JCM Journals.ASM.org

Clinical-Epidemiological Features of 13 Cases of Melioidosis in Brazil

Raimunda S. N. Brilhante,[a] Tereza J. P. G. Bandeira,[a,d] Rossana A. Cordeiro,[a] Thalles B. Grangeiro,[b] Rita A. C. Lima,[a] Joyce F. Ribeiro,[a] Débora S. C. M. Castelo-Branco,[a] Jorge L. N. Rodrigues,[e] Ivo C. B. Coelho,[e] Francisco G. Magalhães,[f] Marcos F. G. Rocha,[a,c] and José J. C. Sidrim[a]

Specialized Medical Mycology Center, Postgraduate Program in Medical Microbiology, Federal University of Ceará, Fortaleza, Ceará, Brazil[a]; Postgraduate Program in Biochemistry, Federal University of Ceará, Fortaleza, Ceará, Brazil[b]; Postgraduate Program in Veterinary Science, State University of Ceará, Fortaleza, Ceará, Brazil[c]; LabPauster Laboratory, Fortaleza, Ceará, Brazil[d]; Postgraduate Program in Public Health, Federal University of Ceará, Fortaleza, Ceará, Brazil[e]; and São Mateus Hospital, Fortaleza, Ceará, Brazil[f]

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 meliodosis 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′-CGATGATCGTTGG CGCTT-3′) and Bp4 (5′-CGTTGTGCCGTATTCCAAT-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′-AGAGTTTGATCCTGGCTCAG-3′) and 1525R (5′-AAGGAGGTGATCCAGCC-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 DYEnamic 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 75% 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-

Received 13 June 2012 Returned for modification 28 June 2012
Accepted 8 July 2012
Published ahead of print 18 July 2012
Address correspondence to Raimunda S. N. Brilhante, brilhante@ufc.br.

doi:10.1128/JCM.01577-12

TABLE 1 Clinical features and epidemiological data of 13 cases of melioidosis in the state of Ceará, Northeastern Brazil, from 2003 to 2011

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

[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.

TABLE 2 Soil type, climate, rainfall, and altitude[a] in the municipalities affected by melioidosis in Ceará from 2002 to 2010

Municipality (case[s][b]), 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
Banabuiú (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
Aracoiaba (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°35′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.

ipalities affected by melioidosis showed various levels of elevation, ranging from 10 to 847 m, similar to what is observed in other countries where the disease is endemic, in which the altitudes range from 5 to 600 m (2).

Additionally, concerning the climate, Tejuçuoca is classified as semiarid, while the other municipalities affected have a mild, semiarid tropical climate (Table 2). The other areas in the world where melioidosis is endemic also have tropical or subtropical climates (9, 10).

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.

ACKNOWLEDGMENTS

There are no conflicts of interest to declare.

This research was supported by CAPES-PNPD (process number 23038.027637/2009-68-2103/2009) and CNPq (process number 562296/2010-7).

REFERENCES

1. **Aardema H, et al.** 2005. Changing epidemiology of melioidosis? A case of acute pulmonary melioidosis with fatal outcome imported from Brazil. Epidemiol. Infect. **133**:871–875.
2. **Corkeron M, Loehr S, Norton R, Nelson P.** 2010. Melioidosis case clusters in a tropical urban setting: association with soil type and geomorphology. Division Symposium 4.2. 19th World Congress of Soil Science, Soil Solutions for a Changing World. Brisbane, Australia 1 to 6 August 2010.
3. **Couto MS, et al.** 2009. A diagnosis of *Burkholderia pseudomallei* directly in a bronchoalveolar lavage by polymerase chain reaction. Microbiol. Infect. Dis. **65**:73–75.
4. **Currie BJ, Jacups SP.** 2003. Intensity of rainfall and severity of melioidosis, Australia. Emerg. Infect. Dis. **9**:1538–1542.
5. **Currie BJ, Ward L, Cheng AC.** 2010. The epidemiology and clinical spectrum of melioidosis: 540 cases from the 20 year Darwin prospective study. PLoS Negl. Trop. Dis. **11**:e900. doi:10.1371/journal.pntd.0000900.
6. **Inglis TJJ, Rolim DB, Sousa AQ.** 2006. Melioidosis in the Americas. Am. J. Trop. Med. Hyg. **75**:947–954.
7. **Inglis TJJ, Rolim DB, Rodriguez JL.** 2006. Clinical guideline for diagnosis and management of melioidosis. Rev. Inst. Med. Trop. Sao Paulo **48**: 1–4.
8. **IPECE.** 2007. Ceará em mapas. Instituto de Pesquisa e Estratégia Econômica do Ceará, Secretaria de Planejamento e Gestão (SEPLAG) Governo do Estado do Ceará. http://www2.ipece.ce.gov.br/atlas/.
9. **Kaestli M, et al.** 2009. Landscape changes influence the occurrence of the melioidosis bacterium Burkholderia pseudomallei in soil in Northern Australia. PLoS Negl. Trop. Dis. **3**:e364. doi:10.1371/journal.pntd.0000364.
10. **Limmathurotsakul D, et al.** 2010. Burkholderia pseudomallei is spatially

distributed in soil in northeast Thailand. PLoS Negl. Trop. Dis. **4**:e694. doi:10.1371/journal.pntd.0000694.
11. **Merritt A, Inglis TJJ, Chidlow G, Harnett G.** 2006. PCR-based identification of *Burkholderia pseudomallei*. Rev. Inst. Med. Trop. Sao Paulo **48**: 239–244.
12. **Miralles IS, et al.** 2004. *Burkholderia pseudomallei*: a case report of a human infection in Ceará, Brazil. Rev. Inst. Med. Trop. **46**:51–54.
13. **Rolim DB, et al.** 2009. Environmental isolates of *Burkholderia pseudomallei* in Ceará State, Northeastern Brazil. Appl. Environ. Microbiol. **75**:1215–1218.
14. **Sidrim JJC, et al.** 2011. Mycotic aneurysm caused by *Burkholderia pseudomallei*: report of a Brazilian strain genetically related to Thai strains. Clin. Microbiol. Infect. **17**:719–721.
15. **Weisburg WG, Barns SM, Pelletier DA, Lane DJ.** 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. **173**:697–703.