

**Identification of rapidly growing mycobacteria by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry**

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Nontuberculous mycobacteria (NTM) are responsible for increasingly common opportunistic infections. Differentiation to species level is important in order to determine clinical significance and initiate adequate treatment. Nowadays, identification of mycobacteria is based mainly on standard biochemical tests and molecular methods, including PCR, sequencing and hybridization assays using specific probes. More recently, mass spectrometry, which compares protein profiles, has been introduced as an identification tool for a great variety of microorganisms. Objectives: The purpose of this study was to evaluate the capability of the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to characterize rapidly growing mycobacteria (RGM) isolated from patients' samples and previously identified through molecular typing methods. Methods: In the present study, strains of RGM isolated during the year 2011 from clinical samples of patients attended at the University Hospital La Fe of Valencia, Spain, were analyzed at the Microbiology Department. NTM were identified by a PCR-based hybridization assay that consists of multiplex amplification and reverse hybridization (GenoType<sup>®</sup> Mycobacterium CM/AS, HAIN Lifescience). For mass spectrometry (MS) identification, an extraction of each isolate was prepared using formic acid and acetonitrile. This extract was analysed in a microflex mass spectrometer (Bruker Daltonik GmbH, Bremen) and the spectra obtained were compared with the MALDI Biotyper 2.0 database, which includes 23 RGM. Results: A total of 16 strains of RGM were identified using the molecular method described, obtaining 3 *Mycobacterium abscessus*, 6 *M. fortuitum* and 7 *M. chelonae*. MS identified 10 (62.5%) strains as mycobacteria and was concordant to species level in 5 (31,3%) of them - 2 *M. abscessus* and 3 *M. fortuitum*. The score value was less than 2,0 in all cases. The identifications and score values obtained are presented in Table 1, together with the best mycobacterial match proposed by the MALDI Biotyper. Conclusions: MS is not an adequate diagnostic tool for RGM by itself nowadays. Score values obtained showed no reliable identification in most cases. However, we believe that in the near future this technique will further improve as the quality of the available mycobacterial databases or the extraction protocols improve.

	HAIN	MALDITOF	Score	Best Mycobacterial Option	(Rank) Score
1	<i>Mycobacterium fortuitum</i>	<i>Mycobacterium fortuitum</i>	1,503	same	1
2	<i>Mycobacterium abscessus</i>	<i>Mycobacterium abscessus</i>	1,757	same	1
3	<i>Mycobacterium fortuitum</i>	<i>Blastomonas ursincola</i>	1,301	none	-
4	<i>Mycobacterium chelonae</i>	<i>Lactobacillus satsumensis</i>	1,398	none	-
5	<i>Mycobacterium chelonae</i>	<i>Cryptococcus neoformans</i>	1,196	none	-
6	<i>Mycobacterium abscessus</i>	<i>Arthrobacter castelli</i>	1,694	<i>M. abscessus</i>	(3) 1,581
7	<i>Mycobacterium fortuitum</i>	<i>Mycobacterium fortuitum</i>	1,243	same	1
8	<i>Mycobacterium chelonae</i>	<i>Gordonia rubripertineta</i>	1,306	none	-
9	<i>Mycobacterium abscessus</i>	<i>Mycobacterium abscessus</i>	1,611	same	1
10	<i>Mycobacterium chelonae</i>	<i>Mycobacterium avium</i>	1,607	same	1
11	<i>Mycobacterium chelonae</i>	<i>Mycobacterium abscessus</i>	1,951	same	1
12	<i>Mycobacterium chelonae</i>	<i>Mycobacterium abscessus</i>	1,565	same	1
13	<i>Mycobacterium fortuitum</i>	<i>Mycobacterium peregrinum</i>	1,981	same	1
14	<i>Mycobacterium chelonae</i>	<i>Mycobacterium abscessus</i>	1,68	same	1
15	<i>Mycobacterium fortuitum</i>	<i>Mycobacterium fortuitum</i>	1,497	same	1
16	<i>Mycobacterium fortuitum</i>	<i>Listeria innocua</i>	1,462	<i>M. fortuitum</i>	(7) 1,301