Application status of MALDI-TOF mass spectrometry in the identification and drug resistance of *Mycobacterium tuberculosis*

Ruixue Zhang[1*], Yin Long[2*], Wenfang He[1], Xiaoke Hao[1], Jiayun Liu[1]

[1]Institute of Clinical Laboratory Medicine, [2]Department of Traditional Chinese Medicine, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, China

*These authors contributed equally to this work.

Correspondence to: Jiayun Liu. Institute of Clinical Laboratory Medicine, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, China. Email: jiayun@fmmu.edu.cn.

Abstract: Characterizing *Mycobacterium tuberculosis* (MTB) and detecting its drug resistance are challenging for clinical laboratory diagnosis, largely due to its slow growth and higher rate of genetic mutation. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a revolutionary technique for the routine identification of microorganisms. In this review, we discuss the application status of mass spectrometry in the identification and drug resistance of *M. tuberculosis*.

Keywords: *Mycobacterium tuberculosis* (MTB); identification; drug resistance; matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS)

Submitted Jan 18, 2014. Accepted for publication Feb 26, 2014.
doi: 10.3978/j.issn.2072-1439.2014.02.19
View this article at: http://www.jthoracdis.com/article/view/2348/2936

Introduction

Tuberculosis (TB) remains one of the most serious health threats facing several parts of the world (1). Methods for identifying *Mycobacterium tuberculosis* (MTB) are conventionally based on biochemical tests, typically require several weeks for adequate growth, and accurate results are often difficult to achieve. Besides the lengthy time required for identification, the emergence and transmission of multiple drug-resistant tuberculosis (MDR-TB) and extensive drug-resistant tuberculosis (XDR-TB) render control and prevention of tuberculosis even more difficult. The MDR phenotype is defined as resistant to at least isoniazid and rifampicin—the two most effective drugs recommended by the WHO for the first-line treatment of TB (2). Thus, the principal problems confront us exist in two respects. The first is to reduce the time and costs associated during the diagnosis of tuberculosis by conventional methods. Another is to speed up the efficiency and accuracy of drug susceptibility and resistance testing.

New strategies have been developed largely using molecular biology tools (3). Techniques based on DNA hybridization are sensitive, fast, and simple, but the available commercial assays (AccuProbe; Gen-Probe, San Diego, CA, USA) are able to identify only four species and two complexes of mycobacteria. Techniques requiring amplification followed by a hybridization step on a solid support are more complete than probes, but commercially available kits are limited to 5 (Geno-Type MTBC; Hain Lifescience GmbH, Nehren, Germany), 16 (Inno-LiPa Mycobacteria v2; Innogenetics, Gent, Belgium), or 30 (GenoType Mycobacterium; Hain Life science GmbH, Germany) species (4). Systems based on sequencing or enzymatic restriction targeting the *hsp65*, *16S rRNA*, *sod*, and *rpoB* genes allow good identification of all mycobacteria at the species level but remain limited to specialized laboratories (5). In addition, they are expensive and time consuming and require highly-qualified operators. More recently, alternatives based on the analysis of mycolic acid by high-performance liquid chromatography (HPLC) or electrospray ionization-tandem mass spectrometry analysis (6) have been proposed. In this review, the authors discuss the application status of mass spectrometry, to the identification and drug resistance of *M. tuberculosis*.

Identification of *M. tuberculosis* using MALDI-TOF MS

The identification of bacteria by mass spectrometry dates back to the 1970s, where researchers first described the use of mass spectrometry to identify bacteria (7). Despite the promise of this work and the development of significant advances in specimen preparation, adoption of this technology by clinical laboratories occurred slowly. Early studies targeted the analysis of polar fatty acids that comprised 5% to 8% of the dry cell weight of bacteria, whereas more recent studies have focused on analysis of basic proteins, primarily in the mass range of 2,000 to 20,000 Da (60% to 70% of the dry cell weight of bacteria).

An overview of MALDI-TOF MS

Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) has emerged as a novel tool for rapid and reliable identification of microorganisms by analysis of protein profiles from either disrupted cells (8) or intact bacterial cells (9). Bacterial colonies can be removed from agar culture plates, mixed with an excess of UV-absorbing matrix, and dried on steel target plates. The dried preparations are then exposed to laser pulses, resulting in energy transfer from the matrix to the nonvolatile analyte molecules, with desorption of the analyte into a gas phase. The ionized molecules are accelerated by electric potentials through a flight tube to the mass spectrometer, with separation of the biomarkers determined by their mass/charge ratio (m/z). The profile of biomarkers acquired by the mass spectrometer system is then compared to profiles of a collection of well-characterized organisms contained in a knowledgebase (10).

Identification of M. tuberculosis by MALDI-TOF MS

Recently, several studies have provided the proof-of-concept that MALDI-TOF MS can also identify mycobacteria (11). Indeed, this technique has emerged over the last few years as a revolutionary technique for the routine identification of bacterial isolates. El *et al.* (12) proposed an original protocol for the MALDI-TOF MS identification of heat-inactivated mycobacteria after dissociation in Tween-20, mechanical breaking of the cell wall, and protein extraction with formic acid and acetonitrile. By applying this protocol to as few as 10^5 colony-forming units of reference isolates of *M.tuberculosis*, *Mycobacterium avium*, and 20 other *Mycobacterium* species, they obtained species-specific mass spectra for the creation of a local database. Using this database, their protocol enabled the identification by MALDI-TOF MS of 87 *M. tuberculosis*, 25 *M.avium* and 12 non-tuberculosis clinical isolates with identification scores ≥2 within 2.5 hours. Thus MALDI-TOF MS appears to be an alternative first-line approach to the routine identification of a large majority of bacteria commonly cultured in the clinical microbiology laboratory. Nevertheless, the experimental conditions must be carefully managed, as the MALDI mass spectra acquired are heavily dependent on both bacterial culture and MALDI sample preparation conditions (13).

In order to address these above problems, Lotz *et al.* (14) engineered a strategy to identify mycobacterial strains using MALDI-TOF MS without cell extraction, and instead based on the choice of a limited number of species-specific profiles. A total of 311 strains belonging to 31 distinct species and 4 species complexes grown in Lowenstein-Jensen (LJ) and liquid [mycobacterium growth indicator tube (MGIT)] media were analyzed to validate the mycobacterial database that was constructed for mycobacterial species isolated from human pathology. Correct identifications were obtained for 97% of strains from LJ and 77% from MGIT media. The result suggested that this system may represent a legitimate alternative for clinical laboratories to identify mycobacterial species.

Machen *et al.* (15) developed two novel protocols for inactivation and extraction to identify 107 Mycobacterium clinical isolates, including *M. tuberculosis* complex, from solid cultures using Vitek matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (bioMérieux, Marcy l'Etoile France). The protocol using heat inactivation with sonication and cell disruption with glass beads resulted in 82.2% and 88.8% species and genus level identification, respectively.

These previously published protocols are relatively complex, involve significant preparation time, and the required materials are not usually present in the typical clinical laboratory. In 2013, Mather *et al.* (16) tested two simplified protein extraction protocols developed at the University of Washington (UW) and by bioMerieux (BMX) using two different mass spectrometry platforms (MALDI Biotyper by Bruker Daltonics; and VITEK MS by bioMérieux). Both extraction protocols included vortexing with silica beads in the presence of ethanol. The commercial Bruker database was also augmented with an in-house database composed of 123 clinical Mycobacterium strains. A total of 198 clinical strains, representing 18 *Mycobacterium* species, were correctly identified to the species level 94.9%

of the time when extracted using the UW protocol and compared to the augmented database. The BMX protocol and VITEK MS system resulted in correct species level identifications for 94.4% of these strains. In contrast, only 79.3% of strains were identified to the species level by the non-augmented Bruker database, although use of a lower identification score threshold (≥1.7) increased the identification rate to 93.9%, with two misidentifications that were unlikely to be clinically relevant. The two simplified protein extraction protocols described in this study are easy to use and can be used to identify commonly encountered Mycobacterium species.

Other methods based on mass spectrometry

In addition, several other methods based on mass spectrometry are being explored. Dang *et al.* (17) developed a 20-compound model to distinguish between MTB and NTM using gas chromatography-mass spectrometry and chemometrics. They reported validation of this model with two independent sample sets, one consisting of 39 MTB and 17 non-tuberculous Mycobacteria (NTM) isolates from the Netherlands, the other comprising 103 isolates (91 MTB and 12 NTM) from South Africa. All of the MTB strains in the 56 Dutch samples were correctly identified and the model had a sensitivity of 100% and a specificity of 94%. For the South African samples the model had a sensitivity of 88% and specificity of 100%. Kataria *et al.* (18) conducted a proteomic analysis of tuberculous meningitis (TBM) cerebrospinal fluid (n=20) with 2-dimensional difference gel electrophoresis (2D-DIGE) and mass spectrometry. They found that arachidonate 5-lipoxygenase may be considered for validation as a potential marker for diagnosis of TBM.

Identification of mycobacteria and other acid-fast organisms by mass spectrometry still poses a considerable challenge. However, as a rapid, relatively inexpensive method for the identification of mycobacteria, this technology may quickly become a widespread application in routine clinical practice.

Detection of drug resistance in *M. tuberculosis* by MALDI-TOF MS

Genetic mutation associated with drug-resistant M.tuberculosis

Bacteria can resist antibiotic actions by the following mechanisms: the production of enzymes that inactivate antibiotic molecules (e.g., β-lactamases and aminoglycoside-modifying enzymes) (19), the hyperproduction or production of novel efflux pumps and other changes in the cell wall (e.g., porin alterations) (20), mutations in target genes [e.g., in ribosomal protein genes or in genes coding for penicillin-binding proteins (PBPs)] (21), the bypass of a metabolic pathway (e.g., the expression of acquired PBPs with a low affinity for antibiotic molecules), and the production of proteins that protect the target site (e.g., quinolone resistance mediated by Qnr) or of target site-modifying enzymes. For *M. tuberculosis*, data have been analyzed to outline a set of SNPs which are appropriate as markers of the RIF and INH resistance of *M. tuberculosis* strains (22).

Detection of first-line drug resistance in M. tuberculosis

Ikryannikova *et al.* (22) developed a novel MALDI-TOF MS-based minisequencing method for the analysis of rifampin and isoniazid resistance of *M.tuberculosis* strains. Jiang *et al.* (23) compared the proteomes of isoniazid-resistant *M. tuberculosis* strains and isoniazid-susceptible strains by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) and MALDI-TOF-MS. The data obtained from peptide mass fingerprinting were used to conduct a protein database search, and the genes of all strains were sequenced. They concluded in all protein spot differences, five protein spots were upregulated in isoniazid-resistant strains and identified as Rv1446c, Rv3028c, Rv0491, Rv2971, and Rv2145 by (MALDI-TOF-MS) and these results suggested that the differentially expressed proteins from isoniazid-resistant strains might be used as potential immunodiagnostic antigens and candidate novel drug targets against drug resistant tuberculosis.

Determination of second-line drug resistance in M. tuberculosis

Simner *et al.* (24) used PCR coupled with electrospray ionization mass spectrometry (PCR-ESI-MS) for detection and identification of Mycobacterium spp. and *M. tuberculosis* complex (MTBC) resistance determinants from solid and broth Middlebrook culture media. The performance of the MDR-TB assay was compared to identification using nucleic acid hybridization probes and *16S rRNA* gene sequencing for 68 MTBC and 97 NTM isolates grown on agar and 107 cultures grown in Bactec MGIT broth. MTBC resistance profiles from the MDR-TB assay were compared to results with the agar proportion method.

The PCR-ESI-MS system correctly identified all MTBC isolates and 97.9% and 95.8% of the NTM isolates from characterized agar cultures and MGIT broth cultures to the species level, respectively. In comparison to the agar proportion method, the sensitivity and specificity for the detection of drug resistance using the MDR-TB assay were 100% and 92.3% for rifampin, 100% and 93.8% for isoniazid, 91.6% and 94.4% for ethambutol, and 100% and 100% for fluoroquinolones, respectively. The MDR-TB assay appears to be a rapid and accurate method for the simultaneous detection and identification of mycobacterial species and resistance determinants of MTBC from culture.

Kumar *et al*. performed a proteomic analysis of *M. tuberculosis* isolates resistant to kanamycin and amikacin using two dimensional gel electrophoresis (2DGE), MALDI-TOF and bioinformatic tools such as BLASTP, InterProScan, KEGG motif scan and molecular docking. The major finding implicates that two hypothetical proteins (Rv3867 and Rv3224) might be playing some crucial role in contributing resistance to second line drugs. Further application in this direction may lead to the development of newer therapeutics against tuberculosis (25).

In brief, MALDI-TOF MS is used to detect the probable proteins or oligonucleotides related to the resistance, based on the specific peptide mass fingerprinting in protein database, and the drug susceptibility of *M. tuberculosis* can be resolved. These results also suggest that the differentially expressed proteins from resistant strains might be used as potential immuno-diagnostic antigens and novel drug candidates against drug resistant tuberculosis.

Future prospects

MALDI-TOF MS has been successfully adapted for the routine identification of mycobacteria with standard protocols for sample preparation and standard approaches to quantify reproducibility. This revolutionary technique allows for easier, cheaper and faster diagnosis of mycobacterial pathogens compared with conventional phenotypic identification methods. However, detection of drug resistance by mass spectrometry is still in the initial stages of exploration, and more efforts should be focused on this aspect in the future.

Acknowledgements

Funding: This work was supported by the National Natural Science Foundation of China (grant number: 81073095).

Disclosure: The authors declare no conflict of interest.

References

1. Zhao Y, Xu S, Wang L, et al. National survey of drug-resistant tuberculosis in China. N Engl J Med 2012;366:2161-70.
2. Lienhardt C, Glaziou P, Uplekar M, et al. Global tuberculosis control: lessons learnt and future prospects. Nat Rev Microbiol 2012;10:407-16.
3. Richter E, Rüsch-Gerdes S, Hillemann D. Evaluation of the GenoType mycobacterium assay for identification of mycobacterial species from cultures. J Clin Microbiol 2006;44:1769-75.
4. Lee AS, Jelfs P, Sintchenko V, et al. Identification of non-tuberculous mycobacteria: utility of the GenoType Mycobacterium CM/AS assay compared with HPLC and 16S rRNA gene sequencing. J Med Microbiol 2009;58:900-4.
5. Woo PC, Lau SK, Teng JL, et al. Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin Microbiol Infect 2008;14:908-34.
6. Song SH, Park KU, Lee JH, et al. Electrospray ionization-tandem mass spectrometry analysis of the mycolic acid profiles for the identification of common clinical isolates of mycobacterial species. J Microbiol Methods 2009;77:165-77.
7. Fournier PE, Drancourt M, Colson P, et al. Modern clinical microbiology: new challenges and solutions. Nat Rev Microbiol 2013;11:574-85.
8. Kemptner J, Marchetti-Deschmann M, Mach R, et al. Evaluation of matrix-assisted laser desorption/ionization (MALDI) preparation techniques for surface characterization of intact Fusarium spores by MALDI linear time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 2009;23:877-84.
9. Eigner U, Holfelder M, Oberdorfer K, et al. Performance of a matrix-assisted laser desorption ionization-time-of-flight mass spectrometry system for the identification of bacterial isolates in the clinical routine laboratory. Clin Lab 2009;55:289-96.
10. Murray PR. What is new in clinical microbiology-microbial identification by MALDI-TOF mass spectrometry: a paper from the 2011 William Beaumont Hospital Symposium on molecular pathology. J Mol Diagn 2012;14:419-23.
11. Hettick JM, Kashon ML, Slaven JE, et al. Discrimination

of intact mycobacteria at the strain level: a combined MALDI-TOF MS and biostatistical analysis. Proteomics 2006;6:6416-25.
12. El Khéchine A, Couderc C, Flaudrops C, et al. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry identification of mycobacteria in routine clinical practice. PLoS One 2011;6:e24720.
13. Croxatto A, Prod'hom G, Greub G. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. FEMS Microbiol Rev 2012;36:380-407.
14. Lotz A, Ferroni A, Beretti JL, et al. Rapid identification of mycobacterial whole cells in solid and liquid culture media by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2010;48:4481-6.
15. Machen A, Kobayashi M, Connelly MR, et al. Comparison of heat inactivation and cell disruption protocols for identification of mycobacteria from solid culture media by use of vitek matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2013;51:4226-9.
16. Mather CA, Rivera SF, Butler-Wu SM. Comparison of the bruker biotyper and vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry systems for identification of mycobacteria using simplified protein extraction protocols. J Clin Microbiol 2014;52:130-8.
17. Dang NA, Kuijper S, Walters E, et al. Validation of biomarkers for distinguishing Mycobacterium tuberculosis from non-tuberculous mycobacteria using gas chromatography-mass spectrometry and chemometrics. PLoS One 2013;8:e76263.
18. Kataria J, Rukmangadachar LA, Hariprasad G, et al. Two dimensional difference gel electrophoresis analysis of cerebrospinal fluid in tuberculous meningitis patients. J Proteomics 2011;74:2194-203.
19. Shakil S, Khan R, Zarrilli R, et al. Aminoglycosides versus bacteria--a description of the action, resistance mechanism, and nosocomial battleground. J Biomed Sci 2008;15:5-14.
20. Gutsmann T, Seydel U. Impact of the glycostructure of amphiphilic membrane components on the function of the outer membrane of Gram-negative bacteria as a matrix for incorporated channels and a target for antimicrobial peptides or proteins. Eur J Cell Biol 2010;89:11-23.
21. Hsieh YC, Su LH, Hsu MH, et al. Alterations of penicillin-binding proteins in pneumococci with stepwise increase in β-lactam resistance. Pathog Dis 2013;67:84-8.
22. Ikryannikova LN, Afanas'ev MV, Akopian TA, et al. Mass-spectrometry based minisequencing method for the rapid detection of drug resistance in Mycobacterium tuberculosis. J Microbiol Methods 2007;70:395-405.
23. Jiang X, Zhang W, Gao F, et al. Comparison of the proteome of isoniazid-resistant and -susceptible strains of Mycobacterium tuberculosis. Microb Drug Resist 2006;12:231-8.
24. Simner PJ, Buckwalter SP, Uhl JR, et al. Identification of mycobacterium species and mycobacterium tuberculosis complex resistance determinants by use of PCR-electrospray ionization mass spectrometry. J Clin Microbiol 2013;51:3492-8.
25. Kumar B, Sharma D, Sharma P, et al. Proteomic analysis of Mycobacterium tuberculosis isolates resistant to kanamycin and amikacin. J Proteomics 2013;94:68-77.

Cite this article as: Zhang R, Long Y, He W, Hao X, Liu J. Application status of MALDI-TOF mass spectrometry in the identification and drug resistance of *Mycobacterium tuberculosis*. J Thorac Dis 2014;6(5):512-516. doi: 10.3978/j.issn.2072-1439.2014.02.19