Am. J. Trop. Med. Hyg., 102(2), 2020, pp. 388–391
doi:10.4269/ajtmh.19-0625
Copyright © 2020 by The American Society of Tropical Medicine and Hygiene

Burkholderia pseudomallei Detection among Hospitalized Patients, Sarawak

Jessica Y. Choi,[1,2] King Ching Hii,[3] Emily S. Bailey,[1,2]* Jia Yun Chuang,[3] Wei Yieng Tang,[3] Edmund Kwang Yuen Wong,[3] Tiana Ti,[3] Kat Siong Pau,[3] Antoinette Berita,[3] Izreena Saihidi,[3] Jakie Ting,[4,5] Tiing-Tiing Chua,[4] Teck-Hock Toh,[4,5] David P. AuCoin,[6] David DeShazer,[7] and Gregory C. Gray[1,2,8,9]*

[1]*Duke Global Health Institute, Duke University, Durham, North Carolina;* [2]*Division of Infectious Diseases, Duke University School of Medicine, Durham, North Carolina;* [3]*Kapit Hospital, Ministry of Health Malaysia, Kapit, Sarawak, Malaysia;* [4]*Clinical Research Center, Sibu Hospital, Ministry of Health Malaysia, Sibu, Sarawak, Malaysia;* [5]*Faculty of Medicine, SEGi University, Kota Damansara, Malaysia;* [6]*School of Medicine, University of Nevada, Reno, Nevada;* [7]*US Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Maryland;* [8]*Global Health Research Center, Duke-Kunshan University, Kunshan, China;* [9]*Emerging Infectious Diseases Program, Duke-NUS Medical School, Singapore*

Abstract. *Burkholderia pseudomallei* infections are prevalent in Southeast Asia and northern Australia and often misdiagnosed. Diagnostics are often neither sensitive nor rapid, contributing up to 50% mortality rate. In this 2018 pilot study, we enrolled 100 patients aged 6 months–79 years from Kapit Hospital in Sarawak, Malaysia, with symptoms of *B. pseudomallei* infection. We used three different methods for the detection of *B. pseudomallei*: a real-time polymerase chain reaction (PCR) assay, a rapid lateral flow immunoassay, and the standard-of-care bacterial culture—the gold standard. Among the 100 participants, 24 (24%) were positive for *B. pseudomallei* by one or more of the detection methods. Comparing the two individual diagnostic methods against the gold standard—bacterial culture—of any positive test, there was low sensitivity for each test (25–44%) but high specificity (93–98%). It seems clear that more sensitive diagnostics or a sensitive screening diagnostic followed by specific confirmatory diagnostic is needed for this disease.

INTRODUCTION

Melioidosis is caused by infection with *Burkholderia pseudomallei*, bacteria often found in soil and freshwater.[1] Southeast Asia and northern Australia[2] have reported the greatest number of cases, particularly related to the amount of rice farming carried out in the region. Epidemiological surveillance in Singapore reported a mean annual incidence rate of 1.7 per 100,000 individuals with a case fatality rate of 39.5%.[3] Similarly, in Cambodia, 40% of respiratory patients and 32% of patients who died during hospitalization were seropositive.[4] A recent systematic review and data synthesis have estimated the global burden of melioidosis as 4.6 million disability adjusted life years or 84.3 per 100,000 people.[5] Another report estimated that there were 165,000 human melioidosis cases per year worldwide and 89,000 of those died.[6]

In Malaysia, the tropical rainforests provide an optimal location for *B. pseudomallei* to grow and proliferate. Each year, an estimated 1,000 cases of melioidosis are officially reported across Malaysia,[7] with case mortality as high as 43%,[8] but true *B. pseudomallei* mortality counts are difficult to estimate.[9]

Seeking to help rural hospitals improve their diagnostic capabilities, we sought to compare three different methods for the detection of *B. pseudomallei*: a real-time PCR (qPCR) assay, a rapid lateral flow assay, and by gold standard bacterial culture.

MATERIALS AND METHODS

Ethics approval and study location. This study was approved by the Medical Research and Ethics Committee of the Ministry of Health, Malaysia, and Duke University and conducted at the 134-bed Kapit Hospital. The hospital is located along the Rajang River in Kapit district, Sarawak state, eastern Malaysia, and primarily accessed by boat.

Subject recruitment and enrollment. Licensed medical officers (MOs) made clinical assessments of melioidosis-suspected patients and enrolled participants based on the following melioidosis-like symptoms: enlarged lymph nodes, tender swelling of glands, splenic or liver lesion by bedside scan, joint pain with swelling, purplish vesicle or bullae in limbs, deep-seated abscess or brain abscess, pneumonia not responding to 48 hours of first-line antibiotic, and severe sepsis with or without shock. Patients were excluded from the study if they had a clear alternative diagnosis other than melioidosis provided by a trained health care professional at Kapit Hospital, had started an antibiotic treatment for melioidosis, or were younger than 6 months. Individuals who met the inclusion criteria were consented (parental consent was required for adolescents aged 6 months–17 years and assent for adolescents aged 12–17 years) and asked to complete a brief questionnaire about their health, living, and working environments.

Subject sample collection. Medical officers collected a single 5.0-mL tube of blood from adults and adolescents aged 12–18 years or a 2.0-mL tube of blood from children younger than 12 years. If participants were willing and able, MOs asked them also to provide urine sample in a sterile urine collection cup. If the MOs deemed it clinically necessary, other bodily fluids such as pus and sputum were also collected and studied.

Laboratory testing. Sample processing procedures were adopted from Houghton et al., 2014.[10] Blood culture specimens were placed immediately into an incubator. Other blood samples were centrifuged at 2,000 × *g* for 10 minutes, and once separated, both serum and urine samples were transferred into new, sterile 2.0-mL cryovial tubes. After sample processing, specimens were immediately used for the Active Melioidosis Detect™ (AMD) rapid test. All serum, urine, and bodily fluid specimens were then stored at –80°C until

*Address correspondence to Emily S. Bailey, Duke Global Health Institute, Duke University, P.O. Box 102359, Durham, NC 27710, E-mail: emily.bailey2@duke.edu or Gregory C. Gray, Duke Global Health Institute, Duke University, Durham, NC, E-mail: gregory.gray@duke.edu.

molecular testing was performed. Each sample was tested using three methods: bacterial culture, qPCR, and a rapid diagnostic test. These methods are described briefly in the following text.

Bacterial culture, considered the gold standard diagnostic technique, was performed for each clinical specimen by Kapit laboratory technicians using standard bacterial isolation techniques. Briefly, the blood sample in BD BACTEC™ (Becton, Dickinson and Company, Dun Laoghaire, Ireland) medium was incubated up to 5 days; then re-cultured on rich media, blood agar and/or chocolate agar, and/or MacConkey agar; and then one pure colony was subcultured on modified Francis media to isolate the pure colony.[11] Basic Gram stain was performed to confirm the presence of *B. pseudomallei*, which was identified with delayed oxidase-positive results. The colony isolated was further confirmed by API 20NE (analytical profile index, Non-*Enterobacteriaceae*) fast identification system. Results of the bacterial culture, Gram stain, and the antibiotic sensitivity were collected approximately 2 weeks after patient admission.

Bacterial DNA was extracted from blood, urine, and other bodily fluids using the TaKaRa NucleoSpin (Takara Bio Inc., Kusatsu, Japan) and examined with a qPCR assay for *B. pseudomallei*.[12,13] PCR was performed using SsoAdvanced Universal Probes Supermix (Bio-Rad, Hercules, CA) by adding 5 μL of extracted DNA to 15 μL of the Supermix kit reaction mixture containing 0.9 μM of each primer and 0.2 μM of the probe. Real-time PCR was carried out at 95°C for 3 minutes followed by 40 cycles at 95°C for 15 seconds and 60°C for 30 seconds.

Samples were also examined using the InBiOS AMD rapid test kit, which was first developed by the University of Nevada team in Nevada, USA, and optimized by InBios International, Inc., Seattle, WA. Fifty microliters of serum or urine sample was combined with 100 μL (approximately two drops) of chase buffer and applied to the AMD sample pad. For pus, sputum, and other viscous bodily fluids, 20 μL was combined with 100 μL of lysis buffer and vortexed. Twenty microliters of the resulting lysate was then combined with 100 μL of chase buffer, and the solution was applied to the AMD sample pad. Each test was allowed to flow for 15 minutes and a digital image was captured. Active Melioidosis *Detect*™ test strips have a reported limit of detection for the *B. pseudomallei* capsular polysaccharides (CPSs) of 0.2 ng/mL.[12,14]

RESULTS

From June 21, 2018, to January 8, 2019, 100 patients meeting the inclusion criteria were enrolled, yielding 100 blood, 97 urine, and 16 other bodily fluid samples (n = 213). Sixteen bodily fluid samples included five pus, seven sputum, two pleural effusion, one pericardial fluid, and one nasal pharyngeal aspirate sample. An additional 56 bodily fluid specimens were collected during routine clinical care. By the standard-of-care culture method, nine blood samples, one urine sample, and 11 bodily fluid samples were positive for *B. pseudomallei.* Using the qPCR method, five blood samples, four urine samples, and five bodily fluid samples were positive, whereas five blood samples, six urine samples, and one bodily fluid sample were positive using the AMD strips. For the purpose of this study, a patient with any evidence of *B. pseudomallei* by one or more of the three detection methods was considered *B. pseudomallei* positive. Based on these detection methods, 24 of 100 patients showed evidence of *B. pseudomallei.* The prevalence detected using each diagnostic method is displayed in Table 1. A table representing positivity by sample type is included in the supplement (Supplemental Table 1).

The results of the two detection methods were evaluated for agreement based on sensitivity, specificity, positive predictive value, and negative predictive value with positivity by any test, the gold standard. Kappa values based on Cohen's kappa interpretation[15] were used to determine the strength of the agreement between any two of the diagnostic methods (Table 2).

DISCUSSION

A total of 24 patients had one or more positive results based on the three diagnostic tests of either one or more of the three specimen types (blood, urine, or bodily fluid). Of the 24 patients whose samples were indicated as infected with *B. pseudomallei*, blood and bodily fluid culture detected the highest number (n = 22) of infections. Only three patients were positive by all the three tests (culture, qPCR, and AMD) in either one or more of three types of specimen. The discrepancy in the numbers of *B. pseudomallei* detection largely is due to the target of each diagnostic method. Whereas the standard-of-care bacterial culture detects viable bacteria in the clinical samples, qPCR detects orf2 of the *B. pseudomallei* type III secretion system gene cluster and AMD detects CPS on the bacteria. A discrepancy in the number of *B. pseudomallei* was also observed (Supplemental Table 1). This may be due to the pathophysiology of the bacteria. *Burkholderia pseudomallei* does not accumulate to high levels in the blood (1 colony forming unit/mL).[16,17] Inside the body, CPS is believed to be shed from abscesses into blood stream. Previous studies have shown that when purified CPS is injected intravenously into mice, the half-life in blood was only 2.9–4.4 hours long.[18] Also, secreted CPS does appear to pass from the blood into the kidneys, where it may accumulate to higher concentrations in urine.[19,20] Serum samples tend to have a low detection rate consequentially.

When comparing the three methods (Table 2) based on Kappa analysis, there was a poor agreement (low sensitivity) among each pair of comparisons (25–44%) and high specificity across the pairs of comparisons (93–98%). Kappa

TABLE 1

Number of positive Burkholderia *pseudomallei* samples by specimen type and diagnostic method among 100 patients in Kapit Hospital from June 21, 2018 to January 8, 2019

Type of test/type of specimen	Culture	Real-time PCR	Active melioidosis detect™
Blood, n (%)	9/99 (9.1%)	5/100 (5.0%)	5/100 (5.0%)
Urine, n (%)	1/63 (1.6%)	4/97 (4.1%)	6/97 (6.2%)
Bodily fluid, n (%)	11/72 (15.3%)	5/16 (31.3%)	1/16 (6.3%)

TABLE 2
Comparison of diagnostic methods (95% CI)

	AMD vs. bacterial culture (*N* = 99)	qPCR vs. bacterial culture (*N* = 99)	AMD vs. real-time PCR (*N* = 100)
Prevalence	9 (5, 15)	18 (11, 27)	12 (7, 20)
Sensitivity	44 (22, 70)	39 (17, 64)	25 (5, 57)
Specificity	98 (93, 99)	94 (86, 98)	93 (86, 97)
Positive predictive value	89 (52, 98)	58 (33, 79)	33 (13, 64)
Negative predictive value	89 (84, 92)	87 (83, 90)	90 (87, 93)
Accuracy	89 (81, 94)	84 (75, 90)	85 (76, 91)
Kappa	0.536	0.375	0.203

AMD = active melioidosis detect™.

analysis suggested that both AMD and PCR were in weak agreement with the bacterial culture and that the AMD and PCR were in minimal agreement.

As no effective vaccines are available, current prevention strategies in melioidosis-endemic regions consist largely of using personal protective gear when working in potentially contaminated water or soil. Antimicrobial therapy is often based on clinical suspicion. In this study, we sought to evaluate the AMD rapid diagnostic test that would potentially provide faster and more sensitive test results than the blood culture which requires 48–72 hours of incubation after patient admission and results in a bacterial yield of only 10–15%. However, the AMD strips exhibited poor sensitivity and high specificity when compared with the bacterial culture results. Although the AMD strips seemed to perform better in urine samples, where higher concentrations of CPS are expected, the test strips did not detect bacteria frequently in serum or other bodily fluids. Our results appear to support previous studies involving the AMD LFI,[21–23] where sensitivity is low when testing serum samples. This is clearly due to the low levels of bacteria found in blood.[16,17] Moving forward, it will be important for clinicians to know which clinical sample types are most useful to test with the AMD. Other studies have shown that the AMD has high sensitivity and specificity when testing in grown blood culture broth and isolated colonies, which should reduce the time to diagnosis by days.[22,24]

Similarly, there was poor detection of bacterial DNA using the real-time PCR method, potentially because of low DNA concentrations. The PCR assay was optimized (sensitivity 80%, specificity 100%) using bacterial isolates; however, clinical isolates led to a much lower sensitivity. Other factors, such as urea in the urine, could also have acted as PCR inhibitors.

To our knowledge, this is the first attempt to field test AMD strips and a real-time PCR assay in Malaysia using clinical samples from patients meeting a case definition of melioidosis that included classical symptoms such as prolonged fever with joint pain and/or abscess. The comparison of three methods of *B. pseudomallei* detection in addition to clinical diagnosis indicates that both molecular/culture detection and clinical diagnosis are important in the accurate diagnosis and treatment of melioidosis. Although this sample size was small (100 enrolled participants with 213 samples), we detected a prevalence of 24% *B. pseudomallei*, indicating the need for further research on sources of this bacterial infection and more accurate methods for detection.

Received August 23, 2019. Accepted for publication October 9, 2019.

Published online November 25, 2019.

Note: Supplemental table appears at www.ajtmh.org.

Acknowledgments: We would like to thank the Director General of Health Malaysia for his permission to publish this article. This work was conducted in partnership with Duke University, the Duke Global Health Institute, Sibu Hospital Clinical Research Center, and SEGi University Sibu Clinical Campus. This study was supported by Duke University discretionary funding (Gray PI) and AMD lateral flow assay donations by David P. AuCoin and InBios International Inc., Seattle, WA. We appreciate the kind donation of capsular polysaccharides (CPS) from Paul Brett from the University of Nevada, Reno as a positive control. We thank Dr. Randy Schoepp (US Army Medical Research Institute of Infectious Diseases, Ft. Dietrick, MD), for connecting us to our collaborators at the University of Nevada. We thank Professor Mohammed Raili Suhaili (SEGi University, Sarawak) for guidance, facility use, and administrative support. We thank Juliana Zemke, Gina Kovalik, and David Chen (of Duke University) for assisting with sample extraction. We thank Kapit Hospital laboratorians Rose Ningkan, Goh Hieng Hua, Ujan Pulo, Emelia Boda, Desiree Wong and Cornelius Jambol for preparing clinical specimens for study. We thank Johnny Goh Keh Tun, Tee Sui Poh, Valerie Toh Wen Ting, Wong Sing Chi and Kong Sing Ling from Kapit Hospital for recruiting study participants. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army.

Disclosure: D. A. reports other from InBIOS International outside the submitted work. D. A. has a patent #9,310,365 method of diagnosing and treating melioidosis issued.

Authors' addresses: Jessica Y. Choi and Emily S. Bailey, Duke Global Health Institute, Duke University, Durham, NC, and Division of Infectious Diseases, Duke University School of Medicine, Durham, NC, E-mails: jessica.choi@duke.edu and emily.bailey2@duke.edu. King Ching Hii, Jia Yun Chuang, Wei Yieng Tang, Edmund Kwang Yuen Wong, Tiana Ti, Kat Siong Pau, Antoinette Berita, and Izreena Saihidi, Kapit Hospital, Kapit, Malaysia, E-mails: drhiikc@gmail.com, jiayunchuang@gmail.com, weiyieng0290@gmail.com, eded3319@hotmail.com, tiana_bunny@hotmail.com, katsiong89@gmail.com, nettehardy@yahoo.com, and izreena88@gmail.com. Jakie Ting and Teck-Hock Toh, Clinical Research Center, Sibu Hospital, Sibu, Malaysia, and Faculty of Medicine, SEGi University, Kota Damansara, Malaysia, E-mails: jakie-ting@hotmail.com and thtoh@yahoo.com. Tiing-Tiing Chua, Clinical Research Center, Sibu Hospital, Sibu, Malaysia, E-mail: chuatt90@gmail.com. David P. AuCoin, School of Medicine, University of Nevada, Reno, NV, E-mail: daucoin@med.unr.edu. David DeShazer, US Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD, E-mail: david.deshazer.civ@mail.mil. Gregory C. Gray, Duke Global Health Institute, Duke University, Durham, NC, Division of Infectious Diseases, Duke University School of Medicine, Durham, NC, Global Health Research Center, Duke-Kunshan University, Kunshan, China, and Emerging Infectious Diseases Program, Duke-NUS Medical School, Singapore, E-mail: gregory.gray@duke.edu.

REFERENCES

1. Foong YC, Tan M, Bradbury RS, 2014. Melioidosis: a review. *Rural Remote Health 14:* 2763.
2. Hassan MRA, Pani SP, Peng NP, Voralu K, Vijayalakshmi N, Mehanderkar R, Aziz NA, Michael E, 2010. Incidence, risk factors and clinical epidemiology of melioidosis: a complex socio-ecological emerging infectious disease in the Alor Setar region of Kedah, Malaysia. *BMC Infect Dis 10:* 302.

3. Heng BH, Goh KT, Yap EH, Loh H, Yeo M, 1998. Epidemiological surveillance of melioidosis in Singapore. *Ann Acad Med Singapore 27:* 478–484.
4. Perumal Samy R, Stiles BG, Sethi G, Lim LHK, 2017. Melioidosis: clinical impact and public health threat in the tropics. *PLoS Negl Trop Dis 11:* e0004738.
5. Birnie E, Virk HS, Savelkoel J, Spijker R, Bertherat E, Dance DAB, Limmathurotsakul D, Devleesschauwer B, Haagsma JA, Wiersinga WJ, 2019. Global burden of melioidosis in 2015: a systematic review and data synthesis. *Lancet Infect Dis 19:* 892–902.
6. Limmathurotsakul D et al., 2016. Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis. *Nat Microbiol 1:* 15008.
7. Nathan S et al., 2018. Melioidosis in Malaysia: incidence, clinical challenges, and advances in understanding pathogenesis. *Trop Med Infect Dis 3:* E25.
8. Kingsley PV, Leader M, Nagodawithana NS, Tipre M, Sathiakumar N, 2016. Melioidosis in Malaysia: a review of case reports. *Plos Negl Trop Dis 10:* e0005182.
9. Trinh TT et al., 2018. Melioidosis in vietnam: recently improved recognition but still an uncertain disease burden after almost a century of reporting. *Trop Med Infect Dis 3:* E39.
10. Houghton RL et al., 2014. Development of a prototype lateral flow immunoassay (LFI) for the rapid diagnosis of melioidosis. *PLoS Negl Trop Dis 8:* e2727.
11. Francis A, Aiyar S, Yean CY, Naing L, Ravichandran M, 2006. An improved selective and differential medium for the isolation of *Burkholderia pseudomallei* from clinical specimens. *Diagn Microbiol Infect Dis 55:* 95–99.
12. Novak RT, Glass MB, Gee JE, Gal D, Mayo MJ, Currie BJ, Wilkins PP, 2006. Development and evaluation of a real-time PCR assay targeting the type III secretion system of *Burkholderia pseudomallei*. *J Clin Microbiol 44:* 85–90.
13. Kaestli M et al., 2012. Comparison of TaqMan PCR assays for detection of the melioidosis agent *Burkholderia pseudomallei* in clinical specimens. *J Clin Microbiol 50:* 2059–2062.
14. Lim C, Peacock SJ, Limmathurotsakul D, 2016. Association between activities related to routes of infection and clinical manifestations of melioidosis. *Clin Microbiol Infect 22:* 79.e1–79.e3.
15. McHugh ML, 2012. Interrater reliability: the kappa statistic. *Biochem Med (Zagreb) 22:* 276–282.
16. Wuthiekanun V, Limmathurotsakul D, Wongsuvan G, Chierakul W, Teerawattanasook N, Teparrukkul P, Day NP, Peacock SJ, 2007. Quantitation of *B. Pseudomallei* in clinical samples. *Am J Trop Med Hyg 77:* 812–813.
17. Wongsuvan G, Limmathurotsakul D, Wannapasni S, Chierakul W, Teerawattanasook N, Wuthiekanun V, 2009. Lack of correlation of *Burkholderia pseudomallei* quantities in blood, urine, sputum and pus. *Southeast Asian J Trop Med Public Health 40:* 781–784.
18. Nualnoi T, Kirosingh A, Pandit SG, Thorkildson P, Brett PJ, Burtnick MN, AuCoin DP, 2016. In vivo distribution and clearance of purified capsular polysaccharide from *Burkholderia pseudomallei* in a murine model. *PLoS Negl Trop Dis10:* e0005217.
19. Nuti DE et al., 2011. Identification of circulating bacterial antigens by in vivo microbial antigen discovery. *MBio 2:* e00136–11.
20. Nualnoi T, Kirosingh A, Basallo K, Hau D, Gates-Hollingsworth MA, Thorkildson P, Crump RB, Reed DE, Pandit S, AuCoin DP, 2018. Immunoglobulin G subclass switching impacts sensitivity of an immunoassay targeting *Francisella tularensis* lipopolysaccharide. *PLoS One 13*, e0195308.
21. Robertson G, Sorenson A, Govan B, Ketheesan N, Houghton R, Chen H, AuCoin D, Dillon M, Norton R, 2015. Rapid diagnostics for melioidosis: a comparative study of a novel lateral flow antigen detection assay. *J Med Microbiol 64:* 845–848.
22. Woods KL, Boutthasavong L, NicFhogartaigh C, Lee SJ, Davong V, AuCoin DP, Dance DAB, 2018. Evaluation of a rapid diagnostic test for detection of *Burkholderia pseudomallei* in the Lao people's Democratic Republic. *J Clin Microbiol 56:* e02002–e02017.
23. Shaw T, Tellapragada C, Ke V, AuCoin DP, Mukhopadhyay C, 2018. Performance evaluation of active melioidosis detect-lateral flow assay (AMD-LFA) for diagnosis of melioidosis in endemic settings with limited resources. *PLoS One 13:* e0194595.
24. Peeters M, Chung P, Lin H, Mortelmans K, Phe C, San C, Kuijpers LMF, Teav S, Phe T, Jacobs J, 2018. Diagnostic accuracy of the InBiOS AMD rapid diagnostic test for the detection of *Burkholderia pseudomallei* antigen in grown blood culture broth. *Eur J Clin Microbiol Infect Dis 37:* 1169–1177.

Copyright of American Journal of Tropical Medicine & Hygiene is the property of American Society of Tropical Medicine & Hygiene and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.