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Evaluation of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Identification of *Mycobacterium abscessus* Subspecies According to Whole-Genome Sequencing

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This study was undertaken to evaluate the utility of matrix-assisted laser desorption ionization–time of flight mass spectrometry with the Vitek MS Plus system for identifying *Mycobacterium abscessus* subspecies in order to facilitate more rapid and appropriate therapy. A total of 175 clinical *M. abscessus* strains were identified by whole-genome sequencing analysis: 139 *Mycobacterium abscessus* subsp. *abscessus* and 36 *Mycobacterium abscessus* subsp. *massiliense*. The research-use-only (RUO) Saramis Knowledge Base database v.4.12 was modified accordingly by adding 40 *M. abscessus* subsp. *abscessus* and 19 *M. abscessus* subsp. *massiliense* reference spectra to construct subspecies SuperSpectra. A blind test, used to validate the remaining 116 isolates, yielded 99.1% ($n = 115$) reliability and only 0.9% ($n = 1$) error for subspecies identification. Among the two subspecies SuperSpectra, two specific peaks were found for *M. abscessus* subsp. *abscessus* and four specific peaks were found for *M. abscessus* subsp. *massiliense*. Our study is the first to report differential peaks 3,354.4 *m/z* and 6,711.1 *m/z*, which were specific for *M. abscessus* subsp. *massiliense*. Our research demonstrates the capacity of the Vitek MS RUO Saramis Knowledge Base database to identify *M. abscessus* at the subspecies level. Moreover, it validates the potential ease and accuracy with which it can be incorporated into the IVD system for the identification of *M. abscessus* subspecies.

Mycobacterium abscessus is one of the most common pathogens isolated from patients with cystic fibrosis and the second most prevalent, rapidly growing nontuberculous mycobacteria (NTM) species causing NTM pulmonary diseases (1, 2). Although it might not be the most virulent NTM pathogen, inherent antibiotic resistance makes it notoriously difficult to treat (3–7). Among multiple resistance mechanisms, resistance to macrolides is noteworthy (8). In this regard, acquired resistance to clarithromycin is always associated with a mutation in the 23S rRNA gene; intrinsic resistance is conferred mainly by the erythromycin methylase (*erm*) gene (3). The *erm* gene plays a significant role in the drug susceptibility and antibiotic resistance of *M. abscessus*. *Mycobacterium abscessus* is divided into three subspecies: *Mycobacterium abscessus* subsp. *massiliense*, *Mycobacterium abscessus* subsp. *bolletii*, and *Mycobacterium. abscessus* subsp. *abscessus* (9). The most meaningful genomic difference between the three subspecies is that *M. abscessus* subsp. *massiliense* possesses an incomplete and inactive *erm* gene, which is 276 bp in length and harbors deletion mutations at two positions (10–13). *M. abscessus* subsp. *massiliense* can acquire clarithromycin resistance as a consequence of a 23S rRNA mutation at position A2058 (12, 14). Due to an inactive *erm* gene, *M. abscessus* subsp. *massiliense* does not exhibit inducible resistance after exposure to macrolides. *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *bolletii*, on the other hand, exhibit inducible resistance (9, 15). As a result, lung diseases caused by different *M. abscessus* subspecies exhibit contrasting responses to macrolide-based antibiotic therapy (1, 8, 11, 16–19). As such, identification of isolates at the subspecies level, combined with antimicrobial susceptibility testing, is very important for clinicians to make proper therapeutic decisions.

Accurate identification of *M. abscessus* at the subspecies level is still complicated. Although PCR can differentiate *M. abscessus* subsp. *massiliense* from *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *bolletii* by identifying the fragmented *erm*(41) gene, it still requires sequence analysis of several housekeeping genes (20, 21). In addition, PCR methods that depend upon gene sequencing are costly. Indeed, whole-genome sequencing (WGS), the most reliable method of identification, is extremely expensive, and the data analysis is time-consuming. Moreover, WGS is not practical in many clinical microbiology laboratories. Therefore, an easier, more rapid, accurate, and cost-effective diagnostic tool is needed to distinguish between *M. abscessus* subspecies.

Reportedly, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) offers an effective method for identifying the *M. abscessus* complex in clinical laboratories (22). However, improvements in MALDI-TOF MS are needed to identify very close taxa that include the *M. abscessus* subspecies (23). A number of studies used spectrum analysis to show that several peaks differentiated *M. abscessus* at the subspe-

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cies level; these findings, however, were inconsistent (23–27). Possibly, these inconsistencies in subspecies profiles were determined by geographic location and related to the horizontal transfer of genes during the evolution of *M. abscessus* worldwide. The analysis of mass spectra provides a new opportunity to increase the range of MALDI-TOF MS application.

In the present study, 175 clinical *M. abscessus* isolates were collected at the Shanghai Pulmonary Hospital and analyzed by WGS. Two subspecies were observed by using the unweighted pair group with arithmetic mean (UPGMA) method. These subspecies were used as references for MALDI-TOF MS identification. We propose that the two subspecies, *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense*, can be directly and automatically identified by updating the spectral database using the Vitek MS Plus system (bioMérieux SA, Marcy l'Etoile, France). Considering the absence of *M. abscessus* subsp. *bolletii* in our collection, it was not covered in our present research but remains an important area for further study.

MATERIALS AND METHODS

Bacteria collection and DNA extraction. (i) Bacteria collection. In this study, 175 clinical *M. abscessus* isolates were obtained from the Shanghai Pulmonary Hospital affiliated with Tongji University. Mycobacterial isolates were obtained from respiratory samples of sputum and bronchoalveolar lavage fluid. These isolates were previously identified as NTM by MGIT960 medium culture and the *p*-nitrobenzoic acid (PNB) test. All isolates were further identified as *M. abscessus* by sequencing the *rpoB* gene. The study was approved by the Ethics Committee of Tongji University and the Shanghai Pulmonary Hospital.

Respiratory samples were transferred to Lowenstein-Jensen (LJ) agar plates after alkali treatment with 4% NaOH. Bacterial smears prepared from the colonies that grew were stained and examined microscopically to identify acid-fast bacteria. The acid-fast positive colonies were isolated and cultured in LJ medium at 37°C for 3 to 7 days (14, 28) and then were further used for DNA extraction and MALDI-TOF MS identification.

(ii) DNA extraction. DNA extraction was performed as described previously with slight modification (29). A 10-μl loopful of bacteria grown in LJ medium was transferred to a microcentrifuge tube that contained 1 ml of TE (10 mM Tris 10, 1 mM EDTA [pH 8.0]) buffer and 250 ml of 0.5-mm glass beads. After vortexing, a 400-μl aliquot of the suspension was mixed with 1 mg/ml lysozyme and incubated overnight at 37°C. Then, 70 μl of sodium dodecyl sulfate (10%) and 10 μl of proteinase K (10 mg/ml) were added, and the mixture was incubated at 65°C for 20 min. A 100-μl solution of 10% *N*-acetyl-*N*,*N*,*N*-trimethyl ammonium bromide (CTAB) and NaCl (0.7 M) was added followed by 100 μl of NaCl (0.5 M) alone, and the mixture was incubated at 65°C for 10 min. Afterward, 750 μl of chloroform-isoamylalcohol (24:1) was added, and the tube was centrifuged at 13,000 rpm for 5 min. A supernatant was generated by the addition of a 60% volume of isopropanol. The tube was then incubated at −20°C for 20 min and centrifuged at 13,000 rpm for 15 min. The supernatant was discarded, and the sediment was washed with 70% ethanol and then dissolved in 200 μl of TE buffer. The genomic DNA recovered in the sediment was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc., West Palm Beach, FL, USA). High-quality DNA samples (optical density at 260/280 nm ($OD_{260/280}$) of 1.8 to 2.0, >6 μg) were used to construct 350- to 450-bp fragment libraries.

Library construction and Illumina HiSeq sequencing. For Illumina pair-end sequencing, at least 3 μg of genomic DNA derived from each strain was used for sequencing library construction. Paired-end libraries with ~400-bp insert sizes were prepared following Illumina's standard genomic DNA library preparation procedure. Purified genomic DNA was sheared into smaller fragments with a desired size using a Covaris focused ultrasonicator (Thermo Fisher Scientific); blunt ends were generated with T4 DNA polymerase. After addition of an A base to the 3′ end of the blunt phosphorylated DNA fragments, adapters were ligated to the ends. The desired fragments were purified by gel electrophoresis and then selectively enriched and amplified by PCR. The index tag was introduced into the adapter at the PCR stage as appropriate, and a library quality test was performed. The qualified Illumina paired-end library was used to conduct Illumina HiSeq sequencing (two 150-bp reads).

Genome assembly and evolution tree construction. Before the SPAdes (v.3.6.0; http://bioinf.spbau.ru/en/spades) software default parameters were used to assemble the genome draft (30), SPAdes was combined with BayesHammer (31) in order to adjust the bases and the program carefully to correct sequence assembly errors and any incomplete insertions. QUAST (v.2.3; http://quast.bioinf.spbau.ru/) was used to evaluate the result of the assembly (32).

MASH (v.1.0.1; https://github.com/marbl/Mash) was used with reference genomes to calculate the distance of strains between subspecies (33). Phylogenetic trees of the subspecies were constructed using the UPGMA method. Established strains ATCC 19977 (*M. abscessus* subsp. *abscessus*), CIP108297 (*M. abscessus* subsp. *massiliense*), and CIP108541 (*M. abscessus* subsp. *bolletii*) served as reference controls (11, 14, 34, 35). H37Rv (*M. tuberculosis*) was used as a separate species reference control.

MALDI-TOF MS. (i) Sample preparation. A protocol developed by bioMérieux and improved by Machen et al. (36) was used to prepare the samples. Colonies were transferred from an LJ agar plate to a microcentrifuge tube that contained 800 μl of 70% ethanol and 250 μl of 0.5-mm glass beads. The microcentrifuge tube was vortexed for 15 min and then incubated at room temperature for 10 min. Subsequently, the mycobacteria were suspended by vortexing 10 s. The suspension was transferred to an empty microcentrifuge tube and centrifuged at 13,000 rpm for 5 min. The supernatant was discarded, and the pellet was dried for 10 min, resuspended in 10 μl of 70% formic acid, and incubated for 2 to 5 min at room temperature. Acetonitrile (10 μl) was added, and the tube was then centrifuged at 10,000 rpm for 3 min. One microliter of supernatant was added to a spot on a disposable target plate. The spot was dried completely, covered with 1 μl of α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution and dried again before loading into the MALDI-TOF MS.

(ii) MALDI-TOF MS acquisition. MALDI-TOF MS measurement of the isolates was performed using the Vitek MS Plus system. The spectra were acquired in linear positive ion mode at a laser frequency of 50 Hz across *m*/*z* 2,000 to 20,000 Da. For each target slide, the *Escherichia coli* reference strain ATCC 8739 was used for instrument calibration according to the manufacturer's specifications. After spectrum acquisition, the data were transferred from the Vitek MS acquisition station to the Saramis analysis server. The data were reported with number of peaks and the highest level matches compared to those for the Saramis 4.12 research-use-only (RUO) database (bioMérieux SA).

(iii) Spectra database upgrading. Spectral data of 175 clinical isolates were collected and analyzed. Subsequently, the spectra from 40 *M. abscessus* subsp. *abscessus* isolates and from 19 *M. abscessus* subsp. *massiliense* isolates were imported into the RUO Saramis database. New folders of subspecies were added under the original *M. abscessus* species in the spectral taxonomy tree, and then the imported spectra were pasted into the respective subspecies folders. SuperSpectra were generated by creating consensus spectra that contained the 40 main peaks, which were found with 100% frequency in each respective subspecies. SuperSpectra were then activated for subsequent automated identification at the subspecies level.

Validation. The updated database included the 59 imported reference spectra and two new subspecies SuperSpectra. It was validated by a blind test of the remaining 116 consecutive collection isolates not used to construct the new SuperSpectra.

RESULTS

Reference identification. NTM, which are resistant to PNB, grow well in LJ medium supplemented with PNB. Our clinical *M. abscessus* strains were first detected by growth in the presence of PNB

FIG 1 Using the UPGMA method, 175 strains of *Mycobacterium abscessus* were divided into two groups consisting of 139 subsp. *abscessus* isolates and 36 subsp. *massiliense* isolates. Established strains ATCC 19977 (*M. abscessus* subsp. *abscessus*), CIP108297 (*M. abscessus* subsp. *massiliense*), and CIP108541 (*M. abscessus* subsp. *bolletii*) served as reference controls; H37Rv (*M. tuberculosis*) was used as a separate species reference control.

and further confirmed by MALDI-TOF MS identification and sequencing of the *rpoB* gene. The DNA was extracted from all 175 strains for WGS.

Whole-genome sequencing results. H37Rv (*M. tuberculosis*) was used as an exterior reference during cluster analysis, while ATCC 19977 (*M. abscessus* subsp. *abscessus*) (37), CIP108297 (*M. abscessus* subsp. *massiliense*), and CIP108541 (*M. abscessus* subsp. *bolletii*) served as the internal reference control strains. The WGS dendrogram revealed two clusters, perfectly congruent with the two subspecies. The 175 isolates consisted of 139 *M. abscessus* subsp. *abscessus* and 36 *M. abscessus* subsp. *massiliense* (Fig. 1).

MALDI-TOF MS results. All 175 isolates were identified as *M. abscessus* (average identification confidence score of >95%) by comparing the collected spectra to the research-use-only (RUO) Saramis Knowledge Base database v.4.12. After expansion of the spectral database and upgrading of the taxonomy tree, the 116 blind-tested strains were differentiated into two subspecies. The identification confidence scores approached 99.9%; 99.1% of the isolates ($n = 115/116$) were correctly identified. The single error, sample A173, was a *M. abscessus* subsp. *massiliense* isolate that was misidentified because it exhibited a pattern with two peaks (4,390 *m*/*z* and 8,782 *m*/*z*) that were characteristic of *M. abscessus* subsp. *abscessus*. None of the strains used to construct the subspecies SuperSpectra was misidentified using our modified database.

For each subspecies, 40 peaks that occurred with 100% frequency were selected for constructing the corresponding SuperSpectra (Fig. 2). Between the two subspecies SuperSpectra, 30 peaks overlapped and 10 peaks were unique. The 30 peaks that were superimposable showed a frequency of >98.3% among all 175 *M. abscessus* isolates; the difference of occurrence rates between the two subspecies was <2.8%. Fourteen of the misalignments exhibited a frequency difference between subspecies of <20.8%, while six peaks showed differences of >95.8%. The specific peak signals are shown in Fig. 3 and 4.

We found two specific peaks at 4,390.4 *m*/*z* and 8,782.0 *m*/*z* for *M. abscessus* subsp. *abscessus* and four specific peaks at 3,354.4

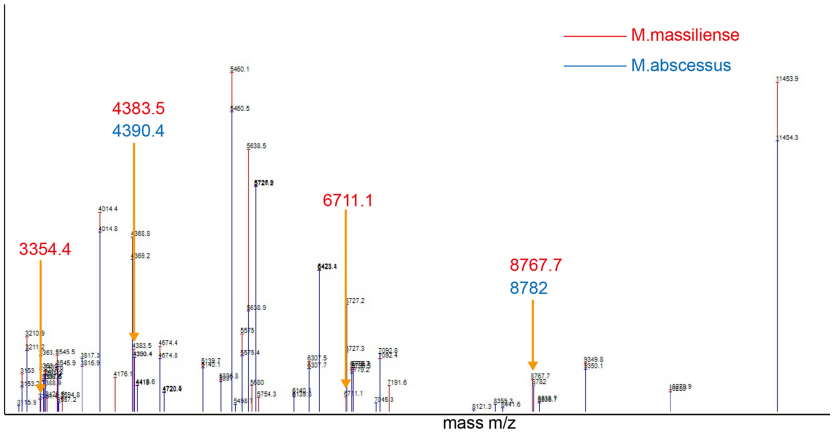

FIG 2 SuperSpectra of *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *abscessus* were generated using Saramis Premium software. Among a combined 40 peaks, 30 peaks overlap, while 10 peaks are unique for each subspecies. Six peaks are regarded as highly specific signals (3,354.4 *m/z*, 4,383.5 *m/z*, 4,390.4 *m/z*, 6,711.1 *m/z*, 8,767.7 *m/z*, and 8,782.0 *m/z*).

m/z, 4,383.5 *m/z*, 6,711.1 *m/z*, and 8,767.7 *m/z* for *M. abscessus* subsp. *massiliense*. Table 1 shows the differential peaks and their frequencies in the two subspecies. Peaks at 3,354.4 *m/z* and 6,711.1 *m/z* were only observed in *M. abscessus* subsp. *massiliense*. In detail, signals at 4,390.4 *m/z* and 8,782.0 *m/z* were found in 137/139 and 138/139, respectively, of the *M. abscessus* subsp. *abscessus* isolates; signals at 4,383.5 *m/z* and 8,767.7 *m/z* were found in 35/36 of the *M. abscessus* subsp. *massiliense* isolates. Signals at 3,354.4 *m/z* and 6,711.1 *m/z* were found in all *M. abscessus* subsp. *massiliense* (36/36); a signal at 3,354.4 *m/z* was detected in only two of the *M. abscessus* subsp. *abscessus* isolates. A signal at 6,711.1 *m/z* was not found among any of the *M. abscessus* subsp. *abscessus* isolates.

DISCUSSION

As an emerging proteomic tool for microbial identification, MALDI-TOF MS is superior in cost and speed. Previous reports showed >84% accuracy in mycobacteria species-level identification (22, 36, 38, 39) but found difficulty in distinguishing closely related (sub)species (40). The present study confirms the accuracy of MALDI-TOF MS in identifying *M. abscessus* at the species level (18, 22, 36, 39–41). Discriminating the subspecies correctly was the real challenge since a quick, reliable, and economic method is still not available (42). The study described herein was undertaken to determine the ability of MALDI-TOF MS to differentiate *M. abscessus* isolates at the subspecies level.

In the present study, all 175 isolates were precisely identified as *M. abscessus* at the species level using the RUO Saramis Knowledge Base. Next, the RUO Saramis Knowledge Base database was expanded with 59 spectra of clinical isolates (40 *M. abscessus* subsp. *abscessus* and 19 *M. abscessus* subsp. *massiliense*) previously identified using WGS and traditional identification methods (18).

Identifications with confidence scores of >90% obtained by activated subspecies-specific SuperSpectra were considered accurate at the subspecies level (43). Expansion of the Saramis Knowl-

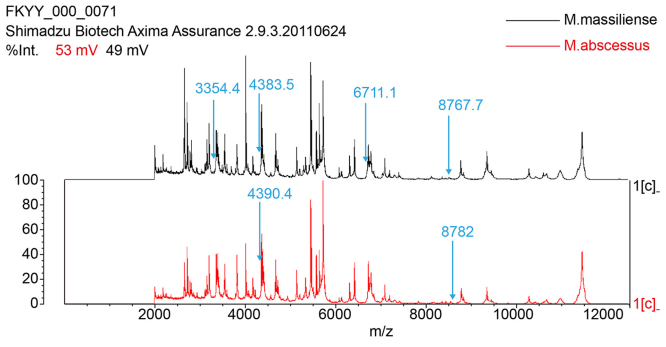

FIG 3 Spectrometric profiles and specific *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *abscessus* peaks were obtained by the Vitek MS Plus system and analyzed with the research-use-only (RUO) Saramis Knowledge Base database v.4.12.

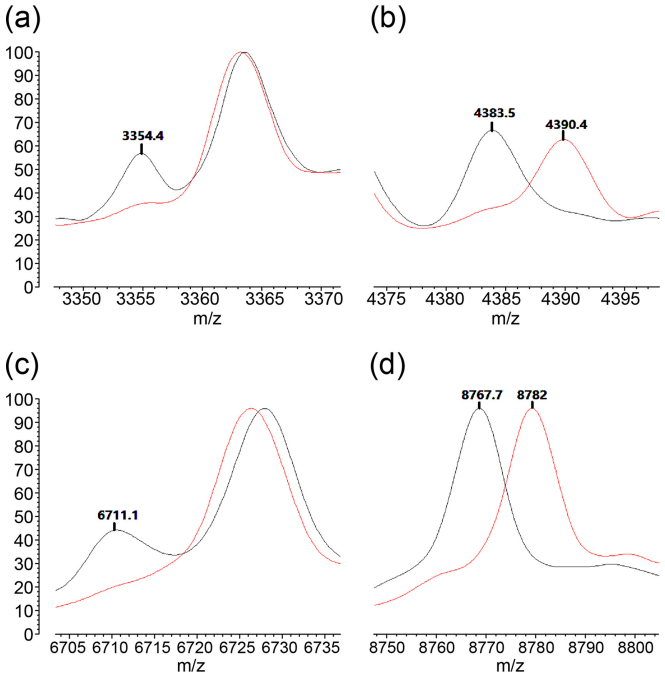

FIG 4 Six peaks differentiate two *M. abscessus* subspecies. The signals at 3,354.4 *m/z* and 6,711.1 *m/z* are specific for *M. abscessus* subsp. *massiliense* (a and c). The signals at 4,383.5 *m/z* and 8,767.7 *m/z* are also specific for *M. abscessus* subsp. *massiliense*; the signals at 4,390.4 *m/z* and 8,782.0 *m/z* are specific for *M. abscessus* subsp. *abscessus* (b and d).

TABLE 1 Six specific peaks analyzed and selected using Saramis Premium software

Subspecies	Detection frequency (*n* [%]) at peak (*m*/*z*) of:					
	3,354.4	4,383.5	4,390.4	6,711.1	8,767.7	8,782.0
M. abscessus subsp. *massiliense* ($n = 36$)	36 (100)	35 (97.2)	1 (2.8)	36 (100)	35 (97.2)	1 (2.8)
M. abscessus subsp. *abscessus* ($n = 139$)	2 (1.4)	2 (1.4)	137 (98.6)	0 (0)	1 (0.7)	138 (99.3)

edge Base database enabled MALDI-TOF MS to discriminate between the *abscessus* and *massiliense* subspecies of *M. abscessus*. To optimize the accuracy of any MALDI-TOF MS identification system, it is critical to add sufficient spectral data to expand the database. This is required to address both unclaimed taxa and claimed taxa in which additional discrimination is desired. Subsequently, a blind test of 116 WGS genotyped isolates revealed 99.1% accuracy and 0.9% error. The single error occurred for isolate A173, *M. abscessus* subsp. *massiliense*, which was misidentified as *M. abscessus* subsp. *abscessus*. This misidentified isolate displayed specific signal patterns characteristic of both subspecies upon further analysis of the peak profiles and mass list, which might have occurred as the result of an ambiguous proteome caused by interspecies lateral gene transfer (44). Recently, this speculation was tested with a classification algorithm based upon 40 total strains composed of 24 *M. abscessus* subsp. *abscessus*, 10 *M. abscessus massiliense* subspecies, and 6 *M*, *abscessus bolletii* subspecies isolates and validated by 49 strains using the MALDI Biotyper (Bruker Daltonics, Bremen, Germany) (24). The improved algorithm reached 94% accuracy with 2% uncertainty and 4% error. Moreover, it accounted for the misidentification of strains that exhibited the biomarkers of both *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense* (24). These hybrid allelic forms and associated proteomes displayed by several *M. abscessus* isolates were reported in previous studies (11, 24, 25, 44). In addition, misidentification of the *M. abscessus* subsp. *massiliense* isolate in the study conducted here may be due in part to the limited number of strains collected and used to construct the *massiliense* subspecies SuperSpectrum. It is obvious from the clinical data, that the incidence of the *massiliense* subspecies in Shanghai, China, is smaller than the incidence of the *abscessus* subspecies. Consequently, optimization of the *M. abscessus* subsp. *massiliense* SuperSpectrum may require the inclusion of more precise MS data. As suggested above, the percentage of *massiliense* subspecies determined among the total *M. abscessus* isolates seems to vary, depending upon geographic location. The ratio of *M. abscessus* subsp. *massiliense* to *M. abscessus* subsp. *abscessus* isolated was much lower in the United States and Europe (9, 23, 24, 45), while the rate was much higher in Japan, South Korea, and Taiwan (5, 14, 46–48). The results of the study presented herein show that 20.6% of *M. abscessus* identified in Shanghai, China, belonged to the *massiliense* subspecies. This rate is closer to that found in the United States and Europe than in other Asian locales.

Several previous studies indicated that, by aligning the specific peak signals, MALDI-TOF MS clearly differentiated *M. abscessus* subsp. *massiliense* from the other two subspecies (23–27). However, discrepancies in the specific peak signals of the three subspecies were reported. The studies of Teng et al. (26) and Suzuki et al. (25), for example, showed results similar to those reported here: specific peaks for *M. abscessus* subsp. *abscessus* at around 4,368 (4,368.24) *m*/*z*, 7,638 (7,639.70, 7,637.24) *m*/*z*, 8,782 (8,783.84, 8,781.77) *m*/*z*, and 9,475 (9,477.48, 9,473.82) *m*/*z*; and specific peaks for *M. abscessus* subsp. *massiliense* at around 4,385 (4,386.24, 4,385.05) *m*/*z*, 7,668 (7,669.20, 7,667.09) *m*/*z*, and 8,769 (8,771.73, 8,767.98) *m*/*z*. In contrast, the groups of Fangous et al. (24) and Panagea et al. (23) found three additional peaks at 2,081 *m*/*z*, 3,123 *m*/*z*, and 3,378 *m*/*z* for *M. abscessus* subsp. *abscessus*; peaks at 3,108 *m*/*z* and 3,378 *m*/*z* were observed for *M. abscessus* subsp. *massiliense*. *M. abscessus* subsp. *bolletii*, on the other hand, was rarely recovered in these studies due to its infrequent occurrence. Studies that described peaks for *M. abscessus* subsp. *bolletii* reported distinctly different results (24, 25). These studies suggested, however, that *M. abscessus* subsp. *bolletii* was so closely related to *M. abscessus* subsp. *abscessus* that discrimination by MALDI-TOF MS was difficult. In our WGS experiment, *M. abscessus* isolates were divided into two clusters in keeping with the observations of other investigators (18). Our results indicated that signals at 4,390.4 or 4,383.5 *m*/*z* and 8,782.0 or 8,767.7 *m*/*z* clearly differentiated *M. abscessus* subsp. *massiliense* from *M. abscessus* subsp. *abscessus* with almost 100% accuracy. This is consistent with the findings of other Asian researchers (25, 26). Peaks around 7,638 *m*/*z* or 7,668 *m*/*z*, described by Taiwanese and Japanese investigators, were not included in our SuperSpectra (26, 27). This suggests that the two peaks in our data set might be ambiguous and not specific enough for identification, which might be due in part to the nature of the culture conditions used. There is no clear evidence, however, that such conditions prominently interfere with MALDI-TOF spectra (18, 25).

M. abscessus isolates collected in Europe and the United States revealed unique peak patterns, i.e., specific signals at 2,081 *m*/*z* and 3,378 *m*/*z* (23, 24). Possibly these occurred as a function of horizontal gene transfer and genetic background differences that influence the evolution of *M. abscessus* worldwide (35, 49). This possibility concurs with the fact that our profiles are similar to others found in Asia but diverge from those found in Europe and the United States (25–27). The 3,354.4 *m*/*z* and 6,711.1 *m*/*z* peaks specific for *M. abscessus* subsp. *massiliense* described herein, however, have not been reported previously. More global MALDI-TOF MS experiments are needed to substantiate the effects of geographic distribution on *M. abscessus* subspecies type.

Mycobacterium abscessus is the most common, rapidly growing mycobacterial species that causes NTM pulmonary disease (11, 50–55). Different subspecies may indicate divergent treatment plans, especially for those plans that involve clarithromycin (5, 28, 46–49, 56). Consequently, development of a fast and accurate technique for the identification of *M. abscessus* subspecies is urgently needed. The present study demonstrated the potential use of the MALDI-TOF MS RUO Saramis Knowledge Base database to identify *M. abscessus* at the subspecies level. Upon verification of its ability to distinguish *M. abscessus* subspecies isolated worldwide, MALDI-TOF MS may be incorporated into the IVD system to ease use and increase diagnostic accuracy (26, 39). Once incorporated, MALDI-TOF MS will contribute significantly to the early treatment of diseases caused by *M. abscessus* subspecies.

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