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Original article

Utility of the MALDI-TOF MS method to identify nontuberculous mycobacteria

Masahiro Kodana [a, 1], Norihito Tarumoto [b, *, 1], Tohru Kawamura [a], Taeko Saito [a], Hideaki Ohno [c], Shigefumi Maesaki [b], Kenji Ikebuchi [a]

[a] Clinical Laboratory Medicine, Saitama Medical University Hospital, Japan
[b] Department of Infectious Disease and Infection Control, Saitama Medical University, Japan
[c] Department of Infectious Disease and Infection Control, Saitama Medical Center, Saitama Medical University, Japan

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ABSTRACT

In comparison to the conventional real-time polymerase chain reaction method (PCR method) or the DNA–DNA hybridization method (DDH method), the utility of NTM identification by the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) method has seldom been reported.

In this study, 75 clinical NTM isolates from our hospital between April 2013 and July 2014 were identified and analyzed using PCR, DDH, and MALDI-TOF MS methods, and the results for the MALDI-TOF MS method were compared with the others. Identification at the species level was in agreement for 71 (94.5%) of the 75 isolates. For further details, identification was possible for 23 (95.8%) of 24 *Mycobacterium avium*, 11 (100%) of 11 *Mycobacterium intracellulare*, and 1 (50%) of 2 isolates mixed with *M. avium and M. intracellulare*. *Mycobacterium ksansasii*, *Mycobacterium abscessus*, *Mycobacterium fortuitum*, *Mycobacterium gordonae*, and *Mycobacterium chelonae* identified by DDH method were same result by MALDI-TOF MS. Additionally, *Mycobacterium mucogenicum*, which could not be identified by the DDH method, was identified by the MALDI-TOF MS method. However, two isolates identified as *Mycobacterium terrae* by DDH method could not be identified by the MALDI-TOF MS method and were determined to be *Mycobacterium arupense* by 16S ribosomal RNA (rRNA) sequence analysis.

The present findings show that, for rare bacterial species, identification is sometimes not possible, but, in most cases, the results of identification by the MALDI-TOF MS method have a high concordance rate with the results of the PCR and DDH methods.

1. Introduction

More than 160 bacterial species of nontuberculous mycobacteria (NTM) are known to exist, but only about 50 are reported to be pathogenic in humans, and the others are environmental bacteria. In Japan, *Mycobacterium avium* complex (MAC) accounts for the majority of infections [1,2]. Unlike pulmonary tuberculosis, human-to-human NTM infections do not occur, but the incidence of these infections is increasing [3–5]. Since treatment and response rates differ depending on the bacterial species, and some are refractory, identification of the bacterial species is clinically important.

In recent years, the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) method is increasingly being applied clinically for bacterial and fungal identification. The MALDI-TOF MS method is a method of identifying target bacterial species by comparing the mass spectral patterns of molecules, mainly ribosomal proteins, specific to bacteria and fungi in a library of known bacterial and fungal strains. The MALDI-TOF MS method is also reported as useful for identifying acid-fast bacteria [6].

Abbreviations: NTM, nontuberculous mycobacteria; MAC, *Mycobacterium avium* complex; PCR, polymerase chain reaction; DDH, DNA–DNA hybridization; MALDI-TOF MS, matrix assisted laser desorption/ionization-time of flight mass spectrometer; SV, score value.

* Corresponding author. 38 Morohongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan. Tel.: +81 49 276 2032; fax: +81 49 276 1188.
E-mail address: n-taru@umin.ac.jp (N. Tarumoto).

[1] Masahiro Kodana and Norihito Tarumoto contributed equally to this paper.

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In our institution, a teaching hospital, sputum samples for acid-fast bacteria testing are cultured on Mycobacteria Growth Indicator Tube (MGIT) medium (BD Diagnostic Systems, Sparks, MD, USA), and if the results are positive, the real-time polymerase chain reaction method (PCR method) is used to identify *Mycobacterium tuberculosis* and MAC. If the results of PCR are negative, the sample is further subcultured on Ogawa medium, and the DNA–DNA hybridization method (DDH method) is used for bacterial species identification. Although the MALDI-TOF MS method may be convenient and cost-effective, whether it has equivalent utility compared to these other conventional methods has seldom been reported. Therefore, this study investigated the utility of the MALDI-TOF MS method for the identification of clinical NTM isolates.

2. Materials and methods

This study was performed using NTM that were isolated and identified from specimens submitted at our hospital between April 2013 and July 2014. The respiratory specimens were pretreated with semi-alkaline protease NALC-NaOH for digestion and decontamination. They were then cultured on a BACTEC MGIT 960 system (BD Diagnostic Systems). Positive cultures on the MGIT medium were analyzed by the PCR method using the Cobas Amplicor MTB test (Roche, Basel, Switzerland) to identify *M. tuberculosis*, *M. avium*, and *Mycobacterium intracellulare*. The quality of spectra are worse by direct use from liquid medium than solid medium and it will be more difficult to identify species [7,8], so 100 μL of MGIT positive culture was sequentially inoculated onto 2% Ogawa medium and cultured at 37 °C for approximately 1–2 weeks. After colony formation, the DDH method using a DDH Mycobacteria kit (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) was performed for no identification by PCR method.

The MALDI-TOF MS method was performed according to the manufacturer's instructions. Colonies that grew on Ogawa medium were suspended with 300 μL of distilled water in a tube. After heating at 95 °C for 30 min, 900 μL of 99.5% alcohol were added, the sample was centrifuged at 13,000 rpm for 2 min, the supernatant was discarded, and the sample was completely dried at room temperature. An equivalent amount of 0.5-mm silica beads and 20 μL of acetonitrile were added to the sediment, and the sample was vortexed for 5 min. Next, 20 μL of 70% formic acid were added, and the sample was vortexed and centrifuged (13,000 rpm, 2 min). Then, 1 μL of the supernatant was placed on a polished steel MSP 48-target plate (Bruker Daltonics, Inc., Billerica, MA) and dried, and 1 μL of matrix was added. After drying, the sample was measured and analyzed by mass spectrometry (MALDI Biotyper 3.1 software and MALDI Biotyper Reference Library version 4.0.0.1 and Mycobacteria Library version 1.0; Bruker Daltonics, Inc.). A score value (SV) of $\geq$2.0 was defined as identification at the species level, an SV of 1.7 to <2.0 as identification at the genus level, and an SV < 1.7 as unable to identify.

For rare bacterial species identified by the MALDI-TOF MS method but that could not be identified by the DDH method, and for those identified by the DDH method but that could not be identified by the MALDI-TOF MS method, sequence analysis of the 16S ribosomal RNA (rRNA) gene was performed. Comparisons were made with sequences from the GenBank database for identification.

This study was approved by the local institutional review boards and ethics committees (No. 14-096).

3. Results

There were 75 NTM isolates, and among these, 37 isolates were identified by the PCR method as *M. avium* or *M. intracellulare* (*M. avium*, 24 isolates; *M. intracellulare*, 11 isolates; 2 isolates positive for both) (Table 1). In addition, among NTM when *M. avium* and *M. intracellulare* were excluded, 36 isolates were identified by the DDH method (*Mycobacterium kansasii*, *Mycobacterium abscessus*, *Mycobacterium fortuitum*, *Mycobacterium gordonae*, *Mycobacterium terrae*, and *Mycobacterium chelonae*). Two isolates could not be identified by either PCR or DDH methods.

Using the MALDI Biotyper system, among all 75 isolates, the SV was $\geq$1.7 in 71 strains (71/75, 94.7%) and $\geq$2.0 in 42 strains (42/75, 56.0%). These included 23 isolates (23/24, 95.8%) and 13 isolates (13/24, 54.2%), respectively, for *M. avium*; and 11 isolates (11/11, 100%) and 6 isolates (6/11, 54.5%), respectively, for *M. intracellulare*. Of the 2 isolates positive for both *M. avium* and *M. intracellulare*, the MALDI-TOF MS method for one isolate was positive for *M. intracellulare*. Among all 37 MAC isolates, SV was $\geq$1.7 for 35 isolates (35/37, 94.6%) and $\geq$2.0 for 19 isolates (19/37, 51.4%). Among all 38 NTM isolates other than MAC, 36 isolates (36/38, 94.7%) and 23 isolates (23/38, 60.5%) could be identified by the MALDI-TOF MS method. For the isolates not identified by either PCR or DDH methods, *M. kansasii* and *Mycobacterium mucogenicum* were identified using the MALDI Biotyper system (SV 1.913 and 1.812, respectively).

Two isolates were identified as *M. terrae* by DDH method, but not identified by MALDI-TOF MS method, so the isolate was sequenced, and the analysis showed 99.9% homology to *Mycobacterium arupense* (790/791 bp) (National Center for Biotechnology Information (NCBI), accession numbers JX119205), and identified as *M. arupense*. Additionally, *M. mucogenicum* was also sequenced because the species was rare in the clinical situation, so the isolate was sequenced, and the analysis showed 100% homology to *M. mucogenicum* and *Mycobacterium phocaicum* (754/754 bp) (NCBI, accession numbers AY859682), and identified as *M. mucogenicum*. One isolate identified as *M. kansasii* by MALDI-TOF MS method but not by DDH method was not sequenced.

4. Discussion

The MALDI-TOF MS method has attracted interest for identification of bacteria because it is rapid, accurate, and has low operational costs [6]. In the present study, the results of the MALDI-TOF MS method for NTM identification were compared with those of the conventional PCR and DDH methods. There was good agreement in the results of identified bacterial species between the MALDI-TOF MS method and the PCR and DDH methods. In addition, the MALDI-TOF MS method successfully identified two isolates that are difficult to identify by the DDH method. One of these, *M. mucogenicum*, is a bacterial species that cannot be detected by the DDH method. This demonstrates the utility of the MALDI-TOF MS method.

However, rarely, some NTM isolates could not be identified by the MALDI-TOF MS method. As a first reason for no identification, it is suspected that the number of the bacterial species to be analyzed might not be enough in the MALDI Biotyper library. Generally, if the registered patterns of the species are few, the identification will be sometimes difficult. In the present study, *M. arupense*, a species related to *M. terrae*, could not be identified by the MALDI-TOF MS method, and it was determined to be *M. arupense* on 16S rRNA sequence analysis. Only one strain of *M. arupense* is registered in the MALDI Biotyper library, so it probably could not be identified, because differences could not be detected in the pattern between the *M. arupense* bacterial strains.

As a second reason, it is suspected that the specimens were got mixed with a lot of egg-based medium to the target plate, possibly as a suppressor of material ionization [9]. This is the problem in the technique, so it may be prevented it by reexamining. In this study,