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

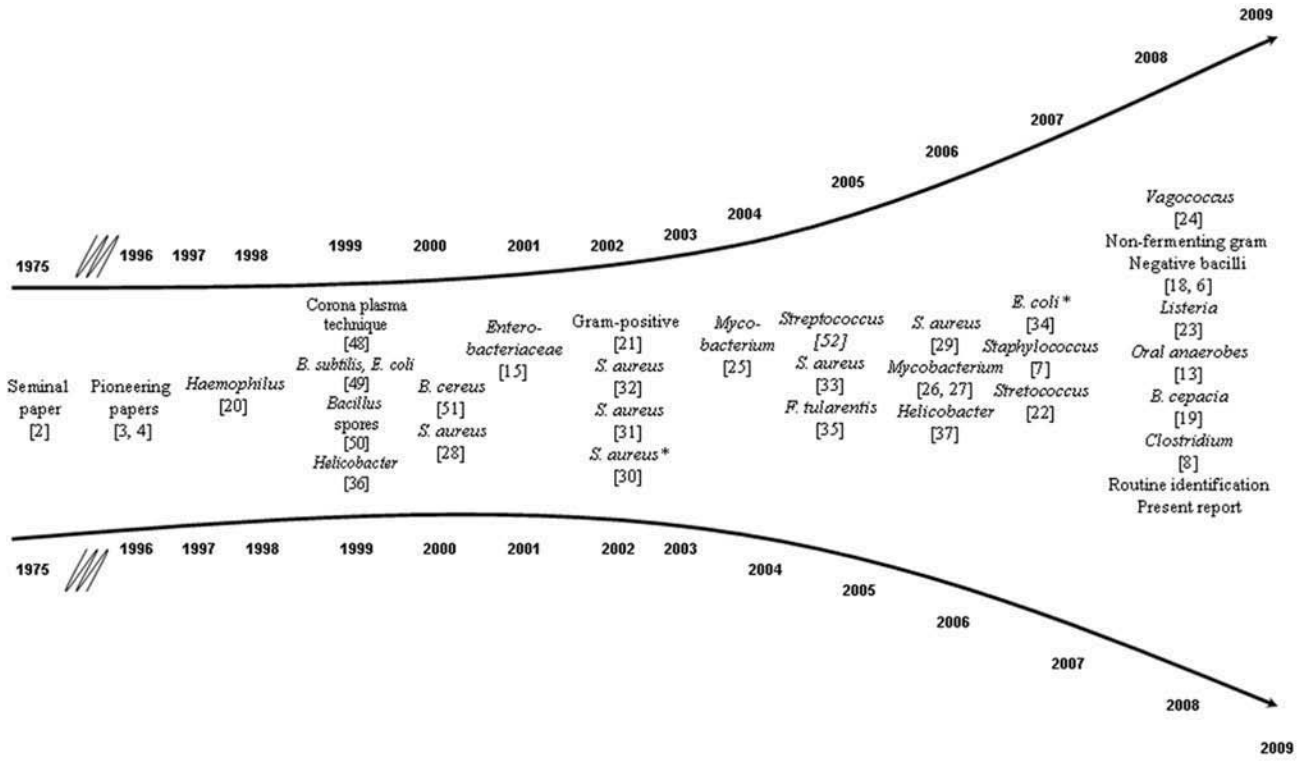

Figure 1. Increasing number of publications related to matrix-assisted laser desorption ionization time-of-flight mass spectrometry applications in medical microbiology. Applications include the identification of isolates, the identification of specific antibiotic-resistance profile, and typing of isolates.

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

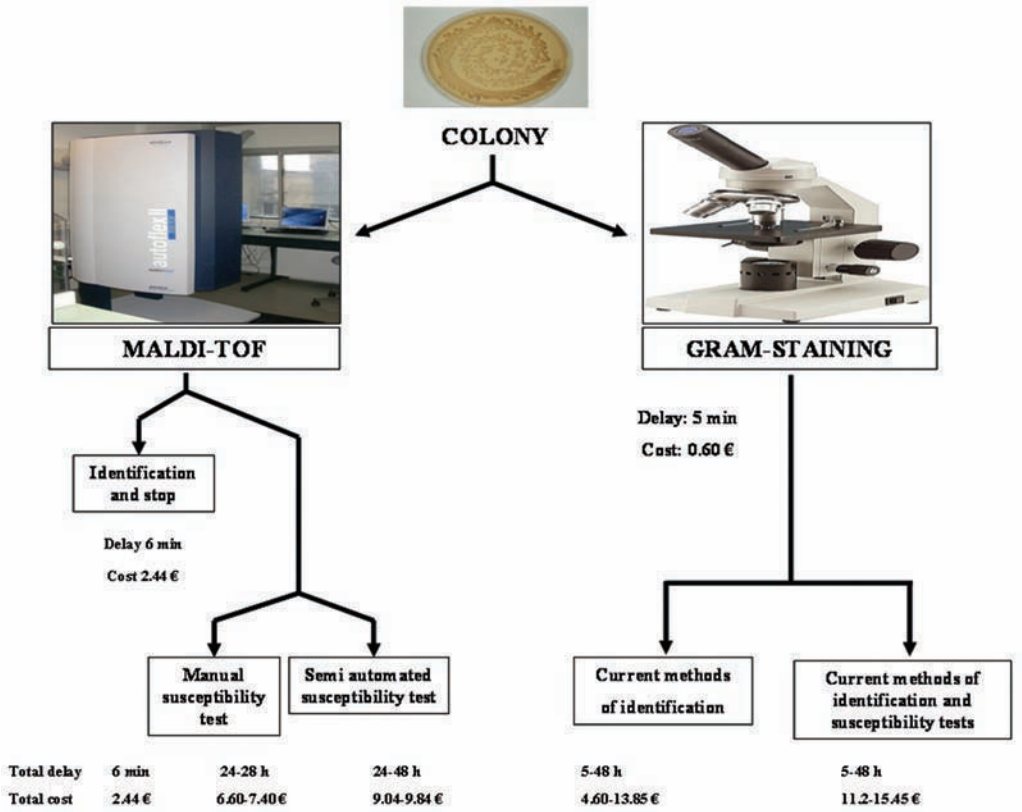

Figure 2. Work flow and delay for matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry identification of bacteria in this study.

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 ⩾5 reference spectra to ⩾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 ⩽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 ⩽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 ⩾10 reference spectra per bacterial species and an identification score ⩽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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