Investigation of Melioidosis Using Blood Culture and Indirect Hemagglutination Assay Serology among Patients with Fever, Northern Tanzania

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Abstract. Prediction models indicate that melioidosis may be common in parts of East Africa, but there are few empiric data. We evaluated the prevalence of melioidosis among patients presenting with fever to hospitals in Tanzania. Patients with fever were enrolled at two referral hospitals in Moshi, Tanzania, during 2007–2008, 2012–2014, and 2016–2019. Blood was collected from participants for aerobic culture. Bloodstream isolates were identified by conventional biochemical methods. Non–glucose-fermenting Gram-negative bacilli were further tested using a Burkholderia pseudomallei latex agglutination assay. Also, we performed B. pseudomallei indirect hemagglutination assay (IHA) serology on serum samples from participants enrolled from 2012 to 2014 and considered at high epidemiologic risk of melioidosis on the basis of admission within 30 days of rainfall. We defined confirmed melioidosis as isolation of B. pseudomallei from blood culture, probable melioidosis as a ≥4-fold rise in antibody titers between acute and convalescent sera, and seropositivity as a single antibody titer ≥40. We enrolled 3,716 participants and isolated non-enteric Gram-negative bacilli in 2510 (68%) of 3716 samples from participants enrolled from 2012 to 2014 and considered at high epidemiologic risk of melioidosis on the basis of admission within 30 days of rainfall. We identified two (0.6%) cases of probable melioidosis, and 57 (17.7%) were seropositive. The absence of confirmed melioidosis from 9 years of fever surveillance indicates melioidosis was not a major cause of illness.

INTRODUCTION

The importance of Burkholderia pseudomallei, the agent of melioidosis, as a cause of illness in Africa is yet to be fully determined. There have been occasional reports of individual cases and a study indicating that melioidosis is present in coastal Kenya.1–4 To our knowledge, there are no published data from Tanzania to indicate whether melioidosis is present,5 but prediction models based on environmental suitability and populations at risk suggest that B. pseudomallei may be endemic in Tanzania.6

Burkholderia pseudomallei infection most commonly results in subclinical or self-limiting symptoms in immunocompetent individuals.7 Acute symptomatic infection with B. pseudomallei typically presents with bacteremia, pneumonia, and localized abscesses. Burkholderia pseudomallei can be cultured from blood in only a proportion of patients with melioidosis, as bacteremia is thought to occur in 40–60%,7 with other patients having localized disease. In diagnostic accuracy studies using latent class models, blood culture has an estimated sensitivity of approximately 60% for melioidosis.8 In culture on solid media, B. pseudomallei can be overlooked as a contaminant, as colonies appear morphologically similar to common contaminants such as Pseudomonas stutzeri.9 Finally, B. pseudomallei can be misidentified as another Gram-negative organism such as Burkholderia cepacia or Chromobacterium violaceum by routinely used identification systems.10,11 and it is not included in standard matrix-assisted laser desorption ionization–time of flight mass spectrometry databases.12 As such, melioidosis may be underappreciated or not recognized through routine diagnostic testing in areas where it is uncommon, or the prevalence is unknown.

Burkholderia pseudomallei indirect hemagglutination assay (IHA) is a widely used serologic test for melioidosis.9 Although use of IHA for the diagnosis of acute melioidosis is limited in endemic countries because of background seropositivity, it remains useful for determining exposure to B. pseudomallei.9 Population seropositivity in turn informs our understanding of the population-level risk of melioidosis. We sought to understand the prevalence of melioidosis and exposure in northern Tanzania through systematic blood culture testing among patients hospitalized with fever, and IHA serology among a high-risk subset of patients.

METHODS

Study setting. We conducted prospective hospital-based fever surveillance studies at Kilimanjaro Christian Medical Centre (KCMC) and Mawenzi Regional Referral Hospital (MRRH) in Moshi, Tanzania, during the periods of September 17, 2007 through August 31, 2008, September 26, 2011 through May 31, 2014, and September 6, 2016 through May 31, 2019. Kilimanjaro Christian Medical Centre is a 630-bed zonal referral, and MRRH is a 300-bed regional hospital. Moshi (population > 180,000) is the administrative center of the Kilimanjaro region (population > 1.6 million) and is situated at an elevation of approximately 890 m above sea level. The climate is tropical, with rainy seasons from March through May and October through December. Agriculture in northern Tanzania includes smallholder systems involving mixed crop and
livestock farming, as well as pastoralism. Continuously irrigated rice farming, an established risk factor for melioidosis, is increasingly practiced by farmers within the area served by our two sentinel site hospitals.13

Study procedures. We prospectively enrolled pediatric and adult inpatients at KCMC and MRRH during each time period, and during the 2012–2014 time period, we also enrolled outpatients. Adolescents and adults, defined as age ≥13 years, were eligible to participate if they had an oral temperature of ≥38.0°C, or, during 2012–2014, a history of fever within the previous 72 hours. Infants and children, defined as age ≥2 months to <13 years, were eligible to participate if they had a history of fever in the past 48 hours, an axillary temperature of ≥37.5°C, or a rectal temperature of ≥38.0°C. We enrolled consecutive eligible inpatients and, during 2012–2014, every second eligible outpatient. Outpatients were included as the primary study sought to describe the incidence of bacterial zoonoses and bloodstream infections, which included diseases that might present to and be managed in the outpatient setting, such as typhoid fever.14,15 All patients were enrolled within 24 hours of admission. After obtaining informed consent, a trained study team member completed standardized case report forms and drew blood for culture and serologic testing. The case report form varied during each time period but included demographic details, symptoms, and use of antibacterial drugs before enrollment. Information regarding rainfall in the 30 days preceding enrollment was obtained for the 2012–2014 period from the Tanzanian production company rainfall station near Moshi.

Blood culture. We drew blood for a single aerobic blood culture from participants at enrollment, which was loaded into the BacT/ALERT 3D microbial detection system (BioMérieux, Marcy-l’Étoile, France), and incubated for up to 5 days. We inoculated 4 mL of blood into BacT/ALERT pediatric FAN aerobic bottles (2007–2008 and 2012–2014) or pediatric PF plus bottles (2016–2019) (BioMérieux) for pediatric participants (aged <13 years) and 10 mL of blood into BacT/ALERT standard aerobic bottles (2007–2008 and 2012–2014) or FA plus bottles (2016–2019) (BioMérieux) for adult participants (aged ≥13 years). Blood cultures were assessed for volume adequacy by measuring the weight before and after inoculation. Adequate blood volume was defined as ±20% of the target blood volume. Bloodstream isolates were identified by conventional methods; following testing on the API20NE (BioMérieux) biochemical identification system, non-glucose-fermenting Gram-negative bacilli were further tested by B. pseudomallei latex agglutination (Mahidol University, Bankok, Thailand) test.9,16

Serology testing. During the 2012–2014 study, we collected blood for acute serum at enrollment and convalescent serum 4–6 weeks later. Blood was allowed to clot for between 30 and 60 minutes. It was then centrifuged for 15 minutes at 1,126–1,455 relative centrifugal force to separate serum. Serum was stored at −80°C. Serum specimens were batch collected blood for acute serum at enrollment and convalescent sera of participants with a reciprocal titer ≥40 on their convalescent serum. We performed B. pseudomallei IHA using antigen pooled from two clinical B. pseudomallei isolates from Southeast Asia and Australia following standard U.S. CDC laboratory protocols.17

Case definitions. A confirmed case of melioidosis was defined as isolation of B. pseudomallei from blood culture, probable melioidosis as a ≥4-fold rise in antibody titers between acute and convalescent sera, and seropositivity as a single reciprocal antibody titer ≥40.18,19

Statistical analysis. Case report form and laboratory data were entered using the Cardiff Teleform system (OpenText, Waterloo, Canada) into an Access database (Microsoft Corporation, Redmond, WA). Descriptive analyses, including median and range for continuous variables and proportions for categorical variables, were performed using Stata, version 16.0 (StataCorp, College Station, TX).

Research ethics. This study was conducted in accordance with the Declaration of Helsinki. It was approved by the KCMC Research Ethics Committee (#295), the Tanzania National Institute for Medical Research National Ethics Coordinating Committee (NIMR1HQ/R.8eNo1. 11/283), Duke University Medical Center Institutional Review Board (IRB#Pro00016134), and the University of Otago Human Ethics Committee (Health (H15/055). Written informed consent was obtained from all participants or their guardians.

RESULTS

We enrolled 3,716 participants who had blood culture performed. Participant characteristics are shown in Table 1. Of note, the median (range) age was 20 (<1, 84) years. The median (range) duration of illness was 4 (1, 120) days. Of 1,752 participants for whom corresponding rainfall data were available, there were 1,472 (84.0%) who presented within 30 days of rain.

Among those with data available, 2,658 (73.1%) of 3,637 participants had adequately filled blood culture bottles. Among pediatric participants, 734 (51.4%) of 1,427 blood culture bottles were adequately filled, and among adults 1,924 (87.1%) of 2,210, blood culture bottles were adequately filled. Of responding participants, 849 (32.6%) of 2,607 reported taking antibacterial drugs before enrollment. Non-enteric

| TABLE 1 Characteristics of participants undergoing blood culture, Tanzania, 2007–2019 (N = 3,716) |
|---|---|---|
| Demographic characteristics | N | n (%) |
| Age (years), median (IQR) | 3,573 | 20.3 (2.0–39.3) |
| Gender, male | 3,668 | 1,833 (50.0) |
| Risk factors | | |
| Rainfall in 30 days before admission (mm), median (IQR) | 1,738 | 24.0 (1.8–67.7) |
| Farming occupation | 1,445 | 261 (18.1) |
| Worked in rice field | 1,445 | 23 (1.6) |
| Self-reported HIV-infected or positive HIV serology | 2,052 | 656 (32.0) |
| Clinical history | | |
| Illness duration (days), median (IQR) | 3,334 | 4 (3–9) |
| Cough | 3,234 | 1,936 (59.9) |
| Dyspnea | 3,227 | 2,216 (67.3) |
| Headache | 2,480 | 1,551 (62.5) |
| Myalgia | 2,371 | 947 (40.0) |
| Rash or cutaneous lesion | 2,076 | 158 (7.6) |

1 IQR = interquartile range; N = number of participants with data available.
Gram-negative bacilli were isolated from the blood culture of five (0.1%) participants. None was determined to be *B. pseudomallei*, so no cases of confirmed melioidosis were identified.

Among 323 participants tested by IHA, the median (range) age was 27 (0–70) years, and the median (range) duration of fever was 4 (1–60) days. Other characteristics of participants tested by IHA are shown in Table 2. Forty-four (13.6%) reported being HIV infected. Two (0.6%) cases of probable melioidosis were identified through paired serology, both of whom survived until follow-up. Neither reported recent travel outside Kilimanjaro region. The first case of probable melioidosis was a 6-year-old boy with a 2-day history of fever, rigors, cough, and dyspnea. The participant and his guardians reported a previous negative HIV test. The presence of other immune-suppressing diseases, including diabetes mellitus, or medications was not ascertained. He reported exposure to standing water around his house and walking barefoot in the mud during the preceding 30 days. A discharge diagnosis of pneumonia was recorded by the treating clinical team. The patient was treated with oral azithromycin. The second case of probable melioidosis was a 36-year-old woman with a 3-day history of fever, rigors, cough, and joint pain. She reported a previous negative HIV test. The presence of other immune-suppressing diseases or medications was not ascertained. She reported no exposure to surface or standing water around her house, and she did not report walking barefoot in the preceding 30 days. She was treated with analgesia and without antimicrobials. A discharge diagnosis of rheumatoid arthritis was recorded by the clinical team.

We identified 57 (17.7%) participants who met the definition for *B. pseudomallei* seropositivity. The highest reciprocal antibody titer was 10,240 (Figure 1). Among participants who were seropositive, 24 (42.1%) were male, and the median (range) age was 23 (< 1, 84) years. Of seropositive participants, 14 (24.6%) reported exposure to surface water compared with 50 (18.8%) seronegative participants ($P = 0.32$), 19 (33.3%) reported the presence of standing water around their house compared with 71 (26.8%) of seronegative participants ($P = 0.32$), and 26 (44.6%) reported walking barefoot in the preceding 30 days compared with 118 (44.4%) of seronegative participants ($P = 0.86$).

**DISCUSSION**

This study did not detect a confirmed case of melioidosis in northern Tanzania despite nearly a decade of blood culture in more than 3,700 patients hospitalized with fever. Melioidosis was unlikely to be a major cause of severe febrile illness in northern Tanzania during the study period. Across three study surveillance periods, we cultured blood of consecutive patients hospitalized with fever and systematically investigated isolates to identify *B. pseudomallei*. The absence of *B. pseudomallei* blood isolates among our patient population does not preclude the occurrence of melioidosis in our region. In Kilifi, Kenya, four *B. pseudomallei* isolates were recovered from > 66,000 blood cultures to provide an estimated annual incidence of 0.2 cases per 100,000 people. Nonetheless, our findings would indicate that if present melioidosis is likely to be a rare cause of bacteremia in northern Tanzania.

Two participants met the case definition for probable melioidosis. Both cases had negative blood cultures but did not have sputum or other potentially relevant samples cultured. Although each probable case reported a clinical illness that was compatible with melioidosis, the clinical features were nonspecific, and both survived the acute illness without receiving antimicrobials active against *B. pseudomallei*. Furthermore, we did not ascertain whether either patient had host risk factors for melioidosis such as diabetes or immune suppression. As IHA has limitations in the diagnosis of melioidosis and is not recommended as a diagnostic test in endemic areas, it is uncertain whether their illness was due to melioidosis.

Almost 20% of participants who underwent IHA testing in our study were seropositive to *B. pseudomallei*. Whether IHA seropositivity indicates exposure to *B. pseudomallei* or an antigenically similar organism such as *Burkholderia mallei* or another *Burkholderia* spp. is uncertain. To our knowledge,
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TABLE 2
Characteristics of participants undergoing Burkholderia pseudomallei indirect hemagglutination testing, Tanzania, 2012–2014 (N = 323)

| Demographic characteristics | n (%) |
|-----------------------------|-------|
| Age (years), median (IQR)   | 27 (5–40) |
| Gender, male                | 142 (44.0) |
| Risk factors                |       |
| Rainfall in 30 days before admission (mm), median (IQR) | 46 (27–72) |
| Farming occupation          | 66 (20.4) |
| Self-reported HIV-infected   | 44 (13.6) |
| Clinical history            |       |
| Fever duration (days), median (IQR) | 4 (2–7) |
| Cough                       | 200 (61.9) |
| Dyspnea                     | 101 (31.3) |
| Headache                    | 217 (67.2) |
| Myalgia                     | 140 (43.3) |
| Rash or cutaneous lesion    | 24 (7.4) |

IQR = interquartile range.

Table 2 shows the characteristics of participants undergoing Burkholderia pseudomallei indirect hemagglutination testing in Tanzania. The table includes information on demographic characteristics, risk factors, and clinical history of the participants. The data suggests that the majority of participants were male, with fever duration ranging from 2 to 7 days, and a high proportion had cough and dyspnea. The prevalence of self-reported HIV-infected participants was also noted.

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