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MALDI-TOF MS in the clinical microbiology laboratory

MALDI-TOF MS no laboratório de microbiologia clínica

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ABSTRACT

Traditional methods for microbial identification are often very laborious and time consuming. A new mass spectrometry based technique, matrix-assisted laser desorption ionization-time of flight (MALDI-TOF), has been described as a rapid, practical and low-cost method for this purpose. In this article, primary and possible future applications of this tool are briefly discussed.

Key words: MALDI-TOF; microbial diagnostics; microbial identification; mass spectrometry.

INTRODUCTION

Phenotypic methods for bacterial identification, including the Gram group, growth characteristics in culture and biochemical tests, remain the tests of choice for performing microbiological analysis in most clinical laboratories worldwide. Although these are traditional methods, they provide answers to the clinician that are often time consuming, reaching several days in the case of fastidious microorganisms[3], hence the need for faster and accurate diagnostic tools. Some molecular techniques such as real-time polymerase chain reaction (RT-PCR), sequencing and microarrays have been successfully deployed in identification, but they are not widespread yet due to some practical limitations.

A mass spectrometry technique, matrix-assisted laser desorption ionization-time of flight (MALDI-TOF), has been used in the analysis of different biomolecules for decades, and its application in routine microbiological diagnosis seems to be promising[1, 21]. After its refinement in recent years, this technique has been described as an effective tool in the identification of Gram-positive, enterobacteriaceae, non-fermentative, anaerobic, mycobacteria and fungi[6, 10, 17, 24, 26, 30].The method is based on protein analysis (the analysis of nucleic acids is also feasible, but rather cumbersome for this purpose) and comparison with available profiles stored in a database[14].

Most studies with this method included strains that had previously been isolated in culture. Nevertheless, some reports admit the possibility of diagnosis directly from clinical specimens, especially blood samples, which would represent a breakthrough in terms of even faster results[3].

METHOD

There are two commercially available systems that have been incorporated in the microbiology area by traditional companies. The first one is MS Vitek (bioMerieux), formerly Shimadzu. The other one is Microflex Biotyper (Bruker), commercialized by Becton Dickinson (BD). Their most distinctive features are related to the operating system, the routine management benefits, size, use of disposable or non-disposable plates, the cost benefits of tests and use of plates, the control quality frequency and noise[27].

The process initializes with the ionization of the sample to be studied, which is accomplished by mixing it with a matrix (alpha-cyano-4-hydroxycinnamic acid) and subsequently exposing it to laser pulses. Thus, the ionized molecules are accelerated by an electrostatic field, passing through a field-free area afterwards and, finally, reaching the mass spectrometry detector. Molecules of different masses and charges "fly" (hence the term time of flight)

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at different speeds. The results are compared with a database, and depending on the spectrum, it is possible to classify the microorganism into genus or species[14].

IDENTIFICATION OF ISOLATED BACTERIA

Seng *et al.*[32] prospectively analyzed 1,660 strains of bacteria identified in parallel through MALDI-TOF and traditional phenotypic methods. 84.1% were accurately identified at the species level and 11.3% at the genus level. According to the authors, no identification (2.8%) and misidentification (1.7%) were due to flaws in the database system. Van Veen *et al.*[36] also analyzed 980 strains prospectively, detecting a 6.8% identification of genus and 92% identification of species. In a retrospective study, Eigner *et al.*[13] studied 1,116 bacterial strains previously identified in clinical routine. MALDI-TOF consistency was 95.2%. Following a prospective study, Bizzini *et al.*[4] obtained correct identification of 95.1 % of 1,278 strains at the species level and 3% at the genus level. Other studies have yielded similar results[7, 29].

Comparing the two available systems in a study that comprised 1,129 samples, there was 92.7% consistency at the species level versus 93.2%, accurately identified by Biotyper or Vitek MS, with no significant statistical difference[27].

As for the test costs, these studies demonstrated that MALDI-TOF was more cost effective in comparison with traditional methods. Even when we take into account the price of the equipment (with depreciation diluted in the first years of use), it is estimated that the cost reaches less than 25% of the conventional identification[7, 29, 32]. Apart from the equipment purchase, the annual maintenance cost becomes more significant rather than the inputs. Results are available on average after only a few minutes, representing a significant difference in comparison with the time required for the results of conventional methods[29, 32].

In general, although adjustments and improvements are required, mainly for some bacteria such as viridans group streptococci, *Shigella* and anaerobes[3], the accuracy of this identification method seems to be considerable, with higher performance and cost effectiveness per identified strain.

IDENTIFICATION OF ISOLATED FUNGI

This technique has been deployed in the characterization of various species of yeast and filamentous fungi. Studies with filamentous fungi are less numerous, probably due to the difficulties related to phenotypic heterogeneity and lack of standardization in protein extraction protocols. Even so, some investigations have demonstrated the success of MALDI-TOF in identifying *Penicillium, Aspergillus, Fusarium* and some dermatophytes[15, 18, 19, 24]. For yeasts, mainly the genus *Candida*, the high identification accuracy has been established[26, 33, 36], and the available databases include reference spectrum of all species commonly found in laboratories.

OTHER APPLICATIONS

After successfully using the tool for the identification of microorganisms already isolated, the major challenge is to perform it directly from clinical samples. Although the reports on microorganism detection by MALDI-TOF directly from positive blood cultures are still relatively scarce[8, 16, 20, 22, 31, 34], the accuracy seems to reach more than 80% when there is an inoculum higher than 10^7 organisms/ml. A preliminary stage of pre-processing extraction and purification should be carried out, which aims at microorganism concentration as well as separation of proteins from leukocytes and erythrocytes that may be interfering factors. To do so, "in house" method or commercial kit (Sepsityper) may be deployed[5, 34].

Some hindrances that must be overcome with the improvement of the method are the possibility of failure in the differentiation between pneumococci and viridans streptococci and the difficulty in identifying two or more organisms in the sample[11], a problem already solved depending on the system and version of the software in use. Furthermore, other applications for this method have been described in the literature, including the assessment of antimicrobial resistance[12, 23, 25] and the detection of virulence factors[2], in addition to the use for classification and grouping with taxonomic or epidemiological purposes[28]. With the advance of this tool and methods of sample refinement prior to the use of MALDI-TOF, other applications as well as the detection of pathogens directly from clinical samples tend to be facilitated[3, 9, 35].

CONCLUSION

Mass spectrometry by MALDI-TOF could revolutionize diagnosis in clinical microbiology, providing accurate, inexpensive and quick identification of microorganisms. Its exact role in the routine laboratory tests remains to be defined, but the technique has the potential to be a major part of microbiological routine, along with traditional laboratory tests. New applications of the method, including the detection of pathogens directly from clinical samples, as well as assessment of virulence factors, antimicrobial susceptibility and even bacterial typing, make MALDI-TOF even more promising. It is expected that the more update the database is, the more accurate the bacterial identification will be.

RESUMO

Os métodos tradicionais para identificação de microrganismos no laboratório clínico muitas vezes são trabalhosos e demorados. Uma nova metodologia, com base em espectrometria de massas, a matrix-assisted laser desorption ionization-time of flight *(MALDI-TOF), é extremamente promissora para utilização na rotina microbiológica, sendo rápida, prática e pouco custosa. Neste artigo, são expostas, de forma breve, as principais aplicações atuais do método, assim como as perspectivas futuras.*

Unitermos: *MALDI-TOF; diagnóstico microbiológico; identificação microbiana; espectrometria de massas.*

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