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Identification of *Burkholderia pseudomallei* by Use of the Vitek Mass Spectrometer

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ABSTRACT *Burkholderia pseudomallei* is the causative agent of melioidosis. This condition most often presents as pneumonia and bacteremia, with mortality rates of 9% to 70%. Therefore, early identification of this organism may aid in directing appropriate management. This study aimed to use the Vitek matrix-assisted laser desorption ionization–time of flight mass spectrometer to create a spectrum for the rapid identification of *B. pseudomallei*. Spectra from 85 isolate cultures were acquired using the Vitek mass spectrometer research mode. A SuperSpectrum was created using peak matching and subsequently activated for analysis of organism identification. All 85 isolates were correctly identified as *B. pseudomallei*. A total of 899 spectra were analyzed and demonstrated a specificity of 99.8%. Eighty-one clinical isolates were used, of which 10 were neuromelioidosis, and no discernible spectrum difference was appreciated. Spectrum acquisition from a single spot was only successful in 374/899 (41%) of isolates. This increased to 100% when 3 spots of the same extract were analyzed. The Vitek mass spectrometer can be used for the rapid identification of *B. pseudomallei* with a high level of specificity.

KEYWORDS *Burkholderia pseudomallei*, matrix-assisted laser desorption ionization, melioidosis, diagnostics, mass spectrometer

The genus *Burkholderia* contains 50 species. Among these, only *B. mallei*, *B. pseudomallei*, *B. cepacia* complex, *B. thailandensis*, and *B. gladioli* are generally recognized as human pathogens (1). These organisms utilize aerobic respiration, are non-spore-forming Gram-negative bacilli, and with the exception of *B. mallei*, are all motile (1).

B. pseudomallei is the causative agent of melioidosis in both animals and humans. It is a saprophytic organism found predominantly in moist soil and water (2, 3). Infection is often associated with an inoculating injury or the inhalation of aerosolized bacteria, which appears to occur more frequently in the wet season or severe weather events such as tropical storms (4–7). The most common presentation of melioidosis is community-acquired pneumonia and bacteremia (5, 8).

Melioidosis is predominantly a disease of the subtropical and tropical regions, endemic largely in northern Australia and South East Asia, including but not limited to Thailand, Vietnam, Laos, Cambodia, Malaysia, Indonesia, Singapore, Papua New Guinea, and the Indian subcontinent (9). The incidence appears to be increasing, and although overall mortality appears to be improving in Australia, mortality rates range from 14% to as high as 42.5% in Northeast Thailand (4, 10). Current estimates suggest 165,000 cases of melioidosis and 89,000 resultant deaths worldwide per year (11).

The identification of *B. pseudomallei* has been an ongoing challenge since its initial description. Apart from colonial morphology recognition and simple bench tests, more robust specific phenotypic characteristics have been utilized. An analytical profile index (API), specifically the bioMérieux API 20NE (bioMérieux, Marcy-l'Etoile, France) is used as

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an adjunct to the diagnosis. Unfortunately, this method, with a turnaround time of approximately 48 h, has demonstrated a 31% misidentification of *B. pseudomallei* in one study and a range of 37% to 99% in others (1, 12). Automated colorimetric-based identification such as Vitek-2 has an improved accuracy; however, it too has a wide margin of error with 63% to 81% accuracy (13). An indirect diagnosis by using serology is an alternative if no microbiological specimens are available or positive. However, serology is less useful in areas of endemicity, as it will not be able to differentiate exposure, past, chronic, or active infection. The indirect hemagglutination assay (IHA) test is performed using an antigen from strains of *B. pseudomallei* sensitized to sheep cells and utilizes unsensitized cells as a control (1). Notably, the combination of IHA and IgM enzyme-linked immunosorbent assay (ELISA) in the diagnosis of acute melioidosis in an area of endemicity demonstrated a sensitivity of 100% and specificity of 95.4%; however, the performance of this test is variable in a low prevalence setting (14). The development of a lateral flow assay which detects capsular polysaccharide via a monoclonal antibody has a reported sensitivity of 99% and specificity of 100% when used on turbid blood cultures (15, 16). The test is easy to perform, relatively inexpensive, and may be a promising rapid diagnostic tool (16). PCR testing of clinical isolates in *B. pseudomallei* culture-positive patients has demonstrated 100% sensitivity and specificity on wound swabs, abscess aspirates, urine, and sputum (17). Notably, the sensitivity of this assay on blood samples ranged from 17% to 74% depending on features of septic shock and likely reflects a constraint in the limit of detection (17, 18). Currently, a major reason for the poor uptake of molecular testing appears to be a limited availability of real-time PCR instrumentation in diagnostic laboratories.

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is a rapid identification system which is widely used in diagnostic microbiology laboratories. The benefits of this method include a short analysis time and high sensitivity and specificity, as well as limited technical and training requirements (19–21). Two commercially available MALDI-TOF MS instruments, namely, the Bruker Microflex Biotyper (Bruker Daltonik GmbH, Bremen, Germany) and bioMérieux Vitek MS (bioMérieux, Marcy-l'Etoile, France), have been approved for clinical use. Neither system's standard diagnostic database includes the reference spectra required for identification of *B. pseudomallei* by MALDI-TOF MS (22, 23). The standard Vitek MS *in vitro* diagnostic (IVD) database can identify the organism to the genus but not species level (24). This is of limited use, as it is not uncommon for *B. pseudomallei* to be misidentified as *B. cepacia* by phenotypic methods. The Vitek MS research use only (RUO) database does include *B. pseudomallei* spectra, but it is not FDA approved nor has it been evaluated in a clinical context (25). Using the Bruker standard database, the result may only be accurate to the genus level or the isolate may be misidentified as *B. thailandensis*. On this platform, the security-relevant library, which includes potential agents of bioterrorism, can identify *B. pseudomallei* (25–27). In-house Bruker MS reference libraries have been created and found to be accurate in the identification of *B. pseudomallei* from both primary isolates and directly from blood cultures (28, 29). This study demonstrated a potential workflow that would decrease the time to identification by up to 24 h. There is no current standard for the minimum number of isolates or strains required for the creation of in-house spectra; however, there is evidence to suggest that 10 or more reference spectra correlate with the greatest identification accuracy (30). A potential drawback to the use of MALDI-TOF MS is the requirement for organism inactivation. Multiple methods have been proposed, including 70% ethanol, formic acid, trifluoroacetic acid, gamma irradiation, centrifugation, and filtration, with various results (31, 32). One study reported a 100% reduction in viable organisms treated with on-plate 70% formic acid (33).

Given the nature of the infection and high mortality rate, a rapid diagnosis is imperative to enable appropriate directed therapy (4, 8). This study aimed to use the Vitek MS RUO mode to create a spectrum for the rapid identification of *B. pseudomallei*.

MATERIALS AND METHODS

The study was performed at the Townsville Hospital, a 600-bed tertiary care center in North Queensland, Australia. Approval for this study was obtained from the Townsville Hospital and Health Service Human Research Ethics Committee (HREC/18/QTHS/3). Isolates of *B. pseudomallei* were obtained from the department of microbiology's stored collection of over 250 clinical isolates, collected from 1990 to 2017. Due to the potential diversity of different *B. pseudomallei* proteomes, 40 random isolates were initially chosen for analysis. All handling of preinactivated isolates occurred in a physical containment (PC) level 3 laboratory. Each isolate was initially plated onto Ashdown's agar (ASH) to ensure pure growth. Subsequently, the cultured organism was plated in duplicates on horse blood agar (HBA), chocolate agar (CHOC), and ASH (bioMérieux, Marcy-l'Etoile, France). Each isolate was then incubated at 35°C in both O_2 and CO_2 environments. Additionally, 24- and 48-h colony growth of each isolate was analyzed.

To minimize the potential risk of laboratory-acquired infection and to maximize protein extraction, the Vitek *Mycobacterium*/Nocardia kit was used for isolate inactivation and extraction according to the manufacturer's instructions. A 1-μl volume of each extracted sample was placed on a target spot on the MALDI-TOF MS acquisition plate. After air drying, 1 μl of α-cyano-4-hydroxycinnamic acid (CHCA) matrix was added to the spot and allowed to crystallize prior to spectrum acquisition. Spectrum acquisition was performed in RUO mode utilizing the Shimadzu Biotech MALDI-MS application (Shimadzu Biotech, Kyoto, Japan). Each isolate from each growth condition was analyzed at least once, totaling a minimum of 12 spectra per isolate. These spectra were then imported into the research database. Data analysis and SuperSpectrum creation were performed using the SARAMIS (Spectral Archive and Microbial Identification System) v4.12 application (bioMérieux, Marcy-l'Etoile, France). Additionally, 8 isolates of *B. thailandensis* were used to create a *B. thailandensis* SuperSpectrum using the aforementioned methodology, with the aim of excluding overlapping mass/charge peaks from the *B. pseudomallei* SuperSpectrum to improve the specificity of identification. Once activation occurred, known isolates were run through the Vitek MS RUO mode and compared to the newly created *B. pseudomallei* SuperSpectrum to assess the accuracy of identification.

RESULTS

A total of 568 spectra were created using 40 isolates. Of the 40 isolates, 36 were from clinical samples, 2 were American Type Culture Collection (ATCC) strains (ATCC 23343 and ATCC 4846), 1 was a National Collection of Type Culture (NCTC) strain (NCTC 13178), and 1 was a Western Australian Culture Collections (WACC) strain (WACC 236/91). A comparison between isolates demonstrated a diversity of both phenotypes and spectra with regard to peaks and intensity (Fig. 1).

There was also substantial intraisolate spectrum variability. This variability did not appear to be specifically influenced by the incubation period, O_2 and CO_2 culture environment, or HBA, CHOC, and ASH media.

Following completion and activation of the SuperSpectrum, the original 40 isolates were rerun, and 32/40 (80%) were identified as *B. pseudomallei*. There were no incorrect organism identifications, as the 8 isolates that were not identified had no genus nor species reported. The specificity of the *B. pseudomallei* SuperSpectrum was assessed by comparing it to the SuperSpectrum of all organisms in the bioMérieux database (bioMérieux, Marcy-l'Etoile, France). Additionally, 8 *B. thailandensis* isolates were prepared, and 220 spectra were acquired with the creation of a *B. thailandensis* SuperSpectrum. None of the spectra were identified as *B. pseudomallei*.

In an attempt to improve the sensitivity of *B. pseudomallei* identification, additional isolates were used to create a second SuperSpectrum. As the initially acquired spectra did not demonstrate substantial variability with regard to culture conditions, an additional 45 isolates were cultured on HBA in 5% CO_2 for 24 h, and then spectra were acquired from colonies performed as per the aforementioned methodology. An additional 286 spectra from these 45 isolates were added to the original data. A new SuperSpectrum using 854 spectra was subsequently created with the aim of improving organism identification. With the additional spectrum acquisitions, a total of 899 spectra were acquired from 85 isolates.

An assessment of the new SuperSpectrum demonstrated correct identification of all 85 (100%) *B. pseudomallei* isolates; however, spectrum acquisition from a single spot was only successful in 374/899 (41%) of isolates. This increased to 100% when 3 spots of the same extract were analyzed. Identification accuracy scores for *B. pseudomallei* isolates ranged from 75.4% to 99.9%. There were only 2 (0.2%) misidentifications when comparing all 899 *B. pseudomallei* spectra to the Vitek MS IVD database. Of these misidentifications, one spectrum was identified as *Yersinia* species with a 78% identi-

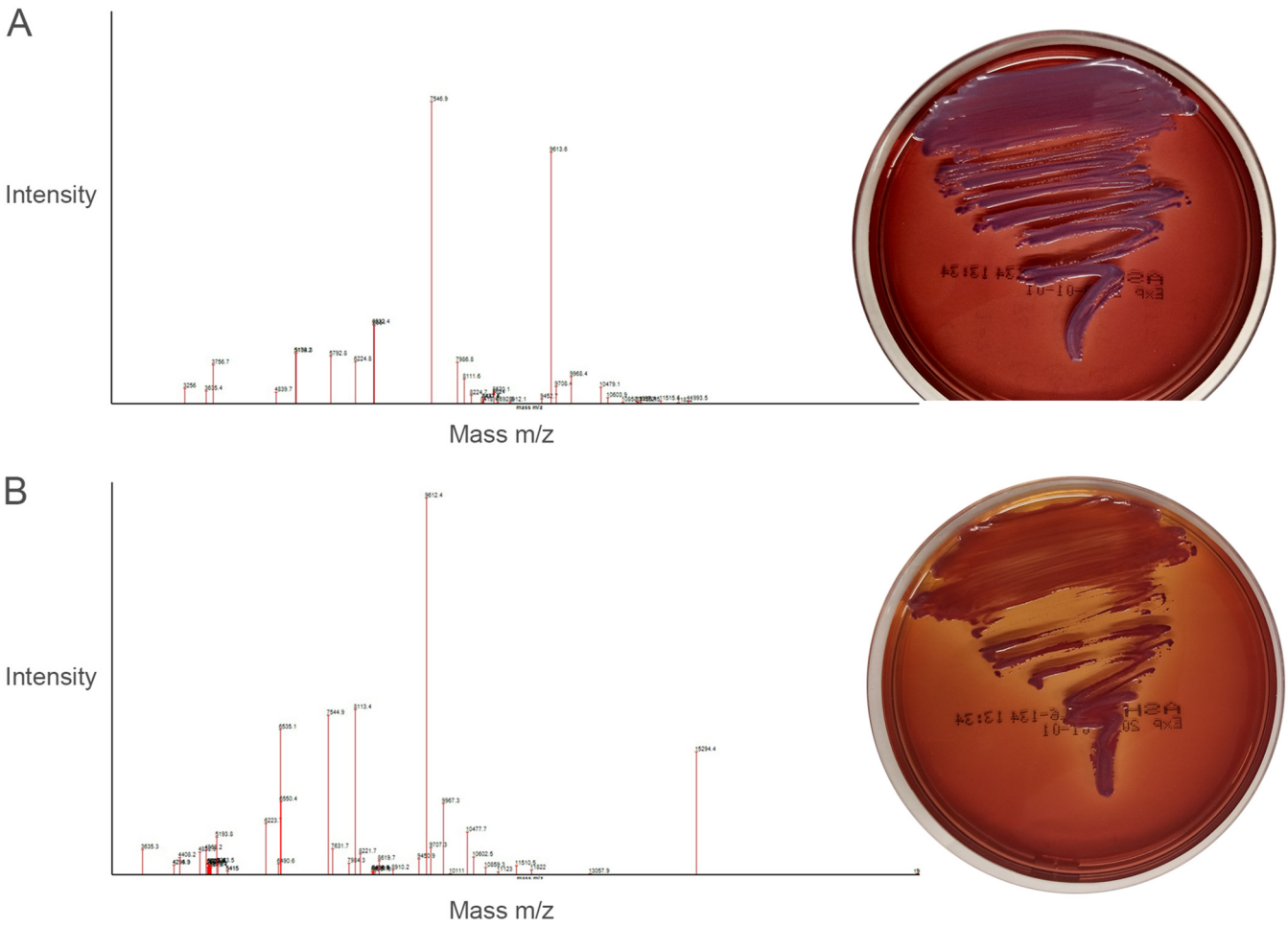

FIG 1 Phenotypic and spectrum comparison between two American Type Culture Collection (ATCC) strains. (A) ATCC 4846; (B) ATCC 23343. Organisms were cultured for 48 h at 35°C in 5% CO_2 on Ashdown agar.

fication score, the second was *Proteus mirabilis* with a score of 75%. Therefore, the specificity for identifying *B. pseudomallei* from a matched spectrum was 99.8%.

The isolates used were reviewed with regard to the clinical source of infection. Of 81 clinical isolates, 27/81 were from pulmonary foci, 13/81 were from an unknown focus, 10/81 from the central nervous system (CNS), 7/81 from joints, 5/81 from skin and soft tissues, 4/81 from lymph nodes, 4/81 from urine, 3/81 from prostates, 3/81 from osteomyelitis, 3/81 from bacteremia, 1/81 from a liver abscess, and 1/81 from a psoas abscess. There was no correlation between spectrum identification and the infection source.

After completion of the evaluation of *B. pseudomallei* identification by the newly created SuperSpectrum, a secondary analysis of spectra was performed in order to compare isolates associated with CNS and non-CNS infections. In total, 10 isolates were from patients with CNS melioidosis. A SuperSpectrum using these isolates was created in order to ascertain the most common mass peaks. In total, 47 peaks were found to have adequate reproducibility between spectra. There were no outlying peaks in the CNS SuperSpectrum that could be used to rapidly differentiate isolates that may have a CNS preponderance.

DISCUSSION

Previous methods of *B. pseudomallei* identification have been plagued by inaccuracy, prolonged turnaround time, and cost (1, 12, 26, 34). Mass spectrometry identification of organisms has increased over time and is now a standard in many laboratories worldwide. Although the initial equipment cost may be high, the laboratory savings for

organism identification has been reported at greater than 50% compared to that with conventional methods (35–37). Additionally, specific identification turnaround time for *B. pseudomallei* may be improved by 16 to 24 h given the current method involves phenotypic analysis. This improved time to diagnosis may improve antimicrobial stewardship and patient outcomes.

This study demonstrates that bioMérieux Vitek MS (bioMérieux, Marcy-l'Etoile, France) can be used for the rapid identification of *B. pseudomallei*. While the use of 40 isolates to create the first SuperSpectrum resulted in 80% isolate identification, the addition of 45 isolates resulted in 100% identification with 99.8% specificity. This information supports the fact that *B. pseudomallei* is phenotypically diverse and, as such, may have a broad potential proteome structure. Our results are surprising with regard to specificity. Previous reports have suggested misidentification with *B. thailandensis*, which was not reproduced in our data. Notably, identification was performed using the Bruker Biotyper MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) instrument (23, 26).

There are several limitations in this study. The current methodology resulted in a low reproducibility of only 41%. Specifically, a single 1-μl spot of the extracted isolate produced inconsistent spectrum results. However, assessing three or more 1-μl spots from the same extract produced 100% identification. This may be due to an imperfect ability to accurately and repeatedly measure the exact volume of colony used in the inactivation-extraction process. It has been reported in other settings that the volume of extraction agents may be adjusted depending on pellet size (38). Arguably, this may be less rigorous than creating a more accurate initial volume, as determining the pellet size accurately would be challenging. The authors acknowledge that performing a spectral analysis in triplicates is not standard practice. However, the additional time and cost in this setting would be of negligible practical consequence and would improve identification to 100% while maintaining a 0.2% false-positive rate. Another limitation of this study is that a *B. mallei* SuperSpectrum was not created to compare to that of *B. pseudomallei*, as previous data suggest that these species may be misidentified (22). However, given that *B. mallei* is a much rarer cause of human disease, this omission may be of limited clinical relevance (1, 39). The authors acknowledge that no specific phenotypic or genetic testing was performed, nor environmental isolates included; therefore, we cannot confirm the diversity of the isolates used. However, the use of 81 clinical isolates representing an array of clinical syndromes may obviate this limitation.

In conclusion, the Vitek MS RUO can be used for the rapid identification of *B. pseudomallei* and will now be considered a supplementary tool in our laboratory workflow. Further research is required with regard to improving the reproducibility of spectrum acquisition and identification. Additionally, this creates the scope to assess the performance of the new SuperSpectrum in identifying *B. pseudomallei* directly from positive blood culture.

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