ORIGINAL ARTICLE

Research on the establishment and application of protein fingerprint spectrum database of *Burkholderia pseudomallei* in Hainan Province China

M. H. Huang, H. Wu, L. L. Liu and X. M. Wang

Department of Clinical Laboratory, Hainan General Hospital, Hainan Affiliated Hospital of Hainan Medical University, China

Abstract

Burkholderia pseudomallei (B. pseudomallei) is the etiologic agent of melioidosis. The aim of this study was to establish a SuperSpectrum of *B. pseudomallei* in Hainan and evaluate its application value in the rapid identification of clinical isolates of *B. pseudomallei*. In total, 99 isolates of *B. pseudomallei* were obtained between 2010-2019 in different regions of Hainan Province, multilocus sequence typing (MLST) was performed, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was applied for spectrum acquisition. A SuperSpectrum was created based on the selection of 80 representative average spectra for 17 isolates of *B. pseudomallei*. Then we validated the SuperSpectra with 82 strains of *B. pseudomallei*, eight strains of *Burkholderia thailandensis*, two strains of *Burkholderia cepacia*, one strain of *Burkholderia cenocepacia* and one strain of *Burkholderia multivorans*, as well as one strain of *Burkholderia gladioli* identified by MLST typing, 16S rRNA gene sequencing and Vitek 2 Compact. The results showed that all samples in this study were confirmed at the species level. Protein fingerprints spectra showed that specific peaks occurred in *B. pseudomallei* from the Hainan region. The result of clustering typing indicated that *B. pseudomallei* and its closely related species could be well classified by MALDI-TOF MS at the protein level.

Keywords: 16S rRNA gene sequencing analysis, *Burkholderia pseudomallei*, MALDI-TOF MS, superspectra, vitek 2 compact
Article published online: 1 September 2021

Corresponding author: X. Wang, Department of Clinical Laboratory, Hainan General Hospital, No. 19 Xiuhua Road, Xiuying District, Haikou, 570311, China
E-mails: huangmeihuifsh@163.com **(M.H. Huang),** syjykwuhua@163.com **(H. Wu),** liu_lingli@126.com **(L.L. Liu),** wxmhn1260@163.com **(X.M. Wang)**

Introduction

Melioidosis is a zoonotic infectious disease caused by *Burkholderia pseudomallei* (*B. pseudomallei*). Acute systemic melioidosis has a case fatality rate of up to 50% even if treated [1]and is the third most common cause of death from infectious diseases [2]. *B. pseudomallei* is widely distributed in water and soil throughout tropical and subtropical zones, and is largely prevalent in northeastern Thailand [3], southern Taiwan [4], and northern Australia [3]. The Hainan Province is an international tourist island and the main epidemic source of melioidosis in China. Melioidosis is mainly distributed in the southwestern coastal cities (counties) of Hainan Province, such as Sanya and Ciudad del Este, and less in the northeastern cities (counties). Due to increased travel activity, international trade, climate change and the potential biothreat of *B. pseudomallei*, an infection caused by *B. pseudomallei* has become a serious problem. Therefore, rapid and accurate identification of the bacteria is of great significance. Currently, the methods of detecting *B. pseudomallei* include primarily biochemical identification, molecular biology methods and mass spectrometry. Rapid biochemical analysis systems, such as API 20NE, Vitek 1 and Vitek 2 database, all contain *B. pseudomallei,* but it takes about 6 h−18 h, and the correct identification rate is unstable (accuracy was 53−98%) [5]. Molecular biology methods, including 16S rRNA, multilocus sequence typing (MLST) [6] and whole-gene sequencing, are highly accurate and sensitive, but because of the high technical requirements and the need for specialized instruments, they cannot be routinely

https://doi.org/10.1016/j.nmni.2021.100938

applied in clinical laboratories. MALDI-TOF MS has the potential to identify pathogens rapidly and reliably, but *B. pseudomallei* is not in the FDA-approved database. Thus, the establishment of localized SuperSpectrum of *B. pseudomallei* is the premise for rapid and accurate identification of the bacteria. In this study, the characteristic fingerprint spectrum of *B. pseudomallei* was acquired by MALDI-TOF MS, and the SuperSpectrum of *B. pseudomallei* was created to realize rapid and correct identification of *B. pseudomallei* to provide technical support for the epidemic outbreak and traceability of melioidosis.

Materials and methods

Bacterial isolates

A total of 99 *B. pseudomallei* were collected from clinical samples of melioidosis patients in different regions of Hainan. In addition, seven *B. thailandensis* isolates were acquired from the soil in parts of Hainan, including Sanya (four strains), Wanning (two strains) and Ledong (one strain). The reference strains of *B. thailandensis*(ATCC700388), *B. cepacia* (ATCC25416) and *B. cenocepacia* (ATCC25608) were all purchased from Beijing Zhongyuan company. Strains of *B. cepacia*, *B. multivorans* and *B. gladioli* were all obtained from clinical isolates of Hainan General Hospital, accounting for one strain each. A total of 17 representative *B. pseudomallei* isolates(all of them were confirmed by MLST and Vitek 2 compact) were selected as the established strains. These 17 strains were collected between 2010–2019, of which eight strains were from male patients, and nine strains were from female patients. And they came from different regions of Hainan Province, such as Haikou, Wenchang, Qionghai, Wanning, etc.

VITEK 2 compact biochemical identification

After recovering all 112 strains stored at −80°C, they were inoculated with quality-control strain *Enterobacter cloacae* ATCC700323 onto the Columbia Sheep Blood Agar Plate (CBA) (Zhengzhou Antu Biological Company, Zhengzhou City, China), MacConkey Agar Plate (MAC) (Zhengzhou Antu Biological Company, Zhengzhou City, China), and Influenza Blood Chocolate Plate (Vancomycin Chocolate Agar Plate, CHA, Zhengzhou Antu Biological Company, Zhengzhou City, China) and incubated in 5% CO_2 at 35°C for 18−24 h. Vitek 2 compact (BioMerieux, French) VT2.R 7.01 of the GN (BioMerieux, French) card was used to identify the colonies grown on three different media, respectively, and the specific operation was conducted according to the standard operating procedure in the instrument manual.

Using 16S rRNA gene sequencing

The identification of 13 strains (eight strains of *B. thailandensis*, two strains of *B. cepacia*, one strain of *B. cenocepacia*, one strain of *B. multivorans and* one strain of *B. gladioli*) used in this study was confirmed at the species level using the primers described by Brett et al. [7–9]. The PCR products were sent to Tianyihui Yuan Company (Guangzhou, China) for sequencing, and the sequences were searched against the GenBank database using the BLAST algorithm(https://blast.ncbi.nlm.nih.gov/Blast.cgi).

MLST sequencing and typing of *B. pseudomallei* pathogenic strains

A total of 99 *B. pseudomallei* pathogenic strains were identified by MLST. The specific methods of MLST analysis referred in detail to the *B. pseudomallei* MLST research scheme established by Godoy *et al.* [10]. The sequencing results of alleles on seven housekeeping genes of each *B. pseudomallei* pathogenic strain were compared with gene sequences in the MLST database to find their respective allele numbers. The composition of all allele numbers was the *B. pseudomallei* sequence type (ST).

MLST sequencing and typing of *B.thailandensis* [10]

A total of seven isolates of *B. thailandensis* other than the reference strain (ATCC700388) were recovered, and their chromosomal DNA was extracted, followed by PCR amplification and sequencing on seven alleles (*ace, dtB, gmhD, lepA, lipA, narK, ndh*). TaKaRa LA Taq with GC buffer system and 50 μl reaction volumes were used for PCR. Amplification conditions: 95°C for 2 min; 30 cycles of 94°C for 40 s, 58°C for 40 s, and 72°C for 40 s; 72°C for 7min. PCR products were sequenced bidirectionally. The sequencing results were collated and submitted to the MLST database (http://pubmlst.org/bpseudomallei/) to obtain the allele sequence number and determine the ST of strains.

MALDI-TOF MS acquisition

A total of 99 *B. pseudomallei* strains and 13 strains (eight strains of *B. thailandensis*, two strains of *B. cepacia*, one strain of *B. cenocepacia*, one strain of *B. multivorans and* one strain of *B. gladioli*) were incubated onto the Columbia Sheep Blood Agar Plate (CBA). All of the plates were inoculated in 5% CO_2 at 37°C for 24 h. The monoclonal colonies were picked up by 1 μl volume of sterile inoculation loop and uniformly spread on the target plate, add 1 μl CHCA matrix solution immediately, and then dried at room temperature to form crystals. The VITEK MS (BioMerieux, French) was calibrated, and then mass spectra data were collected and analysed, as well as identified through SARAMS Premium software (KB version 4.16, BioMerieux, French). After the spectrum acquisition, the data

were transferred from a VITEK MS acquisition station to the Saramis analysis server.

Selection of reference spectra

A total of 100 mass spectra were collected for each established strain, and an average spectrum was generated for the 100 original spectra. SARAMS Premium software was used to transfer the average mass spectrum of *B. pseudomallei* bacteria from each sample and remove the debased spectra, and then aggregate these mass spectra to generate the reference spectra.

Creation of SuperSpectra

According to the specificity, intensity, mass-to-charge ratio (m/z) and other comprehensive parameters of the peak, a SuperSpectra containing all of the characteristic peaks was created. The original Spectra of the SuperSpectra should meet the following conditions: (1) The similarity between different strains of the same species should be 65% or more; (2) the original peak number of the database establishment spectra was controlled between 100 and 200; (3) select 39 specific masses (the common rate of each specific masses was $\geq$80%); (4) the weight of the obtained specific masses was 31, and the sum of the weights was 31 × 39 = 1209 < 1400; (5) the error range of mass number (%) was 0.0013−0.0746 < 0.08; (6) the absolute peak and relative peak intensity (%) range: 0.30−43.5 > 0. The SuperSpectra were activated for subsequent automated identification at the species level after it has been created.

Verification of SuperSpectra for *B. pseudomallei* isolates

For assessing the capability and stability of the newly created SuperSpectra, external validation was performed for the remaining 95 validation isolates. Of the 95 strains, 82 were *B. pseudomallei* strains and their closely related species (eight strains of *B. thailandensis*, two strains of *B. cepacia*, one strain of *B. cenocepacia*, one strain of *B. multivorans* and one strain of *B. gladioli*). The 95 spectra were imported into SARAMS Premium software for pattern matching with the newly created SuperSpectrum database, and the obtained identification results were analysed for evaluating the clinical identification capabilities of the VITEK MS super mass spectrum of *B. pseudomallei*.

MALDI-TOF MS dendrogram

To construct the dendrogram, Flexanalysis software (KB version 4.16) was used to adjust the baseline and smoothness of the spectra. Cluster analysis was performed based on a comparison of the similarity of Main Spectra Projection by calculated the pattern matching.

Results

Vitek 2 compact biochemical identification for *B. pseudomallei* and their closely related species

The colonies of *B. pseudomallei* on the Columbia Sheep Blood Agar Plate and Influenza Blood Chocolate Plate were silvery white and glossy. After 72 hours of culture, the colonies showed a typical 'wheel' shape. On MacConkey Agar Plate, due to the decomposition of lactose, the colonies of *B. pseudomallei* turned into red colonies with a little lustre. The number of 150 *B. pseudomallei* strains cultured on three different media that were accurately identified as *B. pseudomallei* was as follows: 81 strains on CBA, 78 strains on MAC and 75 strains on CHA. Additionally, a total of eight strains of *B. thailandensis*, two strains of *B. cepacia* and one strain of *B. gladioli* were all accurately identified. In addition, one reference strain of *B. cenocepacia* and one strain of *B. multivorans* were misidentified as *B. cepacia*.

Molecular biological identification results

Using 16S rRNA gene sequencing, a total of eight strains of *B. thailandensis*, two strains of *B. cepacia*, one strain of *B. cenocepacia*, one strain of *B. multivorans* and one strain of *B. gladioli* were all identified correctly. Through identification and typing of MLST, a total of 99 isolates, including the strains established database, were all *B. pseudomallei* strains, and the most common STs were ST4 (13 strains), ST55 (13 strains), ST50 (seven strains) and ST58 (nine strains). Furthermore, all of the eight isolates of *B. thailandensis* were identified accurately as *B. thailandensis,* and the most common ST was ST345(3 strains).

MALDI-TOF MS identification results

The identification results of 82 isolates of *B. pseudomallei* were consistent with MLST. In addition, eight strains of *B. thailandensis*, two strains of *B. cepacia*, one strain of *B. cenocepacia* and one strain of *B. multivorans*, as well as one strain of *B. gladioli* were all identified accurately. The correct identification rate of the *B. pseudomallei* database was 100%, and the confidence interval was 75.5−99.9%. All of the original spectra obtained before the SuperSpectrum established of 82 *B. pseudomallei* isolates were no match by comparing with the SARAMS Premium database.

Identification of discriminatory peaks

In this study, it was demonstrated that the specific peaks of Hainan were partially different from those of Thailand [3,11], Taiwan [4], Australia [3]and other endemic regions. *B. pseudomallei* isolates in the above areas were all found in 2049 Da, 4410 Da, 5149 Da, 6551 Da and 7169 Da, respectively, see text

(Fig 1). Furthermore, the specific peaks of Hainan were extremely similar to those of *B. thailandensis* (around 2206 Da, 2597 Da, 3112 Da, 3585 Da, 4043 Da, 4115 Da, 4410 Da, 5194 Da, 6226 Da, 7170 Da, 9621 Da and 10,486 Da), see text (Fig 1). Moreover, nine specific peaks (2876 Da, 3275 Da, 3658 Da, 3762 Da, 3997 Da, 4810 Da, 6551 Da, 7526 Da and 8094 Da) have not previously been reported in other regions, which may be unique biomarkers to *B. pseudomallei* strains in Hainan.

Phylogeny, based on MALDI-TOF MS protein profiles

Based on the Saramis dendrogram threshold of 65%, the mass spectrum peaks of 95 *Burkholderia* strains were divided into three groups by the VITEK MS system. These three groups were *B. pseudomallei*, *B. thailandensis* and other closely related species of *Burkholderia*, see text (Fig 2).

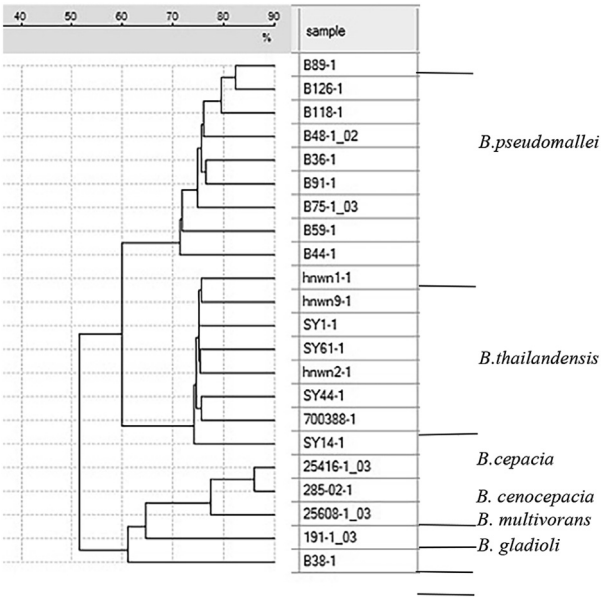

FIG. 2. Clustering of mass spectra of *Burkholderia spp*. Based on the Saramis dendrogram threshold of 65%, the mass spectrum peaks of *B. pseudomallei* and its related species (eight strains of *B. thailandensis*, two strains of *B. cepacia*, one strain of *B. cenocepacia*, one strain *B. gladioli* and one strain *B. multivorans*) were divided into three groups by the VITEK MS system. For details, see text.

Discussion

MALDI-TOF MS is a time-of-flight mass spectrometry technique for microbial identification and typing, which was developed in recent years. It uses a specific mass-to-charge ratio among different bacteria as a biomarker molecule to identify bacteria, which has the advantages of rapid, accurate and high throughput. Moreover, compared with the VITEK 2 Compact automatic microbial analysis system and 16S rRNA sequencing method, MALDI-TOF MS can shorten the identification time of bacteria from 6 h to 48 h to several minutes. However, its identification and typing ability of unknown bacteria must be established in the spectral database with enough known strains to search in order to realize the best matching and true and reliable strain identification results. Due to the apparent geographical distribution characteristics of *B. pseudomallei* and the bacteria is not listed in the current version of FDA approved database. Therefore, the laboratory can establish a high-quality protein fingerprint database of local *B. pseudomallei* strains on the basis of the original database according to the epidemic characteristics of the region to realize the rapid diagnosis of melioidosis.

At present, there are few data on the accurate identification of *B. pseudomallei* by MALDI-TOF MS, which is still in the Basic research stage and has not been widely used in clinical practice. Thus, we need to acquire more data to provide a

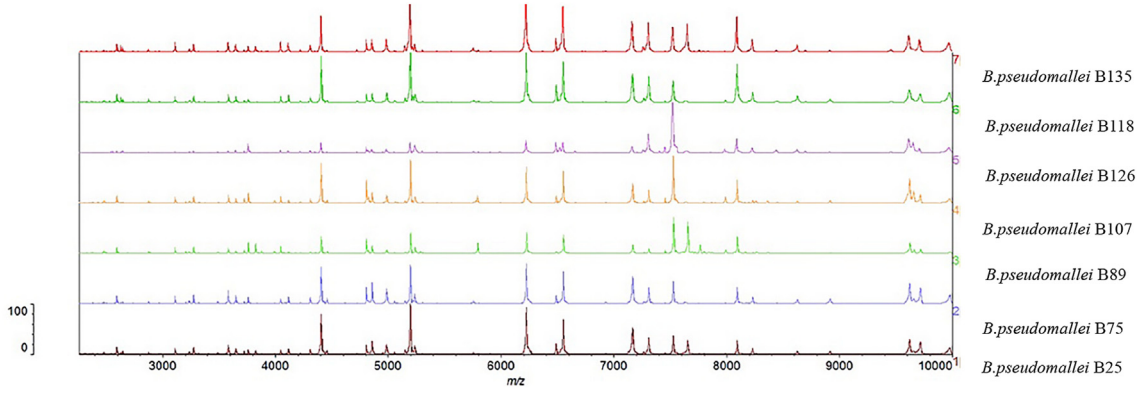

FIG. 1. Characteristic MALDI-TOF MS spectra of *B. pseudomallei*. The vertical axis shows relative intensities of ions, and the horizontal axis shows mass to charge ratio (m/z) or masses of ions (Da).

theoretical basis for the comprehensive application of this technology in clinical practice in the future. Since *B. pseudomallei* bacteria is not contained in the commercialized database, a localized *B. pseudomallei* database should be established first to identify *B. pseudomallei* strains by mass spectrometry. The first published study of its application to *Burkholderia spp*. was in 2012. Karge *et al.* [12] established the database using 10 *B. pseudomallei* strains and 17 *B. mallei* strains. Then 9 *B. pseudomallei* strains (including two strains of the database established) and 16 *B. mallei* strains (including 12 strains of the database established) were used for verification, with an accuracy of 100% [12]. The limitation of the above study was that there were fewer strains within the database for construction and verification, and the validation of strains contains the established strains, which resulted in less representative. Since then, several related studies have been published. Wang *et al.* [4] used 5 *B. pseudomallei* strains from National Taiwan University Hospital and Peking Union Medical College Hospital to establish a database in 2016. Additionally, they verified the newly created database with 57 *B. pseudomallei* strains and 30 *B. cepacia* complex strains and 30 *B. putida* strains, in which *B. pseudomallei* can be correctly identified, and *B. cepacia* and *B. putida* were not identified mistakenly for *B. pseudomallei* [4]. The largest evaluation of *B. pseudomallei*, to date, has been undertaken using 26 strains of *B. pseudomallei* from Thailand, Laos, Cambodia, Australia and 21 other *Burkholderia* strains to construct the database [3]. They then tested the accuracy of the new database for the identification of 581 *B. pseudomallei* strains, 19 *B. mallei* strains, 6 *B. thailandensis* strains and 23 other strains [3]. All of the 581 *B. pseudomallei* were correctly identified, with 100% sensitivity and specificity [3]. In 2018, Li et al. [13] used 10 strains of *B. pseudomallei* and 10 strains of *B. thailandensis* strains to establish a database, and selected 20 strains of *B. pseudomallei*, 20 strains of *B. thailandensis*, 20 strains of *B. cepacia* and 20 strains of *B. multivorans* identified the newly established database, and the accuracy was 100%.

The characteristic fingerprint spectrum was acquired, and the construction of the SuperSpectrum database was done by collecting data of 17 *B. pseudomallei* isolates from different times and regions in this study. What is more, the database was verified by 95 *Burkholderia spp*., and the results indicated that the confidence level of *B. pseudomallei* was 75.5−99.9% and that accurate identification of *B. pseudomallei* can be realized through MALD-TOF MS. The results demonstrated that the identification accuracy rate of *B. pseudomallei* was 100%, the confidence interval was 75.5−99.9%. Our findings confirm that the quality of the self-built laboratory database was ideal, rapid and reliable for the identification of *B. pseudomallei* and its related species. This advantage is particularly significant for the prevention and control of infectious diseases caused by the 'bioterrorist bacteria', such as *B. pseudomallei*, which can prompt the laboratory staff to take personal protection as soon as possible. Moreover, this patient sample and its culture could be operated in strict accordance with the relevant biosafety level requirements to reduce the risk of laboratory infection. Compared with the previously reported, *B. pseudomallei* strains from different regions of Hainan Island were selected to construct the database in this study, and the number of strains for establishing and verifying the database was more than ever before (except Suttisunhakul et al. [13] 2017 report), has very good representativeness and stability. In addition, our self-built database spectra can not only be saved and implanted into bioMerieux's mass spectrometer database system or other institutions but also the data sharing can improve the identification rate of such bacteria. What is more, a total of 95 *Burkholderia spp*. strains were clustered and typed according to the spectra characteristics of different strains while establishing the mass spectrum database of *B. pseudomallei* bacteria in this study. On the one hand, the result of mass spectrometry clustering typing was more consistent with the category of molecular biology typing; on the other hand, it was similar to the result reported in other literatures, indicating that mass spectrometry technology can be well applied to the clustering typing of bacteria and provides technical support for the prevention and traceability of the outbreak and epidemic of *B. pseudomallei* bacteria.

SARAMS Premium software was used to analyse the spectral data of *B. pseudomallei* bacteria and its related species statistically in order to understand whether the protein fingerprint spectra of *B. pseudomallei* strains in Hainan was different from other endemic areas. Our observation that the specific peaks of 2049 Da, 4410 Da,5194 Da, 6551 Da and 7169 Da were the same as the specific peaks of *B. pseudomallei* isolates from endemic regions such as Thailand [3,11], Taiwan [3]and Australia [3] and had an extremely high similarity (a total of 12 mass spectra peaks between 2206 Da, 2597 Da,3112 Da,3585 Da, 4043 Da, 4115 Da, 4410 Da, 5194 Da, 6226 Da, 7170 Da, 9621 Da and 10,486 Da were identical) with *B. thailandensis* isolates from different areas of this province. However, the peaks of 2876 Da, 3275 Da, 3658 Da, 3762 Da,3997 Da, 4810 Da, 6551 Da, 7526 Da and 8094 Da were significantly different from those of other regions, which may be unique biomarkers of *B. pseudomallei* in Hainan. It was found that there was no significant difference in the peak of protein fingerprint of *B. pseudomallei* of different sequence types (ST).

Conclusion

Our findings show that MALDI-TOF MS is an efficient and robust tool for the rapid identification of *B. pseudomallei* and their closely related species after the self-created *B. pseudomallei* database was established. Therefore, MALDI-TOF MS has a high application value in the clinical diagnosis of melioidosis so that patients can be treated timely and effectively and take appropriate hospital infection control measures to prevent the spread of the epidemic. Additionally, it could strengthen biosafety management to avoid laboratory-acquired infections.

Conflict of interest

The authors declare that they have no conflict of interest.

Transparency declaration

This work was funded by the Key Research and Development Program of Hainan Province, China (No. ZDYF2018113, Grant:500,000¥), Hainan Natural Science Foundation, China (No. 817319, Grant:100,000¥).

Authors contributions

MeiHui Huang, Hua Wu, LingLi Liu and XuMing Wang isolated bacteria and performed the laboratory measurements. MeiHui Huang and XuMing Wang made substantial contributions to conception and design and drafted the manuscript. All authors read and approved the final manuscript.

References

[1] Deris ZZ, Hasan H, Siti Suraiya MN. Clinical characteristics and outcomes of bacteraemic melioidosis in a teaching hospital in a northeastern state of Malaysia: a five-year review. J Infect Dev Ctries 2010;4(7):430–5.

[2] Kanoksil M, Jatapai A, Peacock SJ, Limmathurotsakul D. Epidemiology, microbiology and mortality associated with community-acquired bacteremia in northeast Thailand: a multicenter surveillance study. PLoS One 2013;8(1):e54714.

[3] Suttisunhakul V, Pumpuang A, Ekchariyawat P, Elrod MG, Turner P, Currie BJ, et al. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for the identification of Burkholderia pseudomallei from Asia and Australia and differentiation between Burkholderia species. PLoS One 2017;12(4):e0175294.

[4] Wang H, Chen YL, Teng SH, Xu ZP, Xu YC, Hsueh PR. Evaluation of the bruker biotyper matrix-assisted laser desorption/ionization time-of-FlightMass spectrometry system for identification of clinical and environmental isolates of Burkholderia pseudomallei. Front Microbiol 2016;7:415.

[5] Podin Y, Kaestli M, McMahon N, Hennessy J, Ngian HU, Wong JS, et al. Reliability of automated biochemical identification of Burkholderia pseudomallei is regionally dependent. J Clin Microbiol 2013;51(9):3076–8.

[6] Wang XM, Zheng X, Liu LL, Zhou XJ, Kuang HH, Guo HL, et al. Multilocus sequence typing of clinical isolates of Burkholderia pseudomallei collected in Hainan, a tropical island of southern China. Am J Trop Med Hyg 2016;95(4):760–4.

[7] Brett PJ, DeShazer D, Woods DE. Burkholderia thailandensis sp. nov., a Burkholderia pseudomallei-like species. Int J Syst Bacteriol 1998;48(Pt 1):317–20.

[8] Lynch KH, Dennis JJ. Development of a species-specific Fur gene-based method for identification of the Burkholderia cepacia complex. J Clin Microbiol 2008;46(2):447–55.

[9] Bauernfeind A, Schneider I, Jungwirth R, Roller C. Discrimination of *Burkholderia gladioli* from other *Burkholderia* species detectable in cystic fibrosis patients by PCR. J Clin Microbiol 1998;36(9):2748–51.

[10] Godoy D, Randle G, Simpson AJ, Aanensen DM, Pitt TL, Kinoshita R, et al. Multilocus sequence typing and evolutionary relationships among the causative agents of melioidois and glanders,Burkholderia pseudomallei and Burkholderia mallei. J Clin Microbiol 2003;41(5):2068–79.

[11] Niyompanich S, Jaresitthikunchai J, Srisanga K, Roytrakul S, Tungpradabkul S. Source- identifying biomarker ions between environmental and clinical Burkholderia pseudomallei using whole-cell matrix-assisted laser desorption/ionization time-of-flight Mass spectrometry (MALDI-TOF MS). PLOS One 2014;9(6):e99160.

[12] Karger A, Stock R, Ziller M, Elschner MC, Bettin B, Melzer F, et al. Rapid identification of Burkholderia Mallei and Burkholderia pseudomallei by intact cell Matrix-assisted Laser Desorption/Ionisation mass spectrometric typing. BMC Microbiol 2012;12:229.

[13] Li J, Hu W, Zhang Li, Li M, Rao C, Lu W. Evaluation of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for identifying Burkholderia pseudomallei and Burkholderia thailandensis isolates. Eur J Clin Microbiol Infect Dis 2019;38(1):191–6.