Digging for melioidosis

New insights into the epidemiology and pathophysiology

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CHAPTER 5

CLINICAL, ENVIRONMENTAL AND SEROLOGIC SURVEILLANCE STUDIES OF MELIOIDOSIS IN GABON, 2012—2013
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The tier-1 classed bioterror threat agent *Burkholderia pseudomallei* is an environmental Gram-negative bacillus and the causative agent of melioidosis. Isolated reports on *B. pseudomallei* in soil and animals in East and West-Africa suggest that melioidosis may be more widely distributed than previously thought. Given its equatorial tropical climate, we hypothesized that *B. pseudomallei* could be present in the Central African country of Gabon. We conducted a seroprevalance study, set up microbiology facilities at a large clinical referral center and prospectively screened all cultures derived from febrile patients for *B. pseudomallei* and related species; and determined whether this organism could be isolated form Gabonese soil. We identified both *B. pseudomallei* and *B. thailandensis* in the environment and discovered a novel *B. pseudomallei* sequence type causing lethal septic shock. Our data suggest that melioidosis is an emerging infectious disease in Central Africa but is unrecognized because of the lack of diagnostic microbiology facilities.
INTRODUCTION

The tier 1 biothreat agent *Burkholderia pseudomallei* is an environmental Gram-negative bacillus and the cause of melioidosis, a disease characterized by sepsis, pneumonia and abscess formation in virtually any organ.\(^1\)\(^-\)\(^3\) *Burkholderia thailandensis* is closely related to *B. pseudomallei* but rarely causes disease in humans or animals and is usually distinguished from *B. pseudomallei* by its ability to assimilate arabinose.\(^4\)\(^-\)\(^6\) Melioidosis mainly affects individuals who are in regular contact with soil and water and is associated with a mortality of up to 40% in resource poor environments. Southeast Asia and tropical Australia are the major endemic regions for melioidosis.\(^1\)\(^-\)\(^2\) The northern tip of the Northern Territory in Australia and northeast Thailand represent hot spots, with annual incidence rates of up to 50 cases per 100,000 people.\(^1\)\(^,\)\(^7\)

The emergence of melioidosis in Brazil is an example of increasing recognition in areas where the disease is probably endemic and has become apparent as a result of enhanced awareness and diagnostics.\(^1\)\(^,\)\(^8\) Human *B. pseudomallei* infection has been reported from Malawi, Nigeria, The Gambia, Kenya and Uganda; human cases appear to be few and isolated in Africa, although this could be due to both under recognition and underreporting.\(^1\)\(^,\)\(^9\)\(^-\)\(^12\) A limited number of published reports on the isolation of *B. pseudomallei* from soil and animals in East and West Africa, however, does suggest that melioidosis could be widely distributed across this region.\(^13\)\(^-\)\(^14\)

Given the equatorial tropical distribution of *B. pseudomallei* and *B. thailandensis*, we hypothesized that these bacteria are present in the Central African country of Gabon, potentially causing disease. By conducting a seroprevalence study, an environmental survey and setting up microbiology facilities for *B. pseudomallei* detection at a large referral hospital, we detected *B. pseudomallei* in soil and as a cause of lethal infection in Gabon. *B. thailandensis* has not been previously reported from Central Africa but environmental sampling revealed that this organism was also present in Gabon.
METHODS

Study sites and populations
The study was performed in Moyen-Ogooué and Ngounié provinces (combined population 162,000) in central Gabon, which covers an area of 56,285 square kilometers, and predominantly consists of dense primary rain forest. A seroepidemiological surveillance study was performed using 304 serum samples from healthy non-febrile school children (age 12-20 years) living in and around Lambaréné, the capital of Moyen-Ogooué province; these children also participated in a chemoprophylaxis study for malaria as described.15 A prospective analysis of community-acquired bloodstream infections was performed at Albert Schweitzer Hospital (HAS) in Lambaréné (population 24,000), located in the Central African rainforest on the river Ogooué. The rainy season starts in October and ends in June (including a short dry season in December/January). Mean annual rainfall is 1,981 mm (78 inches), which is equivalent to mean rainfall in northeast Thailand.16 Studies were approved by Centre National de la Recherche Scientifique et Technologique (CENAREST), Libreville, and the scientific review committee of the Centre de Recherches Médicales de Lambaréné, HAS, Lambaréné, Gabon.

Prospective analysis of community-acquired bloodstream infections
To obtain data on the prevalence and causes of community-acquired bloodstream infections in Lambaréné we prospectively monitored all blood cultures taken from admitted febrile patients in the HAS for one year (June 1, 2012–May 31, 2013) using BacT/Alert PF (bioMérieux, Marcy l’Etoile, France). Criteria for taking blood cultures were left to the discretion of the treating physician. Technicians and staff of the clinical microbiology laboratory received additional training on sample handling and processing.17,18 All oxidase-positive Gram-negative bacteria that were not Pseudomonas aeruginosa were tested for B. pseudomallei using methods for subculture and identification as described below. Antimicrobial susceptibilities were determined by E-test (bioMérieux) on Mueller-Hinton-agar (bioMérieux); when available, breakpoints were defined as described.19

B. pseudomallei antibody detection by indirect haemagglutination assay
Presence and titer of antibodies to B. pseudomallei were determined by the indirect-haemagglutination-assay (IHA) as described,20,21 using pooled
antigens prepared from two Thai \textit{B. pseudomallei} isolates. An antibody titer of $\geq 1:40$ was used as the cut-off value for seropositivity.\textsuperscript{22}

\textbf{Soil sampling study}

Soil sampling for the presence of \textit{B. pseudomallei} was based on consensus guidelines and direct culture of soil in enrichment broth was performed.\textsuperscript{17,23} Eight sites were selected on the basis of local maps, consultations with inhabitants, extensive inspection throughout Moyen-Ogooué (6x) and Ngounié (2x) provinces and known factors associated with the presence of \textit{B. pseudomallei} namely wet soil (e.g. rice field) or land use (e.g. farmers with goats).\textsuperscript{17} Within each sampling area (50x50 sq. meters) a fixed interval sampling grid was used to collect 100 samples per field at a distance of 5 m from each other. For each sample, 10 grams of soil was collected from 30 cm depth, stored away from direct sunlight and processed within three hours. Isolation of potential \textit{Burkholderia sp.} from soil was done as described.\textsuperscript{17,23} In brief, 10 gram of soil was diluted in 10 mL of TBSS-C50 broth containing colistin and crystal violet and vortexed for 30 seconds before incubation at $\sim 42^\circ$C for 48 hours. 10 uL of supernatant was subcultured onto Ashdown-agar and incubated and examined every 24 hours for seven days. \textit{B. pseudomallei} was identified by colony-morphology, positive oxidase test, inability to assimilate arabinose, antibiotic susceptibility pattern (\textit{B. pseudomallei} is generally resistant to gentamicin and colistin while susceptible to amoxicillin/clavulanic acid\textsuperscript{1,2}), API 20NE (bioMérieux), and Bps specific latex-agglutination test.\textsuperscript{18,24,25} Positive results were confirmed with molecular analysis (see below). Soil type was determined by standard lithological and pedological analysis of sediments; for this purpose, two extra samples were collected per site from 30 cm depth.\textsuperscript{26} Sediment properties were compared to the (geomorphological) location as described in the recently published Soil Atlas of Africa.\textsuperscript{26}

\textbf{Genetic and phylogenetic analysis}

Genomic DNA was extracted using Qiagen DNeasy (Qiagen, Valencia, CA) to perform multilocus sequence typing (MLST).\textsuperscript{27} Primers used to amplify fragments of the seven housekeeping genes were identical to those described at the Burkholderia MLST site (http://bpseudomallei.mlst.net/misc/info2.asp). For isolate \textit{B. thailandensis} D50 (see below) primer narK-up was replaced by nark-upAMC 5’-tctctactcgtgcgctgggg-3’. Sequences of the seven gene fragments of African isolates were concatenated and combined with those from a selection of 971 STs representing all \textit{B. pseudomallei}, \textit{B. thailandensis}, and \textit{B. pseudomallei}.
mallei and B. thailandensis isolates in the B. pseudomallei MLST database. Concatenated sequences were aligned and analyzed in MEGA-6. A phylogenetic tree was constructed using Neighbor-Joining algorithm using the Kimura 2-parameter model. Bootstrap test was for 500 repetitions. Whole-genome sequencing (WGS) was performed using the MiSeq platform (Illumina, San Diego, CA) as described.⁹
RESULTS

Prospective analysis of community-acquired bloodstream Infections
A one-year prospective study was conducted of community-acquired bloodstream infections in the HAS, which admits about 6,000 patients annually. A total of 941 bacterial blood cultures were taken from patients admitted with a febrile illness, of which 77 (8.2%) were positive. Eight (10.0%) bloodstream infections were due to *Escherichia coli*, the most prevalent isolate overall together with *Staphylococcus aureus* (6; 7.8%) and *Salmonella enterica* (6; 7.8% - 5 of which were non-typhoidal *Salmonella*). Other isolates that were isolated at least 5 times included *Streptococcus pneumoniae* (5; 6.5%), *Klebsiella pneumoniae* (5; 6.5%) and *Enterobacter* sp. (5; 6.5%). *B. pseudomallei* was isolated from one patient (1.4%), who is described below.

CA S E  R E P O R T
A 62-year-old Gabonese lady was admitted in January with a seven day history of fever, cough, weakness, headache, vomiting, and a painful knee. There were no complaints of cough or shortness of breath. She had poorly controlled diabetes mellitus and was taking glibenclamide. There was no history of cardiopulmonary or renal disease, was receiving no long-term medications besides glibenclamide, and was a non-smoker. She was a retired school teacher, but still engaged in family farming. Physical examination revealed a blood pressure of 160/90 mmHg, a pulse of 130 beats per minute and a temperature of 40.5°C. A wound with underlying abscess formation on the right leg was present, together with diffuse tenderness of the right knee with warmth, erythema, and limitation of active and passive range of motion due to pain and effusion. Neurologic, cardiovascular, and respiratory examinations revealed no abnormalities. Laboratory findings obtained on admission showed an elevated blood sugar of 24 mmol/L, but creatinine (0.85 mg/dL), white-cell count (9,800 x 10³ per cubic millimeter), and haemoglobin (9.2 gm/dL) levels were within normal ranges. No other blood or urine test or (chest)radiography was performed. On day one treatment was initiated with amoxicillin/clavulanic acid as empiric treatment for sepsis. On day two the abscess was incised and drained and on day three antibiotic therapy was switched to ceftriaxone. Cultures taken from blood, wound, and synovial fluid all showed identical Gram-negative rods which were initially classified as *Pseudomonas* sp. No other pathogens were detected. Her clinical condition proceeded to deteriorate, and she died on day eight after admission from septic shock. A post-mortem examination was not performed. After death, the *Pseudomonas* sp. was classified as *B. pseudomallei* (termed patient strain Gb100), which was confirmed by MLST and WGS (see below). This isolate was later determined to be susceptible to trimethoprim-sulfamethoxazole, amoxicillin/clavulanic acid, ceftriaxone, and meropenem (Table 1).
Table 1  Antibiotic susceptibility of B. pseudomallei and B. thailandensis strains from Gabon

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>B. pseudomallei patient strain</th>
<th>B. pseudomallei soil strain C2</th>
<th>B. thailandensis soil strain C50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>4 a</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>Tobramycin a</td>
<td>4 a</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1</td>
<td>0.75</td>
<td>1.0</td>
</tr>
<tr>
<td>Moxifloxacin b</td>
<td>1.0</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Meropenem</td>
<td>4</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>8</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>TMP/SMX</td>
<td>1/19</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Amoxi/clav</td>
<td>8/2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Pip/taz</td>
<td>32/32 b</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>8</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>4 б</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>n/a e</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
</tr>
</tbody>
</table>

Bacterial isolates were tested for their susceptibility to antimicrobial agents. Minimum inhibitory concentration (MICs; mg/L) were determined by E-test on Mueller-Hinton-agar. When available breakpoints were defined as described. a Breakpoint for gentamicin was used, b Breakpoint for ciprofloxacin used. c Breakpoint available for piperacillin only. d Breakpoint for doxycycline was used. e Intrinsic resistance. TMP/SMX denotes trimethoprim-sulphamethoxazole, Amox/clav is amoxicillin/clavulanic acid, Pip/taz is piperacillin/tazobactam.

Seroepidemiology study

The presence and titer of antibodies to B. pseudomallei was determined in 304 children healthy school children age 12-20 years who lived in Moyen-Ogooué province, of whom 143 (47.0%) were male. Details of this cohort of healthy non-febrile children have been described previously. For-thirty children (14.1%) had a detectable IHA titer; values ranged from 1:10 to 1:80 (median 1:10, interquartile range [IQR] 1:10–1:20). Five children (1.6%) had an IHA titer of ≥1:40, which has been used as cut-off value for seropositivity. Of note, none of the children had an IHA titer ≥1:160, which is used in several centers in Thailand to support a diagnosis of melioidosis in patients with clinical features consistent with this diagnosis.
Environmental surveillance

We next conducted an extensive environmental survey targeted around the residences of children included in the serosurvey study, the identified patient, and from land types associated with *B. pseudomallei* in other geographic locations, namely wet soil (e.g. rice field) or land use (e.g. farmers with goats). Based on this information, eight soil sampling locations in Moyen-Ogooué and Ngounié provinces were identified (Figure 1). Soil analysis revealed that the predominant soil type in this area was a ferralsol, which is red and yellow weathered soil, with the exception of the samples taken near Mouila village from a rice paddy, where the soil was defined as gleysol (clay, a hydric soil saturated with groundwater for long enough periods to develop a characteristic gleyic color pattern; Table 2). *B. pseudomallei* was isolated from 21 (3%) of 800 soil samples taken from three (38%) of eight sample sites; the maximum number of positive samples for one site was 14 (14%; Table 2). The biochemical profile of all isolates was in accordance with *B. pseudomallei* (API 20NE code 1156576). The antibiogram of *B. pseudomallei* soil strain C2 is shown in Table 1.

Isolation of *Burkholderia thailandensis*

*B. thailandensis*, which can coexist with *B. pseudomallei* in the soil, is gener-ally considered avirulent as it does not cause overt disease and has previously been reported from Southeast Asia and Australia. We now identify *B. thailandensis* to be present in the soil of Gabon (see below; Figure 2). Of interest, this strain, termed *B. thailandensis* soil strain D50, was positive on *Bps* latex-agglutination. This Gabonese *B. thailandensis* strain, with API 20NE code 1157577, was susceptible to trimethoprim-sulphamethoxazole, amoxicillin/clavulanic acid, ceftazidime, and meropenem (Table 1).
(A) Gabon, showing location of the 8 sites from which soil was sampled to test for the presence of *B. pseudomallei*, July 2012–September 2012. (B) Soil sampling site no. H, a rice field near Mouila village.
<table>
<thead>
<tr>
<th>Nearest village</th>
<th>Elevation (m)</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Land use</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Lambaréné, Albert Schweitzer Hospital</td>
<td>34</td>
<td>S 00°40'40.5</td>
<td>E 010°23'49.7</td>
<td>Football pitch</td>
</tr>
<tr>
<td>B Lambaréné, Adouma</td>
<td>14</td>
<td>S 00°40'50.2</td>
<td>E 010°23'31.5</td>
<td>Riverbed that is dry most of the year</td>
</tr>
<tr>
<td>C Makouké</td>
<td>20</td>
<td>S 00°28'30.8</td>
<td>E 010°24'34.7</td>
<td>Cattle ranch</td>
</tr>
<tr>
<td>D Lambaréné, Adiwa</td>
<td>8</td>
<td>S 00°41'06.0</td>
<td>E 010°23'43.5</td>
<td>Next to school (with Bps IHA positivity)</td>
</tr>
<tr>
<td>E Lambaréné, Petit Paris</td>
<td>35</td>
<td>S010°24'24.4</td>
<td>E 010°25'20.7</td>
<td>Cattle ranch</td>
</tr>
<tr>
<td>F Fougamou</td>
<td>88</td>
<td>S 01°18'40.3</td>
<td>E 010°37'14.4</td>
<td>Savannah, grassland</td>
</tr>
<tr>
<td>G Massika II</td>
<td>55</td>
<td>S 00°40'40.7</td>
<td>E 010°23'51.4</td>
<td>Football pitch</td>
</tr>
<tr>
<td>H Mouila</td>
<td>92</td>
<td>S 01°51'27.8</td>
<td>E 011°02'37.7</td>
<td>Rice paddy</td>
</tr>
<tr>
<td>Soil type</td>
<td>Soil description</td>
<td>% Sample holes positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td>-------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferralsol</td>
<td>Yellowish brown, clay fluvial sediments, not strongly humic, some gravels, poorly sorted sediment, decalcified, Ap-horizon.</td>
<td>14%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferralsol, clay, orange, dry</td>
<td>Brownish yellow, clay fluvial sediments, moderately humic, some gravels, Ap-horizon with strong indicators for human interference.</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferralsol, Orange, little stones, hard, rocky, less hard, orange</td>
<td>Yellowish brown, clay fluvial sediments, not strongly humic, some gravels, poorly sorted sediment, decalcified, Ap-horizon.</td>
<td>4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferralsol</td>
<td>Brownish yellow, clay fluvial sediments, moderately humic, some gravels, Ap-horizon with strong indicators for human interference.</td>
<td>3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Savannah / ferralsol</td>
<td>Yellowish grey, well sorted clay, weakly humic.</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Savannah / ferralsol</td>
<td>Yellowish grey, well sorted clay, weakly humic.</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferralsol</td>
<td>Reddish brown, clay fluvial sediments, not strongly humic, sediment, decalcified, Ap-horizon.</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gleysol</td>
<td>Greyish yellow clay with ferric concretions, gleyic features, probably connected to rice cultivation</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Phylogenetic tree of *Burkholderia pseudomallei* and *B. thailandensis* strains from Gabon, 2012–2013. Phylogenetic analysis by multilocus sequence typing amplification (MLST) of isolate Gb100 (from 62-year-old patient who died of melioidosis), *B. pseudomallei* soil isolate C2 (sample collected at site C), and *B. thailandensis* soil isolate D50 (sample collected at site D), together with sequence types representing all *B. pseudomallei* and *B. thailandensis* isolate accessible in the MLST database. Phylogenetic tree was constructed by using the neighbor-joining algorithm with the Kimura 2-parameter model. Bootstrap test was for 500 repetitions. Sequence type labels were omitted for simplicity. Position of the isolates from Gabon, including their closest relatives, are indicated.
Genetic Analysis of Gabonese Burkholderia strains

The three isolates from Gabon contained previously described MLST alleles but belonged to novel sequence types (ST). The patient isolate Gb100 (ST1127) and soil isolate C2 (ST1128) were single locus variants and showed one nucleotide difference in the narK sequence only. Patient isolate Gb100 was also a single locus variant of ST707 (a single nucleotide substitution in ndh). The only B. pseudomallei strain with ST707 in the database was isolated in 2010 from a patient in the UK, six weeks after returning from a trip to Nigeria. The soil isolate C2 ST1128 was a single locus variant of ST7 (a single nucleotide substitution in ndh) and ST879 (single nucleotide substitution in lipA). ST7 was represented by two isolates in the MSLT database both isolated in 1963 from patients in Vietnam. The B. pseudomallei ST879 strain was isolated in 2011 from a patient in Spain, who had returned from a trip through Madagascar and 14 countries in West Africa. The soil isolate D50 (ST1126) was a single locus variant of ST73. This ST is represented in the database by two B. thailandensis strains, one isolated from a foal in France and one isolated from the environment in Kenya. Phylogenetic analysis of the Gabonese isolates together with 971 STs drawn from the MLST database using the aligned concatenated sequences of the seven loci in the Neighbor-Joining algorithm with the Kimura 2-parameter model showed that the patient isolate and soil isolate C2, found near the community of the patient, grouped together with seven different STs representing 9 B. pseudomallei strains isolated in Cambodia (two strains), Vietnam (two strains), Niger, Nigeria, Spain (imported), France (two strains; one imported), and UK (imported) (Figure 2). Again, both the patient isolate Gb100 and the soil isolate C2 are most closely related to ST879. The soil isolate D50 was found to group together with three different STs representing four B. thailandensis strains isolated from Kenya, France, USA and Cambodia. Using this approach, we showed that the closest relatives of the strain that infected and eventually killed our patient were ST879 and the strain isolated from soil around her community. Our WGS sample data has been submitted to a project that is undertaking WGS on a large number of B. pseudomallei isolates from around the world. This approach is anticipated to offer superior resolution of the global phylogeny.
This study has confirmed the presence of *B. pseudomallei* in the environment in Gabon, and detected a human case of melioidosis in Central-Africa. In addition, we isolated *B. thailandensis* during environmental sampling in this part of the world. The low rate of antibody seropositivity in healthy children combined with the low prevalence of *B. pseudomallei* isolated from blood cultures in a local hospital, however, suggest that melioidosis is rare in this setting.

Only four out of the 13 human melioidosis cases acquired in Africa that have been reported in the literature were PCR confirmed. We now show with phylogenetic analysis that the newly identified patient isolate Gb100 groups with a *B. pseudomallei* isolate from a Spanish patient who had travelled across West Africa and Madagascar. Of interest, *B. pseudomallei* seropositivity was reported in a WHO investigation into an outbreak of severe pneumonia in the northeastern of the Democratic Republic of Congo (personal communication Eric Bertherat, MD, Control of Epidemic Diseases, WHO Geneva). Notably however, in that study some of the melioidosis seropositive cases were later diagnosed to have plague, calling into question the value of serology based testing in this setting.

The predominant soil type in the sites at which *B. pseudomallei* was isolated from the soil was similar to the one in which *B. pseudomallei* strains were found in Cambodia. The low positivity rate for *B. pseudomallei* per site points towards a relative low abundance of *B. pseudomallei* in Gabonese soil when compared to highly endemic areas in Southeast Asia and Australia. The true distribution of melioidosis in Africa remains uncertain, but we now can expand this area towards the Central African country of Gabon.

The genus *Burkholderia* contains more than 30 species, of which *B. pseudomallei* and *B. mallei* are considered the most pathogenic. The closely related *B. thailandensis* coexists with *B. pseudomallei* in the soil in Southeast Asia and Australia and is generally considered avirulent. The isolation of *B. thailandensis* from Gabonese soil extends our knowledge of the geographic distribution of this species. Of interest, this strain was positive on *Bps* latex-agglutination, which is in line with a previous report of a Thai *B. thailandensis* strain with a *Bps*-like capsular polysaccharide variant that also tested positive with the *Bps* latex-agglutination assay. Our phylogenic analysis shows a divergence between the Gabonese strain and the original *B. thailandensis* E264 from Thailand, which is the most
Evidence of the presence of this bacterium in Africa will have implications for bacterial identification in clinical laboratories, diagnostic serology assays, and environmental studies.

Our study has several limitations. *B. pseudomallei* serology can be misleading, with false positivity a major concern. Clearly there is the need for an accurate, inexpensive, simple serological assay to assess exposure to *B. pseudomallei*. In the interim, however, serological evidence of exposure should be based on assays with known sensitivity and specificity against culture confirmed melioidosis and to our understanding the IHA is best for melioidosis at the moment. Given the nature of working in a resource poor environment, only limited information is available on the clinical case; e.g. no imaging was performed to investigate the presence of deeper abscesses. Lastly, regarding the environmental study, *B. pseudomallei* is known for its capacity to survive in water and has been reported to be present in the air during severe weather events; we, however, did not investigate this in the present study. Furthermore, we cannot dismiss the possibility of sample error during soil sampling although guidelines for environmental sampling of *B. pseudomallei* were followed.

In summary, we have identified both *B. pseudomallei* and *B. thailandensis* in the Gabonese environment and discovered a novel *B. pseudomallei* sequence type that can cause lethal septic shock. It is likely that *B. pseudomallei* is an under-recognized cause of disease in Central-Africa. We propose that melioidosis does occur in Central-Africa but is unrecognized because of the lack of diagnostic microbiology facilities.

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REFERENCES


