Evaluation of the Bruker Biotyper Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry System for Identification of Clinical and Environmental Isolates of Burkholderia pseudomallei

He Wang¹, Ya-Lei Chen², Shih-Hua Teng³, Zhi-Peng Xu¹, Ying-Chun Xu¹* and Po-Ren Hsueh*⁺

¹ Department of Clinical Laboratory, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China, ² Department of Biotechnology, National Kaohsiung Normal University, Kaohsiung, Taiwan, ³ Department of Graduate Institute of Biomedical Sciences, Chang Gung University, Tao-Yuan, Taiwan, ⁴ Departments of Laboratory Medicine and Internal Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan

**Burkholderia pseudomallei** is not represented in the current version of Bruker Biotyper matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) system. A total of 66 isolates of *B. pseudomallei*, including 30 clinical isolates collected from National Taiwan University Hospital (NTUH, \(n = 27\)) and Peking Union Medical College Hospital (PUMCH, \(n = 3\)), and 36 isolates of genetically confirmed strains, including 13 from clinical samples and 23 from environmental samples, collected from southern Taiwan were included in this study. All these isolates were identified by partial 16S rDNA gene sequencing analysis and the Bruker Biotyper MALDI-TOF MS system. Among the 30 isolates initially identified as *B. pseudomallei* by conventional identification methods, one was identified as *B. cepacia* complex (NTUH) and three were identified as *B. putida* (PUMCH) by partial 16S rDNA gene sequencing analysis and Bruker Biotyper MALDI-TOF MS system. The Bruker Biotyper MALDI-TOF MS system misidentified 62 genetically confirmed *B. pseudomallei* isolates as *B. thailandensis* or *Burkholderia* species (score values, 1.803–2.063) when the currently available database (DB 5627) was used. However, using a newly created MALDI-TOF MS database (including *B. pseudomallei* NTUH-3 strain), all isolates were correctly identified as *B. pseudomallei* (score values >2.000, 100%). An additional 60 isolates of genetically confirmed *B. cepacia* complex and *B. putida* were also evaluated by the Bruker Biotyper MALDI-TOF MS system using the newly created database and none of these isolates were identified as *B. pseudomallei*. MALDI-TOF MS is a versatile and robust tool for the rapid identification of *B. pseudomallei* using the enhanced database.

**Keywords:** Burkholderia pseudomallei, *B. thailandensis*, matrix-assisted, laser desorption/ionization time-of-flight mass spectrometry, 16S rDNA gene sequencing analysis, enhanced database
INTRODUCTION

Melioidosis is a tropical and subtropical infectious disease caused by Burkholderia pseudomallei, a Gram-negative, aerobic, motile rod-shaped bacterium that is widely distributed in rice field soil, and in stagnant water throughout the tropics (Hsueh et al., 2001; Currie et al., 2008; Lau et al., 2014). B. pseudomallei is also a major cause of community-acquired septicaemia and pneumonia in adults in the Asia-pacific region, particularly in northeast Thailand (Peto et al., 2014). In Taiwan, the first case of melioidosis was reported in 1985 in a man who acquired the disease after aspirating river water in a near-drowning accident in the Philippines (Lee et al., 1985). Since then several sporadic and epidemic cases have been reported (Hsueh et al., 2001; Ko et al., 2007; Su et al., 2011; Chen et al., 2013, 2014). Previous studies clearly showed that the disease was endemic in Taiwan and demonstrated that all clinical isolates were arabinose non-assimilators (Hsueh et al., 2001; Chen et al., 2013). Other studies have reported high concentrations of ambient B. pseudomallei during typhoon season in regions of Taiwan (Ko et al., 2007; Su et al., 2011; Chen et al., 2014). Several typhoon-related melioidosis epidemics have also been reported (Ko et al., 2007; Su et al., 2011). There is also evidence that melioidosis can be transmitted to humans via environmental aerosols contaminated with B. pseudomallei (Chen P. S. et al., 2015).

In Mainland China, melioidosis was first reported in 1990 (Yang, 2000) and is now known to be endemic to several tropical provinces, including Hainan, Guangdong, and Guangxi (Chen H. et al., 2015; Fang et al., 2015; Zheng et al., 2015). Multilocus sequence typing (MLST) disclosed that B. pseudomallei ST562 is dominant in southern China as well as in Australia and Taiwan and that its wide-ranging presence might be due to recent spread caused by transmission between regions (Chen H. et al., 2015). Whole-genome sequencing of B. pseudomallei has been conducted for isolates obtained from melioidosis in Mainland China (strain BPC006) and Taiwan (strain vgh07; Fang et al., 2012; Chen Y. S. et al., 2015).

MALDI-TOF MS is increasingly being used in clinical microbiology laboratories to identify bacterial isolates to the species level and the technique is expected to further accelerate the routine identification of suspicious isolates (Bizzini and Greub, 2010; Inglis et al., 2012; Lau et al., 2012; Niyompanic et al., 2014; Jang et al., 2015; Lasch et al., 2015). Because diseases due to B. pseudomallei are uncommon in North America and Europe, B. pseudomallei are not included (but B. thailandensis was included) in the reference spectra of the Bruker Biotype and Vitek MS libraries (SARAMIS database) (Jang et al., 2015).

In the present study, we evaluated the ability of the Bruker Biotype MALDI-TOF MS system to accurately identify genetically confirmed B. pseudomallei that were recovered from patients and environmental sources.

MATERIALS AND METHODS

Bacterial Isolates

A total of 66 isolates of B. pseudomallei, including 43 clinical isolates and 23 environmental isolates, were collected for study. Among these isolates, 27 were recovered from patients with bloodstream infections who were treated at National Taiwan University Hospital (NTUH, a 2500-bed university-affiliated hospital in northern Taiwan) during the period 1998–2014 (Table 1), and three isolates were recovered from patients treated at Peking Union Medical College Hospital (PUMCH), Beijing, China. The other 36 isolates of B. pseudomallei, which had been identified by sequencing of the 16S rRNA and flagellar genes (Chen et al., 2013), included 13 isolates from various clinical samples obtained from patients treated at several hospitals in southern Taiwan and 23 isolates obtained from different environmental sources during the period 1994–2013 (Table 2; Chen et al., 2013). Cultures and analysis of B. pseudomallei isolates were manipulated in the Mycobacteriology Laboratory at NTUH, a biosafety level 3 laboratory, and followed the biosafety level 3 precaution. Identification of these isolates were initially based on conventional biochemical methods and commercial identification systems, including API (API 20E) and Vitek 2 (ID-GN card) (bioMérieux, Marcy l’Etoile, France). Arabinose assimilation testing was performed for all genetically confirmed B. pseudomallei isolates as reported previously (Hsueh et al., 2001).

Identification of Isolates by Gene Sequencing Analysis

Partial 16S rRNA gene sequencing of all 66 isolates was performed using two primers, 8FPL (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492RPL (5′-GTACCTTGGTTACATCCT-3′; Cheng et al., 2015). The sequences (1425 bp) obtained were compared with published sequences in the GenBank database using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast).

Identification of Isolates by the Bruker Biotype MALDI-TOF System

Samples of the 66 isolates were prepared for analysis by the Bruker Biotype MALDI-TOF MS system as previously described (Cheng et al., 2015). B. thailandensis E264 (ATCC700388) was included as the control strain. All isolates were inoculated onto Trypticase soy agar with 5% sheep blood (blood agar plates, BAP; Becton Dickinson Microbiology Systems Sparks, MD, USA) and incubated in 5% CO2 at 37°C for 18 to 24 h. Two to three colonies were transferred to a 1.5-ml screw-cap Eppendorf tube containing 50 µl of 70% formic acid. After incubation for 30 s, 50 µl of acetonitrile (Sigma-Aldrich) was added. The suspension was centrifuged at 13,000 rpm for 2 min, and then 1.0 µl of the supernatant was applied to a 96-spot polished steel target sample and dried. Measurements were performed with the Bruker Biotype MALDI-TOF MS system using FlexControl software with Compass Flex Series version 1.3 software and a 60-Hz nitrogen laser (337 nm wavelength). Spectra ranging from 2000 to 20,000 m/z were analyzed using the MALDI Biotype system’s automation control and the current Bruker
TABLE 1 | Results of 16S rRNA sequencing analysis and Bruker Biotyper MALDI TOF MS for the identification of 26 isolates of B. pseudomallei recovered from patients who were treated at National Taiwan University Hospital (NTUH).

| No. (NTUH) | Year of isolation | Identification by 16S rRNA sequencing analysis | Identification by Bruker Biotyper MALDI TOF MS system |
|------------|-------------------|-----------------------------------------------|---------------------------------------------------|
|            |                   | Species (% of identity) | Accession no. (best match) | Organism (best match) | Score value | Organism (best match) | Score value |
| 1.         | 1998              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 2.063       | B. pseudomallei       | 2.15        |
| 2.         | 1998              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.913       | B. pseudomallei       | 2.228       |
| 3.         | 1998              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 2.036       | B. pseudomallei       | 2.318       |
| 4.         | 1999              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.974       | B. pseudomallei       | 2.257       |
| 5.         | 1999              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.993       | B. pseudomallei       | 2.332       |
| 6.         | 2000              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.907       | B. pseudomallei       | 2.161       |
| 7.         | 2000              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.907       | B. pseudomallei       | 2.117       |
| 8.         | 2000              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.899       | B. pseudomallei       | 2.266       |
| 9.         | 2000              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.938       | B. pseudomallei       | 2.075       |
| 10.        | 2000              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.934       | B. pseudomallei       | 2.063       |
| 11.        | 2001              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.933       | B. pseudomallei       | 2.055       |
| 12.        | 2001              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.996       | B. pseudomallei       | 2.252       |
| 13.        | 2001              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.808       | B. pseudomallei       | 2.017       |
| 14.        | 2001              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.941       | B. pseudomallei       | 2.043       |
| 15.        | 2001              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.936       | B. pseudomallei       | 2.09        |
| 16.        | 2001              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 2.016       | B. pseudomallei       | 2.077       |
| 17.        | 2001              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.803       | B. pseudomallei       | 2.113       |
| 18.        | 2001              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.898       | B. pseudomallei       | 2.235       |
| 19.        | 2003              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.946       | B. pseudomallei       | 2.27        |
| 20.        | 2003              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.865       | B. pseudomallei       | 2.147       |
| 21.        | 2004              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.971       | B. pseudomallei       | 2.308       |
| 22.        | 2004              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 2.002       | B. pseudomallei       | 2.303       |
| 23.        | 2005              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 1.968       | B. pseudomallei       | 2.221       |
| 24.        | 2008              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 2.013       | B. pseudomallei       | 2.221       |
| 25.        | 2014              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 2.013       | B. pseudomallei       | 2.176       |
| 26.        | 2014              | B. pseudomallei (100)  | CP004043.1                    | B. thailandensis     | 2.013       | B. pseudomallei       | 2.307       |

Biotyper 3.1 software and library (database [DB] 5627 with 5627 entries). Identification scores of ≥2.000 indicated species-level identification, scores of 1.700 to 1.999 indicated genus-level identification, and scores of <1.700 indicated no reliable identification. B. pseudomallei is not listed in the current Bruker Biotyper MALDI-TOF MS database.

Clustering Analysis and Main Spectra Projection by the Bruker Biotyper MALDI TOF System

A clustering analysis of 26 isolates collected from NTUH was performed using ClinProTools 3.0 (Bruker Daltonics GmbH, Bremen, Germany; Cheng et al., 2015). Dendrograms from the MALDI Biotyper data of 26 genetically well-characterized B. pseudomallei isolates were obtained and cluster groups with a default critical distance level of 850 were identified. Isolates from different cluster groups were selected for main spectra projection (MSP; database entrance) creation using MALDI Biotyper software (Bruker Daltonics). The database generated using the isolates selected from different cluster groups was blindly tested against the 26 B. pseudomallei isolates from NTUH.

External validation was performed for another 36 B. pseudomallei isolates collected from southern Taiwan, 30 isolates of B. pseudomallei (all >90% probability) by the Vietk 2 ID-GN card, four (one for NTUH and three from PUMCH) were identified as non-B. pseudomallei by partial 16S rDNA sequencing analysis. Among these four isolates, one was identified as B. cepacia complex (NTUH) and three

RESULTS

Among the 30 clinical isolates collected from clinical microbiology laboratories at the NTUH and PUMCH that were initially identified as B. pseudomallei (all >90% probability) by the Vietk 2 ID-GN card, four (one for NTUH and three from PUMCH) were identified as non-B. pseudomallei by partial 16S rDNA sequencing analysis. Among these four isolates, one was identified as B. cepacia complex (NTUH) and three
TABLE 2 | Identification of 36 isolates of *B. pseudomallei* obtained from clinical specimens and environmental sources by Bruker Biotyper MALDI-TOF MS with the NTUH-3 strain.

| No | Year of isolation | Source | Type of infection or environmental sources | Identification results by Bruker Biotyper MALDI TOF MS system (NTUH-3 strain) |
|----|------------------|--------|------------------------------------------|------------------------------------------|
| 27 | 1994             | Human  | Mycotic aneurysm (thoracic aorta)         | 2.545                                   |
| 28 | 1995             | Human  | Septic pulmonary emboli                    | 2.375                                   |
| 29 | 1996             | Human  | Osteomyelitis, subcutaneous abscess        | 2.259                                   |
| 30 | 1996             | Human  | Pneumonia                                  | 2.405                                   |
| 31 | 1996             | Human  | Osteomyelitis                              | 2.509                                   |
| 32 | 1996             | Human  | Primary bacteremia                         | 2.529                                   |
| 33 | 1996             | Human  | Hepatosplenic abscess                      | 2.482                                   |
| 34 | 1996             | Human  | Hepatosplenic abscess                      | 2.398                                   |
| 35 | 1996             | Human  | Hepatosplenic abscess                      | 2.449                                   |
| 36 | 1998             | Human  | Hepatic abscesses                          | 2.567                                   |
| 37 | 2001             | Human  | Mycotic aneurysm (thoracic aorta)         | 2.517                                   |
| 38 | 2001             | Human  | Septicemia                                 | 2.318                                   |
| 39 | 2001             | Human  | Multiple abscesses                         | 2.506                                   |
| 40 | 2011             | Soil   | Farm-1 (60 cm below surface)               | 2.470                                   |
| 41 | 2011             | Soil   | Farm-1 (30 cm below surface)               | 2.626                                   |
| 42 | 2011             | Soil   | Lawn (10 cm below surface)                 | 2.508                                   |
| 43 | 2011             | Soil   | Lawn (60 cm below surface)                 | 2.436                                   |
| 44 | 2011             | Soil   | Lawn (30 cm below surface)                 | 2.461                                   |
| 45 | 2011             | Water  | Pond                                       | 2.537                                   |
| 46 | 2012             | Soil   | Farm-1 (60 cm below surface)               | 2.425                                   |
| 47 | 2012             | Soil   | Farm-1 (60 cm below surface)               | 2.509                                   |
| 48 | 2012             | Soil   | Farm-1 (10 cm below surface)               | 2.511                                   |
| 49 | 2012             | Soil   | Farm-1 (10 cm below surface)               | 2.315                                   |
| 50 | 2013             | Aerosols | Primary school                              | 2.307                                   |
| 51 | 2013             | Aerosols | Primary school                              | 2.479                                   |
| 52 | 2013             | Aerosols | Primary school                              | 2.385                                   |
| 53 | 2013             | Soil   | Farm-1 (60 cm below surface)               | 2.457                                   |
| 54 | 2013             | Soil   | Farm-1 (60 cm below surface)               | 2.462                                   |
| 55 | 2013             | Soil   | Farm-1 (60 cm below surface)               | 2.413                                   |
| 56 | 2013             | Soil   | Farm-1 (60 cm below surface)               | 2.573                                   |
| 57 | 2013             | Soil   | Farm-1 (60 cm below surface)               | 2.517                                   |
| 58 | 2013             | Soil   | Farm-1 (60 cm below surface)               | 2.509                                   |
| 59 | 2013             | Water  | Pond                                       | 2.561                                   |
| 60 | 2013             | Water  | Pond                                       | 2.27                                    |
| 61 | 2013             | Water  | Pond                                       | 2.436                                   |
| 62 | 2013             | Water  | Pond                                       | 2.595                                   |

These isolates were identified as *B. pseudomallei* by sequencing of the 16S rRNA and flagellar genes (6).

were identified as *B. pseudomallei* (PUMCH) by partial 16S rDNA sequencing analysis.

The Bruker Biotyper MALDI-TOF MS system misidentified all the 62 isolates of genetically confirmed *B. pseudomallei* as *B. thailandensis* (seven isolates with a score value ≥2.000) or *Burkholderia* species (19 with score values ranging from 1.803 to 1.996). However, arabinose assimilation testing showed that all 62 isolates were arabinose non-assimilators, indicating that these isolates were not *B. thailandensis*. Clustering analysis of the 26 genetically confirmed isolates of *B. pseudomallei* collected from NTUH by the Bruker Biotyper MALDI-TOF MS system identified five cluster groups (Figure 1).

Because *B. pseudomallei* was not listed in the current Bruker Biotyper MALDI-TOF MS database (DB5627), spectra of five isolates, namely NTUH-3, NTUH-13, NTUH-14, NTUH-16, and NTUH-17, were selected from each cluster group for MSP (database entrance). The database generated using the five isolates was blindly tested against the spectra of the remaining 57 isolates (21 from NTUH and 36 from southern Taiwan). The best identification scores were found according to the...
MADI-TOF MS for Burkholderia pseudomallei

This study revealed several important findings. First, the Bruker Biotyper MALDI-TOF MS system failed to correctly identify clinical and environmental isolates of *B. pseudomallei* because *B. pseudomallei* is not listed in the current version of FDA-cleared Bruker library. Although there is a "security-relevant library" which contains *B. pseudomallei*, this library is not available in most clinical microbiological laboratories. Second, when we included the *B. pseudomallei* NTUH-3 strain only into the current library, all isolates of *B. pseudomallei* could be identified *B. pseudomallei* by the Bruker Biotyper MALDI-TOF MS system with a correct identification rate of 100%. Third, the control strain of *B. thailandensis* was still identified as *B. thailandensis* as the best match organism when the enhanced database was used. Finally, all isolates obtained from environmental sources were confirmed to be *B. pseudomallei* not *B. thailandensis*.

Identification of *B. pseudomallei* poses difficulties in clinical microbiology laboratories. Although, *B. pseudomallei* is included in the API 20NE, the Vitek 1, and the Vitek 2 databases, the accuracy of identification by these systems varies (Lau et al., 2015). Misidentification of *B. pseudomallei* as other *Burkholderia* species such as *B. cepacia* complex and *B. putida* as well as *Pseudomonas aeruginosa* is common using these commercial systems (around 20%; Lowe et al., 2006; Deepak et al., 2008; Zong et al., 2012; Lau et al., 2015). Furthermore, commercial bacterial identification kits might fail to differentiate between *B. pseudomallei* and a closely related species such as *B. thailandensis*, although >99% of cases of melioidosis are caused by *B. pseudomallei* (Lau et al., 2015). The issue of misidentification of *B. pseudomallei* is of importance for patient care as well as laboratory safety.

Traditionally, *B. pseudomallei* can be distinguished from *B. thailandensis* by arabinose assimilation (Lau et al., 2015). Genotypic differentiation between *B. pseudomallei* and *B. thailandensis* can be achieved by specific PCR-based identification using *B. pseudomallei*-specific gene targets, such as the Type III secretion system and Tat-domain protein and sequencing of gene targets of 16S rRNA and groEL (Lau et al., 2015).

MALDI-TOF MS, a revolutionary technique for pathogen identification, has been shown to be a potentially useful tool for rapid identification of *B. pseudomallei*, although existing databases require optimization by adding reference spectra for *B. pseudomallei* (Inglis et al., 2012; Lau et al., 2012; Niyompanich et al., 2014; Lasch et al., 2015). Jang et al. reported a case of multifocal aneurysms in the aortic arch of the thoracic aorta and pseudoaneurysm in the abdominal aorta and the inferior area of the superior mesenteric artery caused by *B. pseudomallei* (Jang et al., 2015). Colonies from positive blood and tissue were compatible with the presumptive identification of *B. pseudomallei*. However, using the Vitek 2 system, the blood isolate was identified as *B. pseudomallei* and the tissue isolate was identified as *B. cepacia* complex. The blood isolate was identified as *B. thailandensis* with a score value of 1.901 by the Bruker Biotyper MALDI-TOF MS system (Jang et al., 2015). Lau et al. used the Bruker database extended with *B. pseudomallei* reference strains, three *B. thailandensis* isolates were misidentified as *B. pseudomallei*. In this study, the reference strain of *B. thailandensis* was correctly identified with enhanced Bruker database. More isolates of *B. thailandensis* isolates are needed for verify the accuracy of this new database.

Recently, bioMerieux recognized misidentification of *B. pseudomallei* as an important issue and addressed the problem
by altering the algorithm parameters. The new parameters are included in the most recent software release (version 4.03 for Vitek 2 60/XL and version 2.01 for Vitek 2 Compact; Lowe et al., 2006).

Interestingly, in this study all four isolates initially identified as *B. pseudomallei* by the Vitek 2 ID-GN card were identified as *B. cepacia* complex or *B. pudita* by 16S rRNA sequencing analysis. In areas with high endemicity of melioidosis like Taiwan, laboratory staff tends to report the results of *B. pseudomallei* isolation without further clarification. In contrast, most clinical microbiologists in Beijing often recheck the identification of *B. pseudomallei* because of the low incidence of melioidosis in northern China (Yang, 2000; Currie et al., 2008; Fang et al., 2015; Zheng et al., 2015).

**SUMMARY**

In areas where this organism is endemic, such as in South Asia and Northern Australia, identification of *B. pseudomallei* is not usually problematic (Currie et al., 2008; Lau et al., 2015).
With increased international travel and threats of bioterrorism, recognition, and accurate identification of these organisms is important (Lau et al., 2015). The use of automated identification systems, including MALDI-TOF MS, in the clinical microbiology laboratory is becoming common as the pressure of cost containment impacts staff resources. In this study, using our newly created database, all *B. pseudomallei* isolates were correctly identified to the species level using the Bruker Biotyper MALDI-TOF MS system. These findings suggest that MALDI-TOF MS is a versatile and robust tool for the rapid identification of *B. pseudomallei* isolates. Expansion of commercially available databases with pathogens endemic in different regions is crucial to improve the usefulness of MALDI-TOF MS. However, this successful application of MALDI-TOF can only be regarded as pilot study, due to the small sample size, which needs independent validation before it can be offered as routine technique in the clinic.

**AUTHOR CONTRIBUTIONS**

HW, PH, and YX conceived and designed the experiments, performed the experiments, analyzed the data, and wrote the paper. YC, ST, ZX performed the experiments and analyzed the data. HW, YC, ST, ZX, PH, YX read and approved the final version of the manuscript.

**ACKNOWLEDGMENTS**

This study was conducted at Taoyuan General Hospital and four privately run respiratory care wards in Northern Taiwan.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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