Rapid identification of nontuberculous mycobacteria directly from positive primary MGIT cultures by MALDI-TOF MS

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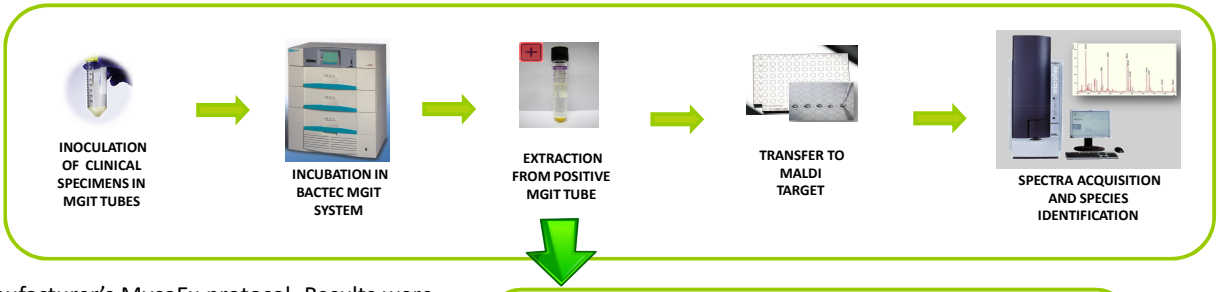
Introduction

Over the last years, nontuberculous mycobacteria (NTM) have emerged as important human pathogens. Accurate and rapid mycobacterial species identification is needed for successful diagnosis, treatment, and management of infections caused by NTM. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was demonstrated to effectively identify mycobacteria isolates subcultured from solid or liquid media rather than new positive cultures. The aim of the present study is to develop a rapid method for direct identification of NTM from primary MGIT cultures by MALDI-TOF MS.

Materials and Methods

A total of 20 positive MGIT broths, collected from February to July 2021, were examined by the Bruker Biotyper system with Mycobacteria Library v 2.0 (Bruker Daltonics). Extraction was performed within 24 h after automated growth detection by MGIT.

Protein extraction was carried out by the manufacturer's MycoEx protocol. Results were compared with those obtained by the Line probe assay GenoType Mycobacterium CM/AS/NTM-DR (Hain LifeScience).



EXTRACTION PROTOCOL

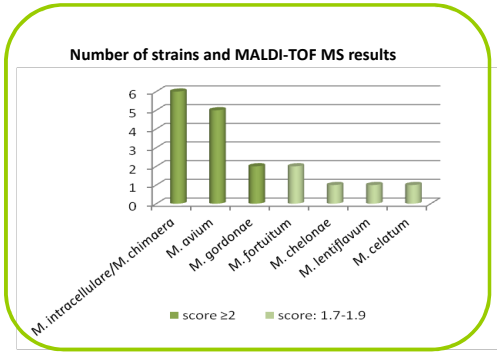
1. Resuspend mycobacterial biomass in 300 µl H_2O
2. Add 900 µl EtOH
3. Centrifugate and remove EtOH
4. Allow the pellet to dry
5. Add 40 mg 0.5 mm zirconia/silica beads
6. Add 25 µl acetonitrile
7. Vortex for 1 min
8. Add 25 µl 70% formic acid
9. Centrifuge at max speed for 2 min
10. Pipette 1 µl of supernatant onto the MALDI target

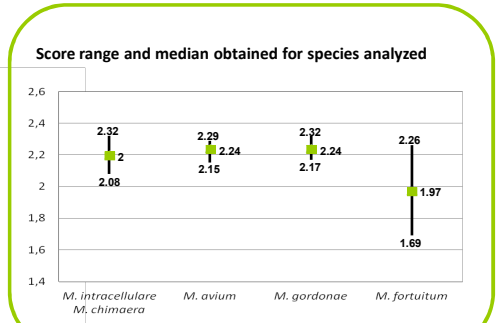
Results

Our results showed concordant identification for all the mycobacteria isolated. In particular, the molecular test identified the mycobacteria as *M. avium* (n. 5), *M. intracellulare* (n. 3), *M. chimaera* (n. 3), *M. gordonae* (n. 2), *M. fortuitum* (n. 2), *M. tuberculosis complex* (n. 2), *M. chelonae* (n. 1), *M. lentiflavum* (n. 1) and *M. celatum* (n. 1). All identifications based on MALDI-TOF MS had scores >1.7 and were concordant with the molecular identifications. MALDI-TOF MS cannot differentiate between *M. intracellulare* and *M. chimaera*, two closely related potentially pathogenic species of NTM that are members of the *M. avium* complex.

Number of strains identified

Species identification	Genotype	MALDI-TOF MS
M. avium	5	5
M. intracellulare	3	6
M. chimaera	3	
M. gordonae	2	2
M. fortuitum	2	2
M. chelonae	1	1
M. lentiflavum	1	1
M. celatum	1	1





Conclusions

Although a small number of strains and a limited diversity of mycobacterial species were analysed, our results indicate that MALDI-TOF MS could represent a useful routine diagnostic tool for rapid identification of mycobacterial species directly from primary liquid culture. The MALDI-TOF MS is a reliable method that could provide a shorter and less time-consuming workflow than Line probe assay for NTM infection diagnosis.