Review

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Mass spectrometry: a revolution in clinical microbiology?

Abstract

Recently, different bacteriological laboratory interventions that decrease reporting time have been developed. These promising new broad-based techniques have merit, based on their ability to identify rapidly many bacteria, organisms difficult to grow or newly emerging strains, as well as their capacity to track disease transmission. The benefit of rapid reporting of identification and/or resistance of bacteria can greatly impact patient outcomes, with an improvement in the use of antibiotics, in the reduction of the emergence of multidrug resistant bacteria and in mortality rates. Different techniques revolve around mass spectrometry (MS) technology: matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), PCR combined with electrospray ionization-mass spectrometry (PCR/ESI-MS), iPLEX MassArray system and other new evolutions combining different techniques. This report emphasizes the (r)evolution of these technologies in clinical microbiology.

Keywords: clinical microbiology; iPLEX MassArray; MALDI-TOF MS; mass spectrometry; PCR/ESI-MS.

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Introduction

Despite the extensive use of antibiotics and vaccination programs, infectious diseases, particularly bacterial infections, remain a major cause of morbidity and mortality worldwide. One of the great challenges of microbiology for the coming years remains the development of new antimicrobial agents. Indeed, because of the massive and often untimely use of antibiotics, pathogenic bacteria have developed resistance mechanisms against most classes of antibiotics currently available. This trend has been particularly dramatic over the past 20 years. The possible transmission of genes encoding mechanisms of resistance between different bacterial species has led to the emergence, particularly in the hospital, of multidrug and pain resistant bacteria that led to increasing difficulties in therapeutic management. This major public health problem also faces a challenging reality: the virtual withdrawal of the pharmaceutical industry from the development of new antibiotics. No blockbuster drug is being promoted [1, 2]. Given this reality, the development of new therapeutic strategies should be considered. One strategy is the best use of antibiotics. In this way the recent (r)evolution in clinical microbiology approach could help to improve this problem. Different solutions have increased: first the molecular diagnostic methods (e.g., 16S ribosomal RNA sequencing, real-time PCR for detection of selected genes) which is classically used in parallel with routine bacteriological methods; and second mass spectrometry (MS) which is propelling us into a new era far beyond the classical bacteriology of Louis Pasteur. These promising new broad-based techniques have merit, since they can rapidly identify many bacteria, including organisms that are difficult to culture or new emerging strains. They also can be used as epidemiological tools to follow disease transmission. The benefit of rapid reporting of isolation and/or identification of resistance of bacteria can potentially impact patient outcome, improve the use of antibiotics and

reduce the emergence of multidrug resistant bacteria and mortality rates.

The aim of this review is to describe the different solutions using MS recently developed and to evaluate their impact on rapidity of diagnosis and on the prognosis of infectious diseases.

The MALDI-TOF MS technology

Principle (Figure 1)

The first description concerning the use of MS in bacteriology was in 1975 [3]. The technology was developed to study the biomarker profiles of some bacterial species. It used the ionization by fast atom bombardment and the association of gas chromatography and MS. The difficulty was to detect the release of the ribosomal and membrane proteins without destroying them in order to analyze the protein profiles and obtain mass pattern spectra. This encouraging study was not followed by any other development until 1996. At this time, the first matrix-assisted laser desorption/ionization time-of-flight MS (MALDI-TOF MS) experiment was successful in identifying bacteria directly from whole colonies based on protein content [4, 5]. However, the most important evolution corresponded to the system of detection by soft ionization techniques such as MALDI and electrospray ionization (ESI) allowing the analysis of biomolecules and large organic molecules, which tend to be fragile when ionized by the old other conventional ionization methods [6]. The continual development of the hardware provided increasing accuracy and resolution of the different proteins, and the MALDI-TOF MS was being used for the identification of bacteria in research settings [4]. Following this period, the new approaches for species identification were developed involving the use of a different matrix. The change of matrix allowed the ionization of mainly ribosomal proteins, which are more conserved than surface proteins [7]. This was considered to be more reliable for routine identification of bacterial species, as culture conditions seemed to have little effect on the results of identification [8]. Therefore, the MS became a revolutionary tool for bacteriology laboratories.

Matrix-assisted laser desorption/ionization involves the principle of the co-crystallization of the sample with a matrix. The couple sample-matrix is irradiated with photons of a laser whose wavelength is in the absorption band of the matrix. This radiation causes the ionization in the gas phase of molecules of the sample and matrix (Figure 1). The ions formed are then accelerated and sent in a vacuum flight tube where they are separated according to their speed. This speed depends itself of a mass/charge ratio peak (m/z). Ions characterized by a high m/z fly more slowly than those with lower m/z. The approach of protein identification is based on the accurate mass measurement of a group of peptides derived from a protein by sequence-specific proteolysis. Proteins of different amino acid sequence produce a series of peptides masses, which can be detected by the detector. The spectrum of identified peptide masses is unique for a protein specific to a bacterial species. The peptide profiles are generated from direct ionization of an intact colony or a bacterial protein extract after manual extraction. The spectrum obtained is then compared with spectra contained in the database according to the algorithm-specific software used. Identification occurs after a protein's spectral signature is correlated to a database of spectra collected from reference strains. The results are returned with a scoring system, which appears to be conservative enough to avoid false-positive identifications with both systems [9, 10]. Different systems (software/database) have been created for routine identification of bacteria: the Bruker instrument provides its own solution, MALDI BioTyper (software, bioinformatic and database); the Shimadzu instrument uses also its own software (Launchpad) and the SARAMIS database developed by AnagnosTec GmbH and recently acquired by BioMérieux; Andromas (a French start-up) provides a different type of database and software for routine bacteriology, compatible with either Bruker or Shimadzu hardware. These databases currently available for both systems need to be optimized for certain species but are large and contain up to 2000 species (including bacteria, yeast and mycobacteria), with over 3000 spectra. Their performance is in any case higher than phenotypic identification systems [11–13]. We could note that no statistically significant difference was identified between the platforms for clinically relevant bacteria [11, 14].

As we previously noted, MS needs the use of a matrix to facilitate the ionization of proteins. They allow a burst of microorganisms and the release of proteins that migrate performing a true chromatography. Depending on the matrix, we obtain a spectrum of proteins of specific molecular weight ranges. Alpha-4-cyano-4-hydroxycinnamic acid (HCCA) induces the formation of small spherical crystals with more uniform distribution. It is not suitable for some taxons and requires fewer laser shots and allows the production of 80 to 150 peaks per spectrum [15]. The UV absorbing matrices used were found to be highly specific to bacterial Gram type: HCCA for Gram-negative bacteria and

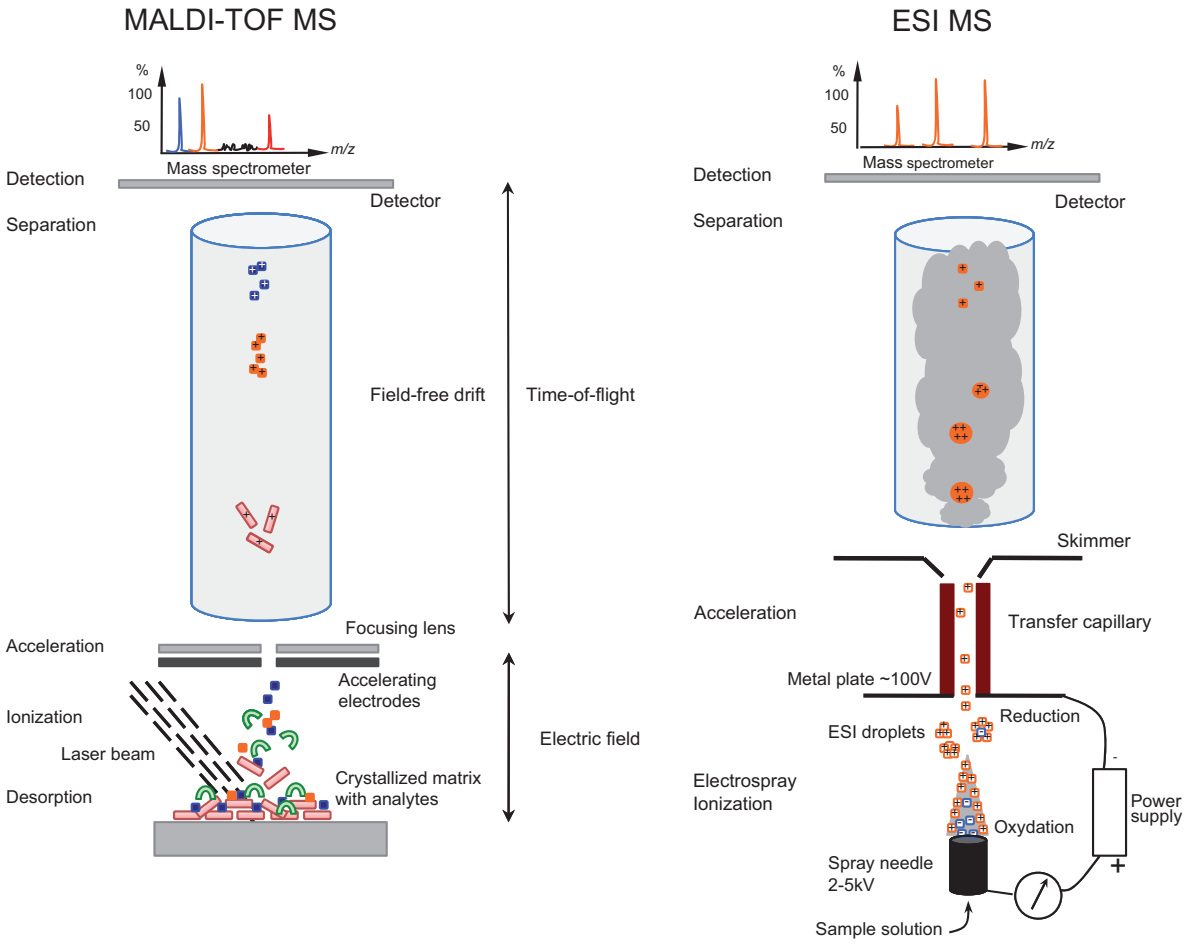

Figure 1 Principle of MALDI-TOF MS and ESI-MS identification of bacteria.
For MALDI-TOF, laser impact causes thermal desorption of ribosomal proteins of bacteria embedded in matrix material and applied to the target plate (analytes shown as red, light blue, and orange spheres, the matrix is given as green spheres). In an electric field, ions are accelerated according to their mass and electric charge. The drift path allows further separation and leads to measurable differences in time-of-flight of the desorbed particles that are detected on top of the vacuum tube. From the time-of-flight, the exact mass of the polypeptides can be calculated. For ESI, the DNA amplicons are dissolved in a solvent and injected in a conductive capillary, where high voltage is applied, resulting in the emission of aerosols of charged droplets of the sample. The latter are sprayed through compartments with diminishing pressure, resulting in the formation of gas-phase multiple-charged analyte ions, which then are detected by spectrometer.

5-chloro-2-mercaptobenzothiazole for Gram-positive bacteria [16]. The latter matrix system enhances the sensitivity of the analysis of bacterial endotoxins (lipid A) by more than 100-fold and provides tolerance to high concentrations of reagents (such as sodium dodecyl sulphate, sodium chloride and calcium chloride) [17]. The 2,5-dihydroxybenzoic acid (DHB) allows the formation of long crystals from the periphery to the center of the deposit. It is suitable for a majority of taxons and requires more laser shots to obtain 100 to 200 peaks per spectrum and many signals whose the m/z is >10 kDa [18]. The manipulation of this matrix is trickier. Finally 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid or SA) is a more recent matrix. It allows the analysis of proteins of higher molecular weight than the HCCA and DHB [19].

Advantages of MALDI-TOF MS (Table 1)

The MALDI-TOF MS is the most promising technology for the present and the future in the microbiology laboratories. This is due to its ability to analyze whole bacterial cells with virtually no sample preparation or no batching and the improvement in the identification time of a positive culture (10–20 s for acquisition of the protein spectra and 15–30 s for the comparison in the databank), starting from a colony (Figure 2).

	MALDI-TOF	PCRESI/MS	iPLEX Mass array	SELDI TOF	LC-ESI-QqQ-MS
Interest	Excellent specificity High throughput: detection of multiple spots[20–67] in one experiment Bacterial detection from urine sample Adapted to bacteriology lab workflow	Excellent sensitivity and specificity Powerful tool for epidemiological investigation	Excellent sensitivity and specificity High throughput: analysis of 96 or 384 samples on a same chip Powerful tool for phylogenetic and epidemiological investigation	High throughput Minimal sample volume required Directly from crude biological samples Peptide sensitivity in the femtomolar range	Excellent specificity (higher than MALDI)
Limits	Requires often culture Limited to bacterial genera and species, more difficult for sub-species Quantification detection difficult	Batching of six samples at a time Need to DNA extraction (which increases the costs) Need a validation	Cost of the equipment	Not link to MS (no peptide identification) Low mass resolution Low mass accuracy	Research tool No bacterial application Not adapted for lab workflow Cost of the equipment
Complexity of data analysis	Very easy	Moderate	High	High	High
Time to result	1–2 min after culture (18 h)	4–6 h without culture	Few seconds after PCR (4 h)	Min to h	Min to h
Molecules detected	Proteins, glycopeptides, oligonucleotides, carbohydrates	PCR amplicons	PCR amplicons	Native proteins, peptides	Molecules, peptides
Mono/polymicrobial detection	Monomicrobial	3–4 bacteria	Polymicrobial	Polymicrobial	Polymicrobial
Detection of new organism	Possible but limited	Yes and also virus	Yes and families of virus	Yes	Yes
Running costs	Low	High	High	High	Low

Table 1 Comparison between the different technologies using mass spectrometry in bacteriology.

MALDI-TOF MS is also widely used because of its high accuracy, low running cost and low maintenance needs. Indeed this technology requires only the medium to grow the organism and a small quantity of matrix. It has already replaced most of the biochemical tests currently used for bacterial identification in routine laboratories (e.g., catalase, oxydase, identification card or API gallery, latex test, agglutination tests) because it does not require prior knowledge about the organism [68]. This new method has now proven to be reliable and safe for the identification of the clinically relevant bacteria (e.g., *Enterobacteriacae*, the non-fermenting bacteria, staphylococci or streptococci) [12, 69–72]. Continuous improvement of the database is performed and updates are released every 3–6 months. In this way, the identification of most rarely isolated bacteria such as some potential bioterrorism agents (*Brucella* sp. [73], *Coxiella burnetii*, *Francisella tularensis* and *Bacillus anthracis* [74]), some Gram-negative bacilli (*Pasteurellaceae* [75], *Acinetobacter baumannii* group [76, 77], *Yersinia* sp. [78], *Legionella* sp. [79]), anaerobes [80–82], or bacteria difficult to identify after Gram staining (*Leptospira* sp. [83], *Mycobacterium* sp. [84, 85]) have been reported.

All these developments are promising notably since the publication by Gaillot et al. on cost-effectiveness of MS. The authors reported that phasing out of conventional techniques in favor of MS resulted in the overall saving of $177,090 in 1 year [86].

Inconveniences of MALDI-TOF MS (Table 1)

The speedy identification of bacteria by MS clearly presents a major advantage for clinicians in the management of antibiotic treatment. However, even if the protein mass pattern spectra can be analyzed for identification of bacteria to the genus and species level, many results are rarely available to the subspecies level. This means that this tool is not completely efficient to identify all bacteria (e.g., difficulty in distinguishing between *Escherichia coli* and *Shigella* sp.) and does not provide help in epidemiological studies to follow crossed transmission of bacteria. Moreover, the major problem is the incomplete current databases. The composition and quality of these databases is crucial for a correct identification. They still need implementation and expansion [68].

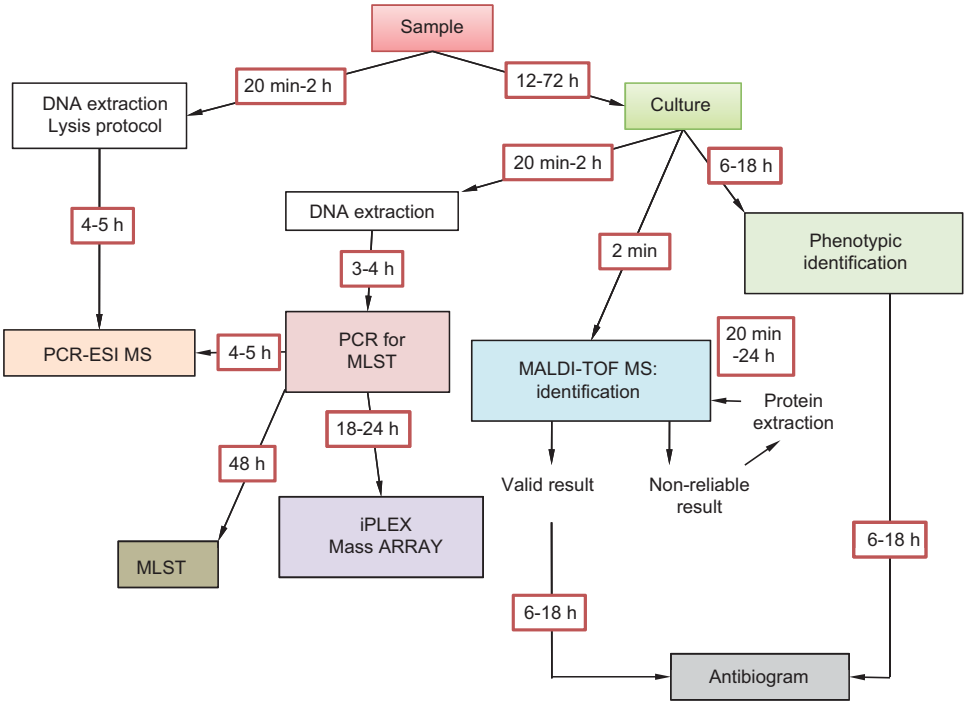

Figure 2 Typical workflow of new and old methods used in a clinical microbiology laboratory. The time to identification, typing and resistance analysis is noted.
MALDI-RE, matrix-assisted laser desorption ionization resequencing; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; MLST, multilocus sequence typing; PCR-ESI MS, electrospray ionization mass spectrometry.

Adjustment of the default MALDI-TOF MS database allowed the identification of all members of the *A. baumannii* group as well as other *Acinetobacter* spp. with similar accuracy, as was reported by Espinal et al. [76].

A large number of bacterial cells are required for identification. Usually a whole intact colony is used for analysis, limiting the ability to rapidly identify microorganisms directly from biological fluids where the bacterial count is expected to be relatively low. Research is currently being performed to mitigate some of these requirements.

Other problems could also be noted: 1) the use of MS is challenged by the high costs of the instruments and by the long period of maintenance (up to 2 days) requiring another solution during this period; 2) this technology must be completely adapted to the new constraints of diagnostic laboratories, in particular, the traceability of all the tests; 3) Anderson et al. has demonstrated the effects of some selective solid-medium type on the rate of identification of bacterial isolates by MS [87]. For example, *Staphylococcus* spp. from colistin-nalidixic acid agar medium exhibit low identification rates whereas the same bacteria from blood medium were perfectly identified. In addition to that, it has also been found that protein extraction enhances identification rates and is recommended for colonies grown on different media. However, this extraction increases the time of the experiment; 4) there is also an important need to obtain isolated bacterial colonies to avoid the growth of microorganisms from potentially contaminated material because the technique's ability to resolve mixtures is lacking; and 5) the uncultured bacteria detected by this technology are not mandatory pathogens and must be evaluated with the clinical signs.

It is probable that all these minor inconveniences could be corrected in the future developments. However, one of the most important objectives support improved antibiotic prescription. In fact, this is one of the missing features of this technology and bacterial cultures are still required for antimicrobial susceptibility testing. Therefore, accurate measures and identification of resistance factors could represent the future evolution of this technology.

New developments of the MS

A great number of developments have been made recently to improve the detection limit based on genus and species identification. The MS is now used not only to detect endogenous peptide/proteins to identify bacteria but in the new evolutions, MS enables the improvement of immunological/PCR detection methods. Moreover, the protein biomarkers that are measured in MS of microorganisms are highly expressed proteins responsible for housekeeping functions, such as ribosomal, chaperone, and transcription/translation factor proteins [88–92]. Based on this detection, new markers have been found and specific databases are being developed for the identification of specific resistance or virulence factors with MALDI-TOF MS technology.

Detection of resistance

Recently some resistance markers to one or more antimicrobial agents have been detected by MALDI-TOF MS. Reports suggest that MS has the ability to differentiate methicillin-susceptible *S. aureus* from methicillin-resistant *S. aureus* (MRSA) strains [93, 94], and also detect carbapenem resistance activity based on the detection of degradation of β-lactam antimicrobials [20, 21, 95]. Basically, the MS follows the enzymatic hydrolysis of the antimicrobial agent. The sensitivity and specificity of this approach is high (97% and 98%, respectively) [21] and the results are available in <3 h [95]. However, this approach must be validated in routine laboratories to consider replacing the conventional techniques (such as cefoxitin disks, or PCR to detect the *mecA* gene). We can speculate that the time frame of MS can be further shortened as suggested by Hooff et al. [20].

Even if the detection of subtle protein alterations will probably be difficult to assess by MALDI-TOF MS, recently, some reports have demonstrated it is possible such as the detection of *rpoB* mutations in *Brucella* sp. [22]. There have also been reports of the detection of bacterial enzymes targeting antibiotics, such as β-lactamases or carbapenemases in *E. coli* and *A. baumannii* [21, 23–25, 95], the CfiA carbapenemase in *Bacteroides fragilis* [26]. Other resistance mechanisms recently reported to be detected by this technology including porin defects and expression of efflux pumps [27].

The ability to use MS technology to rapidly detect the resistance mechanisms produced by a bacterial pathogen will be a key element that will revolutionize clinical microbiology.

Detection of virulence factors

Differences in virulence profiles for bacterial isolates can be based on the selective determination of the presence or absence of *m/z* peaks in the MALDI-TOF MS instrument.

This approach allows not only the identification of the bacterial species, but also to show the presence of some key surface-associated molecules or some well-known virulence factors involving a rapid management of the infection. In this way, the MALDI-TOF MS technology could detect a *m/z* peak specific to Panton-Valentine leukocidin-producing *S. aureus* strains, a well-known virulence factor in the development of acute severe *S. aureus* infection [28]. However, a recent publication calls into question the previous work. Szabados et al. claim that protein peaks of 4448 and 5302 Da are not associated with the presence of Panton-Valentine leukocidin [29].

Some efforts have been made to find biomarkers to differentiate between infectious and non-infectious causes of systemic inflammatory response syndrome (SIRS). Discriminatory peaks have been detected suggesting a direct link between infectious-related protease activity and a sepsis-specific diagnostic pattern for discrimination of patients with SIRS [30]. An interesting example is represented by the detection of the staphylococcal delta-toxin which was found associated with the acute infection [31].

Detection and identification of quorum sensing signals, immune-modulatory proteins and the binding of host factors including antibodies are targets for future research in this field.

Detection directly from samples

Molecular methods have revolutionized clinical microbiology. Indeed a growing range of rapid diagnostic tests that can be performed at the point-of-care has been implemented such as real-time PCR assays [32]. These tools considered to be expensive and time-consuming have clearly evolved and represented a suitable evolution for routine identification. For example, different tests (e.g., GeneXpert™ system or BD GeneOhm™ StaphSR) have been developed for rapid management of contagious diseases such as *Clostridium difficile*, *Bordetella pertussis* or *Neisseria meningitidis* (33, 34). These tests also allow MRSA to be detected from different sample types (e.g., blood, skin and soft tissue, nasal swabs) and to prevent unjustified prescriptions (e.g., detection of Enterovirus or *Streptococcus agalactiae* in pregnant women [35, 36]).

In this way, the use of MALDI-TOF MS for microorganisms' identification in clinical samples has become essential in the future development of this technology. Some reports have been published recently to detect microorganisms directly from blood or urine samples [37–47]. Methods (combining centrifugation steps, the use of serum separator tubes or ammonium chloride lysis) for the processing of positive blood culture samples have been proposed to increase the sensibility of the technique but especially in the case of direct testing of other materials (e.g., urine specimens), consensus still has not been reached [37–47]. The analytical sensitivity in blood culture varied between 66% and 76% with a major precision in the identification of Gram-negative bacteria (around 90%) compared to Gram-positive bacteria (<50%). It is of note that current MALDI-TOF MS data software analysis is not able to reliably identify all microorganisms present in mixed cultures. Direct identification of pathogens in urine samples has also been evaluated. The results are not yet satisfactory, the most promising result was obtained for urine containing more than 100,000 CFU/mL [44] and other developments still seem necessary. Different protocols have been used (e.g., concentration step, membrane filtration and magnetic separation) to improve the sensitivity of MS [43, 48]. The use of automation of urine analysis (e.g., urines flow cytometry) in the laboratory in order to eliminate negative samples might render downstream use of MALDI-TOF MS more efficient.

All these evolutions are attractive for the future. Vlek et al. have demonstrated that the direct performance of MALDI-TOF MS on positive blood culture broths reduced the time until species identification by 28.8 h and was associated with an increased proportion of patients receiving an adequate antibiotic treatment within 24 h [49].

PCR/ESI-MS (Table 1)

Other MS solutions have been recently developed to increase the interest of MS technology. The PLEX-ID system (Abbott™) is a nearly fully automated system that associates broad-spectrum PCR (targeting ribosomal and housekeeping protein genes) with electrospray ionization-MS (ESI-MS) (Figure 1) [10]. It delivers broad microbial screening to semiquantitatively identify all organisms present in a sample. It is capable of running multiple human identification targets such as mitochondrial DNA, short-tandem repeat (STR) or single-nucleotide polymorphism (SNP). The principle is to measure the *m/z* of amplicons, generated by multiplex PCRs that target several loci within bacterial or fungal genomes. The method targets both conserved and species-specific genetic regions to identify microbes based on amplicon base compositions relative to a known database of microorganisms. To date this tool has demonstrated its ability to directly detect and identify bacteria [50] and associated antibiotic resistance genes, such as drug-resistance *M. tuberculosis*, carbapenemase-producing *A. baumannii* or *K. pneumoniae* and

quinolone resistance in *A. baumannii* from isolates [51–55] (Figure 2). Indeed specific primers may be added to the assay to screen the *mecA* gene for methicillin resistance, the *vanA* and *vanB* genes for vancomycin resistance in enterococci, and the bla_{KPC} gene for resistance to carbapenems [56–58]. Most recently, the method produced highly accurate results when used to identify bacterial and yeast pathogens directly from the clinical specimen (blood culture) [59], in particular, to detect *Erlichia* isolates [60].

It is important to highlight the technology also offers extended utility for epidemiological surveillance and infection control [51, 52, 54]. It provides quick results (<6 h) and can identify mixtures of up to three to four microorganisms but requires the batching of six samples at a time [10].

In contrast, the first and most limiting step of this technique is DNA extraction from clinical samples. This induces an increase of the costs (2 to 3 times compared to MALDI-TOF) due to the cost of consumables, software package and the need to use DNA extraction reagents (buffers, enzymes, and primers) for PCR.

Although this technology needs to be validated more extensively, there are some recent publications on the detection of *Aspergillus terreus* from bronchio-alveolar lavage [61] and a panel of respiratory viruses from nasopharyngeal aspiration that represents an important target for the future in clinical microbiology [62].

iPLEX MassARRAY® system (Table 1)

The MassARRAY iPLEX single-nucleotide polymorphism (SNP) typing platform uses and the MS technology coupled with single-base extension PCR to analyze amplicons of PCR for rapid and accurate molecular identification of microorganisms [63]. This system is commercialized by Sequenom™ (San Diego, CA, USA).

The assay consists of an initial locus-specific PCR reaction, followed by single base extension using mass-modified dideoxynucleotide terminators of an oligonucleotide primer which anneals immediately upstream of the polymorphic site of interest. Using MALDI-TOF MS, the different mass of the extended primer identifies the SNP allele. The starting point of the protocol is the amplification of a target region of interest. T7- and SP6- promoter tagged primers are used to amplify the template. After treatment, in vitro transcription provides RNA transcripts which are base-specifically cleaved. The resulting RNA cleavage products are analyzed by MALDI-TOF MS.

The spectra are compared with the simulated spectra of the reference sequences as published for MLST. Due to the distinct mass of each nucleotide base, the results are as good as those of conventional dideoxy sequencing [64].

This technology has two main applications in clinical microbiology (Figure 2): the comparative sequence analysis, and the SNP genotyping. These two approaches provide a powerful tool in phylogenetic investigation, epidemiology (molecular typing) and surveillance of crossed transmission of bacteria [63, 65, 84]. An interesting example using the MassARRAY technology is the study performed by Syrmis et al. [63] related with the genotyping of MRSA.

MassARRAY iPLEX is more efficient than a sequencing method; however, the analysis by MALDI-TOF MS is much faster than the analysis by capillary electrophoresis, requiring a few seconds for the former one and up to several minutes for the latter one [85]. The iPLEX assay is suitable for high-throughput analysis, as either 96 or 384 samples can be analyzed on the same chip. The major drawback of this technology lies in the requirement for specific equipment and the cost of this equipment.

Other technologies

Other systems for future applications could be developed. One solution includes the surface enhanced laser desorption/ionization time-of-flight (SELDI-TOF) MS system (Table 1). This technology is a specific MALDI-TOF application that combines a chip-based chromatographic enrichment of proteins with TOF-MS [66]. The combination of SELDI (to generate protein profiles and identify significant peaks from large sample sets) and MALDI (to obtain sequence identity of significant peaks) can be extremely powerful for the rapid identification and validation of biomarkers. The SELDI technology incorporates sample prefractionation and binding to the active surface of a 'ProteinChip' array providing more information about the protein of interest than just size, with inferences based on the surface chemistry of the 'chip' (e.g., hydrophobic, reverse-phase, cation-exchange). This design feature of SELDI markedly decreases the complexity of protein-rich fluids such as serum and permits quantitative comparisons of peak intensities between samples using large sample sets [67, 96]. SELDI platforms are specifically designed for the rapid high-throughput comparative analysis of multiple biological samples, increasing the chance of finding proteins with consistently altered expression during disease development, progression or following treatment [96]. The technology allows assessment of the performance of individual biomarkers and to evaluate combinations of biomarkers with potential diagnostic.

The two main limitations of SELDI are its relative imprecision in its assignment of molecular mass to any given peak [67] and its high cost. Very few studies are available concerning bacterial detection increasing the detection limit to the subspecies [66, 97].

A second solution is the liquid chromatography coupled to electrospray ionization triple quadrupole (LC-ESI-QqQ) MS, LC coupled to ESI-Q-TOF (LC-ESI-Q-TOF MS) or MALDI triple quadrupole coupled to MALDI-TOF (Table 1). The LC-ESI-QqQ in selected or multiple reactions way has been used for routine detection of small molecules including metabolites and drugs [31]. More recently, the LC-ESI-QqQ has been suggested as a replacement for classical ELISAs for the quantitation of proteins in complex matrices [98]. The MALDI triple quadruple measures enzyme-mediated, time-dependent hydrolysis of the β-lactam ring structure of penicillin G and ampicillin and inhibition of hydrolysis by clavulanic acid for clavulanic acid susceptible β-lactamases. This assay represents the basis for future investigations of β-lactamase activity in various bacterial strains [20]. These MS technologies have already been used in research settings extensively and it will be just a brief time before this technology can be introduced in the routine clinical microbiology laboratories. Noteworthy, the most popular routine diagnostic procedure to date was developed two decades ago to facilitate the analysis of solid next to the customary volatile compounds [99]. The technology must be used on liquid and could detect proteins especially peptides. It is completely adapted to quantification of proteins, but, until now, no development has been made in clinical microbiology.

The future: fad or real revolution?

As pointed out in the present review and in previously published papers, MS has clearly revolutionized bacteriological diagnostics. Indeed, currently the delay between the collection of the specimen and the result of the bacterial culture is a great hindrance to the clinician (Figure 2) [10]. For better use of antibiotics and control of the antimicrobial resistance, a rapid identification of the involved pathogens is of the greatest importance for effective patient management. Indeed, it can reduce the empirical use of broad-spectrum antibiotic therapy to a more narrow specific treatment. However, even if it is promising, MS technology still has limitations which are being overcome. When they are finally resolved, we can definitely speak of a 'revolution'. MS will become a tool for the detection of microbial subtyping, antimicrobial susceptibility testing and virulence factors directly in the samples to guide the clinician in his choice of treatment. Indeed, in parallel, molecular biology seems to be more efficient especially at the point-of-care organization and the syndrome panel solutions.

In conclusion, MS is the future of microbiology: helping clinicians with accurate identification of microorganisms will contribute to timely decision-making for most infectious diseases, resulting in the optimal use of antibiotics, the decrease of multidrug resistant bacteria, the reduction of length and costs of hospitalization.

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References

1. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 2009;48:1–12.
2. Livermore DM. Bacterial resistance: origins, epidemiology, and impact. Clin Infect Dis 2003;36:S11–23.
3. Anhalt JP, Fenselau C. Identification of bacteria using mass-spectrometry. Anal Chem 1975;47:219–25.
4. Claydon MA, Davey SN, Edwards-Jones V, Gordon DB. The rapid identification of intact microorganisms using mass spectrometry. Nat Biotechnol 1996;14:1584–6.
5. Holland RD, Wilkes JG, Rafli F, Sutherland JB, Persons CC, Voorhees KJ, et al. Rapid identification of intact whole bacteria based on spectral patterns using matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 1996;10:1227–32.

6. Sauer S, Kliem M. Mass spectrometry tools for the classification and identification of bacteria. Nat Rev Microbiol 2010;8:74–82.
7. Suh MJ, Limbach PA. Investigation of methods suitable for the matrix assisted laser desorption/ionization mass spectrometric analysis of proteins from ribonucleoprotein complexes. Eur J Mass Spectrom 2004;10:89–99.
8. van Veen SQ, Claas EC, Kuijper EJ. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. J Clin Microbiol 2010;48:900–7.
9. Wolk DM, Dunne WM Jr. New technologies in clinical microbiology. J Clin Microbiol 2011;49:S62–7.
10. Emonet S, Shah HN, Cherkaoui A, Schrenzel J. Application and use of various mass spectrometry methods in clinical microbiology. Clin Microbiol Infect 2010;16:1604–13.
11. Cherkaoui A, Hibbs J, Emonet S, Tangomo M, Girard M, Francois P, et al. Comparison of two matrix-assisted laser desorption ionization-time of flight mass spectrometry methods with conventional phenotypic identification for routine identification of bacteria to the species level. J Clin Microbiol 2010;48:1169–75.
12. Benagli C, Rossi V, Dolina M, Tonolla M, Petrini O. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for the identification of clinically relevant bacteria. PLoS One 2011;6:e16424.
13. Saffert RT, Cunningham SA, Ihde SM, Jobe KE, Mandrekar J, Patel R. Comparison of Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometer to BD Phoenix automated microbiology system for identification of gram-negative bacilli. J Clin Microbiol 2011;49:887–92.
14. Veloo AC, Knoester M, Degener JE, Kuijper EJ. Comparison of two matrix-assisted laser desorption ionisation-time of flight mass spectrometry methods for the identification of clinically relevant anaerobic bacteria. Clin Microbiol Infect 2011;17:1501–6.
15. Zhu X, Papayannopoulos IA. Improvement in the detection of low concentration protein digests on a MALDI TOF/TOF workstation by reducing alpha-cyano-4-hydroxycinnamic acid adduct ions. J Biomol Tech 2003;14:298–307.
16. Evason DJ, Claydon MA, Gordon DB. Exploring the limits of bacterial identification by intact cell-mass spectrometry. J Am Soc Mass Spectrom 2001;12:49–54.
17. Zhou P, Altman E, Perry MB, Li J. Study of matrix additives for sensitive analysis of lipid A by matrix-assisted laser desorption ionization mass spectrometry. Appl Environ Microbiol 2010;76:3437–43.
18. Ishida Y, Madonna AJ, Rees JC, Meetani MA, Voorhees KJ. Rapid analysis of intact phospholipids from whole bacterial cells by matrix-assisted laser desorption/ionization mass spectrometry combined with on-probe sample pretreatment. Rapid Commun Mass Spectrom 2002;16:1877–82.
19. Fagerquist CK, Garbus BR, Williams KE, Bates AH, Harden LA. Covalent attachment and dissociative loss of sinapinic acid to/from cysteine-containing proteins from bacterial cell lysates analyzed by MALDI-TOF-TOF mass spectrometry. J Am Soc Mass Spectrom 2010;21:819–32.
20. Hooff GP, van Kampen JJ, Meesters RJ, van Belkum A, Goessens WH, Luider TM. Characterization of β-lactamase enzyme activity in bacterial lysates using MALDI-mass spectrometry. J Proteome Res 2012;11:79–84.
21. Hrabák J, Walková R, Studentová V, Chudácková E, Bergerová T. Carbapenemase activity detection by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2011;49:3222–7.
22. Sandalakis V, Psaroulaki A, De Bock PJ, Christidou A, Gevaert K, Tsiotis G, et al. Investigation of rifampicin resistance mechanisms in Brucella abortus using MS-driven comparative proteomics. J Proteome Res 2012;11:2374–85.
23. Kempf M, Bakour S, Flaudrops C, Berrazeg M, Brunel JM, Drissi M, et al. Rapid detection of carbapenem resistance in Acinetobacter baumannii using matrix-assisted laser desorption ionization-time of flight mass spectrometry. PLoS One 2012;7:e31676.
24. Sparbier K, Schubert S, Weller U, Boogen C, Kostrzewa M. Matrix-assisted laser desorption ionization-time of flight mass spectrometry-based functional assay for rapid detection of resistance against β-lactam antibiotics. J Clin Microbiol 2012;50:927–37.
25. Camara JE, Hays FA. Discrimination between wild-type and ampicillin-resistant Escherichia coli by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Anal Bioanal Chem 2007;389:1633–8.
26. Wybo I, De Bel A, Soetens O, Echahidi F, Vandoorslaer K, Van Cauwenbergh M, et al. Differentiation of cfiA-negative and cfiA-positive Bacteroides fragilis isolates by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2011;49:1961–4.
27. Cai JC, Hu YY, Zhang R, Zhou HW, Chen GX. Detection of OmpK36 porin loss in Klebsiella spp. by Matrix-assisted laser desorption/ionization-time of flight mass spectrometry. J Clin Microbiol 2012;50:2179–82.
28. Bittar F, Ouchenane Z, Smati F, Raoult D, Rolain JM. MALDI-TOF-MS for rapid detection of staphylococcal Panton-Valentine leukocidin. Int J Antimicrob Agents 2009;34:467–70.
29. Szabados F, Becker K, von Eiff C, Kaase M, Gatermann S. The matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS)-based protein peaks of 4448 and 5302 Da are not associated with the presence of Panton-Valentine leukocidin. Int J Med Microbiol 2011;301:58–63.
30. Kiehntopf M, Schmerler D, Brunkhorst FM, Winkler R, Ludewig K, Osterloh D, et al. Mass spectrometry-based protein patterns in the diagnosis of sepsis/systemic inflammatory response syndrome. Shock 2011;36:560–9.
31. van Belkum A, Welker M, Erhard M, Chatellier S. Biomedical mass spectrometry in today's and tomorrow's clinical microbiology laboratory. J Clin Microbiol 2012;50:1513–7.
32. Cohen-Bacrie S, Ninove L, Nougairède A, Charrel R, Richet H, Minodier P, et al. Revolutionizing clinical microbiology laboratory organization in hospitals with in situ point-of-care. PLoS One 2011;6:e22403.
33. Best EL, Fawley WN, Parnell P, Wilcox MH. The potential for airborne dispersal of Clostridium difficile from symptomatic patients. Clin Infect Dis 2010;50:1450–7.
34. Daskalaki I, Hennessey P, Hubler R, Long SS. Resource consumption in the infection control management of pertussis exposure among healthcare workers in pediatrics. Infect Control Hosp Epidemiol 2007;28:412–7.
35. Ninove L, Nougairede A, Gazin C, Zandotti C, Drancourt M, de Lamballerie X, et al. Comparative detection of enterovirus RNA

in cerebrospinal fluid: GeneXpert system vs. real-time RT-PCR assay. Clin Microbiol Infect 2011;17:1890–4.
36. Gavino M, Wang E. A comparison of a new rapid real-time polymerase chain reaction system to traditional culture in determining group B streptococcus colonization. Am J Obstet Gynecol 2007;197:388.e1–4.
37. Schubert S, Weinert K, Wagner C, Gunzl B, Wieser A, Maier T, et al. Novel, improved sample preparation for rapid, direct identification from positive blood cultures using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. J Mol Diagn 2011;13:701–6.
38. Köhling HL, Bittner A, Müller KD, Buer J, Becker M, Rübben H, et al. Direct identification of bacteria in urine samples by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and relevance of defensins as interfering factors. J Med Microbiol 2012;61:339–44.
39. Klein S, Zimmermann S, Köhler C, Mischnik A, Alle W, Bode KA. Integration of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in blood culture diagnostics: a fast and effective approach. J Med Microbiol 2012;61: 323–31.
40. Juiz PM, Almela M, Melción C, Campo I, Esteban C, Pitart C, et al. A comparative study of two different methods of sample preparation for positive blood cultures for the rapid identification of bacteria using MALDI-TOF MS. Eur J Clin Microbiol Infect Dis 2012;31:1353–8.
41. Kok J, Thomas LC, Olma T, Chen SC, Iredell JR. Identification of bacteria in blood culture broths using matrix-assisted laser desorption-ionization Sepsityper™ and time of flight mass spectrometry. PLoS One 2011;6:e23285.
42. Schmidt V, Jarosch A, März P, Sander C, Vacata V, Kalka-Moll W. Rapid identification of bacteria in positive blood culture by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Eur J Clin Microbiol Infect Dis 2012;31:311–7.
43. Ferreira L, Sánchez-Juanes F, Porras-Guerra I, García-García MI, García-Sánchez JE, González-Buitrago JM, et al. Microorganisms direct identification from blood culture by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Clin Microbiol Infect 2011;17:546–51.
44. Ferreira L, Sánchez-Juanes F, González-Avila M, Cembrero-Fuciños D, Herrero-Hernández A, González-Buitrago JM, et al. Direct identification of urinary tract pathogens from urine samples by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2010;48:2110–5.
45. La Scola B, Raoult D. Direct identification of bacteria in positive blood culture bottles by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. PLoS One 2009;4:e8041.
46. Prod'hom G, Bizzini A, Durussel C, Bille J, Greub G. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for direct bacterial identification from positive blood culture pellets. J Clin Microbiol 2010;48:1481–3.
47. Stevenson LG, Drake SK, Murray PR. Rapid identification of bacteria in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2010;48:444–7.
48. Guo K, Li L. Differential 12C-/13C-isotope dansylation labeling and fast liquid chromatography/mass spectrometry for absolute and relative quantification of the metabolome. Anal Chem 2009;81:3919–32.
49. Vlek AL, Bonten MJ, Boel CH. Direct matrix-assisted laser desorption ionization time-of-flight mass spectrometry improves appropriateness of antibiotic treatment of bacteremia. PLoS One 2012;7:e32589.
50. Baldwin CD, Howe GB, Sampath R, Blyn LB, Matthews H, Harpin V, et al. Usefulness of multilocus polymerase chain reaction followed by electrospray ionization mass spectrometry to identify a diverse panel of bacterial isolates. Diagn Microbiol Infect Dis 2009;63:403–8.
51. Perez F, Endimiani A, Ray AJ, Decker BK, Wallace CJ, Hujer KM, et al. Carbapenem-resistant Acinetobacter baumannii and Klebsiella pneumoniae across a hospital system: impact of post-acute care facilities on dissemination. J Antimicrob Chemother 2010;65:1807–18.
52. Wang F, Massire C, Li H, Cummins LL, Li F, Jin J, et al. Molecular characterization of drug-resistant Mycobacterium tuberculosis isolates circulating in China by multilocus PCR and electrospray ionization mass spectrometry. J Clin Microbiol 2011;49:2719–21.
53. Massire C, Ivy CA, Lovari R, Kurepina N, Li H, Blyn LB, et al. Simultaneous identification of mycobacterial isolates to the species level and determination of tuberculosis drug resistance by PCR followed by electrospray ionization mass spectrometry. J Clin Microbiol 2011;49:908–17.
54. Schuetz AN, Huard RC, Eshoo MW, Massire C, Della-Latta P, Wu F, et al. Identification of a novel Acinetobacter baumannii clone in a US hospital outbreak by multilocus polymerase chain reaction/electrospray-ionization mass spectrometry. Diagn Microbiol Infect Dis 2012;72:14–9.
55. Hujer KM, Hujer AM, Endimiani A, Thomson JM, Adams MD, Goglin K, et al. Rapid determination of quinolone resistance in Acinetobacter spp. J Clin Microbiol 2009;47:1436–42.
56. Ecker DJ, Sampath R, Li H, Massire C, Matthews HE, Toleno D, et al. New technology for rapid molecular diagnosis of bloodstream infections. Expert Rev Mol Diagn 2010;10:399–415.
57. Wolk DM, Blyn LB, Hall TA, Sampath R, Ranken R, Ivy C, et al. Pathogen profiling: rapid molecular characterization of Staphylococcus aureus by PCR/electrospray ionization-mass spectrometry and correlation with phenotype. J Clin Microbiol 2009;47:3129–37.
58. Hall TA, Sampath R, Blyn LB, Ranken R, Ivy C, Melton R, et al. Rapid molecular genotyping and clonal complex assignment of Staphylococcus aureus isolates by PCR coupled to electrospray ionization-mass spectrometry. J Clin Microbiol 2009;47: 1733–41.
59. Kaleta EJ, Clark AE, Johnson DR, Gamage DC, Wysocki VH, Cherkaoui A, et al. Use of PCR coupled with electrospray ionization mass spectrometry for rapid identification of bacterial and yeast bloodstream pathogens from blood culture bottles. J Clin Microbiol 2011;49:345–53.
60. Eshoo MW, Crowder CD, Li H, Matthews HE, Meng S, Sefers SE, et al. Detection and identification of Ehrlichia species in blood by use of PCR and electrospray ionization mass spectrometry. J Clin Microbiol 2010;48:472–8.
61. Modi DA, Farrell JJ, Sampath R, Bhatia NS, Massire C, Ranken R, et al. Rapid identification of Aspergillus terreus from bronchoalvelolar lavage fluid by PCR and electrospray-ionization with mass spectrometry (PCR/ESI-MS). J Clin Microbiol 2012;50:2529–30.
62. Chen KF, Rothman RE, Ramachandran P, Blyn L, Sampath R, Ecker DJ, et al. Rapid identification viruses from nasal

pharyngeal aspirates in acute viral respiratory infections by RT-PCR and electrospray ionization mass spectrometry. J Virol Methods 2011;173:60–6.
63. Syrmis MW, Moser RJ, Whiley DM, Vaska V, Coombs GW, Nissen MD, et al. Comparison of a multiplexed MassARRAY system with real-time allele-specific PCR technology for genotyping of methicillin-resistant Staphylococcus aureus. Clin Microbiol Infect 2011;17:1804–10.
64. Honisch C, Chen Y, Mortimer C, Arnold C, Schmidt O, van den Boom D, et al. Automated comparative sequence analysis by base-specific cleavage and mass spectrometry for nucleic acid-based microbial typing. Proc Natl Acad Sci USA 2007;104:10649–54.
65. Morelli G, Song Y, Mazzoni CJ, Eppinger M, Roumagnac P, Wagner DM, et al. Yersinia pestis genome sequencing identifies patterns of global phylogenetic diversity. Nat Genet 2010;42:1140–3.
66. Kiehntopf M, Melcher F, Hänel I, Eladawy H, Tomaso H. Differentiation of Campylobacter species by surface-enhanced laser desorption/ionization-time-of-flight mass spectrometry. Foodborne Pathog Dis 2011;8:875–85.
67. Issaq HJ, Veenstra TD, Conrads TP, Felschow D. The SELDI-TOF MS approach to proteomics: protein profiling and biomarker identification. Biochem Biophys Res Commun 2002;292:587–92.
68. Bizzini A, Greub G. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification. Clin Microbiol Infect 2010;16:1614–9.
69. Cherkaoui A, Emonet S, Fernandez J, Schorderet D, Schrenzel J. Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid identification of Beta-hemolytic streptococci. J Clin Microbiol 2011;49:3004–5.
70. Conway GC, Smole SC, Sarracino DA, Arbeit RD, Leopold PE. Phyloproteomics: species identification of Enterobacteriaceae using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Mol Microbiol Biotechnol 2001;3:103–12.
71. Mellmann A, Bimet F, Bizet C, Borovskaya AD, Drake RR, Eigner U, et al. High interlaboratory reproducibility of matrix-assisted laser desorption ionization-time of flight mass spectrometry-based species identification of nonfermenting bacteria. J Clin Microbiol 2009;47:3732–4.
72. Carbonnelle E, Beretti JL, Cottyn S, Quesne G, Berche P, Nassif X, et al. Rapid identification of staphylococci isolated in clinical microbiology laboratories by matrix assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2007;45:2156–61.
73. Lista F, Reubsaet FA, De Santis R, Parchen RR, de Jong AL, Kieboom J, et al. Reliable identification at the species level of Brucella isolates with MALDI-TOF-MS. BMC Microbiol 2011;11:267.
74. Murray PR. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry: usefulness for taxonomy and epidemiology. Clin Microbiol Infect 2010;16:1626–30.
75. Kuhnert P, Bisgaard M, Korczak BM, Schwendener S, Christensen H, Frey J. Identification of animal Pasteurellaceae by MALDI-TOF mass spectrometry. J Microbiol Methods 2012;89:1–7.
76. Espinal P, Seifert H, Dijkshoorn L, Vila J, Roca I. Rapid and accurate identification of genomic species from the Acinetobacter baumannii (Ab) group by MALDI-TOF MS. Clin Microbiol Infect 2011 Oct 13. [Epub ahead of print].
77. Ecker JA, Massire C, Hall TA, Ranken R, Pennella TT, Agasino Ivy C, et al. Identification of Acinetobacter species and genotyping of Acinetobacter baumannii by multilocus PCR and mass spectrometry. J Clin Microbiol 2006;44:2921–32.
78. Stephan R, Cernela N, Ziegler D, Pflüger V, Tonolla M, Ravasi D, et al. Rapid species specific identification and subtyping of Yersinia enterocolitica by MALDI-TOF mass spectrometry. J Microbiol Method 2011;87:150–3.
79. He Y, Chang TC, Li H, Shi G, Tang YW. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry and database for identification of Legionella species. Can J Microbiol 2011;57:533–8.
80. Fedorko DP, Drake SK, Stock F, Murray PR. Identification of clinical isolates of anaerobic bacteria using matrix-assisted laser desorption ionization-time of flight mass spectrometry. Eur J Clin Microbiol Infect Dis 2012;31:2257–62.
81. Justesen US, Holm A, Knudsen E, Andersen LB, Jensen TG, Kemp M, et al. Species identification of clinical isolates of anaerobic bacteria: a comparison of two matrix-assisted laser desorption ionization-time of flight mass spectrometry systems. J Clin Microbiol 2011;49:4314–8.
82. Knoester M, van Veen SQ, Claas EC, Kuijper EJ. Routine identification of clinical isolates of anaerobic bacteria: matrix-assisted laser desorption ionization-time of flight mass spectrometry performs better than conventional identification methods. J Clin Microbiol 2012;50:1504.
83. Djelouadji Z, Roux V, Raoult D, Kodjo A, Drancourt M. Rapid MALDI-TOF mass spectrometry identification of Leptospira organisms. Vet Microbiol 2012;159:544.
84. Wang J, Chen WF, Li QX. Rapid identification and classification of Mycobacterium spp. using whole-cell protein barcodes with matrix assisted laser desorption ionization time of flight mass spectrometry in comparison with multigene phylogenetic analysis. Anal Chim Acta 2012;716:133–7.
85. Bouakaze C, Keyser C, Gonzalez A, Sougakoff W, Veziris N, Dabernat H, et al. Matrix-assisted laser desorption ionization-time of flight mass spectrometry-based single nucleotide polymorphism genotyping assay using iPLEX gold technology for identification of Mycobacterium tuberculosis complex species and lineages. J Clin Microbiol 2011;49:3292–9.
86. Gaillot O, Blondiaux N, Loïez C, Wallet F, Lemaître N, Herwegh S, et al. Cost-effectiveness of switch to matrix-assisted laser desorption ionization-time of flight mass spectrometry for routine bacterial identification. J Clin Microbiol 2011;49:4412.
87. Anderson NW, Buchan BW, Riebe KM, Parsons LN, Gnacinski S, Ledeboer NA. Effects of solid-medium type on routine identification of bacterial isolates by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2012;50:1008–13.
88. Misra SK, Milohanic E, Aké F, Mijakovic I, Deutscher J, Monnet V, et al. Analysis of the serine/threonine/tyrosine phosphoproteome of the pathogenic bacterium Listeria monocytogenes reveals phosphorylated proteins related to virulence. Proteomics 2011;11:4155–65.
89. Fuchs G, Diges C, Kohlstaedt LA, Wehner KA, Sarnow P. Proteomic analysis of ribosomes: translational control of mRNA populations by glycogen synthase GYS1. J Mol Biol 2011;410:118–30.
90. Hotta Y, Sato J, Sato H, Hosoda A, Tamura H. Classification of the genus Bacillus based on MALDI-TOF MS analysis of ribosomal proteins coded in S10 and spc operons. J Agric Food Chem 2011;59:5222–30.

91. Han X, Hu Q, Ding S, Chen W, Ding C, He L, et al. Identification and immunological characteristics of chaperonin GroEL in Riemerella anatipestifer. Appl Microbiol Biotechnol 2012;93:1197–205.
92. Dieppedale J, Sobral D, Dupuis M, Dubail I, Klimentova J, Stulik J, et al. Identification of a putative chaperone involved in stress resistance and virulence in Francisella tularensis. Infect Immun 2011;79:1428–39.
93. Edwards-Jones V, Claydon MA, Evason DJ, Walker J, Fox AJ, Gordon DB. Rapid discrimination between methicillin-sensitive and methicillin resistant Staphylococcus aureus by intact cell mass spectrometry. J Med Microbiol 2000;49:295–300.
94. Majcherczyk PA, McKenna T, Moreillon P, Vaudaux P. The discriminatory power of MALDI-TOF mass spectrometry to differentiate between isogenic teicoplanin-susceptible and teicoplanin-resistant strains of methicillin-resistant Staphylococcus aureus. FEMS Microbiol Lett 2006;255:233–9.
95. Burckhardt I, Zimmermann S. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 hours. J Clin Microbiol 2011;49:3321–4.
96. Ndao M, Rainczuk A, Rioux MC, Spithill TW, Ward BJ. Is SELDI-TOF a valid tool for diagnostic biomarkers? Trends Parasitol 2010;26:561–7.
97. Seibold E, Bogumil R, Vorderwülbecke S, Al Dahouk S, Buckendahl A, Tomaso H, et al. Optimized application of surface-enhanced laser desorption/ionization time-of-flight MS to differentiate Francisella tularensis at the level of subspecies and individual strains. FEMS Immunol Med Microbiol 2007;49:364–73.
98. Fortin T, Salvador A, Charrier JP, Lenz C, Lacoux X, Morla A, et al. Clinical quantitation of prostate-specific antigen biomarker in the low nanogram/milliliter range by conventional bore liquid chromatography-tandem mass spectrometry (multiple reaction monitoring) coupling and correlation with ELISA tests. Mol Cell Proteomics 2009;8:1006–15.
99. Vestal ML. The future of biological mass spectrometry. J Am Soc Mass Spectrom 2011;22:953–9.

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