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***Burkholderia pseudomallei* in Water Supplies, Southern Thailand**

To the Editor: Melioidosis is an infectious disease caused by the environmental gram-negative bacillus *Burkholderia pseudomallei*, which is present in northern Australia and across much of Asia (1,2). In Thailand, melioidosis is highly endemic to the northeast, where most infected persons are agricultural farmers with repeated environmental exposure (3). Melioidosis is infrequently reported from southern Thailand, although a cluster of 6 cases occurred in Phangnga Province after the December 2004 tsunami (4). Given the infrequency of reported cases, a cluster of 11 persons with melioidosis on Koh Phangan (an island in the Gulf of Thailand) during January–March 2012 (5) led to an investigation. Three case-patients were foreign tourists; 8 Thai case-patients were from 7 different villages throughout the island, and none were agricultural workers (5). Three cases were fatal; water inhalation was suspected as a route of infection in a fatal case in a neonate who was born in a birthing pool outside of a hospital (online Technical Appendix Table 1, <http://wwwnc.cdc.gov/EID/article/20/11/14-0832-Techapp1.pdf>). The lack of history for environmental exposure, such as farming, led to the hypothesis that water was the source of infection. After a request by Koh Phangan Hospital and the Thai Ministry of Public Health, an environmental survey was conducted for *B. pseudomallei* in water supplies on the island.

In March 2012, we randomly collected water from accessible water supplies in local residences and hotels from all 14 villages on Koh Phangan. A total of 190 samples were collected (range 10–18 samples per village, Figure) for culture, genotyping, and analysis (online Technical Appendix).

Isolates from 3 persons who died (a single bacterial colony saved from each person) from Koh Phangan were also available for genotyping and analysis. 26 (14%) of 190 samples were culture positive for *B. pseudomallei*. The positivity rate did not differ by source of the water sample: spring (5 [28%] of 18 samples), well (17 [13%] of 127), and tap water (4 [9%] of 45; $p = 0.16$, Fisher exact test). Of the 26 samples, 16 (62%), 9 (34%), and 1 (4%) were from local residences, hotels, and an ice cream shop, respectively. Positive water samples were distributed across the island (Figure). The median quantitative *B. pseudomallei* count was 30 CFU/L (range <10–11,300 CFU/L). The quantitative count did not differ by sample source ($p = 0.16$, Kruskal-Wallis test), and the sample with the highest quantitative count (11,300 CFU/L) was from well water. Of the 26 samples, only 1 was from a source that was consumed as drinking water.

We identified 12 multilocus sequence types (STs): 10 STs from water samples and 2 different STs from 3 clinical isolates (online Technical Appendix). The most frequent ST (ST1117, 10 isolates) was widely distributed across the island (Figure; online Technical Appendix Table 2). Phylogenetic analysis showed 12 genetically diverse STs identified on Koh Phangan and separate clusters of the clinical and environmental isolates (online Technical Appendix Figure).

Public tap water contaminated with *B. pseudomallei* has been reported previously in northeastern Thailand (6). The country's National Tap Water Quality Assurance Program does not include *B. pseudomallei* (7), a situation that warrants review. A combination of filtration and chlorination is recommended for treatment of village tap water systems in Thailand, but recent studies report that the quality of village tap water is suboptimal (8). Chlorination with sufficient contact time and free available chlorine can kill *B. pseudomallei* (9,10).

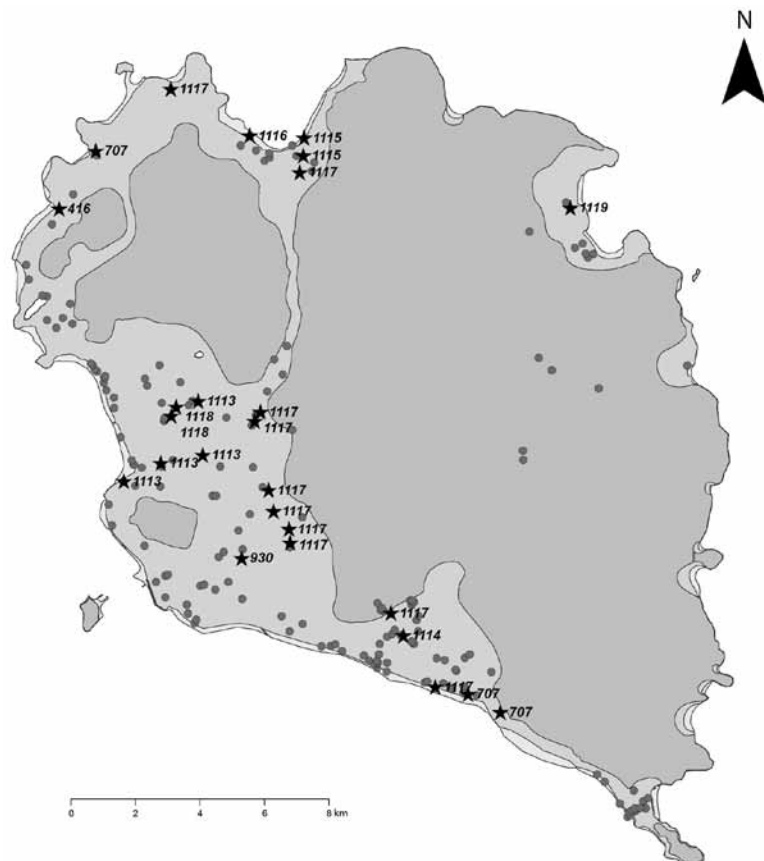


Figure. Location and multilocus sequence types of *Burkholderia pseudomallei* from water supplies on Koh Phangan, Thailand, 2012. A total of 190 water samples are indicated on the map. Twenty-six samples that were culture positive for *B. pseudomallei* are shown by black stars together with the sequence type, and 164 samples that were culture negative for *B. pseudomallei* are indicated as gray dots.

We reported our findings to Koh Phangan Hospital, Koh Phangan Public Health Office, and the Thai Ministry of Public Health. Our findings led to advice being provided by Thai Ministry of Public Health to every water treatment plant, household, and hotel on Koh Phangan in April 2012 to appropriately chlorinate water before general consumption. We recommend that residents and tourists to this island drink bottled or boiled water to prevent melioidosis and other waterborne infectious diseases.

Our finding that drinking water contained *B. pseudomallei* provides evidence for ingestion as a route of infection. Other routes include skin inoculation and inhalation, but we have no evidence from the clinical history to

support this, other than possible inhalation in the case of the neonate born in a birthing pool. We did not find matching genotypes in water supplies and human samples. Possible explanations include the following: the considerable genetic diversity of *B. pseudomallei* found in water in this study and elsewhere (6), the small sample size, and that fact that we genotyped a single colony per sample when the sample could contain multiple genotypes.

Culture-positive water samples originated from different water sources and were distributed across the island; the genotyping results were consistent with endemic infection and ruled out a single outbreak. Soil sampling and a case-control study on Koh Phangan might provide a more

extensive analysis of activities associated with development of melioidosis in this setting.

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Antimicrobial Drug-Resistant Bacteria Isolated from Syrian War-Injured Patients, August 2011-March 2013

To the Editor: Soft-tissue injuries sustained during wars are subject to environmental contamination and, thus, to a high risk for infection. Efforts to describe the epidemiology of war-associated infections are complicated by difficult access to patients, limited availability of microbiology support, and widespread empirical antimicrobial drug use. Nevertheless, identifying the relevant pathogens is critical because war-associated injuries commonly become infected and antimicrobial drug-resistant bacteria are well-described in these injuries, including those in the Middle East (1-3).

The Médecins Sans Frontières (MSF) surgical project in Amman, Jordan, was initially developed for war-injured Iraqis needing surgical reconstruction or management of chronic osteomyelitis. Infection management is based on organism-directed antimicrobial agents and wide surgical resection of involved tissue. The proximity of this project to the Syrian conflict provided an opportunity to describe microbiologic features of infections caused by war-associated injuries in Syrians, who may be at increased risk for infection-associated complications because of exclusion from care in official health systems. We describe a cross-sectional series of 61 Syrian orthopedic patients who had suspected infections, as determined on the basis of surgical samples obtained intraoperatively.

Syrian patients admitted to the MSF clinic underwent initial surgical exploration of wounds; if infection was suspected, ≥ 3 intraoperative samples (bone, fibrous tissue,

fluid) were obtained for culture and transported (at 4°-8°C) within 2 h to the laboratory at Ibn al-Haytham Hospital in Amman. Patients who were treated with antimicrobial drugs within 2 weeks before admission were excluded from analysis.

We retrospectively reviewed data for patients admitted during August 1, 2011-March 31, 2013. Data were collected from databases and individual charts in Amman and analyzed by using Stata 12 (<http://www.stata.com/stata12/>). This study was deemed exempt from additional ethical approval by the MSF review board because it involved routinely collected data.

We defined a multidrug-resistant (MDR) isolate as 1) extended-spectrum β -lactamase-expressing *Enterobacteriaceae*; 2) *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates resistant to at least 1 agent in 3 antimicrobial categories typically used for treatment; or 3) methicillin-resistant *Staphylococcus aureus* (MRSA). Pathogen identification was conducted by using conventional methods and the API system (bio-Mérieux, Durham, NC, USA). Antimicrobial drug susceptibility testing was conducted by using the MicroScan Walk-Away System (Dade Behring, West Sacramento, CA, USA).

During the study period, 870 patient consultations were conducted, of which 345 (40%) were for patients from Syria. At the initial operating room evaluation, infection was suspected in 61 (18%) Syrians. These patients had a median age of 26 years (interquartile range 22-34); 98% were male. The median time from injury to admission was 5 months (interquartile range 1.2-8.1), but for 27 (44%) patients, the time from injury to admission was >6 months. The 2 most common injuries were gunshot wounds (32 patients [52%]) and wounds from explosions (20 patients [33%]). The dominant injury was located in an upper extremity in 14 (23%) patients and a lower extremity in 47 (77%) patients.

Burkholderia pseudomallei in Water Supplies on Resort Island, Southern Thailand

Technical Appendix

Technical Appendix Table 1. Clinical characteristics of 3 fatal cases and sequence types of *Burkholderia pseudomallei* isolates

Case-patient	Age	Sex	Nationality	Occupation	Underlying disease	Sequence type
1	5 d	M	Russian	Newborn	Natural childbirth in an unknown source of water and without routine medical interventions	385
2	51 y	M	French	Tourist	Chronic liver disease	164
3	49 y	M	Thai	Labourer	None	164

Water Sample Collection, Culture, Genotyping, and Phylogenetic Tree Analysis

We collected 1.5 L water from the main water supply at each location. Householders were asked whether the water supply was used for drinking and, if so, whether it was treated before consumption. If the water supplies were treated (by filtration or boiling) before drinking, only treated water samples were collected for culture. The location of each water sample was recorded by using EpiCollect (1), and a map was drawn using ArcMap implemented with ArcGIS 10.0 for desktop (ESRI, Redlands, CA, USA).

For each water sample, 1, 10, and 100 mL were each passed through a 0.45- μ m filter to obtain a quantitative bacterial count. The remaining 1.4 L was passed through 2.5 g of sterile diatomaceous earth (Celite; World Minerals Corporation, San Jose, CA, USA) to obtain a sensitive, qualitative method (2). Filters were cultured on Ashdown agar, and diatomaceous earth was cultured in selective broth (15 mL of threonine-basal salt plus colistin broth) (2). Broth was incubated at 40°C in air for 48 h, after which 10 μ L of the upper layer was streaked onto an Ashdown agar to achieve single colonies. All Ashdown agar plates were incubated at 40°C in air, and examined every 24 h for 7 d. If enrichment broth cultures were positive for *Burkholderia pseudomallei* but filter cultures were negative, the quantitative count was defined as <10 CFU/L.

A single colony of *B. pseudomallei* isolated from each water sample was selected for genotyping by using multilocus sequence typing (MLST). MLST was undertaken as described previously (3). A maximum-likelihood tree was reconstructed from concatenated

sequences of 7 MLST loci by using PhyML version 3.0.1 (4). The CLC Main Work Bench version 7.0 was used to edit and display the tree (QIAGEN, Valencia, CA, USA).

We compared genotypes of *B. pseudomallei* from 26 water specimens and 3 melioidosis cases from Koh Phangan. Sequence types (STs) of the clinical isolates (ST385 [n = 1] and ST164 [n = 2]) have been identified previously from water in southern Thailand (ST385) (5), and soil in northeastern Thailand (ST164) (6). Three of 10 STs from water samples have been identified previously from water in southern Thailand (ST416), and human case-patients from Cambodia (ST930) and Nigeria (ST707). The remaining 7 STs were novel (ST1113 to ST1119). A total of 12 STs were identified, including 10 STs from water samples and 2 different STs from 3 clinical isolates.

A maximum-likelihood tree of *B. pseudomallei* isolates from Koh Phangan was constructed, together with all of the unique STs in the public MLST database (<http://bpseudomallei.mlst.net/>) (Technical Appendix Figure 1). The tree showed that the 12 STs identified on Koh Phangan were genetically diverse and fell into 6 clusters. The major cluster included 19 isolates from 5 novel STs (ST1113, ST1115–ST1118), suggesting that these related genotypes dominate on this island. The clinical and environmental isolates did not cluster together.

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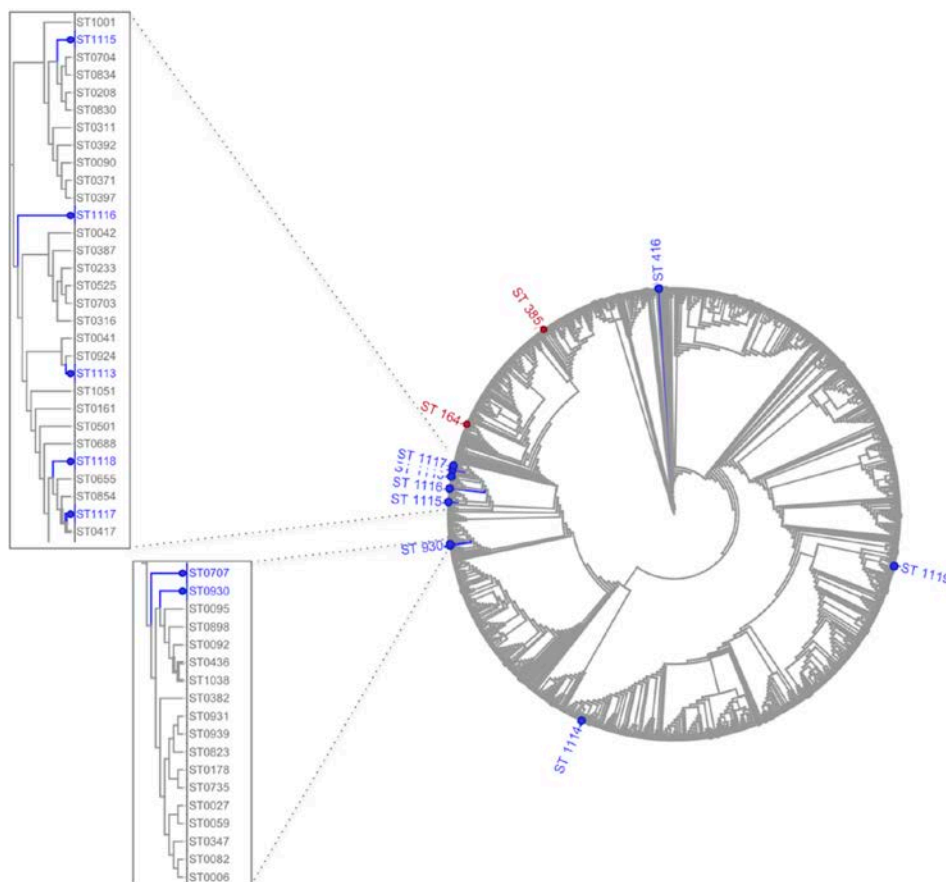
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Technical Appendix Table 2. Multilocus sequence types of *Burkholderia pseudomallei* from water supplies on Koh Phangan, Thailand, 2012

Sequence type	No.			Total
	Tap water	Well water	Spring water	
416	0	1	0	1
707	0	1	2	3
930	0	1	0	1
1113	0	4	0	4
1114	0	1	0	1
1115	1	0	1	2
1116	0	1	0	1
1118	0	2	0	2
1119	0	0	1	1
1117	3	6	1	10
Total	4	17	5	26



Technical Appendix Figure. Phylogenetic tree of *Burkholderia* spp. A maximum likelihood tree was reconstructed from concatenated nucleotide sequences of 7 multilocus sequence type loci. The isolates included were *B. pseudomallei* from Koh Phangan island, Thailand, as described in this study, together with 1 representative of each of the sequence types (STs) held in the public *Burkholderia* MLST database (<http://bpseudomallei.mlst.net/>). Blue dots indicate STs found in water supplies; red dots indicate STs isolated from the 3 patients with fatal melioidosis.