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Review

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry in clinical microbiology: An updating review

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ABSTRACT

In the last years, Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry gained the attribute of gold-standard method for microbial identification. A rich scientific literature has been produced to evalutate its performance in gram-positive, gram-negative, anaerobic bacteria, and also difficult and exigent pathogens identification, included mycobacteria, yeasts, and molds. Typing in PubMed "MALDI-TOF mass spectrometry" at the date of August 1st 2019, about 14.468 articles can be found. Typing "MALDI-TOF identification" or "MALDI-TOF and microbiology" or "MALDI-TOF and infection" the number of artcicles is reduced to 5747, 3720 and 1746, respectively. In this review, an update of the most important findings reported during last ten years has been provided, confirming the central role of this technology in microbiology.

1. Introduction

Efforts made in clinical microbiology have been ever represented by the need of new methodologies allowing timely microorganism identification and prompt antimicrobial therapy administration. Classical culture and identification approaches requires at least 36–48 h for a clinical report, thus delaying patients management especially in case of severe infections where the rapid treatment significantly impact on survival. In the last ten years, new rapid approaches have been proposed to speed up time for diagnosis, thus revolutioning the clinical microbiology.

Molecular based techniques not requiring culture growth of microorganisms have represented an advantage over current methods, but since they were difficult to adapt to any kind of clinical microbiology laboratory the large scale diffusion could be limited. In the last years, beside these, Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS), by MALDI source and the time-of-flight (TOF) combination has largely revolutioned microbial identification in routine microbiology laboratory, gaining the attribute of gold-standard method of identification. It resulted a rapid, simple and reliable way to identify gram-positive, gram-negative, anaerobic bacteria and difficult or exigent pathogens such as mycobacteria, yeasts, and molds (Angeletti et al., 2017).

The first application of mass spectrometry to bacterial identification dated back to 1975 (Anhalt and Fenselau, 1975), but this first approach was not completely successful because bacteria were not identified at species level since only lipid could be obtained. In the 1980s, with the advent of soft ionization techniques, polypeptides analysis was possible (Tanaka et al., 1988) and in 1996, Holland et al. (1996) developed MALDI-TOF MS by whole bacterial cells (Holland et al., 1996).

A rich scientific literature has been produced to evalutate its performance in microbial identification as well as in antimicrobial susceptibility phenotype.

Typing in PubMed "MALDI-TOF mass spectrometry" at the date of August 1st, 2019, about 14.468 articles can be found, but typing "MALDI-TOF identification" or "MALDI-TOF and microbiology" or MALDI-TOF and infection" the number of artcicles is reduced to 5747, 3720 and 1746, respectively. In Fig. 1 is reported the number of articles published per year from 2010 to 2019 found by typing "MALDI-TOF identification", "MALDI-TOF and microbiology" and "MALDI-TOF and infection" that is almost the same for the first two research typing and slightly lower for the last (Fig. 1). This is conceivable thinking that a continuous scientific production has been dedicated to these fields during the last decade especially for the application of MALDI-TOF MS in the clinical microbiology. MALDI-TOF MS is based on Mass Spectrometry through which it is possible to detect the mass to-charge ratio (m/z) and to produce specific and characterizing spectra, representing a unique mass spectral fingerprint of the microorganisms within few minutes (Angeletti et al., 2017).

Mass spectra are obtained starting from a small amount of cells

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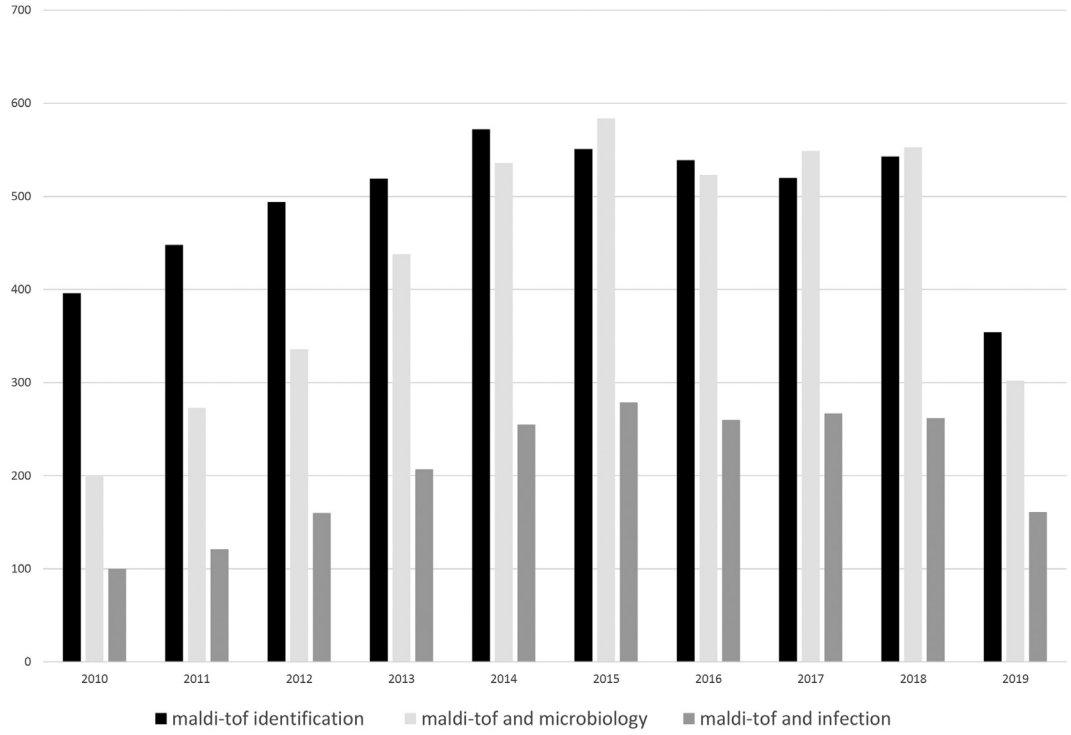

Fig. 1. Number of articles published per year from 2010 to 2019 found by typing "Maldi-tof identification", "Maldi-tof and microbiology" and "Maldi-tof and infection".

(about 10^5–10^6 cells that is spotted on a target plate along with a chemical matrix for protein extraction, the α-cyano-4-hydroxy-cinnamic acid (CHCA), that is fundamental for ionization.

Mass spectra, consisting of a peak list with *m/z* values and related intensities, can be determined automatically by laser sample spot scanning. The laser usually is 50 Hz emitting and analyze a single spot in < 30 s. Species identification can be achieved comparing the mass fingerprint with a database including include spectra from all relevant microorganism accounting for the most important human infections. (Welker and Moore, 2011). Larger is the number of spectra representing each specie more reliable is the identification. The reliability is guaranteed by the "score value" computed during spectra analysis (Welker and Moore, 2011).

In some cases, spectra quality can be improved by pretreatment of samples with ethanol-formic acid (FA) enhancing protein extraction especially in case of yeasts, some gram-positive cocci and *Mycobacterium* spp. Typical sample preparation methods and MALDI-TOF procedure is schematized in Fig. 2. MALDI-TOF direct application on clinical samples represented a new challenge in clinical microbiology routine because it really decreases time to results providing early identification and prompt target treatment improving patient clinical management. The application is in most cases limited by the low number of microorganisms in the microbiological sample limiting accurate spectra acquisition. Identification can be improved by sample enrichment as in case of positive blood cultures where the cell biomass is generally enough to allow identification after sufficient concentration and purification, pre-treatments made before spectra acquisition (Fothergill et al., 2013; Nonnemann et al., 2013; Leli et al., 2013).

Recently, other protocols, based on quick-spin and wash and gradient centrifugation, have been proposed to provide "short methods". These methods starts from an aliquot of positive blood culture spotted directly on the agar plate followed by a short incubation period of about 4–6 h. The fine colonies growing are used as cell biomass for further MALDI-TOF identification (Köck et al., 2017; Altun et al., 2015; Curtoni et al., 2017). This rapid approach for sepsis diagnosis represents a great revolution because especially in bloodstream infections the tempestive identification of the causal pathogen represents a key factor with significant impact on patient survival (Seymour et al., 2016; Kumar et al., 2006).

When using direct identification methods it has to be remembered that for reliable identification approximately at least 10^4 cells of biomass are required to achieve a spectrum of sufficient quality (Hsieh et al., 2008). Another useful direct protocol application is for urine samples from suspected urethritis because it can contain sufficient numbers of bacteria ($> 10^3$ cells/mL) that can be further concentrated starting from larger volumes (> 10 mL). A limitation in this case could be the presence of aspecific peaks derived from defensing proteins that could interfere producing not reliable spectra acquisition (Köhling et al., 2012).

In general, the direct identification could be impaired by polimicrobial contamination or infections, which requires the based culture approach allowing different colonies subculture and microorganisms identification starting from pure colonies.

2. MALDI-TOF MS microbial identification and clinical management

Since MALDI-TOF introduction in the clinical microbiology, microbial identification registered a significant improvement not only for timely microorganism identification but also for the ability to easily identify species that with the classical methodology remained unknown (Theparee et al., 2017; Seng et al., 2013; Hou et al., 2019). Recently, a case of *Methylobacterium radiotolerans* bacteraemia in a patient with end-stage renal failure was reported. *Methylobacterium radiotolerans* is a fastidious environmental microorganism occasionally found in clinical samples especially in immunocompromised subjects. Despite its difficult and time-consuming identification by classical methods, authors showed how MALDI-TOF MS provided reliable and rapid identifcation of the pathogen (Cordovana et al., 2019). Aslani et al., by MALDI-TOF besisdes the most common yeasts species such as *Candida albicans*, *Candida tropicalis* and *Candida glabrata*, identified from the oral cavity of cancer patients also less common species as *Candida dubliniensis*,

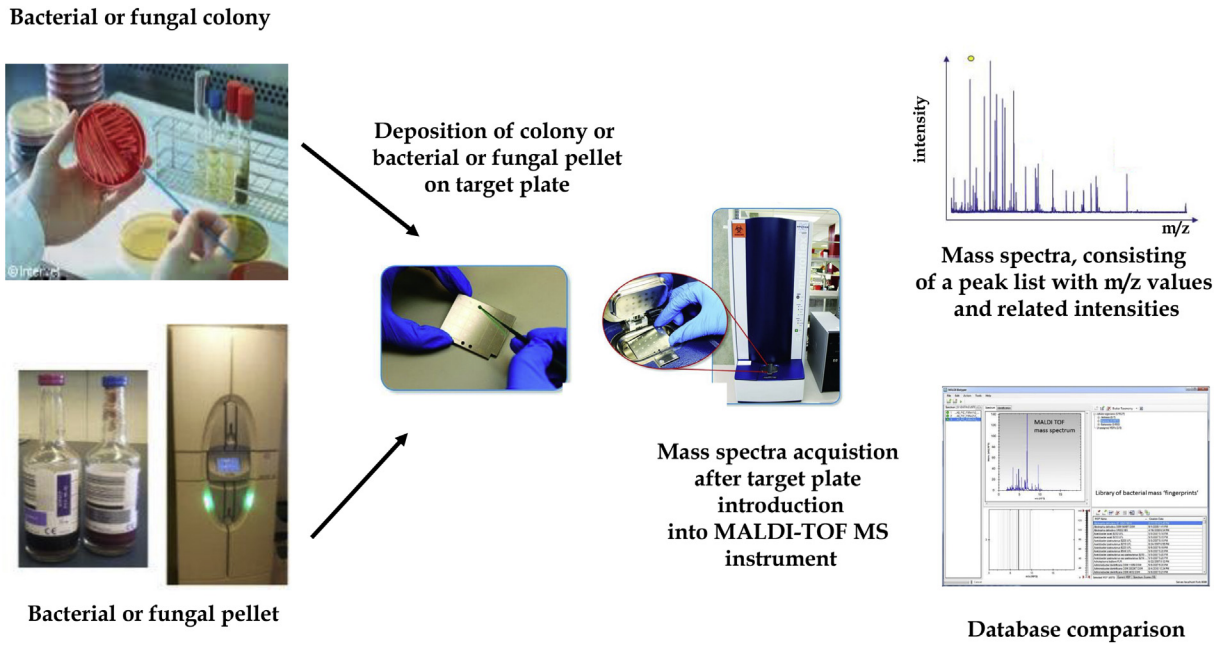

Fig. 2. Typical sample preparation methods and MALDI-TOF procedure.

Kluyveromyces marxianus also known as *Candida kefyr*. Furthermore, uncommon yeast species as *Saprochaete capitata*, *Saccharomyces cerevisiae*, *Clavispora lusitaniae* and *Pichia kluyveri* were isolated in the clinical samples collected from the oral lesion of these patients (Aslani et al., 2018).

The introduction of MALDI-TOF in clinical microbiology represented a great improvement for different challenging organisms by the continuous enhancement of available spectra database.

Among gram-negative nonfermenters, the genus *Acinetobacter* represents a paradigma of taxonomic evolution: many new species are emerging with consequent problems in reliable identification. MALDI-TOF database can be updated and easily adapted to this taxonomic changes, in fact it is possible to distinguish within the *Acinetobacter baumannii* group different species as *A. baumannii, A. pittii, A. nosocomialis, A. seiferti and A. dijkshoornie*, thus providing a detailed epidemiological report and targeted therapy strategies (Li et al., 2018).

Within the HACEK (*Haemophilus parainfluenzae, H. aphrophilus, Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, Kingella kingae*) group, by the introduction of MALDI-TOF some species identification was improved, as *Eikenella, Kingella and Haemophylus*. It is of particular interest the ability to discriminate between *H.influenzae* and *H. haemolyticus*, less pathogen (Couturier et al., 2011).

A significant improvement was obtained also in the identification of *Mycobacteria* spp. A limit for the identification is represented by long incubation time requiring until three weeks. To overcome this limit, nucleic acid hybridization or amplification based methodolgy have been proposed decreasing turnaround time to 24–28 h, thus allowing a rapid diagnosis of microorganisms included in *Mycobacterium tuberculosis* complex. Besides nucleic acid based technologies, MALDI-TOF has been proposed as a reliable tool for *Myobacteria* identification. The most common commercial MALDI-TOF MS systems available for clinical microbiology laboratories are from bioMérieux (Marcy l'Etoile France) and Bruker Daltonics (Bremen, Germany) with dedicated protocols for *Mycobacteria* identification (Hou et al., 2019). As recently reported, MALDI-TOF correctly identified as 92% of the *M. tuberculosis* and 68% of *M. bovis* strains (Psaroulaki and Chochlakis, 2018a, 2018b).

Samli et al., in a study comparing different assyas for *M. tuberculosis* and non-tuberculosis *Mycobacterium* species, as nucleic acid hybridization, immunochromatographic test and MALDI-TOF showed the ability to identify 100% of *M. tuberculosis* strains in less tha one hour and at lower cost by MALDI-TOF. Despite this, MALDI-TOF performance was lower in case of non-tuberculous *Mycobacteria* where the percentage of correct identification descreased to 38.5% (Samli and Ilki, 2016). In a recent report, authors provided evidence that by applying freezing and homogenizing, easier protein extraction of non-tubercuolous *Mycobacteria* was achieved, improving identification rates (Rodriguez-Temporal et al., 2018). It has been also reported the possibility to discriminate different species within the *Mycobacterium avium* complex avoiding misidentification between *M. intracellualre* and *M. chimera* and improving patient management (Samli and Ilki, 2016). Recently, Lecorche et al. reported the ability of MALDI-TOF to discriminate *M. chimaerae* and *M. intracellulare* from *M. avium* but not from each other (Lecorche et al., 2018).

After MALDI-TOF MS introduction, a great improvement was obtained for yeast and fungi identification. MALDI-TOF showed the ability to discriminate very similar species as *Candida albicans* from *Candida dubliniensis, Candida pararugosa* from *Candida rugosa* or *Candida parapsilosis* from *Candida orthopsilosis*. Furthermore, some new species have been described as emerging pathogen like *Candida auris* or *Candida famata* (Patel, 2019). For yeast, the correct identification rates ranged from 92.5 to 98.8% (Chen et al., 2013; Wang et al., 2016; Chao et al., 2014). Filamentous fungi characterized by considerable phenotypic variants have shown large heterogeneity of protein spectra and for this reason, the identification reliability resulted highly dependent on growth conditions and on the part of mycelium collected for the analysis. Despite this, filamentous fungi can be identified by MALDI-TOF MS with reliable identification rates ranging from 95.4% to 98.8% allowing also identification of closely related species (Becker et al., 2014; Gautier et al., 2014; Normand et al., 2017a, 2017b).

By MALDI-TOF MS improved identification of anaerobe with faster turn around time has been achieved, despite the more challenging slow growth and incubation conditions. However, identification of the most important species can remain difficult because the number of database entries is low limiting the reliability of the available database. Efforts have been made to enrich databases, submitting new mass spectra validated by 16S rDNA sequencing (Veloo et al., 2018; Boiten et al., 2018; Fontanals et al., 2018).

Recently, the European Network for the Rapid Identification of Anaerobes (ENRIA) elaborated a specific project where seven European

laboratories provided spectra from clinical isolates to enrich databases. The project was in collaboration with one of the commercial manufacturer of MALDI-TOF MS, the Bruker Daltonics (Bremen, Germany) so that ENRIA strains were added to the db6903 database of Bruker MALDITOF MS Biotyper system. The enriched databse was further validated obtaining an increasing rate of strains identified at high confidence from 71.1% to 79.2% and a significant decrease of the percentage of not identified strains from 12% to 7.3%. (Veloo et al., 2018).

3. The impact of rare species identification on the clinical workflow in the era of MALDI-TOF MS

MALDI-TOF MS introduction determined a significant improvement in microbial identification with very high percentage of success ranging from 90% to 100% also for fastidious organisms, as previously described. Ever and ever more protocols are described for sample treatment leading to specific mass spectra able to clearly identifiy at high level of confidence more and more micooorganisms causing human infections. Spectra database are constantly revised to improve identification rates. In this panorama, the identification of unexpected species never described before in clinical samples is not uncommon. Recently, the first case of bacteriemia by *Methylobacterium radiotolerans*, a fastidious environmental gram-negative bacillus occasionally isolated in humans, especially in case of immunosuppression or in presence of intravascular device, has been described. *Methylobacterium radiotolerans* was promptly identified by MALDI-TOF MS directly from positive blood culture shortening the time to report of three days than conventional methods (Cordovana et al., 2019). In the oral cavity of cancer patients during antineoplastic treatment, uncommon yeast species were identified by MALDI-TOF MS many of which with reduced susceptibility to antifungal treatment (Aslani et al., 2018). MALDI-TOF ability to achieve identification even in case of “difficult” microorganisms that is also the reason of its success, lie on the principle that mass spectra identification is based on housekeeping proteins, exactly ribosomal proteins, and it is independent from the metabolic pathway (Welker and Moore, 2011). Potentially all micoorganisms could be identified and when identification is not reliable it depends only on database completedness (Rodríguez-Sánchez et al., 2014). Despite this important advance, identification of new species impose the clinician to provide a correct interpretation and correlation of the microbiological finding with the clinical condition of the patient. From this unexpected ability of MALDI-TOF MS, the need for appropriate antimicrobial therapy and discussion of more and more case of uncommon species involved in clinical infections have emerged. Recently, some authors reported the possibility to identify by MALDI-TOF MS, *Nocardia* isolates using direct spotting and an updated database. In this study, the importance of correct identification at the species level was represented by the ability to evidence strains as *Nocardia pseudobrasiliensis* fully resistant to ceftriaxone, imipenem and amikacin, *Nocardia abscessus* resistant to imipenem in 69% of cases and *Nocardia otitidiscaviarum* always resistant to ceftriaxone (Marín et al., 2018).

Recently, MALDI-TOF MS ability to identify *Moraxella* species other than *Moraxella catarrhalis* has been reported. The possibility to identify all *Moraxella* species remained unresolved for years until the era of MALDI-TOF. Authors reported the reliable identification of all *Moraxella* species isolated from ocular specimens as agents of keratitis (*M. lacunata, M. nonliquefaciens, and M. osloensis*), conjunctivitis and blebitis (*M. nonliquefaciens*). The same authors discussed the problem, after a correct identification, to provide a correct antimicrobial treatment through adequate antimicrobial susceptibility test (Takahashi et al., 2019).

4. MALDI-TOF successful identification: Mass spectra databse quality

The reliable identification depends mostly by database integrity. During last years, many efforts have been done to enrich and provide more and more new mass spectra for each specie to be added to the growing database. Reacher are database more reliable is the identification of the analysed strain. Nowadays, MALDI-TOF MS databases are generally robust and complete, especially commercial databases, providing rapid and efficient identification of the most common clinical strains. However, all mass spectra included in the most used clinical database represent a small fraction of the exisisting microbial species that could potentially cause human infections. For this reason MALDI-TOF spectra database are continuously evolving and can never be considered fully complete. This is well represented by the microbial diversity described in the modern concept of the microbiota that has recently caught the interest of many scientists playing a central role also in the pathophysiology of many diseases (Lagier et al., 2016; Rajilić-Stojanović and de Vos, 2014).

Recently, Ji et al. analysed MALDI-TOF MS ability to define the microbiota of bronchoalveolar lavage fluid in comparison with the reference method based on next generation sequencing (NGS). As expected some bacteria, mainly anaerobes, failed to be identified by MALDI-TOF because not growing on conventional culture media, thus a lower number of species were identified compared to NGS. Conversely, MALDI-TOF produced reliable identification of some common microorganisms that were not sequenced by NGS. The study showed how culture could be a determinant step for some strains, especially those less represented in the microbial community. Authors concluded that MALDI-TOF and 16SrRNA NGS have to be considered complementary techniques in microbiota composition definition (Sung et al., 2018).

5. Antimicrobial susceptibility: Resistant strains identification

Besides identification, the detection of resistant strains is fundamental to provide a prompt and tailored antimicrobial therapy especially in case of bloodstream infections. MALDI-TOF MS was used at this aim to produce a resistance profile complementary to the standard techniques by using different approaches applied with success to gram-negative bacteria and *Staphylococcus aureus* Psaroulaki and Chochlakis, 2018a, 2018b).

The principal alternative methodologies used include whole-cell mass spectrometry (WCMS); detection of microbial growth in presence of antimicrobial and detection of antibiotic degradation.

The first approach is based on the detection of resistance proteins falling within the mass range availabale for WCMS. This approach is successfully used for methicillin-resistant *Staphylococcus aureus* strains. In these strains, the presence of the cassette chromosome mec (SCCmec) is often associated with a protein of the spectra at m/z 2415 Da (Josten et al., 2014; Rhoads et al., 2016).

The detection of microbial growth in presence of antimicrobial is based on bacteria exposition to antibiotics and, after incubation, MALDI-TOF mass spectra analysis. Resistant strains phenotype results in different spectra profile compared to the not exposed strain (Sakarikou et al., 2017).

By MALDI-TOF is possible to evidence resitant strains detecting antibioctics degradation. This approach has been used especially for carbapenemase activity. Carbapenemase determine antibiotic modification resulting in a mass shift from the normal antibiotic mass if hydrolysis of the carbapenem is present (+18 Da) or if a further decarboxylation happens (−26 Da) (Sparbier et al., 2012; Hrabák et al., 2012).

Recently, Giordano and Barnini proposed a new algorithm based on MALDI-TOF technology to identify *Klebsiella pnemumoniae* colistin resistant strains. By this rapid approach, resistant strains were correctly identifed in 91% of cases and susceptible strains in 73% (Giordano and Barnini, 2018).

6. MALDI-TOF and mass spectra clustering analysis

MALDI-TOF mass spectrometry has been used not only for identification or for antimicrobial susceptibility testing but also to determine the relatedness between different strains especially within the same species. The use of dedicated software as ClinProTools or Saramis able to analyze and compare the mass spectra peaks composition of each strain represents a useful tool. By producing a clustering model based on different mathemathical algorithms, it is possible to discriminate between various clonal lineages and establish their relatedness.

By typing in PubMed "MALDI-TOF clustering" at the date of August 1st, 2019, about 630 articles can be found. This methodology has been used especially for nosocomial outbreaks investigation. By typing "MALDI-TOF clustering and nosocomial infections" at the date of August 1st, 2019, about 22 articles can be retrieved.

In 2018, De Florio et al. used MALDI-TOF mass specyrometry to characterize *Enterobacter* strains isolated within a nosocomial setting. Authors built a class dendrogram of isolates loaded on two different MALDI-TOF platform by the ClinProTool software (Bruker Daltonics, Bremen, Germany) and the Saramis software (bioMérieux, Marcy l'Etoile France). In both cases, two major clusters were evidenced including strains from patients admitted to general surgery and geriatric wards, thus demonstrating a clonal route of transmission within this hospital wards (De Florio et al., 2018).

Khennouchi et al. already showed the use of MALDI-TOF mass spectrometry for epidemiological typing (Khennouchi et al., 2015). They performed epidemiological investigation of Algerian *Enterobacter* srains by MALDI-TOF and multilocus sequence typing (MLST). At MALDI-TOF clustering, three clusters corresponding to different geographical regions and species were evidenced. Interestingly, a specific clade of *E. cloacae* isolates from the urology ward clustered together in the MALDI-TOF dendrogram, suggesting a potential outbreak (Khennouchi et al., 2015).

Kaur et al. reported the improved identification of *Chryseobacterium* species, an emerging pathogen causing nosocomial infections, that were isolated during a frame period of four months from patients admitted in the same ward. By MALDI-TOF clustering, strains were clearly grouped into three different clusters, showing the ability to differentiate them upon their relatedness (Kaur et al., 2017).

MALDI-TOF dendrogram was used for epidemiological investigation to confirm a nosocomial outbreak by multidrug-resistant *Corynebacterium striatum* strains in Belgium. Authors compared MALDI-TOF with other molecular methodology used in outbreak description and concluded that dendrogram, clearly identifying the outbreak-related strains from the not related, could represent a reliable and rapid epidemiological tool for outbreak confrimation in nosocomial setting (Verroken et al., 2014).

Since 2013, MALDI-TOF identification and clustering was described a promising tool not only for strains idenfication but also for epidemiological investigation. Berrazeg et al. showed as MALDI-TOF clearly identified clusters of *Klebsiella pneumoniae* MDR strains associated with specific traits and different epidemiological distribution from a geographical as well as a seasonal point of view. These particular phenotypes were detected and confirmed by building a mass spectra dendrogram evidencing different clusters of strains, corresponding to different phenotypes and geographical or seasonal distributions.

The successful use of MALDI-TOF MS for rapid identification of nosocomial outbreaks has been described also for *A. baumannii* isolates. In this study, Mencacci et al. showed that MALDI-TOF clustering has the ability to accurately identify outbreak related strains earlier than DNA-based techniques (Mencacci et al., 2013).

MALDI-TOF clustering was also used for fungal nosocomial outbreak investigation. Pulcrano et al. compared mass spectroscopy with PCR-based technique and concluded that MALDI-TOF represents a reliable tool for identification and surveillance of strains spreading within the nosocomial setting (Pulcrano et al., 2012).

7. MALDI_TOF MS clustering and phylogenetic analysis: Complementary tools for outbreak description

MALDI-TOF clustering was compared with phylogenetic analysis an approach based on gene sequence homology. Batah et al., described an outbreak of *Serratia marcescens* strains isolated in Eastern Algeria and compared mass spectra clustering with phylogenetic analysis applied to four encoding genes *gyrB, rpoB, infB, and atpD*. In this study, a cluster of ESBL strains isolated in the same ward was showed by phylogenetic analysis as well as by MALDI-TOF clustering. Both methods were comparable for the outbreak identification (Batah et al., 2015).

Interestingly, another study compared MALDI-TOF dendrogram with Multi Locus Sequence Typing (MLST) and phylogenetic analysis of the *bla-kpc* gene conferring resistance to carbapenem. In this study, authors analysed *Klebsiella pnumoniae* MDR strains characterized by carbapenem resistance. MALDI-TOF clustering showed two distinct clusters different for temporal split and antimicrobial susceptibility phenotype. Strains of the two clusters were not discriminated as different strains by MLST resulting all ST 512. Phylogenetic analysis applied to the *bla-kpc* gene confirmed the genetic diversity of strains included in the two different clusters. Authors concluded that MALDI-TOF is a reliable tool for rapid epidemiological investigation in nosocomial setting that can be coupled to phylogenetic analysis for clonal strains identification (Angeletti et al., 2015a). Same authors used a similar approach to characterize clinical *Candida albicans* isolates with comparable results (Angeletti et al., 2015b).

Taking into account that MALDI-TOF clustering and phylogenetic analysis are respectively based on the concepts of similarity and homology, isolates clustering together in MALDI-TOF dendrogram are those strains displaying the highest percentage of similarity, whereas isolates clustering together in a phyogenetic tree are strains sharing the same ancestor.

Based upon the available studies, the complementary nature of information derived by MALDI-TOF clustering and phylogenesis can be used to better describe nosocomial infection in terms of rapid epidemiological information (MALDI-TOF) and further genetic confirmation by phylogenetic analysis. In case of a suspected outbreak of fearsome pathogens, such as multi-drug resistant microorganisms, MALDI-TOF could be immediately applied by dendrogram analysis to evidence strains clusters and possible transmission route. Phylogenetic analysis could complete this first result by phylogeography determination of clustering strains to better describe the resistant strains movement and to evaluate if they shared the same ancestor. This is a real good example of how two techniques based on different principles as well as on different molecular techniques, genomic and proteomic, can lead to the better way guaranteeing the most probable result.

8. Conclusions

MALDI-TOF mass spectrometry has changed the identification workflow in microbiology, representing a modern revolution and conquering the area with large diffusion even in developing countries (Bulane and Hoosen, 2017) or in unusual fields (Mewara et al., 2018; Feucherolles et al., 2019). Recently, Mewara et al. used MALDI-TOF to identify the most medically important mosquitos as *Anopheles, Aedes, Culex* and *Armigerus*. A database with species-specific peaks was built for reliable identification (Mewara et al., 2018). Feucherolles et al. reviewed 84 articles about MALDI-TOF application for nematodes identification in human and veterinary fileds. Authors concluded that this approach could be promising for reliable identification also at diagnostic purpouse with future perspective for fecal as well as serum samples analysis in infected subjcets (Feucherolles et al., 2019).

These studies together with the other presented in this review testify the great revolution that MALDI-TOF mass spectrometry introduced in the modern microbiology at any level.

A future important perspective is represented by the completedness

of spectra databases. All microbiologists hope to arrive to a universal database allowing the simultaneous realization of identification and high-quality antimicrobial susceptibility testing in few minutes and more and more cheap.

Declaration of Competing Interest

We declare that we have no conflicts of interest.

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