDI-TOF MS	5	15	10
	Arthrobacter	*Arthrobacter cumminsii*	5	CPI and MALDI-TOF MS	3	2	4
	Brevibacterium	*Brevibacterium luteolum*	1	CPI	1	0	4
		Brevibacterium massiliense	1	MALDI-TOF MS	0	1	2
		Brevibacterium paucivorans	1	MALDI-TOF MS	0	1	3
		Brevibacterium ravenspurgense	1	MALDI-TOF MS	0	1	0
	Corynebacterium	*Corynebacterium auriscanis*	3	CPI	3	0	5
		Corynebacterium coyleae	7	CPI and MALDI-TOF MS	2	5	7
		Corynebacterium fastidiosum	2	MALDI-TOF MS	0	2	0
		Corynebacterium imitans	2	MALDI-TOF MS	0	2	2
		Corynebacterium mucifaciens	5	MALDI-TOF MS	0	5	6
	Microbacterium	*Microbacterium schleiferi*	1	MALDI-TOF MS	0	1	6
	Pseudoclavibacter	*Zimmermannella bifida*	1	CPI	1	0	1
	Varibaculum	*Varibaculum cambriense*	2	CPI	3	9	2
Bacteroidetes	*Alistipes*	*Alistipes finegoldii*	3	CPI and MALDI-TOF MS	0	3	4
	Bacteroides	*Bacteroides cellulosilyticus*	4	MALDI-TOF MS	5	15	2
	Butyricimonas	*Butyricimonas virosa*	1	MALDI-TOF MS	3	2	1
	Porphyromonas	*Porphyromonas somerae*	9	CPI and MALDI-TOF MS	1	0	1
	Prevotella	"*Candidatus* Prevotella conceptionensis"	3	CPI and MALDI-TOF MS	0	1	1
		Prevotella massiliensis	1	CPI	0	1	2
Firmicutes	*Acidaminococcus*	*Acidaminococcus intestini*	2	CPI and MALDI-TOF MS	0	1	2
	Anaerococcus	*Anaerococcus lactolyticus*	3	MALDI-TOF MS	3	40	9
		Anaerococcus octavius	7	MALDI-TOF MS	2	5	3
	Eubacterium	*Eubacterium tenue*	2	MALDI-TOF MS	0	2	6
		Eubacterium yurii	1	MALDI-TOF MS	0	2	10
	Facklamia	*Facklamia languida*	1	CPI	0	5	2
	Peptoniphilus	*Peptoniphilus harei*	95	CPI and MALDI-TOF MS	0	1	7
	Robinsoniella	*Robinsoniella peoriensis*	3	MALDI-TOF MS	1	0	8
	Sporosarcina	*Sporosarcina ginsengisoli*	1	CPI	2	0	1
	Streptococcus	*Streptococcus massiliensis*	4	MALDI-TOF MS	1	2	1
	Turicibacter	*Turicibacter sanguinis*	3	CPI and MALDI-TOF MS	0	4	3
	Veillonella	*Veillonella montpellierensis*	1	MALDI-TOF MS	0	1	3
Fusobacteria	*Leptotrichia*	*Leptotrichia goodfellowii*	1	CPI	1	8	5
		Leptotrichia trevisanii	3	CPI	1	2	3
Proteobacteria	*Acinetobacter*	*Acinetobacter parvus*	2	MALDI-TOF MS	1	0	8
	Comamonas	*Comamonas kerstersii*	2	MALDI-TOF MS	1	1	3
	Enterobacter	*Enterobacter cowanii*	3	MALDI-TOF MS	0	3	9
		Enterobacter kobei	272	MALDI-TOF MS	0	7	10
	Ochrobactrum	*Ochrobactrum grignonense*	1	MALDI-TOF MS	0	2	8
	Pandoraea	*Pandoraea pulmonicola*	31	MALDI-TOF MS	0	1	7
	Paracoccus	*Paracoccus yeeii*	2	CPI and MALDI-TOF MS	1	0	1
	Pseudomonas	*Pseudomonas hibiscicola*	2	MALDI-TOF MS	11	84	4
	Roseomonas	*Roseomonas ludipueritiae*	1	CPI	0	3	4
	Serratia	*Serratia ureilytica*	1	MALDI-TOF MS	1	0	6

[a] List of 48 species of 534 clinical isolates that were identified by phenotypic identification as species that had been rarely reported as human pathogens, with ≤10 reports in PubMed. PID, phenotypic identification; CPI, conventional phenotypic identification (Gram staining, API, Vitek 2 system identification).

spectrum. The second cause was the presence of a low number of spectra in the database that does not allow MALDI-TOF to identify the bacteria in the groups with biodiversity within species. As an example, 10 genera that frequently needed molecular identification in the MALDI-TOF MS period in spite of the presence of some reference spectra were *Streptococcus*, *Corynebacterium*, *Pseudomonas*, *Acinetobacter*, *Actinomyces*, *Staphylococcus*, *Bacillus*, *Enterobacter*, *Enterococcus*, and *Nocardia*.

In addition to the capacity to analyze more isolates as shown in the present study, MALDI-TOF MS has annually identified 2.5 times more species than CPI, identifying 112 species (i.e., 36 species/10,000 isolates) compared with 44 species (i.e., 19 species/10,000 isolates), respectively. This performance of MALDI-TOF MS in annually identifying more species per isolate tested can be explained first by the increasing numbers of colonies analyzed from each clinical sample and a tendency to identify systematically all isolates from a polymicrobial clinical specimen. Second, the MALDI-TOF database is now 10 times larger than the Vitek 2

TABLE 3 Rare bacterial species identified using molecular identification[a]

Phylum	Genus	Bacterial species confirmed by molecular identification	No. of isolates	No. of isolates identified in the CPI period	No. of isolates identified in the MALDI-TOF MS period	No. of reports in PubMed	48 rare species by PID	Presence/absence of species in our MALDI-TOF MS database[b]	Presence/absence of species in MALDI-TOF MS database (Brüker)[b]
Actinobacteria	*Actinomyces*	*Actinomyces europaeus*	3	1	2	9	Yes	Present	Present
		Actinomyces lingnae	1	0	1	1	No	**Absent**	**Absent**
		Actinomyces radingae	5	3	2	10	Yes	Present	**Absent**
		Actinomyces urogenitalis	2	0	2	4	No	Present	**Absent**
	Arthrobacter	*Arthrobacter cumminsii*	5	4	1	4	Yes	Present	**Absent**
	Bifidobacterium	*Bifidobacterium scardovii*	1	1	0	5	No	Present	**Absent**
	Brachybacterium	*Brachybacterium muris*	1	0	1	3	No	Present	**Absent**
		Brachybacterium sacelli	1	0	1	3	No	**Absent**	**Absent**
	Brevibacterium	*Brevibacterium massiliense*	1	1	0	2	Yes	**Absent**	**Absent**
		Brevibacterium otitidis	1	1	0	9	No	**Absent**	**Absent**
		Brevibacterium paucivorans	2	1	1	3	Yes	Present	**Absent**
		Brevibacterium ravenspurgense	1	1	0	0	Yes	Present	**Absent**
		Brevibacterium sanguinis	1	1	0	2	No	Present	**Absent**
		Brevibacterium stationis	1	0	1	10	No	Present	**Absent**
	Corynebacterium	*Corynebacterium argentoratense*	2	0	2	3	No	Present	Present
		Corynebacterium auriscanis	3	3	0	5	Yes	Present	Present
		Corynebacterium confusum	1	0	1	2	No	Present	Present
		Corynebacterium coyleae	4	0	4	7	Yes	Present	Present
		Corynebacterium durum	1	1	0	3	No	Present	**Absent**
		Corynebacterium fastidiosum	1	0	1	0	Yes	**Absent**	**Absent**
		Corynebacterium imitans	1	0	1	2	Yes	Present	Present
		Corynebacterium kroppenstedtii	1	0	1	9	No	Present	Present
		Corynebacterium mucifaciens	3	0	3	6	Yes	Present	Present
		Corynebacterium riegelii	1	0	1	6	No	Present	Present
		Corynebacterium ureicelerivorans	1	0	1	3	No	Present	Present
	Dietzia	*Dietzia cinnamea*	1	1	0	10	No	Present	**Absent**
	Janibacter	*Janibacter hoylei*	1	0	1	2	No	Present	**Absent**
	Microbacterium	*Microbacterium aurum*	2	1	1	5	No	Present	Present
		Microbacterium chocolatum	1	1	0	1	No	**Absent**	**Absent**
		Microbacterium flavum	1	0	1	5	No	Present	**Absent**
	Nesterenkonia	*Nesterenkonia lacusekhoensis*	1	0	1	4	No	Present	**Absent**
	Propionimicrobium	*Propionimicrobium lymphophilum*	2	1	1	3	No	Present	**Absent**
	Trueperella	*Trueperella abortisuis*	1	1	0	5	No	Present	**Absent**
	Zimmermannella	*Zimmermannella bifida*	1	1	0	1	Yes	**Absent**	**Absent**
Bacteroidetes	*Alistipes*	*Alistipes finegoldii*	1	1	0	4	Yes	Present	**Absent**
	Bacteroides	*Bacteroides dorei*	1	1	0	8	No	**Absent**	**Absent**
	Butyricimonas	*Butyricimonas virosa*	2	0	2	1	Yes	Present	**Absent**
	Chryseobacterium	*Chryseobacterium hominis*	1	0	1	4	No	Present	**Absent**
		Chryseobacterium vrystaatense	1	0	1	3	No	**Absent**	**Absent**
	Peptoniphilus	*Candidatus Peptoniphilus massiliensis*	1	0	1	0	No	**Absent**	**Absent**
	Porphyromonas	*Porphyromonas uenonis*	4	4	0	2	No	Present	**Absent**
	Prevotella	"*Candidatus* Prevotella conceptionensis"	1	1	0	1	Yes	Present	**Absent**
	Wautersiella	*Wautersiella falsenii*	2	1	1	4	No	Present	**Absent**
Firmicutes	*Aerosphaera*	*Aerosphaera taetra*	1	1	0	0	No	Present	**Absent**
	Anaerococcus	*Anaerococcus octavius*	2	2	0	3	Yes	Present	**Absent**
	Anaerotruncus	*Anaerotruncus colihominis*	2	1	1	2	No	Present	**Absent**
	Bacillus	*Lysinibacillus massiliensis*	1	0	1	8	No	**Absent**	**Absent**
	Catabacter	*Catabacter hongkongensis*	1	1	0	6	No	**Absent**	**Absent**
	Clostridium	*Clostridium aldenense*	1	0	1	3	No	Present	**Absent**
	Dialister	*Dialister micraerophilus*	1	0	1	3	No	Present	**Absent**
	Granulicatella	*Granulicatella para-adiacens*	1	0	1	2	No	Present	**Absent**
	Peptoniphilus	*Peptoniphilus harei*	3	2	1	7	Yes	Present	**Absent**
	Streptococcus	*Streptococcus criceti*	3	0	3	10	No	Present	Present

(Continued on following page)

TABLE 3 (Continued)

Phylum	Genus	Bacterial species confirmed by molecular identification	No. of isolates	No. of isolates identified in the CPI period	No. of isolates identified in the MALDI-TOF MS period	No. of reports in PubMed	48 rare species by PID	Presence/absence of species in our MALDI-TOF MS database[b]	Presence/absence of species in MALDI-TOF MS database (Brüker)[b]
		Streptococcus massiliensis	2	2	0	1	Yes	Present	Present
		Streptococcus peroris	1	0	1	6	No	Present	Present
	Turicibacter	*Turicibacter sanguinis*	1	1	0	3	Yes	Present	**Absent**
Fusobacteria	*Leptotrichia*	*Leptotrichia trevisanii*	5	4	1	3	Yes	Present	**Absent**
Proteobacteria	*Acetobacter*	*Acetobacter indonesiensis*	2	2	0	9	No	Absent	**Absent**
	Acinetobacter	*Acinetobacter parvus*	1	1	0	8	Yes	Present	Present
		Acinetobacter septicus	5	4	1	3	No	Present	**Absent**
	Aurantimonas	*Aurantimonas altamirensis*	1	0	1	9	No	Present	**Absent**
	Blastomonas	*Blastomonas ursincola*	1	1	0	5	No	Present	Present
	Desulfovibrio	*Desulfovibrio intestinalis*	1	1	0	5	No	**Absent**	**Absent**
	Enterobacter	*Enterobacter kobei*	3	0	3	10	Yes	Present	Present
	Hematobacter	*Hematobacter massiliensis*	3	1	2	2	No	**Absent**	**Absent**
	Pandoraea	*Pandoraea pulmonicola*	3	0	3	7	Yes	Present	Present
	Pantoea	*Pantoea brenneri*	1	0	1	1	No	**Absent**	**Absent**
		Pantoea eucrina	1	0	1	2	No	Present	**Absent**
	Pseudochrobactrum	*Pseudochrobactrum asaccharolyticum*	1	0	1	2	No	Present	**Absent**
	Pseudomonas	*Pseudomonas lurida*	1	0	1	3	No	Present	**Absent**
	Ralstonia	*Ralstonia insidiosa*	1	0	1	5	No	Present	**Absent**
	Roseomonas	*Roseomonas genomospecies 5*	1	1	0	6	No	**Absent**	**Absent**
	Rothia	*Rothia aeria*	1	1	0	8	No	Present	**Absent**
	Serratia	*Serratia nematodiphila*	1	0	1	3	No	**Absent**	**Absent**
	Sphingomonas	*Sphingomonas mucosissima*	1	1	0	2	No	Present	**Absent**

[a] List of 75 rare bacterial species identified using molecular identification; 18 of these species were absent from our MALDI-TOF database, and 57 species from the Brüker database. PID, phenotypic identification; CPI, conventional phenotypic identification (Gram staining, API, Vitek 2 system identification).
[b] ●●●●●●●●.

AQ: K database (bioMérieux, Durham, NC), with 6,213 reference strains compared with 330 reference strains, respectively.

Another benefit of MALDI-TOF MS in routine identification revealed in this study is the reduced need for secondary phenotypic identification, which significantly decreased the cost and time required to provide results to clinicians. Only 50 secondary phenotypic identifications of 110,263 clonal-bacterial isolates tested (i.e., 4.5 reidentifications/10,000 isolates) were required during the MALDI-TOF MS period compared with 620 of 175,999 isolates during the CPI period (i.e., 35.2 reidentifications/10,000 isolates).

TABLE 4 Comparison of time, cost, and level of training required for routine identification of one isolate using the different techniques in our clinical laboratory

Identification technique	Time required for identification of one isolate	Cost (euros)	Level of training
Gram staining	6 min	0.6	Medium to high
API system identification (bioMérieux)	18–48 h	4.6–6	Medium
Vitek 2 system identification (bioMérieux)	5–8 h	5.9–8.23	Medium
Molecular identification by 16S rRNA or *rpoB* sequencing	24 h	137.7	Medium to high
MALDI-TOF MS by AutoFlex II system (Brüker Daltonik)	6–8 min 30 s	1.43	Low to medium
MALDI-TOF MS by MicroFlex LT mass spectrometer (Brüker Daltonik)	1 min 46 s	1.35	Low to medium

Over 3 years of experience in routine identification using MALDI-TOF MS, we observed a rise in the numbers of isolates and species that were identified using MALDI-TOF MS. The ability to expand the database by incorporation of laboratory spectra for bacteria that had been identified previously by molecular techniques has improved the performance of MALDI-TOF MS in identifying human-pathogenic bacteria.

Interestingly, MALDI-TOF MS identified more bacterial species that had been rarely reported as human pathogens than CPI did. A total of 37 of 48 rare species (77%) identified by phenotypic techniques were identified using MALDI-TOF MS. A systematic identification of all colonies derived from clinical samples will increase the capacity to identify more rare species in the future.

We also evaluated the time and cost-effectiveness of MALDI-TOF MS, which reduced by 55-fold and 169-fold the time required for identification and reduced by 5- and 96-fold the cost compared with CPI and gene sequencing, respectively (12). The time required for identification has been newly improved to 1 min 46 s using the MicroFlex LT mass spectrometer (Brüker Daltonik) compared with the AutoFlex II system, which took 6 to 8 min 30 s for identification of one isolate. The cost was evaluated at 1.35

euros for the MicroFlex LT mass spectrometer and 1.43 euros for the AutoFlex II system.

Conclusion. We have shown the effectiveness and performance of MALDI-TOF MS in the identification of clinical isolates and bacterial species in routine bacterial identification in a clinical laboratory over 11 years of study.

The ability of MALDI-TOF MS to identify a large number of bacterial species well is leading many clinical laboratories to abandon traditional phenotypic identification. We have shown that MALDI-TOF MS is not only a powerful tool for routine bacterial identification in the clinical laboratory but also a powerful tool to identify rare bacterial species implicated in human infectious diseases.

This capacity to identify rare species as human pathogens using MALDI-TOF MS could be an alternative to molecular methods in the clinical laboratory. The rapid identification of bacterial species that were rarely or never previously described as pathogens in specific clinical specimens will help us to study the clinical burden due to the emergence of these species as human pathogens and to implement their real-time surveillance.

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Supplementary material, Piseth SENG *et al.*; Identification of rare pathogenic bacteria in a clinical microbiology laboratory: impact of MALDI-TOF mass spectrometry.

Details referring to the Results section:

Table S1. Distribution and identification of the 76 bacterial species identified using only conventional phenotypic identification; 15 of the species were absent from our MALDI-TOF database.

Table S2. Distribution and identification of the 124 bacterial species identified using only MALDI-TOF; 103 of the species were absent from Vitek 2 database and only 21 were present.

Table S3. List of 21 species of 670 isolates that were confirmed by a second phenotypic identification; 3 of these species were absent from our MALDI-TOF database and 18 were present.

Table S4. List of 339 species of 1,273 isolates that were confirmed using molecular identification; 24 of these species were absent from our MALDI-TOF database and 63 from the Brüker database.

Table S5. Distribution of the sources of clinical samples for the 48 rare bacterial species identified by phenotypic identification.

Table S1. Distribution and identification of the 76 bacterial species identified using only conventional phenotypic identification; 15 of the species were absent from our MALDI-TOF database.

Phylum	Genus	Bacterial species identified using only CPI*	N° of isolates	Our MALDI-TOF database
Actinobacteria	*Actinomadura*	*Actinomadura cremea*	1	absent
	Actinomyces	*Actinomyces viscosus*	5	present
	Brevibacterium	*Brevibacterium luteolum*	1	present
	Cellulosimicrobium	*Cellulosimicrobium cellulans*	1	present
	Corynebacterium	*Corynebacterium auriscanis*	3	present
		Corynebacterium bovis	2	present
		Corynebacterium macginleyi	17	present
		Corynebacterium ulcerans	4	present
	Gordonia	*Gordonia sputi*	1	present
	Leifsonia	*Leifsonia aquatica*	3	present
	Microbacterium	*Microbacterium flavescens*	1	present
	Nocardia	*Nocardia abscessus*	1	present
		Nocardia asteroides	1	absent
		Nocardia otitidiscaviarum	1	present
	Pseudoclavibacter	*Zimmermannella bifida*	1	absent
	Varibaculum	*Varibaculum cambriense*	2	present
Bacteroidetes	*Bacteroides*	*Bacteroides eggerthii*	2	present
		Bacteroides stercoris	29	present
	Myroides	*Myroides odoratimimus*	1	present
	Prevotella	*Prevotella loescheii*	1	absent
		Prevotella massiliensis	1	present
Firmicutes	*Bacillus*	*Bacillus coagulans*	1	present
	Clostridium	*Clostridium baratii*	1	present
		Clostridium bifermentans	5	present
		Clostridium histolyticum	1	present
	Dialister	*Dialister pneumosintes*	3	absent
	Facklamia	*Facklamia languida*	1	present
	Lactobacillus	*Lactobacillus acidophilus*	2	present
		Lactobacillus vaginalis	1	present
		Lactococcus lactis subsp. cremoris	3	absent
	Listeria	*Listeria grayi*	1	present
	Peptoniphilus	*Peptoniphilus lacrimalis*	2	present
	Sporosarcina	*Sporosarcina ginsengisoli*	1	absent
	Staphylococcus	*Staphylococcus carnosus*	2	present
		Staphylococcus chromogenes	1	present
		Staphylococcus hyicus	1	present
		Staphylococcus kloosii	3	present
		Staphylococcus lentus	8	present
	Streptococcus	*Streptococcus sobrinus*	1	present
		Streptococcus uberis	1	present
Fusobacteria	*Fusobacterium*	*Fusobacterium varium*	4	present
	Leptotrichia	*Leptotrichia goodfellowii*	1	absent
		Leptotrichia trevisanii	1	present
Proteobacteria	*Aeromonas*	*Aeromonas sobria*	8	present
	Agrobacterium	*Agrobacterium radiobacter*	1	absent

Phylum	Genus	Bacterial species identified using only CPI*	N° of isolates	Our MALDI-TOF database
	Anaerobiospirillum	*Anaerobiospirillum succiniciproducens*	1	absent
	Bordetella	*Bordetella pertussis*	16	present
	Campylobacter	*Campylobacter upsaliensis*	1	present
	Cardiobacterium	*Cardiobacterium hominis*	1	present
	Chromobacterium	*Chromobacterium violaceum*	1	present
	Citrobacter	*Citrobacter sedlakii*	2	present
	Cronobacter	*Cronobacter sakazakii*	11	present
	Escherichia	*Escherichia fergusonii*	1	present
		Escherichia hermannii	9	present
	Helicobacter	*Helicobacter pylori*	1	present
	Ignatzschineria	*Ignatzschineria larvae*	1	absent
	Kluyvera	*Kluyvera intermedia*	1	present
	Mannheimia	*Mannheimia haemolytica*	1	present
	Neisseria	*Neisseria cinerea*	6	present
		Neisseria polysaccharea	1	present
	Pasteurella	*Pasteurella dagmatis*	1	present
		Pasteurella pneumotropica	3	present
	Pseudomonas	*Pseudomonas chlororaphis subsp. aureofaciens*	1	present
		Pseudomonas luteola	4	present
		Pseudomonas oleovorans	1	present
		Pseudomonas syringae	1	present
	Psychrobacter	*Psychrobacter phenylpyruvicus*	1	absent
	Roseomonas	*Roseomonas ludipueritiae*	1	absent
	Salmonella	*Salmonella enterica*	8	present
		Salmonella enterica subsp. arizonae	1	present
		Salmonella enterica subsp. enterica serovar Enteritidis	5	present
		Salmonella enterica subsp. enterica serovar Paratyphi A	3	present
		Salmonella enterica subsp. enterica serovar Typhimurium	3	present
	Serratia	*Serratia plymuthica*	7	present
	Shigella	*Shigella flexneri*	12	absent
		Shigella sonnei	26	absent

CPI*: conventional phenotypic identification (Gram staining, API, Vitek 2 system identification).

Table S2. Distribution and identification of the 124 bacterial species identified using only MALDI-TOF; 103 of the species were absent from Vitek 2 database and only 21 were present.

Phylum	Genus	Bacterial species identified using only MALDI-TOF	N° of isolates	Vitek 2 database
Actinobacteria	*Actinobaculum*	*Actinobaculum massiliense*	1	absent
	Actinomyces	*Actinomyces radicidentis*	2	absent
	Arthrobacter	*Arthrobacter polychromogenes*	1	absent
	Atopobium	*Atopobium minutum*	1	absent
	Bifidobacterium	*Bifidobacterium breve*	7	absent
		Bifidobacterium dentium	1	absent
	Brevibacterium	*Brevibacterium massiliense*	1	absent
		Brevibacterium paucivorans	1	absent
		Brevibacterium ravenspurgense	1	absent
	Collinsella	*Collinsella aerofaciens*	1	absent
	Corynebacterium	*Corynebacterium fastidiosum*	1	absent
		Corynebacterium freneyi	1	absent
		Corynebacterium glutamicum	1	absent
		Corynebacterium imitans	1	absent
		Corynebacterium mucifaciens	3	absent
		Corynebacterium pseudogenitalium	2	absent
		Corynebacterium pseudotuberculosis	1	absent
	Microbacterium	*Microbacterium paraoxydans*	1	absent
		Microbacterium schleiferi	1	absent
	Nocardia	*Nocardia cyriacigeorgica*	1	absent
	Trueperella	*Trueperella pyogenes*	1	absent
Bacteroidetes	*Bacteroides*	*Bacteroides cellulosilyticus*	3	absent
	Butyricimonas	*Butyricimonas virosa*	1	absent
	Capnocytophaga	*Capnocytophaga canimorsus*	2	absent
		Capnocytophaga gingivalis	1	absent
		Capnocytophaga ochracea	1	absent
	Chryseobacterium	*Chryseobacterium gleum*	3	present
	Porphyromonas	*Porphyromonas gingivalis*	1	absent
		Porphyromonas gulae	1	absent
	Prevotella	*Prevotella pallens*	1	absent
	Weeksella	*Weeksella virosa*	2	absent
Firmicutes	*Anaerococcus*	*Anaerococcus lactolyticus*	2	absent
		Anaerococcus octavius	6	absent
		Anaerococcus tetradius	3	absent
	Bacillus	*Bacillus amyloliquefaciens*	1	absent
		Bacillus circulans	3	absent
		Bacillus megaterium	2	absent
		Bacillus mycoides	1	absent
		Bacillus subtilis	2	absent
		Bacillus thuringiensis	3	absent
	Brevibacillus	*Brevibacillus agri*	1	absent
		Brevibacillus brevis	1	absent
	Clostridium	*Clostridium cadaveris*	1	absent

Phylum	Genus	Bacterial species identified using only MALDI-TOF	N° of isolates	Vitek 2 database
		Clostridium celerecrescens	3	absent
		Clostridium chauvoei	1	absent
		Clostridium sphenoides	1	absent
		Clostridium subterminale	1	absent
		Clostridium tetani	2	absent
	Enterococcus	*Enterococcus cecorum*	1	present
		Enterococcus hirae	12	present
		Enterococcus raffinosus	3	present
	Eubacterium	*Eubacterium tenue*	2	absent
		Eubacterium yurii	1	absent
	Gemella	*Gemella sanguinis*	1	present
	Lactobacillus	*Lactobacillus delbrueckii*	8	absent
		Lactobacillus iners	3	absent
		Lactobacillus johnsonii	1	absent
		Lactobacillus mucosae	1	absent
		Lactobacillus murinus	3	absent
		Lactobacillus salivarius	2	absent
	Lactococcus	*Lactococcus garvieae*	2	present
	Leuconostoc	*Leuconostoc mesenteroides*	1	absent
	Mogibacterium	*Mogibacterium timidum*	1	absent
	Paenibacillus	*Paenibacillus amylolyticus*	2	absent
		Paenibacillus barcinonensis	1	absent
	Pediococcus	*Pediococcus acidilactici*	5	present
		Pediococcus pentosaceus	3	present
		Peptococcus niger	1	absent
	Robinsoniella	*Robinsoniella peoriensis*	3	absent
	Ruminococcus	*Ruminococcus gnavus*	1	absent
	Staphylococcus	*Staphylococcus arlettae*	1	present
		Staphylococcus condimenti	2	absent
		Staphylococcus pasteuri	61	absent
		Staphylococcus pettenkoferi	29	absent
		Staphylococcus pseudintermedius	17	absent
		Staphylococcus vitulinus	1	present
	Streptococcus	*Streptococcus canis*	1	present
		Streptococcus cristatus	6	present
		Streptococcus equi	3	absent
		Streptococcus massiliensis	3	absent
		Streptococcus parasanguinis	37	absent
		Streptococcus pyogenes	75	present
		Streptococcus vestibularis	9	present
	Veillonella	*Veillonella dispar*	4	absent
		Veillonella montpellierensis	1	absent
Fusobacteria	*Fusobacterium*	*Fusobacterium periodonticum*	1	absent
Proteobacteria	*Acinetobacter*	*Acinetobacter bereziniae*	34	absent
		Acinetobacter calcoaceticus	4	absent

Phylum	Genus	Bacterial species identified using only MALDI-TOF	N° of isolates	Vitek 2 database
		Acinetobacter parvus	2	absent
		Acinetobacter pittii	84	absent
		Acinetobacter radioresistens	3	absent
		Acinetobacter schindleri	1	absent
	Aggregatibacter	*Aggregatibacter segnis*	1	absent
	Agrobacterium	*Agrobacterium tumefaciens*	2	absent
	Burkholderia	*Burkholderia gladioli*	1	present
		Burkholderia multivorans	10	absent
	Campylobacter	*Campylobacter curvus*	1	absent
	Comamonas	*Comamonas kerstersii*	1	absent
	Desulfovibrio	*Desulfovibrio desulfuricans*	1	absent
		Desulfovibrio fairfieldensis	3	absent
	Enterobacter	*Enterobacter cowanii*	3	absent
		Enterobacter hormaechei	20	absent
		Enterobacter kobei	190	absent
	Haemophilus	*Haemophilus parahaemolyticus*	6	present
	Moraxella	*Moraxella nonliquefaciens*	11	absent
		Moraxella osloensis	9	absent
	Neisseria	*Neisseria perflava*	2	absent
		Neisseria subflava	1	absent
	Ochrobactrum	*Ochrobactrum grignonense*	1	absent
		Ochrobactrum intermedium	1	absent
	Olsenella	*Olsenella uli*	1	absent
	Pandoraea	*Pandoraea pulmonicola*	9	absent
	Pasteurella	*Pasteurella aerogenes*	1	present
		Pasteurella canis	6	absent
	Pseudomonas	*Pseudomonas geniculata*	2	absent
		Pseudomonas hibiscicola	2	absent
		Pseudomonas mosselii	11	absent
		Pseudomonas pseudoalcaligenes	1	present
	Roseomonas	*Roseomonas mucosa*	2	absent
	Serratia	*Serratia ureilytica*	1	absent
	Shigella	*Shigella dysenteriae*	1	absent
	Vibrio	*Vibrio alginolyticus*	3	present
		Vibrio parahaemolyticus	1	present
		Vibrio vulnificus	1	present

Table S3. List of 21 species of 670 isolates that were confirmed by a second phenotypic identification; 3 of these species were absent from our MALDI-TOF database and 18 were present.

Phylum	Genus	Bacteria species needed 2nd PID*	N° of isolates	N° isolates, CPI** period	N° isolates, MALDI period	Our MALDI-TOF database
Actinobacteria	*Corynebacterium*	*Corynebacterium aurimucosum*	1	0	1	present
		Corynebacterium sp. A	52	52	0	absent
		Corynebacterium sp. G	1	1	0	absent
		Corynebacterium striatum	2	0	2	present
		Corynebacterium tuberculostearicum	2	0	2	present
Firmicutes	*Bacillus*	*Bacillus cereus*	1	0	1	present
	Lactobacillus	*Lactobacillus aviarius*	1	1	0	present
	Peptoniphilus	*Peptoniphilus harei*	1	1	0	present
	Streptococcus	*Streptococcus intermedius*	1	1	0	present
		Streptococcus oralis	2	0	2	present
		Streptococcus pneumoniae	1	1	0	present
		Streptococcus sp. 'group G'	596	561	35	absent
	Veillonella	*Veillonella parvula*	1	0	1	present
Fusobacteria	*Fusobacterium*	*Fusobacterium nucleatum*	1	1	0	present
Proteobacteria	*Acinetobacter*	*Acinetobacter guillouiae*	1	0	1	present
	Arthrobacter	*Arthrobacter ramosus*	1	0	1	present
	Citrobacter	*Citrobacter braakii*	1	0	1	present
	Enterobacter	*Enterobacter aerogenes*	1	1	0	present
		Enterobacter kobei	1	0	1	present
	Pseudomonas	*Pseudomonas monteilii*	1	0	1	present
		Pseudomonas nitroreducens	1	0	1	present

PID*: phenotypic identification; CPI**: conventional phenotypic identification (Gram staining, API, Vitek 2 system identification).

Table S4. List of 339 species of 1,273 isolates that were confirmed using molecular identification; 24 of these species were absent from our MALDI-TOF database and 63 from the Brüker database.

Phylum	Genus	Bacterial species confirmed using molecular identification	N° of isolates (n=)	N° of isolates, CPI* period	N° of isolates, MALDI-TOF period	Our MALDI-TOF database	MALDI-TOF database (Brüker)
Actinobacteria	*Actinobaculum*	*Actinobaculum schaalii*	10	5	5	present	present
	Actinomyces	*Actinomyces europaeus*	3	1	2	present	present
		Actinomyces georgiae	3	1	2	absent	absent
		Actinomyces lingnae	1	0	1	absent	absent
		Actinomyces meyeri	1	1	0	present	present
		Actinomyces naeslundii	10	7	3	present	present
		Actinomyces neuii	9	5	4	present	present
		Actinomyces odontolyticus	10	7	3	present	present
		Actinomyces oris	1	0	1	present	absent
		Actinomyces radingae	5	3	2	present	present
		Actinomyces sp oral	2	2	0	absent	absent
		Actinomyces turicensis	7	1	6	present	present
		Actinomyces urogenitalis	2	0	2	present	present
		Actinomyces viscosus	4	2	2	present	present
	Arcanobacterium	*Arcanobacterium bernardiae*	3	1	2	present	present
		Arcanobacterium haemolyticum	4	2	2	present	present
	Arthrobacter	*Arthrobacter cumminsii*	5	4	1	present	present
		Arthrobacter nitroguajacolicus	1	1	0	absent	absent
		Arthrobacter oxydans	1	1	0	present	present
	Atopobium	*Atopobium parvulum*	9	3	6	present	present
		Atopobium vaginae	1	1	0	present	present
	Bifidobacterium	*Bifidobacterium breve*	3	3	0	present	present
		Bifidobacterium scardovii	1	1	0	present	absent
	Brachybacterium	*Brachybacterium muris*	1	0	1	present	present
		Brachybacterium sacelli	1	0	1	absent	absent
	Brevibacterium	*Brevibacterium casei*	3	2	1	present	present
		Brevibacterium massiliense	1	1	0	present	absent
		Brevibacterium otitidis	1	1	0	absent	absent
		Brevibacterium paucivorans	2	1	1	present	present
		Brevibacterium ravenspurgense	1	1	0	present	present
		Brevibacterium sanguinis	1	1	0	present	present
		Brevibacterium stationis	1	0	1	present	absent
	Cellulomonas	*Cellulomonas hominis*	1	0	1	present	absent
	Clavibacter	*Clavibacter michiganensis*	1	0	1	present	present
	Corynebacterium	*Corynebacterium accolens*	2	1	1	present	present
		Corynebacterium afermentans	4	3	1	present	present
		Corynebacterium amycolatum	42	15	27	present	present
		Corynebacterium argentoratense	2	0	2	present	present
		Corynebacterium aurimucosum	10	6	4	present	present
		Corynebacterium auris	1	1	0	present	present
		Corynebacterium auriscanis	3	3	0	present	present
		Corynebacterium bovis	1	1	0	present	present
		Corynebacterium confusum	1	0	1	present	present

Phylum	Genus	Bacterial species confirmed using molecular identification	N° of isolates (n=)	N° of isolates, CPI* period	N° of isolates, MALDI-TOF period	Our MALDI-TOF database	MALDI-TOF database (Brüker)
		Corynebacterium coyleae	4	0	4	present	present
		Corynebacterium diphtheriae	1	1	0	present	present
		Corynebacterium durum	1	1	0	present	present
		Corynebacterium fastidiosum	1	0	1	absent	absent
		Corynebacterium genitalium	5	4	1	absent	absent
		Corynebacterium glucuronolyticum	3	1	2	present	present
		Corynebacterium imitans	1	0	1	present	present
		Corynebacterium jeikeium	13	8	5	present	present
		Corynebacterium kroppenstedtii	1	0	1	present	present
		Corynebacterium macginleyi	2	1	1	present	present
		Corynebacterium minutissimum	4	2	2	present	present
		Corynebacterium mucifaciens	3	0	3	present	present
		Corynebacterium propinquum	9	8	1	present	present
		Corynebacterium pseudodiphtheriticum	10	9	1	present	present
		Corynebacterium pseudogenitalium	1	0	1	present	absent
		Corynebacterium riegelii	1	0	1	present	present
		Corynebacterium simulans	4	2	2	present	present
		Corynebacterium striatum	12	5	7	present	present
		Corynebacterium tuberculostearicum	31	6	25	present	present
		Corynebacterium urealyticum	4	3	1	present	present
		Corynebacterium ureicelerivorans	1	0	1	present	present
	Dermabacter	*Dermabacter hominis*	15	10	5	present	present
	Dietzia	*Dietzia cinnamea*	1	1	0	present	present
	Eggerthella	*Eggerthella lenta*	6	6	0	present	present
	Gardnerella	*Gardnerella vaginalis*	2	2	0	present	present
	Janibacter	*Janibacter hoylei*	1	0	1	present	absent
	Kocuria	*Kocuria rhizophila*	1	1	0	present	present
		Kocuria rosea	1	1	0	present	present
	Kytococcus	*Kytococcus schroeteri*	1	1	0	present	absent
	Microbacterium	*Microbacterium aurum*	2	1	1	present	present
		Microbacterium chocolatum	1	1	0	absent	absent
		Microbacterium flavum	1	0	1	present	present
		Microbacterium liquefaciens	1	1	0	present	present
		Microbacterium oxydans	1	0	1	present	present
		Microbacterium paraoxydans	2	2	0	present	absent
	Micrococcus	*Micrococcus luteus*	4	3	1	present	present
	Nesterenkonia	*Nesterenkonia lacusekhoensis*	1	0	1	present	absent
	Nocardia	*Nocardia abscessus*	6	3	3	present	present
		Nocardia brasiliensis	1	0	1	present	absent
		Nocardia cyriacigeorgica	3	2	1	present	present
		Nocardia farcinica	5	3	2	present	present
		Nocardia otitidiscaviarum	2	2	0	present	present
		Nocardia transvalensis	1	1	0	present	present
	Ochrobactrum	*Ochrobactrum anthropi*	3	0	3	present	present

Phylum	Genus	Bacterial species confirmed using molecular identification	N° of isolates (n=)	N° of isolates, CPI* period	N° of isolates, MALDI-TOF period	Our MALDI-TOF database	MALDI-TOF database (Brüker)
	Olsenella	*Olsenella uli*	1	0	1	present	absent
	Propionibacterium	*Propionibacterium acnes*	19	16	3	present	present
		Propionibacterium avidum	13	12	1	present	present
		Propionibacterium granulosum	2	1	1	present	present
		Propionibacterium propionicum	3	1	2	present	present
		Propionimicrobium lymphophilum	2	1	1	present	present
	Rothia	*Rothia dentocariosa*	4	3	1	present	present
		Rothia mucilaginosa	2	1	1	present	present
	Trueperella	*Trueperella abortisuis*	1	1	0	present	absent
	Turicella	*Turicella otitidis*	1	0	1	present	present
	Zimmermannella	*Zimmermannella bifida*	1	1	0	absent	absent
Bacteroidetes	*Alistipes*	*Alistipes finegoldii*	1	1	0	present	present
	Bacteroides	*Bacteroides dorei*	1	1	0	absent	absent
		Bacteroides fragilis	1	1	0	present	present
		Bacteroides heparinolyticus	3	1	2	present	absent
		Bacteroides ovatus	1	1	0	present	present
		Bacteroides pyogenes	1	1	0	present	absent
	Butyricimonas	*Butyricimonas virosa*	2	0	2	present	present
	Capnocytophaga	*Capnocytophaga canimorsus*	1	1	0	present	present
		Capnocytophaga gingivalis	1	1	0	present	present
		Capnocytophaga sputigena	4	4	0	present	present
	Chryseobacterium	*Chryseobacterium gleum*	1	0	1	present	absent
		Chryseobacterium hominis	1	0	1	present	absent
		Chryseobacterium indologenes	3	3	0	present	present
		Chryseobacterium vrystaatense	1	0	1	absent	absent
	Elizabethkingia	*Elizabethkingia meningoseptica*	1	1	0	present	present
	Parabacteroides	*Parabacteroides distasonis*	1	1	0	present	present
	Peptoniphilus	*Candidatus Peptoniphilus massiliensis*	1	0	1	absent	absent
	Porphyromonas	*Porphyromonas asaccharolytica*	2	2	0	present	present
		Porphyromonas uenonis	4	4	0	present	absent
	Prevotella	*Candidatus Prevotella conceptionensis*	1	1	0	present	absent
		Prevotella bivia	1	1	0	present	present
		Prevotella buccae	1	1	0	present	present
		Prevotella corporis	1	0	1	present	present
		Prevotella intermedia	3	3	0	present	present
		Prevotella loescheii	2	0	2	absent	absent
		Prevotella oralis	1	1	0	present	present
		Prevotella oris	2	2	0	present	present
	Wautersiella	*Wautersiella falsenii*	2	1	1	present	present
Firmicutes	*Abiotrophia*	*Abiotrophia defectiva*	2	1	1	present	present
	Aerococcus	*Aerococcus urinae*	4	4	0	present	present
		Aerococcus viridans	1	0	1	present	present
	Aerosphaera	*Aerosphaera taetra*	1	1	0	present	absent
	Anaerococcus	*Anaerococcus octavius*	2	2	0	present	present
		Anaerococcus prevotii	1	0	1	present	present

Phylum	Genus	Bacterial species confirmed using molecular identification	N° of isolates (n=)	N° of isolates, CPI* period	N° of isolates, MALDI-TOF period	Our MALDI-TOF database	MALDI-TOF database (Brüker)
		Anaerococcus vaginalis	3	2	1	present	present
	Anaerotruncus	*Anaerotruncus colihominis*	2	1	1	present	absent
	Bacillus	*Bacillus cereus*	18	12	6	present	present
		Bacillus circulans	2	1	1	present	present
		Bacillus coagulans	2	1	1	present	present
		Bacillus flexus	1	1	0	present	present
		Bacillus licheniformis	6	2	4	present	present
		Bacillus megaterium	1	1	0	present	present
		Bacillus mycoides	1	1	0	present	present
		Bacillus pumilus	1	0	1	present	present
		Bacillus simplex	4	2	2	present	present
		Bacillus subtilis	1	0	1	present	present
		Lysinibacillus massiliensis	1	0	1	present	absent
	Catabacter	*Catabacter hongkongensis*	1	1	0	absent	absent
	Clostridium	*Clostridium aldenense*	1	0	1	present	present
		Clostridium botulinum	2	2	0	present	absent
		Clostridium celerecrescens	1	0	1	present	present
		Clostridium clostridioforme	2	2	0	present	present
		Clostridium hathewayi	2	2	0	present	present
		Clostridium lituseburense	1	1	0	present	absent
		Clostridium perfringens	1	1	0	present	present
		Clostridium ramosum	1	0	1	present	present
		Clostridium subterminale	1	0	1	present	present
		Clostridium tertium	3	3	0	present	present
	Dialister	*Dialister micraerophilus*	1	0	1	present	present
	Enterococcus	*Enterococcus avium*	5	2	3	present	present
		Enterococcus casseliflavus	1	0	1	present	present
		Enterococcus faecalis	6	5	1	present	present
		Enterococcus faecium	4	0	4	present	present
		Enterococcus gallinarum	1	1	0	present	present
		Enterococcus raffinosus	1	0	1	present	present
	Erwinia	*Erwinia rhapontici*	1	1	0	present	present
	Finegoldia	*Finegoldia magna*	6	6	0	present	present
	Flavonifractor	*Flavonifractor plautii*	5	5	0	present	absent
	Gemella	*Gemella haemolysans*	2	0	2	present	present
		Gemella morbillorum	2	1	1	present	present
	Granulicatella	*Granulicatella adiacens*	3	1	2	present	present
		Granulicatella elegans	1	1	0	present	present
		Granulicatella para-adiacens	1	0	1	present	absent
	Lactobacillus	*Lactobacillus casei*	3	3	0	present	present
		Lactobacillus fermentum	1	1	0	present	present
		Lactobacillus gasseri	3	1	2	present	present
		Lactobacillus jensenii	1	1	0	present	present
		Lactobacillus plantarum	1	1	0	present	present
		Lactobacillus rhamnosus	11	9	2	present	present

Phylum	Genus	Bacterial species confirmed using molecular identification	N° of isolates (n=)	N° of isolates, CPI* period	N° of isolates, MALDI-TOF period	Our MALDI-TOF database	MALDI-TOF database (Brüker)
		Lactobacillus sakei	1	0	1	present	present
		Lactobacillus vaginalis	1	1	0	present	absent
		Lactobacillus zeae	2	2	0	present	present
		Lactococcus garvieae	1	0	1	present	present
	Leclercia	*Leclercia adecarboxylata*	4	4	0	present	present
	Leuconostoc	*Leuconostoc lactis*	1	1	0	present	present
		Leuconostoc mesenteroides	1	0	1	present	present
	Listeria	*Listeria monocytogenes*	2	2	0	present	present
	Lysinibacillus	*Lysinibacillus sphaericus*	1	1	0	present	present
	Parvimonas	*Parvimonas micra*	7	7	0	present	present
	Peptoniphilus	*Peptoniphilus harei*	3	2	1	present	present
		Peptoniphilus indolicus	1	0	1	present	present
		Peptoniphilus lacrimalis	1	1	0	present	absent
	Ruminococcus	*Ruminococcus gnavus*	1	1	0	present	present
	Sporosarcina	*Sporosarcina globispora*	1	1	0	present	present
	Staphylococcus	*Staphylococcus aureus*	7	5	2	present	present
		Staphylococcus capitis	1	1	0	present	present
		Staphylococcus caprae	3	0	3	present	present
		Staphylococcus cohnii	4	1	3	present	present
		Staphylococcus epidermidis	13	9	4	present	present
		Staphylococcus equorum	1	0	1	present	present
		Staphylococcus haemolyticus	1	0	1	present	present
		Staphylococcus hominis	2	0	2	present	present
		Staphylococcus intermedius	1	1	0	present	present
		Staphylococcus lugdunensis	1	0	1	present	present
		Staphylococcus pettenkoferi	2	0	2	present	present
		Staphylococcus saccharolyticus	2	0	2	present	present
		Staphylococcus saprophyticus	2	0	2	present	present
	Streptococcus	*Streptococcus anginosus*	21	17	4	present	present
		Streptococcus australis	1	1	0	present	present
		Streptococcus constellatus	22	16	6	present	present
		Streptococcus criceti	3	0	3	present	present
		Streptococcus cristatus	1	1	0	present	present
		Streptococcus dysgalactiae	5	1	4	present	present
		Streptococcus gallolyticus	12	9	3	present	present
		Streptococcus gordonii	8	3	5	present	present
		Streptococcus infantarius	4	4	0	present	present
		Streptococcus infantis	9	3	6	present	present
		Streptococcus intermedius	15	13	2	present	present
		Streptococcus lutetiensis	2	1	1	present	present
		Streptococcus massiliensis	2	2	0	present	present
		Streptococcus mitis	44	17	27	present	present
		Streptococcus mutans	5	5	0	present	present
		Streptococcus oligofermentans	4	4	0	absent	absent
		Streptococcus oralis	30	9	21	present	present

Phylum	Genus	Bacterial species confirmed using molecular identification	N° of isolates (n=)	N° of isolates, CPI* period	N° of isolates, MALDI-TOF period	Our MALDI-TOF database	MALDI-TOF database (Brüker)
		Streptococcus parasanguinis	8	4	4	present	present
		Streptococcus pasteurianus	1	0	1	present	present
		Streptococcus peroris	1	0	1	present	present
		Streptococcus pneumoniae	10	3	7	present	present
		Streptococcus pseudopneumoniae	2	0	2	present	present
		Streptococcus pyogenes	3	1	2	present	present
		Streptococcus salivarius	9	6	3	present	present
		Streptococcus sanguinis	7	5	2	present	present
		Streptococcus sp oral	7	7	0	absent	absent
		Streptococcus thermophilus	8	5	3	present	present
		Streptococcus vestibularis	4	4	0	present	present
	Turicibacter	*Turicibacter sanguinis*	1	1	0	present	absent
	Veillonella	*Veillonella parvula*	1	1	0	present	present
	Weissella	*Weissella cibaria*	1	1	0	absent	absent
Fusobacteria	*Fusobacterium*	*Fusobacterium naviforme*	2	2	0	present	present
		Fusobacterium necrophorum	6	6	0	present	present
		Fusobacterium nucleatum	12	12	0	present	present
		Fusobacterium periodonticum	1	1	0	present	absent
	Leptotrichia	*Leptotrichia trevisanii*	5	4	1	present	absent
Proteobacteria	*Acetobacter*	*Acetobacter indonesiensis*	2	2	0	absent	absent
	Achromobacter	*Achromobacter xylosoxidans*	33	27	6	present	present
	Acinetobacter	*Acinetobacter baumannii*	1	1	0	present	present
		Acinetobacter calcoaceticus	7	1	6	present	present
		Acinetobacter haemolyticus	1	0	1	present	present
		Acinetobacter johnsonii	1	0	1	present	present
		Acinetobacter junii	7	3	4	present	present
		Acinetobacter lwoffii	2	2	0	present	present
		Acinetobacter parvus	1	1	0	present	present
		Acinetobacter pittii	4	0	4	present	present
		Acinetobacter schindleri	2	1	1	present	present
		Acinetobacter septicus	5	4	1	present	absent
		Acinetobacter ursingii	12	3	9	present	present
	Actinobacillus	*Actinobacillus ureae*	1	1	0	present	present
	Aeromonas	*Aeromonas veronii*	1	1	0	present	present
	Aggregatibacter	*Aggregatibacter aphrophilus*	7	5	2	present	present
		Aggregatibacter segnis	1	1	0	present	present
	Agrobacterium	*Agrobacterium tumefaciens*	2	0	2	present	present
	Aurantimonas	*Aurantimonas altamirensis*	1	0	1	present	absent
	Blastomonas	*Blastomonas ursincola*	1	1	0	present	present
	Bordetella	*Bordetella bronchiseptica*	3	2	1	present	present
		Bordetella holmesii	1	0	1	present	present
	Brevundimonas	*Brevundimonas diminuta*	3	2	1	present	present
		Brevundimonas vesicularis	1	0	1	present	present
	Brucella	*Brucella melitensis*	2	2	0	present	absent
	Burkholderia	*Burkholderia cenocepacia*	1	0	1	present	present

Phylum	Genus	Bacterial species confirmed using molecular identification	N° of isolates (n=)	N° of isolates, CPI* period	N° of isolates, MALDI-TOF period	Our MALDI-TOF database	MALDI-TOF database (Brüker)
		Burkholderia cepacia	2	1	1	present	present
		Burkholderia fungorum	1	1	0	present	present
		Burkholderia multivorans	4	2	2	present	present
	Campylobacter	*Campylobacter fetus*	1	1	0	present	present
		Campylobacter jejuni	1	1	0	present	present
		Campylobacter lari	1	1	0	present	present
		Campylobacter rectus	1	1	0	present	present
		Campylobacter ureolyticus	3	3	0	present	present
	Delftia	*Delftia tsuruhatensis*	7	5	2	present	absent
	Desulfovibrio	*Desulfovibrio fairfieldensis*	3	0	3	present	absent
		Desulfovibrio intestinalis	1	1	0	absent	absent
	Eikenella	*Eikenella corrodens*	9	7	2	present	present
	Enterobacter	*Enterobacter aerogenes*	3	2	1	present	present
		Enterobacter cancerogenus	2	0	2	present	present
		Enterobacter cloacae	7	1	6	present	present
		Enterobacter hormaechei	3	1	2	present	present
		Enterobacter kobei	3	0	3	present	present
	Erwinia	*Erwinia tasmaniensis*	1	1	0	present	present
	Escherichia	*Escherichia coli*	6	4	2	present	present
	Grimontia	*Grimontia hollisae*	1	1	0	present	present
	Haematobacter	*Haematobacter massiliensis*	3	1	2	absent	absent
	Haemophilus	*Haemophilus influenzae*	2	1	1	present	present
		Haemophilus parainfluenzae	4	4	0	present	present
	Ignatzschineria	*Ignatzschineria larvae*	3	3	0	absent	absent
	Inquilinus	*Inquilinus limosus*	7	6	1	present	present
	Kingella	*Kingella kingae*	5	5	0	present	present
	Klebsiella	*Klebsiella oxytoca*	2	1	1	present	present
		Klebsiella pneumoniae	9	4	5	present	present
	Moraxella	*Moraxella catarrhalis*	6	6	0	present	present
		Moraxella lacunata	1	1	0	present	present
		Moraxella nonliquefaciens	1	1	0	present	present
		Moraxella osloensis	3	0	3	present	present
	Morganella	*Morganella morganii*	2	1	1	present	present
	Neisseria	*Neisseria canis*	1	1	0	present	present
		Neisseria cinerea	3	1	2	present	present
		Neisseria flavescens	2	0	2	present	present
		Neisseria gonorrhoeae	1	0	1	present	present
		Neisseria mucosa	1	1	0	present	present
		Neisseria sicca	2	1	1	present	present
	Pandoraea	*Pandoraea pnomenusa*	1	1	0	present	present
		Pandoraea pulmonicola	3	0	3	present	present
	Pantoea	*Pantoea ananatis*	5	2	3	present	present
		Pantoea brenneri	1	0	1	absent	absent
		Pantoea eucrina	1	0	1	present	absent
	Pasteurella	*Pasteurella canis*	5	1	4	present	present

Phylum	Genus	Bacterial species confirmed using molecular identification	N° of isolates (n=)	N° of isolates, CPI* period	N° of isolates, MALDI-TOF period	Our MALDI-TOF database	MALDI-TOF database (Brüker)
		Pasteurella multocida	11	10	1	present	present
	Pseudochrobactrum	*Pseudochrobactrum asaccharolyticum*	1	0	1	present	present
	Pseudomonas	*Pseudomonas aeruginosa*	47	28	19	present	present
		Pseudomonas alcaligenes	5	3	2	present	present
		Pseudomonas fluorescens	1	0	1	present	present
		Pseudomonas lurida	1	0	1	present	absent
		Pseudomonas mendocina	10	0	10	present	present
		Pseudomonas mosselii	1	0	1	present	present
		Pseudomonas oleovorans	1	1	0	present	present
		Pseudomonas oryzihabitans	4	2	2	present	present
		Pseudomonas putida	2	0	2	present	present
		Pseudomonas rhodesiae	1	0	1	present	present
		Pseudomonas stutzeri	2	1	1	present	present
		Pseudomonas syringae	1	0	1	present	present
	Rahnella	*Rahnella aquatilis*	1	0	1	present	present
	Ralstonia	*Ralstonia insidiosa*	1	0	1	present	present
		Ralstonia mannitolilytica	2	0	2	present	present
	Roseomonas	*Roseomonas genomospecies 5*	1	1	0	present	absent
		Roseomonas mucosa	3	3	0	present	present
	Rothia	*Rothia aeria*	1	1	0	present	present
	Salmonella	*Salmonella enterica*	1	0	1	present	present
	Serratia	*Serratia nematodiphila*	1	0	1	absent	absent
		Serratia rubidaea	2	0	2	present	present
	Slackia	*Slackia exigua*	3	3	0	present	present
	Sphingomonas	*Sphingomonas mucosissima*	1	1	0	present	present
		Sphingomonas paucimobilis	1	1	0	present	present
	Stenotrophomonas	*Stenotrophomonas maltophilia*	11	9	2	present	present

CPI*: conventional phenotypic identification (Gram staining, API, Vitek 2 system identification).

Table S5. Distribution of the sources of clinical samples for the 48 rare bacterial species identified by phenotypic identification.

Phylum	Genus	Bacteria species	Clinical samples	N° of Isolates
Actinobacteria	*Actinobaculum*	*Actinobaculum massiliense*	Joint bone infection	1
	Actinomadura	*Actinomadura cremea*	Biopsy	1
	Actinomyces	*Actinomyces europaeus*	Abscess	1
			Biopsy	3
			Joint bone infection	2
			Skin wound	4
			Surgical drainage	2
		Actinomyces radicidentis	Bloodstream	3
		Actinomyces radingae	Abscess	8
			Biopsy	2
			Bloodstream	1
			Cardiac pacemaker	2
			Skin wound	1
			Surgical drainage	6
	Arthrobacter	*Arthrobacter cumminsii*	Biopsy	1
			Bloodstream	2
			Skin wound	1
			Surgical drainage	1
	Brevibacterium	*Brevibacterium luteolum*	Skin wound	1
		Brevibacterium massiliense	Bloodstream	1
		Brevibacterium paucivorans	Bloodstream	1
		Brevibacterium ravenspurgense	Skin wound	1
	Corynebacterium	*Corynebacterium auriscanis*	Ear	1
			Skin wounds	1
			Skin wound	1
		Corynebacterium coyleae	Bloodstream	4
			Urinary tract infection	3
		Corynebacterium fastidiosum	Biopsy	2
		Corynebacterium imitans	Bloodstream	2
		Corynebacterium mucifaciens	Bloodstream	2
			Cerebrospinal fluid	3
	Microbacterium	*Microbacterium schleiferi*	Cornea	1
	Pseudoclavibacter	*Zimmermannella bifida*	Bloodstream	1
	Varibaculum	*Varibaculum cambriense*	Abscess	1
			Surgical drainage	1
Bacteroidetes	*Alistipes*	*Alistipes finegoldii*	Bloodstream	3
	Bacteroides	*Bacteroides cellulosilyticus*	Biopsy	1
			Bloodstream	2
			Surgical drainage	1
	Butyricimonas	*Butyricimonas virosa*	Joint bone infection	1
	Porphyromonas	*Porphyromonas somerae*	Biopsy	3
			Cardiac pacemaker	1
			Joint bone infection	3
			Surgical drainage	2
	Prevotella	*Candidatus Prevotella conceptionensis*	Sinusitis	3

Phylum	Genus	Bacteria species	Clinical samples	N° of Isolates
		Prevotella massiliensis	Bloodstream	1
Firmicutes	*Acidaminococcus*	*Acidaminococcus intestini*	Abscess	1
			Surgical drainage	1
	Anaerococcus	*Anaerococcus lactolyticus*	Bloodstream	1
			Surgical drainage	2
		Anaerococcus octavius	Biopsy	1
			Bloodstream	1
			Joint bone infection	2
			Pleural biopsy	1
			Sinusitis	1
			Surgical drainage	1
	Eubacterium	*Eubacterium tenue*	Bloodstream	1
			Pericardial effusion	1
		Eubacterium yurii	Sinusitis	1
	Facklamia	*Facklamia languida*	Abscess	1
	Peptoniphilus	*Peptoniphilus harei*	Abscess	8
			Aortic aneurysm	1
			Biopsy	32
			Bloodstream	10
			Bone marrow culture	1
			Joint bone infection	15
			Joint-Bone	2
			Sinusitis	2
			Skin wound	2
			Surgical drainage	22
	Robinsoniella	*Robinsoniella peoriensis*	Joint bone infection	2
			Skin wound	1
	Sporosarcina	*Sporosarcina ginsengisoli*	Bloodstream	1
	Streptococcus	*Streptococcus massiliensis*	Bloodstream	2
			Peritoneal fluid	1
			Skin wound	1
	Turicibacter	*Turicibacter sanguinis*	Bloodstream	2
			Peritoneal fluid	1
	Veillonella	*Veillonella montpellierensis*	Surgical drainage	1
Fusobacteria	*Leptotrichia*	*Leptotrichia goodfellowii*	Surgical drainage	1
		Leptotrichia trevisanii	Bloodstream	3
Proteobacteria	*Acinetobacter*	*Acinetobacter parvus*	Bloodstream	1
			Sputum	1
	Comamonas	*Comamonas kerstersii*	Bloodstream	2
	Enterobacter	*Enterobacter cowanii*	Biopsy	1
			Sinusitis	1
			Urinary tract infection	1
		Enterobacter kobei	Abscess	3
			Biliary drainage	3
			Biopsy	8
			Bloodstream	54
			Bone marrow culture	1

Phylum	Genus	Bacteria species	Clinical samples	N° of Isolates
			Bronchoalveolar lavage	11
			Oral ulcer	1
			Conjunctiva	1
			Dialysis catheter	1
			Ear newborn	1
			Human semen	1
			Intrauterine device	1
			Joint bone infection	7
			Nasal swabs	1
			Peritoneal fluid	2
			Pharynx newborn	1
			Pleural biopsy	1
			Pulmonary biopsy	1
			Skin wound	40
			Sputum	15
			Surgical drainage	10
			Tracheobronchial aspiration	18
			Umbilical cord bloodstream	1
			Urinary tract infection	89
	Ochrobactrum	*Ochrobactrum grignonense*	Bloodstream	1
	Pandoraea	*Pandoraea pulmonicola*	Bloodstream	2
			Bronchoalveolar lavage	5
			Pleural biopsy	1
			Sputum	17
			Tracheobronchial aspiration	6
	Paracoccus	*Paracoccus yeeii*	Bloodstream	2
	Pseudomonas	*Pseudomonas hibiscicola*	Biopsy	1
			Sputum	1
	Roseomonas	*Roseomonas ludipueritiae*	Bloodstream	1
	Serratia	*Serratia ureilytica*	Skin wound	1

Conclusions générales et perspectives

Notre travail a permis de conclure que la spectrométrie de masse MALDI-TOF peut être utilisée en première intention dans l'identification bactérienne avant la coloration de Gram ou d'autres techniques d'identifications phénotypiques basées sur les caractéristiques chimiques des bactéries. La performance et la rentabilité de cette technique de MALDI-TOF en routine pour l'identificaiton bactérienne dans des laboratoires cliniques, ont été confirmées par plus de 300 publications scientifiques. Beaucoup de laboratoires cliniques ont, comme nous, adopté la spectrométrie de masse MALDI-TOF à la place de toutes les techniques d'identification phénotypiques traditionnelles pour le diagnostic de routine directement sur les colonies bactériennes et/ou sur des prélèvements cliniques.

En analysant les données de plus de 3 ans de l'usage en routine la spectrométrie de masse MALDI-TOF sur notre expérience d'identification bactérienne des 11 dernières années, nous avons montré que cette technique est efficace et parfaitement adaptée pour le diagnostic courant dans le laboratoire de microbiologie clinique. Le MALDI-TOF a une capacité d'identification rapide et efficace un grand nombre d'espèces bactériennes avec un coût/efficacité très rentable. La possibilité d'augmenter la base de données par incorporation des spectres des espèces bactériennes de laboratoire précédemment identifiées par la biologie moléculaires, a amélioré la performance de MALDI-TOF dans l'identification des pathogènes humains.

Nous avons également prouvé que MALDI-TOF est un outil puissant pour identifier les espèces bactériennes rarement impliquées dans les maladies infectieuses humaines. Cette technique (MALDI-TOF) pourrait être une alternative aux méthodes moléculaires dans le laboratoire clinique.

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