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Comparison of Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry with VITEK2 Gram positive system for identification of beta hemolytic Streptococci

Yesim Besli[1]*, Meltem Ayas[2], Onur Karatuna[3], Isın Akyar[1]
[1] Acibadem University School of Medicine Medical Microbiology Department, Acibadem Labmed Clinical Laboratories, Istanbul, Turkey. [2] Acibadem Labmed Clinical Laboratories, Istanbul, Turkey. [3] Acibadem University School of Medicine, Medical Microbiology Department, Istanbul, Turkey.

Abstract

Background: Matrix–assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is known as a successful tool for bacterial identification and suitable for clinical diagnostics. The aim of our study was to evaluate diagnostic accuracy of MALDI-TOF MS system and VITEK2 Gram Positive Identification (GP ID) system for the identification of Beta Hemolytic Streptococci (BHS).

Materials and Methods: A total 148 BHS which were isolated from various clinical specimens were included in the study. Isolates were identified by Bruker Biotyper 3.4 and VITEK2 GP ID coupled to the agglutination test. Identification results for Bruker Biotyper 3.4 and VITEK2 GP ID systems were classified in three different categories as follows: correct identification, low level of discrimination, no identification/misidentification. Identification results of BHS were evaluated according to these categories.

Results: BHS isolates were defined as Streptococcus pyogenes (n: 69), *Streptococcus agalactiae* (n: 59), *Streptococcus dysgalactiae* serogroup C (n: 5), and *Streptococcus dysgalactiae* serogroup G (n: 15) according to latex agglutination assay. MALDI-TOF MS analysis of 148 BHS isolates yielded correct identification for 89.9% of *S. pyogenes*, 64.4% of *S. agalactiae*, and 95.0% *S. dysagalactiae* serogroup C and G; while VITEK2 GP ID gave correct identification for 62.3% of *S. pyogenes*, 67.8% of *S. agalactiae*, and 65.0% of *S. dysagalactiae* serogroup C and G.

Conclusions: MALDI-TOF MS-based identification provides faster and more accurate identification of beta-hemolytic streptococcal species than VITEK2 GP ID system.

Key words: *MALDI-TOF, VITEK, Beta Hemolytic Streptococci, S. pyogenes, S. agalactiae, S. dysagalactiae*

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***Corresponding Author:** Yeşim Beşli, Acıbadem Labmed Clinical Laboratories, Microbiology Department, Istanbul, Turkey. **Phone:** +90 216 500 4745;
E-mail: yesim.besli@acibademlabmed.com.tr
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Introduction

Streptococcus pyogenes and *Streptococcus agalactiae* are two species of beta-hemolytic streptococci (BHS) are simply identified by conventional methods and serogrouping (1-3). As for the rest, there are some challenges. Because *Streptococcus dysgalactiae* can contain both Lancefield groups C and G, and rarely A. Different species have been shown to contain Lancefield groups C and G, also (1,4).

However Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) is presented as a reliable tool to use in clinical laboratories, results for beta-hemolytic streptococci have been conflicting (5-7). The VITEK2 Gram Positive Identification (GP ID) system is a more commonly used bacterial identification tool in medical laboratories. In this system identification is primarily based on biochemical tests measuring carbon source utilization, inhibition and resistance, and enzymatic activities, and identification of BHS by VITEK2 requires supplemental tests (8, 9).

In order to evaluate diagnostic accuracy of two different systems, we compared identification results of MALDI-TOF MS system with VITEK2 GP ID system.

Material and methods

Bacterial isolates and conventional identification

A total of 148 BHS strains which isolated from throat (n: 74), urine (n: 43), female genital tract specimens (n: 20), skin and soft tissue samples (n: 6), lower respiratory tract specimens (n: 3), and blood (n: 2) sent to the Central Microbiology Department of Acibadem Labmed Clinical Laboratories, were included in the study. Colonies with pinpoint to medium size, and translucent hemolysis zone (beta-hemolysis) on 5% sheep blood agar plates were defined as BHS. Conventional methods such as gram stain, bacitracin and trimethoprim/sulfamethoxazole susceptibility test, PYR (pyrrolidonylarylamidase activity) test, and CAMP (Christie, Atkins, and Munch-Peterson) test were used in identification (1-3). Strains were stored in brain heart infusion broth with glycerol at -80°C, and were subcultured on 5% sheep blood agar plates, and incubated 5% CO2 at 35°C for 18–24 h. Lancefield grouping of those strains were achieved by the latex agglutination kit. Identification of the strains was also performed simultaneously by MALDI TOF MS (microflex LT, Bruker, Bremen, Germany) and VITEK 2 systems (bioMérieux, USA).

Latex agglutination assay

Lancefield Serogroups of isolates were determined by STREP Test Kit (Plasmatec, UK) which containing the Streptococcus group specific antiserum. According to the manufacturer's instructions; the purely isolated 2-5 BHS colonies were suspended in 300 µL of extraction enzyme and incubated for 15 min at 37 °C. Then 50 µL of this suspension was dropped on one drop of the reagent containing latex beads coated with the antibody against each group polysaccharide antigen which had previously dropped onto the test cards. Rotational movements were performed by orbital shaker (OS-20, Orbital Shaker, Biosan Ltd., Latvia) to determine visible agglutination of the latex particles.

MALDI-TOF

Bruker Daltonics Microflex LT system was used and measurements were performed according to manufacturer's recommended settings. Obtained spectra were analyzed using Maldi Biotyper Automation Control and Bruker Biotyper software version 3.4 (Bruker Daltonics, Bremen, Germany). MALDI TOF target plates were inoculated by applying a small amount of a single freshly grown overnight colony directly onto a ground steel MALDI TOF target plate in a thin film. The microbial film was then overlaid with 1.5 µL of a MALDI matrix (a saturated solution of -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, Bremen, Germany). 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.

Vitek 2 identification

According to the manufacturer's instructions; the inoculum suspension was prepared from the overnight, pure cultures that were grown on 5% sheep blood agar (bioMérieux). Bacterial suspensions were prepared extemporaneously by suspending bacterial isolates in 0.45% saline to the equivalent of a 0.5–0.63 McFarland turbidity standard with the VITEK-2 Densichek instrument. The ID-GP identification cards, that is a 64-well plastic card that includes 43 tests, were inserted the each tubes and data were analyzed using VITEK-2 database, version 06.00.014.

Interpretation Criteria

Identification results were classified in three different categories. For MALDI-TOF identification criteria used in our analysis were as follows: (i) correct identification (correct identification, with a score of 2.00 - 3.00, at the species level); (ii) low level of discrimination (correct identification, with a score of 1.70 to 1.99, at the genus level), (iii) no identification/misidentification (identification with a score of <1.70, or no identification). As for VITEK2 system, (i) correct identification is defined as identification to the species level as the only choice with a ≥ 93% probability; (ii) low level of discrimination (either identification to the genus level or a low level of discrimination between two or more species, including the correct species); (iii) no identification/misidentification (the species identified with the GP identification card was different from that identified by conventional identification and latex agglutination or organism was unidentified) (10-12).

Results

A total of 148 isolates of BHS were classified as group A (n: 69), group B (n: 59), group C (n: 5), group G (n: 15) by the latex agglutination method.

MALDI-TOF MS yielded correct identification to the species level in 89.9% (n: 62) of *S. pyogenes*, 64.4% (n: 38) of *S. agalactiae*, 95.0% (n: 19) of *S.dysagalactiae*, and low level of discrimination in 10.1% (n: 7) of *S. pyogenes*, 35.6% (n: 21) of S. agalactiae isolates. Overall MALDI-TOF MS provided identification to the species level in 80.4% (n: 119) of BHS and low level of discrimination in 18.9% (n: 28) of BHS while it failed to identify 0.7% (n: 1) of BHS.

VITEK2 GP system could correctly identify 62.3% (n: 43) of *S. pyogenes*, 67.8% (n: 40) of *S. agalactiae*, 65.0% (n: 13) of *S. dysagalactiae* isolates to the species level in. It provided low level of discrimination in 26.1% (n: 18) of *S .pyogenes*, 25.4% (n: 15) of *S. agalactiae*, 5.0% (n: 1) of *S. dysagalactiae* isolates. Identification to the species level in 64.9% (n: 96) of BHS and low level of discrimination in 22.9% (n: 34) of BHS was achieved by VITEK 2 GP, however 12.2% (n: 18) of BHS isolates were not identified or misidentified by this system (Table 1).

Table 1. Comparison of identification results of MALDI-TOF Mass Spectrometry with VITEK2 GP Identification for Beta hemolytic Streptococci.

	MALDI-TOF Mass Spectrometry			**VITEK2 GP Identification**		
Lancefield Group	Correct Identification	Low level of discrimination	No identification / Misidentification	Correct Identification	Low level of discrimination	No identification / Misidentification
A (n: 69)	*S. pyogenes* (n: 62)	*S. pyogenes* (n: 7)	-	*S. pyogenes* (n: 43)	*S. pyogenes* (n: 13) *S. pyogenes* / *S. dysgalactiae subsp. equisimilis* (n: 2) *S. pyogenes* / *S. agalactiae (n: 1)* *S. pyogenes* / *S. dysgalactiae subsp. equisimilis* / *S. dysgalactiae subsp. dysgalactiae* (n: 1) *S. pyogenes* / *S. suis* I / *S. suis* II (n: 1)	*Kocuria rosea* (n: 1) *Kocuria rhizophila* (n: 1) Unidentified microorganism (n: 5) *S. suis* I (n: 1)
B (n: 59)	*S. agalactiae* (n: 38)	*S. agalactiae* (n: 21)	-	*S. agalactiae* (n: 40)	*S. agalactiae* / *S. dysgalactiae subsp. equisimilis* (n: 7) *S. agalactiae* (n: 4) *S. agalactiae* / *S. dysagalactiae subsp. equisimilis* *S. constellatus* (n: 2) *S. agalactiae* / *S. constellatus subsp. constellatus* (n: 1) *S. agalactiae* /*S. dysagalactiae* / *S. constellatus subsp. constellatus* (n: 1)	*Pediococcus pentosaceus* (n: 2) Unidentified microorganism (n: 1) *S. sanguinis* (n: 1)
C (n: 5) and G (n:15)	*S.dysagalactiae* (n: 19)	-	Unidentified microorganism (n: 1)	*S. dysagalactiae* subsp. *equisimilis* (n: 6) *S. dysagalactiae* (n: 3) *S. dysagalactiae* / *S. dysgalactiae* subsp. *equisimilis* (n: 3) *S. dysgalactiae* subsp. *equisimilis* / *S. dysgalactiae* subsp. *dysgalactiae* (n:1)	*S. dysagalactiae* subsp. *equisimillis* / *S. pseudoporcinus* (n:1)	Unidentified microorganism (n:2) *S. pluranimalium* (n:1) *S. ovis* (1) *S. suis* I (n:2)
Total (n: 148)	119	28	1	96	34	18

n: Number of isolates.

Discussion

MALDI-TOF is concerned as a reliable and cost-effective method for bacteria identification in the microbiology laboratory, even so there are conflicts in identification results of BHS by this method (7, 11, 13-16). On the other hand VITEK 2 GP system is one of the respectable systems by providing reliable results for the identification of GP cocci under clinical microbiology laboratory conditions (8, 12). In the present study, identification by MALDI-TOF was achieved 99.3% of BHS in general, although low level of discrimination was observed at 10.1% of *S. pyogenes* and 35.6% of *S. agalactia*. VITEK2 GP identified 87.8% of all BHS but it gave lower than 93% probability rates for 26.1% of S. *pyogenes*, 25.4% of *S. agalactiae*. 95.0% of BHS which were classified Group C or G, were identified as *S. dysagalactiae* by MALDI-TOF. On the other hand, 65.0% of these isolates were identified as different subspecies of *S. dysagalactiae* by VITEK 2 GP system. Similarly, Cherkaoui et al. also compared MALDI-TOF MS with VITEK 2 GP system for the identification of 386 BHS. All of the isolates were identified with high confidence scores using MALDI-TOF, while only 85% of them were identified with high confidence using VITEK2 GP system (14). But identification results for BHS by MALDI-TOF varies based on low number of analyzed strains, preparation methods, differences in the amount of bacteria applied to the MALDI-TOF target, and the identification criteria used in interpreting MALDI-TOF results (2, 7, 11, 13-17). In the studies that are included higher number of BHS correct identification rates are 94-100% in *S.pyogenes*, 75-100% in S.agalactiae, and 93-100% in *S. dysagalactiae* isolates for MALDI-TOF identification (13, 14, 17). Also correct identification of bacteria using MALDI-TOF depends on the reference spectra present in the database, and the database of MALDI-TOFF system is regularly updated with spectra from species (7). Database analyzed spectra of the BHS in the present study is newer than the studies mentioned before (7, 9, 13-17). However the database we used does not include a major change for BHS, our correct identification rates were considerably high by using MALDI-TOFF. Besides, the VITEK 2 GP identification system is redesigned to achieve greater accuracy in the identification of Gram-positive cocci in routine laboratory (12). In this study, 87.8% of the BHS isolates were correctly identified by using VITEK2 GP identification system without application of any further additional tests.

Even both of MALDI-TOF and VITEK2 GP systems were identified all Lancefield group C (n: 5) and G (n: 15) isolates as *S. dysagalactiae*, we could not verify these isolates are *S. dysagalactia*. Since it is hard to differentiate species belonging to the Lancefield groups C and G streptococci phenotypically, it requires either molecular methods or standardized phenotypic identification systems (18, 19).

Conclusion

Our results have emphasized that MALDI-TOF MS is powerful tool for the identification of BHS. Considering that MALDI-TOF MS is cost effective in long term use, user friendly. It is appropriate in routine clinical microbiology laboratories in identifying BHS, as well most of clinically important bacteria.

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