Identification of Nontuberculous Mycobacteria in MGIT by Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry

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Abstract

Background: Although Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been shown to correctly identify 97% of *Mycobacterium* species cultured on solid media, it is unclear how well MALDI-TOF MS performs in identifying species of nontuberculous mycobacteria (NTM) cultured using the BACTEC Mycobacteria Growth Indicator Tube (MGIT) liquid broth system.

Material/methods: Of the 151 consecutive MGIT-positive cultures, 53 (35.1%) were *M. tuberculosis* complex (MTBC) isolates and were excluded from the study. Therefore, a total of 98 (64.9%) NTM isolates were used to compare the performance of the Bruker Biotyper MALDI-TOF MS system with that of the BluePoint MycoID *plus* kit (Bio Concept Corporation, Taiwan), conventional identification methods and sequencing analysis of the 16S rRNA gene for species-level identification.

Results: The most prevalent species were *M. intracellulare*, followed by *M. abscessus* complex, *M. avium* complex and *M. avium*. Among the 98 NTM isolates, 86 (87.8%) and 44 (44.9%) isolates were correctly identified to the species level by the BluePoint MycoID *plus* kit and Bruker Biotyper MALDI-TOF MS system, respectively. Bruker Biotyper MALDI-TOF MS system correctly identified 15 (75.0%) of 20 *M. abscessus* complex, 6 (50.0%) of 12 *M. avium*, 13 (56.5%) of 23 *M. intracellulare*, 3 (21.4%) of 14 *M. avium* complex, 1 (33.3%) of 3 *M. fortuitum*, 4 (66.7%) of 6 *M. gordonae*, 1 (33.3%) of 3 *M. kansasii*, 2 (100%) of 2 *M. mageritense*, 0 (0%) of 1 *M. neoaurum*, and 0 (0%) of 1 *M. szulgai*. A total of 15 (15.3%) isolates were falsely identified as MTBC by the Bruker Biotyper MALDI-TOF MS system and all had low confidence scores (<1.700).

Conclusions: The overall correlation between Bruker Biotyper MALDI-TOF MS system and the other reference methods in identifying species of NTM cultured in the flagged BACTEC MGIT liquid broth media was 44.9%. Further optimization of the procedure in liquid media inoculated with clinical specimens is needed to improve the rate of identification

Introduction

The BluePoint MycoID *plus* kit (Bio Concept Corporation, Taiwan), an identification technique based on reverse hybridization of polymerase chain reaction products with an oligonucleotide membrane array, has been demonstrated to be able to identify 2 NTM complexes, 18 NTM species/groups and *Mycobacterium tuberculosis* complex.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is increasingly being used in laboratories to rapidly *Mycobacterium* species.

The purpose of this study was to evaluate the performance of MALDI-TOF MS in identifying MGIT-positive cultures inoculated with clinical specimens of NTM by comparing the accuracy of identification with that of identification by the BluePoint MycoID *plus* kit, conventional biochemical and phenotypic methods, and nucleic acid sequencing.

Materials & methods

Clinical specimens and identification

During June to July 2014, a total of 151 consecutive positive MGIT cultures from 151 patients were collected. A total of 98 NTM isolates were further identified with the BluePoint MycoID *plus* kit (Bio Concept Corporation, Taiwan) according to the instructions supplied by the manufacturer [13].

Mass spectra acquisition

Sequencing analysis

Sequencing analysis of the 16S rRNA gene was performed using two primers F (5'-GAAGAGTTTGATCMTGGCTC-3' and R (5'-GCGTGGACTACCAGGGTATC-3'). The resulting sequence was used for a BLAST search [13] and the percentages of sequence similarity >99% were used as the final identification

TABLE 2 Performance of species identification by MALDI-TOF MS

Species	% of concordant identification			
	MALDI confidence scores			All
	<1.700	1.700-1.999	⩾2.000	
M. abscessus complex	44.4 (4/9)	100.0 (5/5)	100.0 (5/5)	73.7 (14/19)
M. abscessus complex & *M. intracellulare*		0.0 (0/1)		0.0 (0/1)
M. avium	50.0 (5/10)	100.0 (1/1)	0.0 (0/1)	50.0 (6/12)
M. fortuitum	0.0 (0/1)		50.0 (1/2)	33.3 (1/3)
M. gordonae	66.7 (4/6)			66.7 (4/6)
M. intracellulare	47.1 (8/17)	100.0 (4/4)	100.0 (1/1)	59.1 (13/22)
M. kansasii	33.3 (1/3)			33.3 (1/3)
M. mageritense	100.0 (2/2)			100.0 (2/2)
M. neoaurum	0.0 (0/1)			0.0 (0/1)
M. szulgai	0.0 (0/1)			0.0 (0/1)
M. avium complex	16.7 (2/12)	50.0 (1/2)		21.4 (3/14)
No mycobacteria detected	0.0 (0/7)	0.0 (0/1)		0.0 (0/8)
Total	37.7 (26/69)	78.6 (11/14)	77.8 (7/9)	47.8 (44/92)

MALDI-TOF MS: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

TABLE 1 Species identification by the BluePoint MycoID *plus* kit and MALDI-TOF MS

Final identification	BluePoint MycoID *plus* kit[a]	MALDI-TOF MS	No. of isolates			
			MALDI confidence scores			
			<1.700	1.700-1.999	≥2.000	All
M. abscessus complex	*M. abscessus* complex	*M. abscessus subsp. abscessus*	3	3	5	11
		M. abscessus subsp. bolletii	1	2		3
		M. celatum	**1**			**1**
		M. duvalii	**1**			**1**
		M. immunogenum	**1**			**1**
		M. mageritense	**1**			**1**
		M. neworleansense	**1**			**1**
M. abscessus complex & *M. intracellulare*	*M. abscessus* complex & *M. intracellulare*	***M. abscessus subsp. bolletii***		**1**		**1**
M. avium	*M. avium*	*M. avium subsp. avium*	3	1		4
		M. avium subsp. paratuberculosis	1			1
		M. avium subsp. silvaticum	1			1
		M. abscessus subsp. abscessus	**1**			**1**
		***M. chimaera intracellulare* group**			**1**	**1**
		M. moriokaense	**1**			**1**
		***M. tuberculosis* complex**	**3**			**3**
		M. fortuitum subsp. fortuitum			1	1
M. fortuitum	*M. fortuitum*	***M. tuberculosis* complex**	**1**			**1**
	No mycobacteria detected	***M. mageritense***			**1**	**1**
		M. gordonae	4			4
M. gordonae	*M. gordonae*	***M. setense***	**1**			**1**
		M. chelonae	**1**			**1**
	***Mycobacterium* species**	*M. chimaera intracellulare* group	8	4	1	13
M. intracellulare	*M. intracellulare*	***M. duvalii***	**1**			**1**
		M. fortuitum* subsp. *fortuitum	**1**			**1**
		M. hiberniae	**1**			**1**
		M. poriferae	**1**			**1**
		M. stomatepiae	**1**			**1**
		***M. tuberculosis* complex**	**4**			**4**
		M. kansasii	1			1
M. kansasii	*M. kansasii*	***M. boenickei***	**1**			**1**
	No mycobacteria detected	***M. szulgai***	**1**			**1**
M. mageritense	***Mycobacterium* species**	*M. mageritense*	2			2
M. neoaurum	***Mycobacterium* species**	***M. tuberculosis* complex**	**1**			**1**
M. szulgai	*M. szulgai*	***M. duvalii***	**1**			**1**
M. avium complex	*M. avium* complex	*M. chimaera intracellulare* group	1	1		2
		M. abscessus* subsp. *abscessus	**1**			**1**
		M. austroafricanum	**1**			**1**
		M. chelonae	**2**			**2**
		M. chitae	**1**			**1**
		M. noviomagense	**1**			**1**
	M. fortuitum	*M. chimaera intracellulare* group	1			1
	No mycobacteria detected	***M. duvalii***	**1**			**1**
		M. gordonae	**1**			**1**
	***Mycobacterium* species**	***M. chitae***	**1**			**1**
		M. duvalii	**1**			**1**
		M. gordonae		**1**		**1**
No mycobacteria detected	No mycobacteria detected	***M. chelonae***	**1**			**1**
		***M. chimaera intracellulare* group**	**1**			**1**
		M. duvalii	**1**			**1**
		M. fortuitum subsp. acetamidolyticum	**1**			**1**
		M. gordonae		**1**		**1**
⩾	⩾	***M. tuberculosis* complex**	**3**			**3**

[a]Isolates marked in bold indicate discrepant results between the results from the BluePoint MycoID *plus* kit/MALDI-TOP MS and final identification. MALDI-TOF MS: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry