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Editorial note:

There was a significant upsurge of cases of melioidosis in Hong Kong in 2022, especially in the Kowloon region, raising public awareness to the condition. In this issue of the Topical Update, Drs. Kristine Luk, May Lee and WK To share their experience in investigating and managing the cases. We welcome any feedback or suggestion. Please direct them to Dr. Janice Lo (e-mail: janicelo@dh.gov.hk), Education Committee, The Hong Kong College of Pathologists. Opinions expressed are those of the authors or named individuals, and are not necessarily those of the Hong Kong College of Pathologists.

Melioidosis: an urban outbreak in Hong Kong

Dr. Kristine LUK, Dr. May LEE and Dr. Wing Kin TO
Consultant Microbiologists, Department of Pathology, Princess Margaret Hospital, Hospital Authority

Introduction

Melioidosis is a disease of humans and animals resulting from infection with the aerobic Gram-negative bacillus *Burkholderia pseudomallei*, which is ubiquitous throughout the subtropics and tropics, particularly in Southeast Asia and northern Australia[1]. *B. pseudomallei* is a resilient environmental saprophyte and widely distributed in soil and fresh surface water in endemic regions. Although its optimal temperature of survival ranges between 24 and 32°C[2], it can resist temperature extremes, acidic and alkaline conditions, and is able to persist in distilled water for 16 years[3]. Percutaneous inoculation, ingestion, and inhalation of contaminated soil or water are well recognized modes of transmission of melioidosis[4]. One in 4,600 seroconversion-associated exposures results in clinical disease, and 4% of exposures results in latent infection[5]. The incubation period varies from 1 to 21 days (average 9 days)[6] with the majority (85%) of patients having acute presentation[6]. Melioidosis was first reported in Myanmar in 1911[7]. Hong Kong is considered an endemic area given the environmental suitability for *B. pseudomallei* and the earliest report of human melioidosis could be dated back to 1984[8]. The seropositive rate among patients in a chest hospital was reported to be 14% in a study performed in 1987[9] and the majority seropositive subjects had no travel history to endemic areas. An increasing trend of a total of 61 cases were identified in the last two decades[10]. Hong Kong has seen a mysterious spate of melioidosis cases since August 2022, with a cluster emerging in the Sham Shui Po (SSP) district. Melioidosis has been included as a statutory notifiable infectious disease in Hong Kong (under Cap. 599) since 11th November 2022. At the time of writing, a total of 51 cases of melioidosis have been diagnosed since 2015 in Kowloon West Region. In this article, we would share our experiences in the clinical features,

epidemiology and laboratory diagnosis of melioidosis[11].

Clinical features

Among the 51 patients who had their first episode of culture-proven melioidosis diagnosed from Jan 2015 to May 2023, the median age of the patients was 71 years (range, 42-94 years) and 39 (76.5%) of them were male. Worldwide the median age of affected patients is 50 with a male predominance ranging from 58.5% to 84%[12]. Possible explanations include an increased exposure to contaminated soil or water through high-risk occupations, such as agricultural or construction activities; or there is a higher prevalence of risk factors such as smoking or alcohol excess among the male patients. Diabetes mellitus is the most common comorbidity among our patients, contributing 56.9% of cases. This is also in concordance with other case series[12]. Diabetes mellitus impairs immune function by decreasing chemotaxis, phagocytosis, cytokine response, and bacterial killing by polymorphonuclear leukocytes[13,14]. Specifically, the release of the neutrophil signaling chemokine IL-8 from lung epithelial cells is delayed and diabetics are therefore at greater risk of infection by inhalation[15].

Thirty-seven patients (73%) had chest infection, of which 27 (73%) patients presented with multi-lobar pneumonia, 23 (62%) had concomitant bacteremia and 14 (38%) had mediastinal involvement. The overall case fatality rate was 27.5%. In our case cohort, there was a higher percentage of chest infection but a comparable mortality rate when compared with the cases previously reported in Hong Kong (42.6% pneumonia and 31% mortality[10]). Less than 22% of patients had exposure history (6 patients worked near construction sites; 2 patients had travel history to Thailand; 2 had history of farming; and 1 was a sewage worker). Six patients were at the ages of nineties at the time of diagnosis and two were nursing home residents. In fact, the residential address of 43 patients (84.3%) was in the SSP district within an estimated area of 2.5 km^2.

Given the clinical presentation and epidemiological information, inhalation of aerosols containing a higher bacterial load during typhoons and rainstorms was therefore suspected to cause the sudden upsurge of cases in the SSP district. Higher lethality and shorter incubation period of aerosol inhalation of *B. pseudomallei* were demonstrated by animal models[16,17], and rainfall two weeks before presentation was an independent risk factor for pneumonia, septic shock and death[18]. Increased transport of the organism in eroded topsoil via the rise in the water table during period of heavy rainfall[19] and severe weather events and wind are associated with dispersal of bacteria contaminated aerosol[20]. *Lau SK et al*[21] demonstrated the presence of *B. pseudomallei* DNA in 6.8% of soil samples collected in the oceanarium; and it was significantly correlated with ambient temperature and relative humidity. Additionally, *Chen et al*[22] successfully detected *B. pseudomallei* DNA in 80 to 100% of air samples with significant correlation with the rainfall and the presence of typhoons. Furthermore, *Currie et al*[23] cultured *B. pseudomallei* from air samples taken outside the residence of a patient with mediastinal melioidosis, and whole genome sequencing confirmed the linkage between the isolates in the air sample and the patient sample. From 9th to 12th August 2022, there were 4 culture proven melioidosis cases (three *B. pseudomallei* isolates were recovered from blood culture while one was isolated from a sputum sample) and all patients resided in the SSP district. Preceding the presentation of the cases, the Amber Rain warning and the typhoon signal-3 (Wulan) were hoisted for 3 days and 2 days, respectively[24]. On 15th August 2022, 1 out of 8 air samples (1,000 L each) taken at a podium near a construction site in SSP recovered viable *B. pseudomallei*, which was phylogenetically clustered with 27 patient isolates with less than 0.07% core genome difference[11]. It belonged to a new multi-locus sequence type (MLST) ST-1996 and was identified as early as in a patient sample collected in 2016, suggesting that *B. pseudomallei* may have persisted in the nearby environment, dispersal of which has been aggravated by reduction in vegetation in the area and extreme weather events due to climate change. Furthermore, the admission dates of cases were

strongly associated with the rainfall and the hoisting of tropical cyclone warning signals[11].

Genitourinary system was the second most commonly (17.6%) involved (five patients had prostatic abscess; four patients had urinary tract infection). Melioidosis patients also presented a wide clinical spectrum: peritonsillar abscess, skin and soft tissue infection, bone and joint infection, continuous ambulatory peritoneal dialysis (CAPD) peritonitis, organ abscess (renal, liver and spleen), pericarditis, mycotic aneurysm and meningitis. Eleven patients (21.6%) had multiple sites of infection and four patients (7.8%) had relapse of infection, with a range of 5 months to 3 years. One patient had defaulted oral eradication therapy while two patients had doxycycline as the oral eradication drug due to intolerance to trimethoprim-sulfamethoxazole. In an Australian study, the recurrence rate was reported at 5.7% with a median time to relapse of 9.4 months[25]. Relapse is commonly associated with poor compliance to antimicrobial treatment or eradication regimen containing either doxycycline or amoxicillin-clavulanate[26].

Laboratory Diagnosis

Culture

The culture of *B. pseudomallei* from blood, respiratory secretions, urine, cerebrospinal fluid, pus, and wound swabs remains the diagnostic gold standard. *B. pseudomallei* grows well on most routine laboratory media, such as blood, chocolate and MacConkey agars, revealing smooth, creamy colonies with a metallic sheen on blood agar. They are small Gram-negative bacilli with bipolar staining giving them a safety pin appearance. This is due to central accumulation of polyhydroxybutyrate granules, which do not retain the staining reagents[27]. As a consequence of prior antimicrobial treatment of the patients and presence of normal flora in non-sterile specimens, the overall sensitivity of culture has been reported at 60.2% only[28]. In our cohort, 32 patients (62.7%) had bacteremia, which has been found in 38 to 73% of melioidosis cases in other series[12]. In another study using the BacT/alert automated blood culture system (bioMérieux, Marcy ÍEtoile, France), 93% of isolates could be detected within 48 hours of incubation, with a mean time of 23.9 hours to signal positive[29]. Among the nine patients having genitourinary infection, however, only three of them had positive urine culture while additional four patients had pyuria. Urine samples are normally inoculated into cystein-lactose-electrolyte-deficient (CLED) agar for 24 hours incubation per our laboratory protocol and this may account for the low rate of isolation of *B. pseudomallei.* For patients with suspected genitourinary tract infection and sterile pyuria, request should be made to the laboratory for urine culture using nutrient agar for prolonged incubation. Notably, *B. pseudomallei* isolation in urine is consistent with renal parenchymal infection and not passive filtration into the urine[30].

Ashdown's medium, which contains trypticase soy agar with 4% glycerol, 4 mg/L gentamicin, 0.1% crystal violet and 1% neutral red, is the most widely used selective medium for improved isolation of *B. pseudomallei*[31]. Pinpoint, flat, dry, and wrinkled purple colonies are characteristic. It is able to grow at 42ºC and is positive for oxidase activity and motility. However, gentamicin may have inhibitory effects on the growth of *B. pseudomallei*, and incubation should be prolonged for at least 96 hours. Of note, rare gentamicin-susceptible strains from Sarawak, Malaysia, have been described[32]. Subsequently, a modified Ashdown's agar including norfloxacin, ampicillin, and polymyxin B (NAP-A) was evaluated to have improved selectivity but equal recovery of *B. pseudomallei*[33]. The use of an enrichment broth with Ashdown's medium and colistin (500,000 U/L) for incubation at 37ºC for 48 hours followed by inoculating into Ashdown's medium may further increase the yield, though with a compromise of increasing the time to identification[34]. In response to the surge of melioidosis cases, of which the diagnosis of 4 patients was delayed in the second hospital admission 3 to 6 weeks later, Ashdown's agar has been routinely added for the plating of respiratory specimens from the Caritas Medical Centre, whose catchment is in SSP district. An additional 6 undiagnosed patients were identified through the surveillance culture by Ashdown's agar (0.25% of specimens, unpublished data). Due to the non-specific clinical presentation of melioidosis, clinicians should request specific *B. pseudomallei*

culture for patients who present with severe community-acquired pneumonia or for those with risk factors such as diabetes mellitus or exposure history. Furthermore, during heavy rainfall or typhoon season, the routine addition of a selective medium to enhance the isolation of *B. pseudomallei* in respiratory specimens should also be considered.

Identification

Even with presumptive bench speciation, confirmation of identification of *B. pseudomallei* poses challenges in the clinical microbiology laboratory. Commercial bacterial identification system using conventional biochemical tests, namely API 20NE (bioMérieux, Marcy L'Etoile, France) and the Vitek 2 (bioMérieux, Marcy L'Etoile, France) system, may misidentify *B. pseudomallei* as *Chromobacterium violaceum*[35] and *B. cepacia* complex[36], respectively. The Active Melioidosis Detect (AMD; InBios International, USA) is a commercial lateral flow assay (LFA) detecting *B. pseudomallei* capsular polysaccharide (CPS) by a monoclonal antibody. *Houghton RL et al* reported a sensitivity of 98.7% and a specificity of 97.2% when using this LFA on cultured isolates, with a lower limit of detection of approximately 2 ng/ml[37]. LFA is easy to perform and can provide a result in 15 min with a low cost; therefore, is appealing to resource-limited laboratories. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), nonetheless, is potentially useful for the rapid and accurate identification of *B. pseudomallei*, provided an in-house spectrum incorporating adequate strains of *B. pseudomallei* and *B. thailandensis* is constructed[38, 39]. Of note, neither commercially available MALDI-TOF instruments, Bruker Microflex Biotyper (Bruker Daltonik GmBH, Bremen, Germany) and bioMérieux Vitek MS (bioMérieux, Marcy L'Etoile, France), has the routine diagnostic database including the reference spectra required for identification of *B. pseduomallei*[40,41]. There are five conserved biomarkers specific for *B. pseudomallei*[42]. In the Bruker security-relevant library, the mass peak at a mass/charge ratio 6551 differentiates *B. thailandensis* from *B. mallei* and *B. pseudomallei*[42]. PCR testing of *B. pseudomallei* isolates is another option for confirmatory identification. The type III secretion system gene clusters, in particular, cluster 1 (T3SS-1), *orf2*, and *orf11*, can discriminate *B. pseudomallei* from other *Burkholderia* species[43,44]. The difference between the 16S rRNA gene sequences of *B. pseudomallei* and *B. thailandensis* is strikingly low at approximately 1%, and sequencing of *B. pseudomallei* unique gene target (*gro*EL) thus offers a better differentiation[45]. In our laboratory, real-time PCR[43] is adopted to confirm the identification of *B. pseudomallei* colonies with compatible morphotype before the final report issued by the reference laboratory. The rapid molecular confirmation of melioidosis essentially facilitates risk communication and subsequent public health actions.

Direct Molecular Detection

Given the non-specific clinical presentation and the high mortality of melioidosis, and the relatively poor yield of culture, a sensitive and specific PCR test that can detect *B. pseudomallei* directly from clinical specimens is imperative to aid early directed therapy. *Meumann EM et al* reported the overall sensitivity and specificity of the T3SS-1 real-time PCR assay on urine, sputum, wound swabs, and drained pus to be 73.2% and 89.2%[46], respectively. In particular, sputum represents a better sample than blood for PCR detection, due to the higher bacterial load[47]. A study on spiked blood demonstrated a 95% probability of detection of *B. pseudomallei* at a concentration of 8.4×10^3 CFU/ml[43]. T3SS-1 real-time PCR test[43] was performed on culture positive samples in our laboratory (5 sputum and 1 blood culture); all were positive with cycle threshold (Ct) values ranging from 31.8 to 39.1 (unpublished data).

Serology

The serodiagnosis of melioidosis is difficult, due to a lack of commercial assays and high background seropositivity rates in endemic regions. In addition, serological tests generally have lower sensitivity than culture as 19-26% of culture-confirmed melioidosis cases never seroconverted[48,49]. Nevertheless, it can be a useful adjunct to the diagnosis of chronic melioidosis

and neuro-melioidosis, when the negative predictive value of culture is low. The serum indirect hemagglutination assay (IHA), using poorly defined antigens from strains of *B. pseudomallei* adsorbed to sheep red blood cells, has been routinely performed in endemic areas and its cutoff values suggestive of infection are based on background seropositivity in the population (e.g., a cutoff titre of ≥ 1:80 in Thailand[50] and ≥ 1:40 in Australia[51]) Alternatively, IgM and IgG enzyme-linked immunosorbent assay (ELISA) using inactivated cell suspension, recombinant hemolysin-coregulated protein (HcP) type VI secretion system or recombinant GroEL protein have been described with sensitivities ranging from 90-93.7% and specificities ranging from 88.3-100%[12]. The serum of 18 patients were sent to Queen Mary Hospital for melioidosis antibody test (in-house ELISA antibody test using whole cell antigens, personal communication). Nine patients were both IgM and IgG positive (9 days to 10 weeks after onset) and one patient demonstrated seroconversion 17 days after onset of symptoms. Three patients with onset less than 14 days were IgM positive but IgG negative; on the contrary, one patient was only IgG positive 5 weeks after presentation. Possibly due to early presentation for less than 7 days, two patients were both IgM and IgG negative. Further studies on the performance characteristics of serological tests, time frame of the melioidosis antibody response and the relative importance of IgM and IgG detection are warranted.

Antimicrobial Susceptibility Testing

Meropenem (MEM) and ceftazidime (CAZ) are the first-line antimicrobials for the intensive phase of treatment, while trimethoprim-sulfamethoxazole (TMP-SMX), doxycycline (DOX), and amoxicillin-clavulanic acid (AMC) are used for eradication therapy[12]. Currently, the Clinical and Laboratory Standards Institute (CLSI) only has interpretative breakpoints of imipenem (IMI), CAZ, TMP-SMX, DOX, and AMC for a broth dilution method[52], while the European Committee on Antimicrobial Susceptibility Testing (EUCAST) also provides breakpoints for interpretation of zone diameters of the commonly used antimicrobials, including MEM[53]. In general, our isolates were susceptible to most used antimicrobials [MIC_{90}: MEM, 2 ug/ml; IMI 2 ug/ml; CAZ 4 ug/ml; TMP-SMX 2 ug/ml; DOX 1 ug/ml; AMC 4 ug/ml; Etest (Liofilchem ®, Italy)], except 3 isolates being non-susceptible to TMP-SMX (MIC 4 ug/ml) and 2 isolates non-susceptible to MEM (MIC 4 ug/ml). The uncommon resistance to first-line antimicrobial therapy is consistent with overseas data[12].

Laboratory Safety

B. pseudomallei has been designated a Tier 1 select agent by the US Centers for Disease Control and Prevention (CDC)[54]. To date, there have been two documented laboratory-acquired infections[55,56]. The first case was a 48-year-old laboratory staff who cleaned up a centrifuge spill of *B. pseudomallei* culture with bare hands[55] and the second case was a 33-year old laboratory staff who performed antimicrobial drug susceptibility testing on a *B. pseudomallei* isolate[56]. They developed symptoms of pulmonary melioidosis 3 and 4 days later after exposure, respectively. Inhalation of an infectious aerosol was thought to be the likely route of infection. Clinical diagnostic laboratories functioning at biosafety level 2 (BSL2) may isolate *B. pseudomallei* from a variety of sample types. Good laboratory practices will prevent most laboratory accidents involving exposure to *B. pseudomallei*. Specimen inoculation and transfer of bacterial isolates should be performed within a biosafety cabinet; a gown, gloves, and a respiratory mask should be worn during sample centrifugation[54]. A study demonstrated 100% reduction in viable organism when on-plate 70% formic acid was applied before processing for MALDI-TOF MS[56]. Besides, *Gassiep I et al* did not find any *B. thailandensis* (an avirulent substitute of *B. pseudomallei*) in air samples during 78 laboratory handling events, including plate opening, oxidase testing, and McFarland suspension creation[57]. Of 30 laboratory scientists handing *B. pseudomallei* on 1,267 occasions outside a biosafety cabinet, no infections or seroconversions were documented[57]. The existing evidence suggests that the risk of laboratory-acquired melioidosis is low. For high-risk exposure incident, e.g., generation of aerosol during sonication outside a biologic safety cabinet, 21 days prophylaxis of TMP-SMX may be considered[12].

References

[1]Limmathurotsakul D, Golding N, Dance DA, et al. Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis. Nat Microbiol 2016; Jan 1;1.

[2]Tong S, Yang S, Lu Z, He W. Laboratory investigation of ecological factors influencing the environmental presence of *Burkholderia pseudomallei.*Microbiol Immunol 1996; 40:451–3.

[3]Pumpuang A, Chantratita N, Wikraiphat C, et al. Survival of *Burkholderia pseudomallei* in distilled water for 16 years. Trans R Soc Trop Med Hyg 2011; 105:598–600.

[4]Hsueh PT, Huang WT, Hsueh HK, et al. Transmission modes of melioidosis in Taiwan. Trop Med Infect Dis 2018; 28;3(1).

[5]Cheng AC, Wuthiekanun V, Limmathurotsakul D, Chierakul W, Peacock SJ. Intensity of exposure and incidence of melioidosis in Thai children. Trans R Soc Trop Med Hyg 2008; 102:S37–S39.

[6]Wiersinga, W., Virk, H., Torres, A. et al. Melioidosis. Nat Rev Dis Primers 2018;4:17107.

[7]Whitmore, A. An Account of a Glanders-like Disease occurring in Rangoon. J Hyg 1913; 13:1–34.1.

[8]So SY, Chau PY, Leung YK, et al. First report of septicaemic melioidosis in Hong Kong. Trans R Soc Trop Med Hyg 1984; 78:456-9.

[9]So SY, Chau PY. Melioidosis: a serological survey in a tuberculosis sanatorium in Hong Kong Trans R Soc Trop Med Hyg 1987; 81:1017-9.

[10]Lui G, Tam A, Tso EYK, et al. Melioidosis in Hong Kong. Trop Med Infect Dis 2018; 25:3.

[11]Wu WG, Shum MHH, Wong ITF, et al. Probable airborne yransmission of *Burkholderia pseudomallei* causing an urban outbreak of melioidosis during typhoon season in Hong Kong, China. Emerg Microbes Infect. 2023; 12:2204155.

[12]Gassiep I, Armstrong M, Norton R. Human Melioidosis. Clin Microbiol Rev 2020; 33:e00006-19.

[13]Geerlings SE, Hoepelman AI. Immune dysfunction in patients with diabetes mellitus (DM). FEMS Immunol Med Microbiol 1999; 26:259–6265.

[14]Graves DT, Kayal RA. Diabetic complications and dysregulated innate immunity. Front Biosci 2008; 13:1227–1239.

[15]Chanchamroen S, Kewcharoenwong C, Susaengrat W, Ato M, Lertmemongkolchai G. Human polymorphonuclear neutrophil responses to *Burkholderia pseudomallei* in healthy and diabetic subjects. Infect Immun 2009; 77:456–463.

[16]West TE, Myers ND, Liggitt HD, et al. Murine pulmonary infection and inflammation induced by inhalation of *Burkholderia pseudomallei*. Int J Exp Pathol 2012; 93:421-8.

[17]Titball RW, Russell P, Cuccui J, et al. *Burkholderia pseudomallei*: animal models of infection. Trans R Soc Trop Med Hyg 2008; 102:S111-6.

[18]Currie BJ, Jacups SP. Intensity of rainfall and severity of melioidosis, Australia. Emerg Infect Dis. 2003; 9:1538-42.

[19]Ribolzi O, Rochelle-Newall E, Dittrich S, et al. Land use and soil type determine the presence of the pathogen *Burkholderia pseudomallei* in tropical rivers. Environ Sci Pollut Res Int 2016; 23:7828 –39.

[20]Cheng AC, Jacups SP, Gal D, Mayo M, Currie BJ. Extreme weather events and environmental contamination are associated with case clusters of melioidosis in the Northern Territory of Australia. Int J Epidemiol 2006; 35:323–9.

[21]Lau SK, Chan SY, Curreem SO, et al. *Burkholderia pseudomallei* in soil samples from an oceanarium in Hong Kong detected using a sensitive PCR assay. Emerg Microbes Infect 2014;3:e69.

[22]Chen YL, Yen YC, Yang CY, et al. The concentrations of ambient *Burkholderia pseudomallei* during typhoon season in endemic area of melioidosis in Taiwan. PLoS Negl Trop Dis 2014; 8:e2877.

[23]Currie BJ, Price EP, Mayo M, et al. Use of Whole-genome sequencing to link *Burkholderia pseudomallei* from air sampling to mediastinal melioidosis, Australia. Emerg Infect Dis 2015;21:2052-4.

[24]Hong Kong Observatory https://www.hko.gov.hk/en/index.html Accessed on 22 Oct 2022.

[25]Sarovich DS, Ward L, Price EP, et al. Recurrent melioidosis in the Darwin Prospective Melioidosis Study: improving therapies mean that relapse

cases are now rare. J Clin Microbiol 2014; 52:650 – 3.
[26]Chierakul W, Anunnatsiri S, Short JM, et al. Two randomized controlled trials of ceftazidime alone versus ceftazidime in combination with trimethoprim-sulfamethoxazole for the treatment of severe melioidosis. Clin Infect Dis. 2005; 41:1105-13.
[27]Inglis TJ, Sagripanti J-L. Environmental factors that affect the survival and persistence of *Burkholderia pseudomallei*. Appl Environ Microbiol 2006; 72:6865– 75.
[28]Limmathurotsakul D, Jamsen K, Arayawichanont A, et al. Defining the true sensitivity of culture for the diagnosis of melioidosis using Bayesian latent class models. PLoS One 2010; 5:e12485.
[29]Tiangpitayakorn C, Songsivilai S, Piyasangthong N, Dharakul T. Speed of detection of *Burkholderia pseudomallei* in blood cultures and its correlation with the clinical outcome. Am J Trop Med Hyg 1997; 57:96 –9.
[30]Wongsuvan G, Limmathurotsakul D, Wannapasni S, et al. Lack of correlation of *Burkholderia pseudomallei* quantities in blood, urine, sputum and pus. South-east Asian J Trop Med Public Health 2009; 40:781–4.
[31]Ashdown LR. An improved screening technique for isolation of *Pseudomonas pseudomallei* from clinical specimens. Pathology 1979; 11:293-7.
[32]Podin Y, Sarovich DS, Price EP, et al. *Burkholderia pseudomallei* isolates from Sarawak, Malaysian Borneo, are predominantly susceptible to aminoglycosides and macrolides. Antimicrob Agents Chemother 2014; 58:162–166.
[33]Goodyear A, Strange L, Rholl DA, et al. An improved selective culture medium enhances the isolation of *Burkholderia pseudomallei* from contaminated specimens. Am J Trop Med Hyg 2013; 89:973–982.
[34]Cheng AC, Wuthiekanun V, Limmathurosakul D, et al. Role of selective and nonselective media for isolation of *Burkholderia pseudomallei* from throat swabs of patients with melioidosis. J Clin Microbiol 2006; 44:2316.
[35]Inglis TJ, Chiang D, Lee GS, Chor-Kiang L. Potential misidentification of *Burkholderia pseudomallei* by API 20NE. Pathology 1998; 30:62– 4.
[36]Zong Z, Wang X, Deng Y, Zhou T. Misidentification of *Burkholderia pseudomallei* as *Burukholderia cepacia* by the VITEK 2 system. J Med Microbiol 2012; 61:1483-4.
[37]Houghton RL, Reed DE, Hubbard MA, et al. Development of a prototype lateral flow immunoassay (LFI) for the rapid diagnosis of melioidosis. PLoS Negl Trop Dis 2014; 8:e2727.
[38]Lau SK, Tang BS, Curreem SO, et al. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid identification of *Burkholderia pseudomallei*: importance of expanding databases with pathogens endemic to different localities. J Clin Microbiol 2012; 50:3142-3.
[39]Gassiep I, Armstrong M, Norton RE. Identification of *Burkholderia pseudomallei* by use of the Vitek mass spectrometer. J Clin Microbiol 2019; 57:e00081-19.
[40]Karger A, Stock R, Ziller M, 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.
[41]Wang H, Chen YL, Teng SH, et al. 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.* Front Microbiol 2016; 7:415.
[42]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:e99160.
[43]Novak RT, Glass MB, Gee JE, et al. Development and evaluation of a real-time PCR assay targeting the type III secretion system of *Burkholderia pseudomallei*. J Clin Microbiol 2006; 44:85–90.
[44]Thibault FM, Valade E, Vidal DR. Identification and discrimination of *Burkholderia pseudomallei*, *B. mallei*, and *B. thailandensis* by real-time PCR targeting type III secretion system genes. J Clin Microbiol 2004; 42:5871–4.
[45]Woo PC, Woo GK, Lau SK, Wong SS, Yuen KY. Single gene target bacterial identification.

*gro*EL gene sequencing for discriminating clinical isolates of *Burkholderia pseudomallei* and *Burkholderia thailandensi*s. Diagn Microbiol Infect Dis 2002; 44:143-9.
[46]Meumann EM, Novak RT, Gal D, et al. Clinical evaluation of a type III secretion system real-time PCR assay for diagnosing melioidosis. J Clin Microbiol 2006; 44:3028 –30.
[47]Gal D, Mayo M, Spencer E, Cheng AC, Currie BJ. Short report: application of a polymerase chain reaction to detect *Burkholderia pseudomallei* in clinical specimens from patients with suspected melioidosis. Am J Trop Med Hyg 2005; 73:1162–4.
[48]Alexander AD, Huxsoll DL, Warner AR, Jr, Shepler V, Dorsey A. Serological diagnosis of human melioidosis with indirect hemagglutination and complement fixation tests. Appl Microbiol 1970; 20:825– 33.
[49] Harris PN, Ketheesan N, Owens L, Norton RE. Clinical features that affect indirect-hemagglutination-assay responses to *Burkholderia pseudomallei*. Clin Vaccine Immunol 2009; 16:924 –30.
[50]Appassakij H, Silpapojakul KR, Wansit R, Pornpatkul M. Diagnostic value of the indirect hemagglutination test for melioidosis in an endemic area. Am J Trop Med Hyg 1990; 42:248 –53.
[51]Cheng AC, O'Brien M, Freeman K, Lum G, Currie BJ. Indirect hemagglutination assay in patients with melioidosis in northern Australia. Am J Trop Med Hyg 2006; 74:330 –4.
[52]CLSI. 2015. Methods for antimicrobial dilution and disc susceptibility testing of infrequently isolated or fastidious bacteria, 3rd ed. M45. Wayne, PA: Clinical and Laboratory Standards Institute.
[53]European Committee on Antimicrobial Susceptibility Testing 2022. Breakpoint tables for interpretation of MICs and zone diameters, bacteria version 12.0.
[54]Peacock SJ, Schweizer HP, Dance DAB, et al. Management of accidental laboratory exposure to *Burkholderia pseudomallei* and *B. mallei*. Emerg Infect Dis 2008; 14:e2.
[54]Green RN, Tuffnell PG. Laboratory acquired melioidosis. Am J Med 1968; 44:599 – 605.
[55]Schlech WF, Turchik JB, Westlake RE, et al. Laboratory-acquired infection with *Pseudomonas pseudomallei*(melioidosis). N Engl J Med 1981; 305:1133–5.
[56]Cunningham SA, Patel R. Standard matrix-assisted laser desorption ionization-time of flight mass spectrometry reagents may inactivate potentially hazardous bacteria. J Clin Microbiol 2015; 53:2788 –9.
[57]Gassiep I, Bauer MJ, Harris PNA, Chatfield MD, Norton R. Laboratory Safety: Handling *Burkholderia pseudomallei* Isolates without a Biosafety Cabinet. J Clin Microbiol 2021; 59:e00424-21.