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Improvement of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry identification of difficult-to-identify bacteria and its impact in the workflow of a clinical microbiology laboratory

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ABSTRACT

This study evaluates matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) capability for the identification of difficult-to-identify microorganisms. A total of 150 bacterial isolates inconclusively identified with conventional phenotypic tests were further assessed by 16S rRNA sequencing and by MALDI-TOF MS following 2 methods: a) a simplified formic acid-based, on-plate extraction and b) performing a tube-based extraction step. Using the simplified method, 29 isolates could not be identified. For the remaining 121 isolates (80.7%), we obtained a reliable identification by MALDI-TOF: in 103 isolates, the identification by 16S rRNA sequencing and MALDI TOF coincided at the species level (68.7% from the total 150 analyzed isolates and 85.1% from the samples with MALDI-TOF result), and in 18 isolates, the identification by both methods coincided at the genus level (12% from the total and 14.9% from the samples with MALDI-TOF results). No discordant results were observed. The performance of the tube-based extraction step allowed the identification at the species level of 6 of the 29 unidentified isolates by the simplified method. In summary, MALDI-TOF can be used for the rapid identification of many bacterial isolates inconclusively identified by conventional methods.

1. Introduction

The identification of most bacteria from clinical samples is still based on conventional phenotypic, time-consuming methods. For difficult-to-identify bacteria, phenotypic methods might be inconclusive, and the definitive identification is usually performed by sequencing the 16S rRNA gene (Janda and Abbott, 2007). This technique requires 12–24 h for a final identification, since DNA amplification followed by sequencing is performed. It also needs trained personnel, and the price per sample is relatively high—about $25 for both stands in our laboratory. Implementation of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) in the last few years has allowed a rapid, specific, and low-cost identification ($0.5–1 according to Lagacé-Wiens et al., 2012) of most common bacterial and fungal species with clinical interest, within a working shift (Bizzini et al., 2010; Eigner et al., 2009). Many studies have reported the successful identification with MALDI-TOF MS of streptococci and staphylococci (Szabados et al., 2010; Dubois et al., 2010; López Roa et al., 2012; Cherkaoui et al., 2011), Gram-positive rods (Alatoom et al., 2012), members of the Enterobacteriaceae family (Pavlovic et al., 2012; Risch et al., 2010; Steensels et al., 2011), anaerobic bacteria (Culebras et al., 2012; Fedorko et al., 2012), the HACEK group (Couturier et al., 2011), and yeasts such as *Candida* spp. (Spanu et al., 2012). However, information regarding the yield of MALDI-TOF in other difficult-to-identify bacterial isolates is not abundant (Alby et al., 2013; Lau et al., 2013; Melo Oliveira et al., 2013; Seng et al., 2013). The aim of this study was to evaluate the accuracy of MALDI-TOF MS for the identification of bacterial isolates that are difficult to identify by conventional phenotypic methods. For this purpose, 2 different protocols were performed for the treatment of samples prior to MALDI-TOF identification: a simplified, on-plate method that could save time and reagents and a more thorough in-tube method that includes a protein step.

2. Materials and methods

2.1. Setting

Ours is a 1550-bed tertiary referral teaching institution, attending a population of approximately 800,000 inhabitants. Our microbiology laboratory identifies an average of 1900 aerobic and facultative

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Table 1
Comparison of bacterial identification obtained by 16S rRNA sequencing (in files) and by MALDI-TOF MS (in columns) using the in-tube extraction method.

A) List of the Gram-positive, difficult-to-identify microorganisms analyzed

Gram-positive microorganisms	Number of isolates	Concordant ID at the species level Score ≥2.0	Concordant ID at the species level Score 1.7 ≥ × ≥ 2.0	Concordant ID at the species level Score ≤1.7	Concordant ID at the genus level Score 1.7 ≥ × ≥ 2.0	Concordant ID at the genus level Score ≤1.7	Not Reliable ID
Abiotrophia defectiva	1			1			
Actinobaculum schaalii	1	1					
Actinomyces spp.	1						1
Actinomyces urogenitalis	2	2					
Anaerococcus hydrogenalis	2	2					
Arthrobacter spp.	1					1	
Atopobium parvulum	1	1					
Bacillus cereus	4	2	1				1
Bacillus licheniformis	1	1					
Bacillus pumilus	1	1					
Cellulosimicrobium cellulans	2	2					
Corynebacterium accolens	1		1				
Corynebacterium amycolatum	1	1					
Corynebacterium aurimucosum	1		1				
C. mucifaciens	1	1					
Corynebacterium propinquum	1	1					
Corynebacterium simulans	1				1		
Corynebacterium striatum	3	2	1				
Corynebacterium ureicelerivorans	2				2		
D. hominis	4	3					1
Dietzia maris	1		1				
Enterococcus avium	1	1					
Enterococcus casseliflavus	1		1				
Enterococcus faecalis	1	1					
Enterococcus faecium	1	1					
Enterococcus gallinarum	2	2					
Enterococcus gilvus	1	1					
Enterococcus hirae	1	1					
Enterococcus raffinosus	3	2					1
Gemella morbillorum	1	1					
Gemella paradiacens	1						1
Granulicatella adiacens	2	1		1			
Kocuria rosea	1	1					
Lactobacillus paracasei	1	1					
Lactobacillus rhamnosus	1		1				
Leuconostoc lactis	1		1				
Leuconostoc mesenteroides	1	1					
Listeria innocua	1				1		
Listeria monocytogenes	1						1
Nocardia cyriacigeorgica	1				1		
Nocardia veterana	1						1
Nocardia wallacei	1						1
Olsenella uli	2	2					
Paenibacillus spp.	1						1
Propionibacterium avidum	1						1
Rothia mucilaginosa	2		1				1
Staphylococcus aureus	2		2				
Staphylococcus capitis	1	1					
Staphylococcus epidermis	1				1		
Staphylococcus lugdunensis	1	1					
Streptococcus anginosus	1				1		
Streptococcus constellatus	1	1					
Streptococcus dysgalactiae	1	1					
Streptococcus infantis	1				1		
Streptococcus lutetiensis	2	2					
Streptococcus mitis	2				2		
Streptococcus oralis	1		1				
Weissella confusa	1						1
Weissella paramesenteroides	1						1
TOTAL	80	42 (52.5%)	12 (15%)	2 (2.5%)	10 (12.5%)	1 (1.25%)	13 (16.25%)

B) List of the Gram-negative microorganisms

Gram-negative microorganisms	Number of isolates	Concordant ID at the species level Score ≥2.0	Concordant ID at the species level Score 1.7 ≥ *x* ≥ 2.0	Concordant ID at the species level Score ≤1.7	Concordant ID at the genus level Score 1.7 ≥ *x* ≥ 2.0	Concordant ID at the genus level Score ≤1.7	Not reliable ID
Acinetobacter johnsonii	1	1					
Acinetobacter lwoffi	2	2					
Acinetobacter ursingii	1	1					
Actinobacillus ureae	1	1					
Aeromonas hydrophila	1	1					

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