

Short Report: Prevalence of Melioidosis in Patients with Suspected Pulmonary Tuberculosis and Sputum Smear Negative for Acid-Fast Bacilli in Northeast Thailand

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Abstract. The clinical and radiological features of pulmonary melioidosis can mimic tuberculosis. We prospectively evaluated 118 patients with suspected pulmonary tuberculosis who were acid-fast bacilli (AFB) smear negative at Udon Thani Hospital, northeast Thailand. Culture of residual sputum from AFB testing was positive for *Burkholderia pseudomallei* in three patients (2.5%; 95% confidence interval [CI] 0.5–7.3%). We propose that in melioidosis-endemic areas, residual sputum from AFB testing should be routinely cultured for *B. pseudomallei*.

Melioidosis is a serious infectious disease caused by the Tier 1 Select Agent and Gram-negative bacillus, *Burkholderia pseudomallei*.¹ Naturally acquired melioidosis is highly endemic in northeast Thailand where it is the third most common cause of death caused by infectious diseases after human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) and tuberculosis,² and in northern Australia where it is the commonest cause of fatal community-acquired bacteremic pneumonia.³ Melioidosis is also increasingly reported from many countries across Asia, regions of South America, various Pacific and Indian Ocean islands, and some countries in Africa including Nigeria, Gambia, Kenya, and Uganda.¹ Death from melioidosis reaches 80% in those who are not treated with effective antimicrobial drugs.⁴

Melioidosis can manifest with a variety of clinical presentations including sepsis, pneumonia, arthritis, and internal organ abscesses, and has been termed “the great mimicker” because it can be confused with a range of diseases. The most notable example is tuberculosis, with an estimated 10% of melioidosis patients presenting with chronic respiratory symptoms and chest radiography mimicking pulmonary tuberculosis.⁵ In reported cases, failure of clinical improvement after the administration of anti-tuberculosis drugs led to bacteriological culture of sputum, broncho-alveolar lavage, or blood and the detection of *B. pseudomallei*.^{6–8} Although it is clear that melioidosis can mimic clinical features of tuberculosis, patients presenting with suspected tuberculosis in Thailand where melioidosis is highly endemic are not systematically screened for melioidosis. Here, we evaluated the use of culturing sputum samples taken from individuals in Thailand with suspected tuberculosis that were smear negative for acid-fast bacilli (AFB) to detect *B. pseudomallei*.

THE STUDY

A cross-sectional study was conducted at Udon Thani Hospital, Udon Thani, northeast Thailand, between August

2012 and February 2013. Inclusion criteria were adult patients (≥ 18 years of age) with suspected pulmonary tuberculosis who had three consecutive sputum samples taken that were AFB smear negative. Cases were initially identified during daily visits to the routine diagnostic microbiology laboratory. We excluded patients with confirmed pulmonary tuberculosis based on a positive AFB smear or sputum culture positive for *Mycobacterium tuberculosis*. Culture for *M. tuberculosis* was performed by the National Health Security Office (NHSO), Thailand, using both solid and liquid culture medium, according to standard procedures.⁹ Sputum culture for *M. tuberculosis* is recommended by NHSO for the following: 1) patients with suspected multidrug-resistant tuberculosis; including suspected cases who are household contacts of multidrug-resistant tuberculosis cases, individuals with HIV/AIDS, and prisoners, 2) patients with pulmonary tuberculosis who have been treated for 3 months but remain sputum AFB smear positive, and 3) those who relapse with pulmonary tuberculosis, or whose treatment is interrupted for 2 or more months.⁹ All participants were enrolled after they gave written informed consent.

In this study, residual sputum from AFB testing was cultured to detect the presence of *B. pseudomallei* as described previously.¹⁰ In brief, all available residual sputum specimens were collected and inoculated into 7 mL of modified Ashdown broth (containing 10 g/L of typtone soya broth, 40 mL/L of glycerol, 5 mL/L of 0.1% crystal violet, and 50 mg/L of colistin). These were incubated at 37°C in air for 48 hours, after which 10 μ L of the upper layer was streaked onto Ashdown agar.¹¹ Plates were then incubated at 37°C in air and examined daily for 4 days, and colonies of presumptive *B. pseudomallei* identified as described previously.¹⁰ Patients with a sputum culture positive for *B. pseudomallei* were contacted and appropriate treatment of melioidosis initiated as soon as possible. This study was approved by the ethical committees of Udon Thani Hospital, and the Faculty of Tropical Medicine, Mahidol University, Thailand.

A total of 118 patients were enrolled, from whom 278 sputum samples were cultured for *B. pseudomallei*. Three, two, and one sputum specimens were available for culture from 68, 24, and 26 patients. Overall, 77 (65.3%) were male, and the median age was 56 years (interquartile range [IQR] 46–66 years,

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TABLE 1
Details of three patients with sputum culture positive for *Burkholderia pseudomallei*

Gender and age	Underlying disease	Presenting symptoms	Chest x-ray*	Outcome
F 55yr	Diabetes	Cough for 14 days and fever with dyspnea for 1 day	Infiltration in RUL	One day after enrollment, developed high fever and severe dyspnea. Admitted to hospital and treated with parenteral ceftazidime. Died 5 days after admission. Probable cause of death was multiple organ failure caused by melioidosis.
M 70yr	Diabetes and hypertension	Cough and fever for 30 days	Infiltration in RUL and cavity in LUL	Two days after enrollment, developed high-grade fever. Admitted to the hospital and treated with parenteral ceftazidime. Received 14 days of parenteral ceftazidime, followed by 20 weeks of oral eradication treatment, and made a complete recovery.
M 34yr	None	Cough for 30 days and fever for 7 days	Moderately thickened wall cavity with subadjacent infiltration in RUL	No signs of clinical deterioration in review. Received 20 weeks of oral eradication treatment and made a complete recovery.

*RUL = right upper lobe; LUL = left upper lobe.

range 18–82 years). Median duration of symptoms was 30 days (IQR 14–30 days, range 0–180 days).

Of the 118 patients, 3 (2.5%; 95% confidence interval [CI] 0.5–7.3%) were sputum culture positive for *B. pseudomallei*. None of the sputum cultures were positive for *M. tuberculosis*. A clinical description of each melioidosis case is provided in Table 1. Cases 1 and 2 had a rapid clinical deterioration after enrollment, and were admitted to the hospital where they were treated with parenteral ceftazidime. Clinical review of case 3 led to the decision that parenteral treatment of acute melioidosis was unnecessary, and oral treatment of melioidosis was prescribed. Patient 1 died, whereas the other two cases completed melioidosis treatment and recovered (Table 1).

The study has a number of limitations. Not all sputum specimens for AFB smear were available for our study because some samples were completely used for mycobacterial culture, and some were accidentally discarded. Furthermore, we did not evaluate the prevalence of melioidosis in tuberculosis patients who were smear positive. Nonetheless, this is the first study to prospectively evaluate the prevalence of pulmonary melioidosis in patients with suspected pulmonary tuberculosis who are sputum AFB smear negative in Thailand. Our estimated prevalence is much higher than that observed in Port Moresby, Papua New Guinea, in which only 1 of 529 patients with suspected pulmonary tuberculosis had sputum culture positive for *B. pseudomallei*¹²; the prevalence reported here probably represents a minimum estimate in Thailand because the sensitivity of *B. pseudomallei* culture is less than perfect.¹³

Overall, we show that culture for *B. pseudomallei* using residual sputum from AFB testing can provide the correct diagnosis and institution of appropriate antimicrobial therapy for patients with pulmonary melioidosis mimicking tuberculosis. Early diagnosis and treatment can reduce the risk of death caused by melioidosis, and avoids unnecessary treatment with anti-tuberculosis drugs that can lead to adverse drug reactions. The case fatality rate in these three patients (33%) is comparable with the overall case fatality rate in melioidosis patients presenting in northeast Thailand (43%).² We propose that in

melioidosis-endemic areas, residual sputum from AFB testing should be routinely cultured for *B. pseudomallei*.

Received May 23, 2013. Accepted for publication August 9, 2013.

Published online September 23, 2013.

Acknowledgments: We thank Surapong Narenpitak, Kana Kasemsub, Sunchai Phiphitaporn, Jarawan Poonsriaram, and staff at Department of Medicine and Department of Medical Technology Udon Thani Regional Hospital. We are grateful to the patients and staff at Mahidol Oxford Tropical Medicine Research Unit, Mahidol University.

Financial support: This study was funded by the Wellcome Trust of Great Britain (089275/Z/09/Z). D.L. is supported by the Wellcome Trust (090219/Z/09/Z). S.J.P. is supported by the NIHR Biomedical Research Centre.

Disclaimer: None of the authors has a conflict of interest.

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