Human Melioidosis, Malawi, 2011

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A case of human melioidosis caused by a novel sequence type of *Burkholderia pseudomallei* occurred in a child in Malawi, southern Africa. A literature review showed that human cases reported from the continent have been increasing.

Melioidosis is widely distributed in tropical and sub-tropical regions, but data are lacking on this disease in sub-Saharan Africa. We report a case of melioidosis caused by a novel sequence type in a 16-month-old boy from rural Malawi. Increasing reports of melioidosis from Africa indicate a need for further investigation.

Case Report

A 16-month-old boy was seen at Queen Elizabeth Central Hospital (QECH), Blantyre, Malawi, in March 2011 with fever of 18 days’ duration, poor feeding, subcutaneous lesions of 15 days’ duration, and edema of the hands and feet. He had received intravenous (IV) benzylpenicillin and gentamicin for 4 days at a local health center before being referred for persistent fever.

The family lived in a remote village in the Shire Valley near the Malawi–Mozambique border at a latitude of −15°S; daytime temperature average was 27°–29°C, and natural flooding occurs from October through April. The boy’s parents were subsistence maize farmers and kept goats and pigs.

On arrival at QECH, the child was irritable and pale; temperature was 38.7°C, weight 9.8 kg (weight-for-age z score −1.14), and height 79 cm (weight-for-height z score −1.06). He had bilateral dactylitis with arthritis of the metacarpophalangeal and interphalangeal joints of the lateral 3 fingers. Numerous rubberty, tender subcutaneous nodules of ~2 cm diameter were palpable on the face, thorax, and limbs. Overlying hyperpigmentation and weepy ulcerations occurred over some nodules. There was cervical and inguinal lymphadenopathy but no hepatosplenomegaly. Symmetric bipedal pitting edema extended to the knees. Neurologic, cardiovascular, and respiratory examinations revealed no abnormalities.

Laboratory results were as follows: blood glucose 7.8 mmol/L (reference 3.5–7.7 mmol/L), hemoglobin 4.6 g/dL (reference 9.7–15.1 g/dL), leukocyte count 31.9 × 10³/µL (reference 3.9–10.7 × 10³/µL), and erythrocyte sedimentation rate 95 mm/h (reference 3–13 mm/h). Blood smear showed poikilocytes with some tear drops and reticulocytes and was negative for malaria parasites. HIV test (Unigold; Trinity Biotech, Bray, Ireland) and VDRL (Venereal Disease Research Laboratory) test for syphilis were negative. Radiographs of the hand showed bilateral osteolytic reactions in the lateral 3 fingers. Chest radiograph and abdominal ultrasound indicated no abnormalities.

Culture (BacT/Alert PF; bioMérieux, Marcy l’Etoile, France) of blood taken on admission and aspirate of pus from a subcutaneous nodule grew white, oxidase-positive colonies of gram-negative rods, and the biochemical profile (API 20NE; bioMérieux) strongly suggested *Burkholderia pseudomallei* (1556575: *B. pseudomallei* [98.3% identity]). The API profile from the pus isolate (1156154) initially suggested *Chromobacterium violaceum*, a recognized misidentification of *B. pseudomallei*, by API profiling (1). Antimicrobial susceptibility by disk diffusion indicated resistance to gentamicin and susceptibility to co-amoxiclav; colistin disk testing was unavailable. Because *B. pseudomallei* has not been reported from Malawi, we sought to confirm the isolate by real-time PCR, targeting the highly specific type III secretion system (2). DNA was extracted by using a Wizard Genomic Purification kit (Promega, Madison, WI, USA), and real-time PCR was performed on an Applied Biosystems 7900HT (Applied Biosystems, Foster City, CA, USA) by using a technique modified for SYBR green detection (2). This PCR confirmed the identity of the organism as *B. pseudomallei*. Whole-genome sequencing (WGS) was performed by using the MiSeq Personal Sequencer (Illumina, San Diego, CA, USA), which enabled multilocus sequence typing (MLST) (3) and revealed a novel allelic combination (1,3,3,1,5,1,1). This sequence type (ST) has been submitted to the MLST database (http://bpsnodmallei.mlst.net/) and has been assigned MLST ST1008, part of clonal complex 1.

The boy was given chloramphenicol for empiric treatment of systemic bacterial infection before the isolate was identified; the patient was discharged on chloramphenicol and co-amoxiclav. Colonies from blood and pus isolates (API 20NE; bioMérieux) showed ceftriaxone resistance and susceptibility to clindamycin, doxycycline, and rifampin. The isolate was susceptible to ceftazidime, ciprofloxacin, azithromycin, and meropenem, based on the presence of the *cmi* (chloramphenicol resistance) and *tet* (tetracycline resistance) genes.

Patient specimen genotyping confirmed the association of the isolate with *B. pseudomallei* (4). The *cmi* and *tet* genes were identified using the Sanger method. The *mls* (meflamine) gene was amplified using primers MlM and MlM, and the product was sequenced using primer MlM. The presence of the *cmi* and *tet* genes was confirmed using the Sanger method. The isolate was susceptible to ceftazidime, ciprofloxacin, azithromycin, and meropenem, based on the presence of the *cmi* (chloramphenicol resistance) and *tet* (tetracycline resistance) genes.

The MLST of the isolate yielded the sequence type ST1008, part of clonal complex 1. The boy responded to therapy; he was discharged on chloramphenicol and co-amoxiclav.

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identified. In light of the anthropometric values, anorexia, fecal morphology, and symmetric pedal edema, acute kwashiorkor was diagnosed, and nutritional rehabilitation was begun. Pedal edema and anorexia improved after 48 hours. At 96 hours, *B. pseudomallei* infection was diagnosed, and treatment was changed to IV ceftazidime. Fever abated by day 7, and after 30 days of IV ceftazidime, the nodules had involuted and the dactylitis and arthritis had abated by day 7, and after 30 days of IV ceftazidime, the nodules had involuted and the dactylitis and arthritis had resolved. At the family’s request, the child was discharged on a 6-month regimen of cotrimoxazole, rather than the planned 6-week IV regimen for osteomyelitis.

Four weeks after discharge, the child remained well with no fevers and no new lesions; clinical anemia had resolved. At the family’s request, the child was discharged

Conclusions

Melioidosis is acquired through the skin or possibly by inhaling the environmental organism *B. pseudomallei*. It causes a wide spectrum of clinical disease—from localized skin infection to severe acute septicemia—but progresses to disease in only a small proportion of exposed persons (4). *B. pseudomallei* is endemic in Southeast Asia and Northern Australia and typically is distributed from latitude 20°N to latitude 20°S, particularly in association with wet soil (4).

Sporadic cases have been documented in all inhabited continents, but a lack of diagnostic microbiological facilities and systematic studies in many low-income regions limit knowledge of the true distribution of the disease (5), a particular problem in rural sub-Saharan Africa. Although the relationship between human and animal

**Table 1. Melioidosis in animals, Africa**

<table>
<thead>
<tr>
<th>Year (reference)</th>
<th>Country</th>
<th>Animal</th>
<th>Clinical characteristics</th>
<th>Method of identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1936 (6)</td>
<td>Madagascar</td>
<td>Pig</td>
<td>Lymphadenopathy</td>
<td>Passaged through guinea pig (tissue from submaxillary gland)</td>
</tr>
<tr>
<td>1960 (7)</td>
<td>Chad</td>
<td>Goat</td>
<td>Lymphadenopathy</td>
<td>Isolated from mesenteric ganglia</td>
</tr>
<tr>
<td>1960 (8)</td>
<td>Africa*</td>
<td>Camel</td>
<td>Retropharyngeal abscess</td>
<td>Inoculation and sacrifice of guinea pig</td>
</tr>
<tr>
<td>1972 (6)</td>
<td>Niger, Burkina Faso</td>
<td>Pigs (&gt;100 cases)</td>
<td>Abscesses in liver, spleen, and lung in apparently healthy pigs</td>
<td>Not described</td>
</tr>
<tr>
<td>1995 (9)</td>
<td>South Africa</td>
<td>Goat</td>
<td>Mammary gland and renal abscesses</td>
<td>Biochemical and phenotypic characteristics</td>
</tr>
</tbody>
</table>

*Specific country of acquisition was not detailed.

**Table 2. Melioidosis in humans, Africa*"}

<table>
<thead>
<tr>
<th>Year (reference)</th>
<th>Country where acquired (diagnosed)</th>
<th>Details of infected persons</th>
<th>Clinical characteristics</th>
<th>Diagnostic method (source of isolation)</th>
<th>Definitive treatment (duration, wk)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982 (10)</td>
<td>Kenya (Denmark)</td>
<td>Adult</td>
<td>Sepsis</td>
<td>Culture (blood, urine, sputum)</td>
<td>OTC (4); cotrimoxazole</td>
<td>Complete recovery</td>
</tr>
<tr>
<td>1985 (11)</td>
<td>Sierra Leone (Gambia)</td>
<td>Child</td>
<td>Cutaeneous abscesses, osteomyelitis</td>
<td>Culture; indirect hemagglutination (pus)</td>
<td>CHL, TET</td>
<td>Improved; lost to follow-up</td>
</tr>
<tr>
<td>2004 (12)</td>
<td>Mauritius (Mauritius)</td>
<td>Adult with SLE</td>
<td>Sepsis, cellulitis</td>
<td>Culture; API 2ONE (blood)</td>
<td>None</td>
<td>Died (d 9)</td>
</tr>
<tr>
<td>2004 (13)</td>
<td>Madagascar (La Réunion)</td>
<td>Adult smoker</td>
<td>Sepsis, respiratory distress, Septicemia</td>
<td>Culture (blood, BAL fluid)</td>
<td>CAZ; cotrimoxazole (20)</td>
<td>Complete recovery</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adult with CPI</td>
<td></td>
<td>Culture (blood, BAL fluid)</td>
<td>CAZ; cotrimoxazole (20)</td>
<td>Not described</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adult with SLE</td>
<td>Septicemia</td>
<td>Culture (blood, BAL fluid)</td>
<td>cotrimoxazole (20)</td>
<td>Not described</td>
</tr>
<tr>
<td>2006 (14)</td>
<td>Madagascar (La Réunion)</td>
<td>Adult smoker</td>
<td>Pneumonia</td>
<td>Culture; API 2ONE; PCR (BAL fluid)</td>
<td>IPM (2); cotrimoxazole (20)</td>
<td>Resolution at 3 mo</td>
</tr>
<tr>
<td>2010 (15)</td>
<td>Africa† (France)</td>
<td>Adult</td>
<td>M ycotic aneurysm</td>
<td>Culture (blood, arterial tissue)</td>
<td>IPM + CIP (5); cotrimoxazole (20)</td>
<td>Resolution at 6 mo</td>
</tr>
<tr>
<td>2011 (16)</td>
<td>Gambia (Spain)</td>
<td>Adult with diabetes mellitus</td>
<td>Pyomymositis, pneumonia</td>
<td>Culture; PCR (pus, sputum)</td>
<td>CAZ + cotrimoxazole (5); DOX + cotrimoxazole (20)</td>
<td>Complete recovery</td>
</tr>
<tr>
<td>2011 (17)</td>
<td>Nigeria (UK)</td>
<td>Adult with diabetes mellitus</td>
<td>Localized lymphadenopathy</td>
<td>Culture; chromatography; PCR (blood)</td>
<td>Meropenem (1); cotrimoxazole (12)</td>
<td>Lost to follow-up</td>
</tr>
<tr>
<td>2011 (18)</td>
<td>Africa† (Spain)</td>
<td>Adult</td>
<td>Sepsis</td>
<td>Culture (blood)</td>
<td>CAZ + DOX</td>
<td>Not described</td>
</tr>
</tbody>
</table>

*OTC, oxytetracycline; TMP-SXT, trimethoprim–sulfamethoxazole; CHL, chloramphenicol; TET, tetracycline; SLE, systemic lupus erythematosus; BAL, bronchoalveolar lavage; CAZ, ceftazidime; CPI, chronic pulmonary insufficiency; IPM, imipenem; CIP, ciprofloxacin; DOX, doxycycline; MER, meropenem.

†Exposure was in multiple countries on the continent or the specific country of acquisition was not detailed.
infection is not precisely understood, infections of animals have been recognized throughout the continent (Table 1) (6–9). During the past 30 years, 11 cases of human melioidosis acquired in Africa (1 in a child) have been reported in the literature (Table 2 [10–15; 16–18 in online Technical Appendix, wwwnc.cdc.gov/EID/article/19/6/12-0717-Techapp1.pdf]). Three of these cases were PCR confirmed. In many earlier reports, identification was not confirmed by methods that would satisfy modern taxonomists; thus, the true distribution of melioidosis in Africa remains uncertain.

We used eBURST software (19 in online Technical Appendix) to model the relationship between ST1008 and the global MLST database (online Technical Appendix Figure 1). The founder of this branch of clonal complex 1 is ST916 (online Technical Appendix Figure 2), which was isolated from Cambodia. The other STs on this branch, ST186 and ST250, were isolated in Thailand. Although none of the other Africa B. pseudomallei isolates of known MLST are predicted to be in the same subgroup, 2 isolates from human infections that are thought to have occurred in Kenya (ST5 and ST9; online Technical Appendix Figure 2) are in the adjacent subgroup. Thus, these Malawi and Kenya strains might share a recent common ancestor. We have submitted WGS data from our sample 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.

Unfamiliarity with the culture characteristics of B. pseudomallei often has resulted in delays in recognition, identification, diagnosis, and treatment (1). The organism exhibits considerable interstrain and media-dependent variability in colonial morphology (1); its wrinkled appearance in older colonies may result in their dismissal as contaminants. Even relatively expensive biochemical test kits, such as the API20NE, may result in misidentification, as with the pus isolate here, which raises the question about whether the infrequency of the diagnosis is due to rarity of the disease or lack of capacity to identify it. B. pseudomallei might be more widespread than recognized in Malawi and ecologically similar areas of sub-Saharan Africa where the environment is conducive to its growth. Health care in Malawi, as in most of sub-Saharan Africa, is delivered frequently without use of even basic diagnostic facilities, which leads to underdiagnosis of malaria and tuberculosis (20 in online Technical Appendix). In this environment, patients with septicemic melioidosis could have died before all locally available empiric treatments had been tried. B. pseudomallei is resistant to penicillin, gentamicin, and many other antimicrobial drugs used to treat sepsis in the tropics, so diagnosis is necessary for appropriate antimicrobial therapy.

Melioidosis therefore could be underestimated in Malawi and throughout the region. Environmental microbiology and seroprevalence studies are required to gauge the extent of this infection and to guide local and regional health care policy.

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Dr Katangwe is a pediatric registrar at QECH in Blantyre, Malawi. Her research interests are in pediatric infectious diseases, particularly in viral nosocomial infections in African hospitals.

References


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Technical Appendix

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Technical Appendix Figure 1. eBURST diagram of clonal complex 1 *Burkholderia pseudomallei* sequence types (STs) submitted to the global multilocus sequence typing (MLST) database. Diagram shows predicted evolutionary relationship between strains by ST in 7 different alleles. Each circle indicates a particular ST, with the relative size of the circle indicating the number of isolations of that particular ST. Blue circle indicates the predicted founding genotype (ST48); yellow circles indicate predicted subgroup founders; and purple circle indicates ST1008 from the Malawi case described in this report. Lines between STs indicate STs that differ at a single allele. The red square is magnified in Technical
Appendix Figure 2 to illustrate more clearly STs closely related to ST1008. The complete database and the eBURST software can be accessed at http://bpseudomallei.mlst.net/

Technical Appendix Figure 2. Detail of eBURST clonal complex 1 subgroup for ST1008. ST1008 is labeled in magenta. The subgroup founder ST916 (bottom right) was isolated from samples in Cambodia. In the adjacent subgroup are STs (ST5 and ST-9) isolated from infections in humans in Kenya.