Identification of Uncommon Pathogenic Bacteria by MALDI-TOF Mass Spectrometry Using a Custom Library of Siriraj Hospital

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ABSTRACT

Objective: Matrix Assisted Laser Desorption Ionization Time-Of-Flight mass spectrometry (MALDI-TOF MS) is a rapid and cost-effective technique that serves as an alternative method for bacterial identification in microbiology laboratories. This study aimed to determine the performance of MALDI-TOF MS for identifying uncommon and/or fastidious bacteria comparing with automated identification method.

Methods: We observed the performances of original and extended databases after adding of in-house MALDI-TOF MS database for a total of 54 isolates belonging to 16 genera of uncommon and/or fastidious bacteria.

Results: The extended database improved identification of these bacteria significantly except for *Listeria monocytogenes* and *Elizabethkingia meningoseptica*.

Conclusion: This study provides a possibility for identification of uncommon and/or fastidious bacteria by MALDI-TOF MS method.

Keywords: Mass spectrometry; MALDI-TOF; fastidious bacteria; identification (Siriraj Med J 2018;70: 127-130)

INTRODUCTION

Rapid and accurate identification of bacteria can be challenging in a routine laboratory. Conventional methods provide standard results, but they are time consuming and require complicated procedures.[1] The automated Vitek 2 (bioMérieux. Marcy l'Etoile, France) provides identification result faster than conventional method, which is approximately 4-8 hours. However, misidentification may still occur.[2] Thus, molecular techniques, particularly 16S rDNA gene sequencing, have been developed as standard methods for bacteria identification.[3] Recently, MALDI-TOF MS has been developed as a rapid bacterial identification method, taking only 6-8.5 minutes for direct transfer method[4] and only 45 minutes for formic acid extraction method.[5] Several studies have utilized MALDI-TOF MS for identifying Gram-positive and Gram-negative bacteria[6] including, difficult-to-identify or uncommon bacteria.[7,8] Previous studies reported that, after using a combined in-house and original database, the identification rates have been improved.[9,10] Thus, the development of in-house database provided a useful tool for bacterial identification. The aim of this study was to evaluate the ability of a combined Bruker's MALDI-TOF MS database (Bruker Daltonics, Helios, Singapore) and our in-house database (extended database) for species differentiation among uncommon and/or fastidious bacteria.

MATERIALS AND METHODS

Generation of a combined MALDI-TOF MS database

To improve the MALDI-TOF database, uncommon

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and/or fastidious bacterial species to be included in the MALDI-TOF MS database were selected from uncommon and/or fastidious bacterial species isolated at the Faculty of Medicine Siriraj Hospital, Mahidol University (Bangkok, Thailand). According to their availability, between one and three isolates of each species were used. This study was approved by the Siriraj Institutional Ethical Review Board (Si 147/2013). A total of 54 bacterial isolates belonging to 20 species within 16 genera were added to the library including both Gram-positive and Gram-negative bacteria, namely: *Abiotrophia defective* (n=3), *Aggregatibacter actinomycetemcomitans* (n=2), *Campylobacter fetus* (n=3), *Chryseobacterium gleum* (n=3), *Corynebacterium jeikeium* (n=3), *Eikenella corrodens* (n=2), *Elizabethkingia meningoseptica* (n=3), *Enterococcus casseliflavus* (n=3), *Enterococcus gallinarum* (n=3), *Gemella haemolysans* (n=3), *Granulicatella adiacens* (n=3), *Lactococcus formosensis* (n=3), *Leuconostoc lactis* (n=3), *Listeria monocytogenes* (n=3), *Providencia rettgeri* (n=3), *Providencia stuartii* (n=3), *Roseomonas mucosa* (n=1), *Vibrio fluvialis* (n=2), *Vibrio parahaemolyticus* (n=3), and *Vibrio vulnificus* (n=2). Identification of each isolate was confirmed by 16S rDNA sequencing according to previous publications.[11,12] MALDI-TOF MS was performed on a Microflex LT instrument following formic acid extraction according to the manufacturer's instructions (Bruker Daltonics, Bremen, Germany). In-house databases of the 55 isolates of uncommon and/or fastidious bacteria were constructed as previously described.[13] For each isolate, a minimum of 20 spectra were collected using Flexanalysis software version 3.0 (Bruker Daltonics) and used to construct a reference main spectrum profile for adding into an in-house database using the automated function of the BioTyper software (Bruker Daltonics). The in-house database was combined with the Bruker's database (5,627 database entries according to Bruker Daltonics version 3.0) and this combined database was used for subsequent analysis.

Comparisons of the identification efficacy between automated methods and MADI-TOF MS

MALDI Biotyper RTC (real-time classification) software was used for bacterial identification. The parameters were 40 shots/steps of laser beam and collection of 240 shots were employed. In this study, peaks with *m/z* ratios in the range of 4,000–15,000 were used for MALDI-TOF MS identification. As shown in a previous study, the performance of MALDI-TOF MS was increased when this peak range was used.[14] Bacterial protein spectra were analyzed using the combined database. An identification score of ≥ 2.000 and a score within the range 1.700 to 1.999 indicated identification of the species and genus levels, respectively. For automated identification, bacterial isolates were identified by the Vitek 2 system (bioMérieux) according to the manufacturer's instructions. Gram-positive (GP), Gram-negative (GN), anaerobes and *Corynebacterium* (ANC) and *Neisseria-Haemophilus* (NH) identification cards were used in this study. Results were accepted at the species level if scores of more than 93% probability were obtained.[15] Genus-level identification was accepted when results were less than or equal to 93% probability.

RESULTS

The extended database improved bacterial identification by MALDI-TOF MS in comparison to the original database. The extended database identified 15 more strains correctly to the species level (Table 1). This suggested the need for addition of an in-house database as the original (company) database is typically not optimized for endemic microbes of each region. A comparison between the automated method and extended database of MALDI-TOF MS showed that 10 species were identified correctly to the species level only by the MALDI-TOF MS. Eight species were identified correctly to the species level by both methods. *E. meningoseptica* and *L. monocytogenes* were identified to the species level only by automated method. Interestingly, *A. actinomycetemcomitans, C. jeikeium, E. casseliflavus, E. gallinarum* and *G. haemolysans* were specifically identified by MALDI-TOF MS, while automated identification method had limitations for these organisms. Automated method had low discriminatory power for identifications of *E. casseliflavus, E. gallinarum* and *G. haemolysans*. However, yellow pigment production and satellite test in combination with acid production enabled discrimination of *E. casseliflavus/E. gallinarum* and *G. haemolysans/G. sanguinis/G. adiacens*, respectively.[16] Thus, phenotypic test and biochemical testing should be considered to support species identification of automated method.

In contrast, *L. monocytogenes* was more appropriately identified using automated method than MALDI-TOF MS because MALDI-TOF MS technique was insufficient for identifying *Listeria* species. A previous study reported that *Listeria* species including *L. innocua, L. ivanovii, L. monocytogenes, L. seeligeri* and *L. welshimeri* were difficult to be differentiated by MALDI-TOF MS because they were closely related based on their spectra.[17] In addition, MALDI-TOF MS also showed difficulty in discriminating *E. meningoseptica* from other species within the same genus. However, this issue has been proposed to be solved by 10% differential rule. This rule

TABLE 1. Identification of 54 isolates by original and extended databases of MALDI-TOF MS.

Bacterial species	Level of identification (No. of isolates with species/genus/misidentification)		
	Automated method	MALDI-TOF MS original database	MALDI-TOF MS extended database
A. actinomycetemcomitans	0/2/0	1/1/0	**2/0/0
A. defectiva	3/0/0	1/2/0	3/0/0
C. fetus	3/0/0	1/2/0	3/0/0
C. gleum	0/3/0	2/1/0	**3/0/0
C. jeikeium	0/3/0	3/0/0	**3/0/0
E. casseliflavus	0/3/0	3/0/0	**3/0/0
E. corrodens	2/0/0	2/0/0	2/0/0
E. gallinarum	0/3/0	3/0/0	**3/0/0
E. meningoseptica	*3/0/0	0/1/2[a]	1/2/0
G. adiacens	3/0/0	3/0/0	3/0/0
G. haemolysans	0/3/0	3/0/0	**3/0/0
L. formosensis	0/3/0	0/0/3[b]	**3/0/0
L. lactis	0/3/0	0/3/0	**3/0/0
L. monocytogenes	*3/0/0	1/2/0	1/2/0
P. rettgeri	3/0/0	3/0/0	3/0/0
P. stuartii	3/0/0	3/0/0	3/0/0
R. mucosa	0/1/0	1/0/0	**1/0/0
V. fluvialis	0/2/0	0/1/1[c]	**2/0/0
V. parahaemolyticus	3/0/0	3/0/0	3/0/0
V. vulnificus	2/0/0	2/0/0	2/0/0
Total 54 isolates	28/26/0	35/13/6	50/4/0

Notes: MALDI-TOF MS (original database) misidentification results: a) *E. meningoseptica* as *E. miricola*, b) *L. formosensis* as *L. garvieae* and c) *V. fluvialis* as *V. furnissii*; * Species identified better with the automated method; **Species identified better with the extended database.

will exclude any genus or species that show difference values between the top best match and next closest scores of higher than 10%.[8,18] In this study, this rule was not able to be used for the discrimination of *Elizabethkingia* and *Listeria* species because they provided difference values less than 10%. Thus, the resolution of MALDI–TOF MS for those species should be of concern.

DISCUSSION

In summary, our study indicated that MALDI-TOF MS is a potential choice for identification of uncommon and/or fastidious bacteria. However, misidentification or low discrimination results were found for some species when identified by using MALDI-TOF MS. Thus, identification results should be carefully interpreted. Furthermore, some uncommon and/or fastidious bacteria including *Abiotrophia, Aggregatibacter, Gemella, Granulicatella* and *Leuconostoc* grew as tiny colonies. Therefore, in these cases formic acid extraction would be recommended for creating high quality of spectra. In contrast, the identification of the genera *Campylobacter, Chryseobacterium, Corynebacterium, Elizabethkingia, Enterococcus, Lactococcus, Listeria, Providencia, Roseomonas* and *Vibrio*, were reliable when identified by MALDI-TOF using direct transfer method which has been demonstrated (data not shown).

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