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Evaluation of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Rapid Identification of Beta-Hemolytic Streptococci[▽]

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This study was undertaken to evaluate matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) for the rapid identification of beta-hemolytic streptococci. We compared Bruker Biotyper 2.0 with Vitek2 coupled to the agglutination test. MALDI-TOF MS analysis of 386 beta-hemolytic streptococcal isolates yielded high-confidence identification to the species level for all 386 isolates. The Vitek2 gave high-confidence identification to the species level for 88% of *Streptococcus agalactiae* isolates (n = 269/306), 92% of *Streptococcus pyogenes* isolates (n = 48/52), and 39% of isolates of *Streptococcus dysgalactiae* serogroups C and G (n = 11/28).

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is a new technology for species identification based on the protein composition of microbial cells. Although the first descriptions of this method were published more than 10 years ago (5, 8), a wider use in routine microbiology laboratories became possible only recently when successful species identification for different genera was demonstrated (1, 4, 6, 7, 9, 11–14). The most prominent advantages of the technology are speed and low cost (running cost), provided that a quality-controlled database of reference spectra, including all relevant microorganisms, is available. Substantial efforts have led to standardized sample preparation protocols (2), leading to improved reproducibility, databases, and analytical tools (10, 12). These new-generation methods are compared with state-of-the art sequence-based and conventional biochemical identifications in the present study.

We evaluated the discriminative power of MALDI-TOF MS on 386 beta-hemolytic streptococcal isolates (306 isolates of *Streptococcus agalactiae*, 52 isolates of *Streptococcus pyogenes*, 10 isolates of *Streptococcus dysgalactiae* serogroup C, and 18 isolates of *Streptococcus dysgalactiae* serogroup G). Diagnostic accuracy was determined by comparing the MALDI-TOF MS system against conventional phenotypic characterization using the Gram Positive Identification Card (GP ID) of the Vitek2 system coupled to latex agglutination (Bio-Rad, Marnes-la-Coquette, France).

Samples originated from the following sources: gynecological swabs (n = 260), urine (n = 20), respiratory tract (n = 50), wound and skin swabs (n = 36), and blood cultures (n = 20). All suspect isolates were subcultivated on sheep blood agar plates at 37°C with 5% CO_2. After overnight growth, each sample was identified using the Gram Positive Identification card (GP ID) of the Vitek2 instrument coupled to latex agglutination to determine the Lancefield groups.

Bruker Biotyper version 2.0 platform with library V.3.1.1.0 with 3,740 database entries was used for MALDI-TOF MS identification. MALDI target plates were inoculated by applying a small amount of a single freshly grown overnight colony directly onto a ground steel MALDI target plate in a thin film. The microbial film was then overlaid with 1.5 μl of a MALDI matrix (a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile–2.5% trifluoroacetic acid) and allowed to dry at room temperature. The plate was inserted into the source of a MicroFlex MALDI-TOF MS instrument (Bruker Daltonics). Mass spectra were collected from 2,000 to 20,000 Da in linear ion mode, using 240 shots of a 20-Hz nitrogen laser for ionization. All spectra were analyzed using the Bruker Biotyper 2.0 software package and compared to reference spectra for identification. Isolates were tested in duplicate by MALDI-TOF MS and by Vitek2 according to the manufacturer's protocol. For MALDI-TOF MS analysis, species identification (score of ≥2.0), genus identification (score of ≤2.0 but ≥1.7), major error (incorrect genus), minor error (correct genus with incorrect species), and no identification (score of <1.7) were scored. Definite identification at the species level was accepted when the identification by phenotypic biochemical methods exactly matched the identification by MALDI-TOF MS. Whenever the methods yielded discordant results, we performed 16S rRNA gene sequencing as described in a previous publication (3).

MALDI-TOF MS analysis of 386 subcultured beta-hemolytic streptococcal isolates yielded high-confidence identification to the species level for all 386 isolates (score of >2.0) with 100% concordance with the Lancefield group classification as determined by the latex agglutination kit. The Vitek2 gave high-confidence identification to the species level (percent identification [%ID] of >95) for 269/306 isolates of *S. agalac-*

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TABLE 1. Accuracy of MALDI-TOF MS identification of 386 beta-hemolytic streptococcal isolates[a]

Organism group (no. of isolates) and identification parameter	No. of isolates (%) found by:		
	MALDI-TOF MS identification with score of >2.0	Vitek2 identification	16S rRNA gene sequencing
Streptococcus pyogenes (52)			
Species correct	52 (100)	48 (92)	4 (7.7)
Major error	0	2 (3.8)	
Minor error	0	0	
No identification	0	2 (3.8)	
Streptococcus agalactiae (306)			
Species correct	306 (100)	269 (88)	37 (12)
Major error	0	2 (0.7)	
Minor error	0	32 (10.5)	
No identification	0	3 (1.0)	
Streptococcus dysgalactiae (28)			
Species correct	28 (100)	11 (39)	17 (6.1)
Major error	0	7 (25)	
Minor error	0	1 (3.6)	
No identification	0	9 (32)	

[a] Isolates were tested by MALDI-TOF MS (duplicate) and Vitek2. Definite identification was accepted when the identificaton by phenotypic biochemical methods exactly matched the identification by MALDI-TOF MS identification or by 16S rRNA gene sequencing. For MALDI-TOF MS analysis, species identification (score of ≥2.0), genus identification (score of ≤2.0 but ≥1.7), major error (incorrect genus), minor error (correct genus with incorrect species), and no identification (score of ≤1.7) were scored.

tiae (88%), 48/52 isolates of *S. pyogenes* (92%), and 11/28 isolates of *S. dysgalactiae* serogroups C and G (39%). Fourteen isolates were not identified by Vitek2, 11 were incorrectly identified (major error), and 33 minor errors were obtained by Vitek2. The 33 minor errors were as follows: 32 isolates were identified ambiguously (%ID of 50) as *Streptococcus agalactiae* or *Streptococcus dysgalactiae* and one isolate was identified as *Streptococcus pluranimalium.* The 11 incorrect Vitek2 results (major error, %ID of >95) were as follows: six isolates identified as *Gemella haemolysans*, two isolates identified as *Kocuria rosea*, one isolate identified as *Lactobacillus gravieae*, and two isolates identified as *Kocuria varians.* Whenever high-confidence MALDI-TOF MS identification disagreed with Vitek2 results, 16S rRNA gene sequencing resolved identification in favor of the MALDI-TOF MS system. 16S rRNA gene sequencing confirmed MALDI-TOF MS identification in 100% (58 isolates) of the discordant results (Table 1).

Overall, this study demonstrates that a single assay can now routinely identify beta-hemolytic streptococci to the species level in less than 5 min per sample, at minimal cost (cost of reagents), and with high diagnostic accuracy. MALDI-TOF MS-based identification therefore provides much cheaper, faster, and more accurate identification of beta-hemolytic streptococcal species than conventional phenotypic identification methods. This is especially relevant for routine clinical microbiology laboratories, since most results can now be reported 1 day earlier.

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