Clinical-Epidemiological Features of 13 Cases of Melioidosis in Brazil


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The aim of this work was to catalog the clinical and ecoepidemiological characteristics of melioidosis in Brazil. The clinical-epidemiological features of melioidosis in Ceará are similar to those in other regions where the disease is endemic. These findings support the inclusion of this Brazilian state as part of the zone of endemicity for melioidosis.

Burkholderia pseudomallei, a Gram-negative bacillus that naturally inhabits soil and water (10), is the agent of melioidosis, an infectious disease endemic in Southeast Asia and Northern Australia (5). The epidemiology of melioidosis, the ecological conditions related to B. pseudomallei’s growth, and the bacterium’s relationship with environmental factors have been studied (4, 9).

Melioidosis in Brazil has been considered an emerging disease since 2003, when an outbreak was reported in the state of Ceará, Northeastern Brazil (13). From 2003 to 2011, 13 cases of melioidosis have occurred in this state, one of which affected a Dutch tourist who had visited Ceará (1) and was reported by the Dutch government. Therefore, the objective of this work was to catalog the clinical and ecoepidemiological characteristics of these 13 cases of melioidosis.

The 10 strains included in this study were isolated from 10 of 13 clinical cases of melioidosis acquired in the state of Ceará, Brazil. These strains were identified through molecular tests at the Laboratory of Emerging and Reemerging Pathogens (LAPERE) of the Ceará Federal University (UFC). We also gathered data from the Ceará Institute of Research and Economic Strategy (IPECE) (8) on the geoclimatic features of the areas where melioidosis has been reported, aiming at comparing them with those of other countries where the disease is endemic.

To search for new cases of melioidosis, we used the compulsory notification service, the database, and the epidemiological bulletins of the Ceará State Health Secretariat. Also, a clinical guideline proposed by a group of infectious disease physicians interested in melioidosis, partners of our research group, aided the detection of new cases (7).

The diagnostic methods utilized for the detection of the reported cases are described in Table 1, along with their references. To confirm the identification of all B. pseudomallei strains, they were initially identified through the automated Vitek 2 system (bioMérieux, Marcy l’Etoile, France). Later, molecular identification was carried out by PCR, through amplification of the specific 16S-23S spacer region of B. pseudomallei according to the method of Merritt et al. (11), using primers Bp1 (5’-CGATGATCGTTG CGCTT-3’) and Bp4 (5’-CGTTGTCGCCGATTCCAAAT-3’) and the following protocol: 4 min at 94°C, followed by 45 cycles of 30 s at 94°C, 30 s at 50°C and 45 s at 72°C, with a final extension at 72°C for 7 min. The amplification of 300-bp bands allowed the identification of the 10 strains as B. pseudomallei.

The nearly complete 16S rRNA gene was amplified by PCR using the primers 27F (5’-AGAGTTTGATCCTGCTCAG-3’) and 1525R (5’-AAGGAGGTATCCAGGC-3’) (15) according to the following protocol: a denaturation step (2 min at 95°C) was followed by 35 cycles of 1 min at 95°C, 1 min at 62°C, and 1.5 min at 72°C, with a final extension at 72°C for 5 min. PCR products were purified by using the GFX PCR DNA and gel band purification kit (GE Healthcare Life Sciences) and then sequenced. DNA sequencing was performed with the DYNamic ET (energy transfer) terminator cycle sequencing kit (GE Healthcare Life Sciences). The sequences of the 16S rRNA gene (with an average length of about 1,400 bp) were compared to those previously deposited in GenBank database (www.ncbi.nlm.nih.gov/GenBank/index.html) using the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi), which allowed the identification of the 20 strains as B. pseudomallei.

Concerning rainfall (Table 2), all the municipalities of Ceará affected by melioidosis (except Tejuçuoca) have annual rainfall rates greater than 800 mm (8), which is similar to the precipitation levels in the other zones where melioidosis is endemic (4). Of the 13 melioidosis cases, 9 occurred during the rainy season (Table 2), corroborating the findings of a previous work that 73% and 85% of the cases in Northeast Thailand and Northern Australia, respectively, occurred in the wet season (4). Even though the possibility of environmental exposure to the pathogen cannot be excluded, we believe the four cases reported during the dry season in Ceará were a consequence of the reactivation of a latent infection, since the patients also suffered from acute and/or debilitating comorbidities (Table 1).

Regarding altitude, Ceará has hilly regions with elevations above 300 m, rich in waterfalls that are popular tourist and recreation areas. Most of the cases described (9/13) occurred in municipalities ranging from 10 to 260 m high. However, all the munic-
### TABLE 1 Clinical features and epidemiological data of 13 cases of melioidosis in the state of Ceará, Northeastern Brazil, from 2003 to 2011

<table>
<thead>
<tr>
<th>Case</th>
<th>Mo and yr of occurrence</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Clinical features(^a); type of exposure</th>
<th>Treatment</th>
<th>Patient outcome</th>
<th>Laboratory diagnostic methods(^c)</th>
<th>CEMM(^d) strains</th>
<th>Origin (IPECE, 2010)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>February 2003</td>
<td>15</td>
<td>M</td>
<td>Fever, cough, headache and pustules on the limbs, fulminant sepsis, no comorbidity; swimming in a reservoir</td>
<td>No data available</td>
<td>Died</td>
<td>No data available</td>
<td>Not available</td>
<td>Tejuçuoca; 3°59'20&quot;S, 39°34'50&quot;W</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>February 2003</td>
<td>14</td>
<td>F</td>
<td>Fever, cough, headache and pustules on the limbs, fulminant sepsis, no comorbidity; swimming in a reservoir</td>
<td>No data available</td>
<td>Died</td>
<td>GNNF biochemical identification, confirmed by PCR</td>
<td>03-06-033</td>
<td>Tejuçuoca; 3°59'20&quot;S, 39°34'50&quot;W</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>February 2003</td>
<td>10</td>
<td>M</td>
<td>Fever, cough, headache and pustules on the limbs, fulminant sepsis, no comorbidity; swimming in a reservoir</td>
<td>No data available</td>
<td>Died</td>
<td>GNNF biochemical identification, confirmed by PCR</td>
<td>03-06-034</td>
<td>Tejuçuoca; 3°59'20&quot;S, 39°34'50&quot;W</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>February 2003</td>
<td>12</td>
<td>F</td>
<td>Fever, cough, headache and pustules on the limbs, no comorbidity; swimming in a reservoir</td>
<td>No data available</td>
<td>Died</td>
<td>GNNF biochemical identification, confirmed by PCR</td>
<td>03-06-035</td>
<td>Tejuçuoca; 3°59'20&quot;S, 39°34'50&quot;W</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>January 2004</td>
<td>39</td>
<td>M</td>
<td>Genital abscess, sepsis, no comorbidity; squatting in a river washing clothes</td>
<td>No data available</td>
<td>Died</td>
<td>No data available</td>
<td>Not available</td>
<td>Banabuí; 5°18'33&quot;S, 38°55'14&quot;W</td>
<td>13</td>
</tr>
<tr>
<td>6</td>
<td>July 2005</td>
<td>50</td>
<td>M</td>
<td>Community-acquired pneumonia, sepsis, comorbidity diabetes; swimming in a lake</td>
<td>Cefuroxime, erythromycin, gentamicin</td>
<td>Died</td>
<td>API 20NE, confirmed by PCR</td>
<td>Not available</td>
<td>Not available</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>May 2005</td>
<td>30</td>
<td>M</td>
<td>Aspiration pneumonia, sepsis, comorbidity diabetes; vehicle accident and immersion in a river</td>
<td>Imipenem</td>
<td>Died</td>
<td>Vitek 1 system, confirmed by PCR</td>
<td>03-06-037</td>
<td>Aracoiaba; 4°22'16&quot;S, 38°48'51&quot;W</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>April 2008</td>
<td>17</td>
<td>M</td>
<td>Pneumonia, sepsis, comorbidity COPD; bathing in waterfalls</td>
<td>Imipenem</td>
<td>Died</td>
<td>Vitek 2 system, confirmed by PCR</td>
<td>03-06-036</td>
<td>Itapajé; 3°41'12&quot;S, 39°35'10&quot;W</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>November 2009</td>
<td>70</td>
<td>M</td>
<td>Myotic aneurysm, sepsis; unknown</td>
<td>Cefepime</td>
<td>Died</td>
<td>Vitek 2 system, confirmed by PCR</td>
<td>03-06-038</td>
<td>Grana; 4°07'13&quot;S, 40°49'34&quot;W</td>
<td>14</td>
</tr>
<tr>
<td>10</td>
<td>October 2009</td>
<td>50</td>
<td>M</td>
<td>Mediastinal adenopathy, fever, comorbidity diabetes; unknown</td>
<td>Meropenem(^e)</td>
<td>Survived</td>
<td>Vitek 2 system, confirmed by PCR</td>
<td>05-03-008</td>
<td>Case not reported</td>
<td>Case not reported</td>
</tr>
<tr>
<td>11</td>
<td>April 2010</td>
<td>57</td>
<td>M</td>
<td>Splenic abscess, peritonitis, comorbidity sickle cell anemia; unknown</td>
<td>Imipenem(^e)</td>
<td>Died</td>
<td>Vitek 2 system, confirmed by PCR</td>
<td>05-03-009</td>
<td>Ubajara; 3°15'16&quot;S, 40°55'16&quot;W</td>
<td>Case not reported</td>
</tr>
<tr>
<td>12</td>
<td>December 2010</td>
<td>53</td>
<td>M</td>
<td>Pneumonia, sepsis, comorbidity dengue fever; handling and transporting bricks</td>
<td>Imipenem</td>
<td>Died</td>
<td>Vitek 2 system, confirmed by PCR</td>
<td>05-03-010</td>
<td>Pacoti; 4°13'30&quot;S, 38°55'24&quot;W</td>
<td>Case not reported</td>
</tr>
<tr>
<td>13</td>
<td>January 2011</td>
<td>32</td>
<td>M</td>
<td>Adenopathy, fever, comorbidity diabetes; unknown</td>
<td>Meropenem(^e)</td>
<td>Survived</td>
<td>Vitek 2 system, confirmed by PCR</td>
<td>05-03-011</td>
<td>Oca; 4°29'2.7&quot;S, 38°35'48&quot;W</td>
<td>Case not reported</td>
</tr>
</tbody>
</table>

\(^a\) M, male; F, female.

\(^b\) CET, cranioencephalic trauma; COPD, chronic obstructive pulmonary disease.

\(^c\) Performed by the laboratory that first isolated the strain. GNNF, Gram-negative nonfermenting.

\(^d\) CEMM, Specialized Medical Mycology Center.

\(^e\) Eradication therapy with doxycycline sulfamethoxazole-trimethoprim.

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Municipality (case[s]a), latitude and longitude | Soil type | Avg temp (°C) | Avg annual rainfall (mm) | Rainy season | Mo and yr of occurrence(s) | Altitude (m)
--- | --- | --- | --- | --- | --- | ---
Tejuçuoca (1,2,3 and 4); 3°59'20"S, 39°34'50"W | Brown sandy loam, litholic soils, solodic planosol, red-yellow podzolic | 26–28 | 659 | January to April | February 2003 | 140.32
Banabuíú (5); 5°18'35"S, 38°55'14"W | Alluvial soils, litholic soils, solodic planosol, red-yellow podzolic cambissol | 26–28 | 815 | February to April | January 2004 | 100.0
Aracoiba (7); 4°22'16"S, 38°48'51"W | Dystrophic quartzose sands, red-yellow podzolic, alluvial soils, litholic soils solodic planosol | 24–26 | 1,010 | February to April | May 2005 | 107.1
Ubajara (8 and 11); 3°51'16"S, 40°55'16"W | Dystrophic quartzose sands, litholic soils, red-yellow latosol red-yellow podzolic | 24–26 | 1,483 | January to April | April 2008, April 2010 | 847.5
Granja (9); 3°07'13"S, 40°49'34"W | Dystrophic quartzose sands, litholic soils, solodic planosol, red-yellow podzolic, | 24–26 | 1,040 | February to April | November 2009 | 10.75
Itapajé (10); 3°41'12"S, 39°33'10"W | Brown sandy loam, litholic soils, solodic planosol, red-yellow podzolic, | 26–28 | 800 | January to April | October 2009 | 262.2
Pacoti (12); 4°13'30"S, 38°55'24"W | Red-yellow podzolic | 24–26 | 1,558 | February to April | December 2010 | 736.1
Ocara (13); 4°29'27"S, 38°35'48"W | Dystrophic quartzose sands, solodic planosol, red-yellow podzolic | 26–28 | 959 | January to April | January 2011 | 170.2

a Source, IPECE, 2007 (8).
b Case 6 was the Dutch tourist; the municipality visited by the patient was not reported.

The soils from the municipalities affected by melioidosis mainly present a clay-enriched subsoil with low base status and low-activity clay (acrisol) or high base status and high-activity clay (luvisol) or shallow soils (leptosols) (8), resembling those where melioidosis is endemic (2, 9).

The detection of the first cases of melioidosis in Ceará happened in a rural area in the municipality of Tejuçuoca, and it was facilitated because the infection occurred among four children of the same family and the social repercussions were sufficient that the cases were referred to health authorities in the state capital (12). Despite the reported cases and isolation of B. pseudomallei from soil samples from the state (13), clinical suspicion of the disease is still not a routine medical practice. Additionally, the low level of diagnostic expertise of local laboratories in identifying B. pseudomallei may be another limiting factor for the diagnosis of melioidosis in Ceará.

In conclusion, the clinical-epidemiological features of melioidosis in Ceará are similar to those of regions where this disease is known to be endemic. This study contributes to knowledge of the importance of melioidosis in Brazil and to its clinical-epidemiological characterization, supporting the inclusion of the state as part of the zone where the disease is endemic.

REFERENCES
10. Limmathurotsakul D, et al. 2010. Burkholderia pseudomallei is spatially...


