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Cystic Fibrosis Patient with *Burkholderia pseudomallei* Infection Acquired in Brazil[▽]

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***Burkholderia pseudomallei* is rarely isolated from cystic fibrosis patients outside known areas of endemicity. We report the recovery of *B. pseudomallei* from the sputum of a cystic fibrosis patient who lives in Brazil. We highlight the importance of careful attention to unusual nonfermentative gram-negative rods in cystic fibrosis patients.**

CASE REPORT

The patient was a 17-year-old female patient with cystic fibrosis (CF) who lives in Barra dos Bugres, Mato Grosso do Sul, a region located in the tropical area of Brazil, and has never traveled outside the country. This patient has been followed at the Hospital de Clínicas de Porto Alegre, a CF reference center in Porto Alegre, south Brazil.

Despite the diagnosis of CF-related diabetes associated with chronic lung infection by methicillin-susceptible *Staphylococcus aureus* and *Pseudomonas aeruginosa*, her pulmonary disease was well controlled until 2003. The patient had normal lung function (forced expiratory volume in the first second [FEV1] of 102% of predicted) and only minor bronchiectactic changes shown by high-resolution chest computer tomography (CT). After 2004, her pulmonary condition progressively deteriorated, as she presented with frequent respiratory exacerbations and recurrent radiological changes, as well as right upper lobe bronchiectasis. During this period, a nonfermentative gram-negative rod was recovered from her sputum, which was not identified using a semiautomated system (mini-API, ID 32 GN card; bioMeriéux, Marcy l′Etoile, France).

In July 2005, due to an episode of respiratory exacerbation the patient was admitted to hospital again, when a sputum sample was collected for bacteriological examination. The sputum sample was inoculated on MacConkey agar (bioMérieux), chocolate agar (bioMérieux), 5% sheep blood agar (bioMérieux), mannitol salt agar (Oxoid, Basingstoke, United Kingdom), and *Burkholderia cepacia* selective agar (BCSA [Oxoid]) which were incubated at 37°C. After 48 h, dry colonies were observed on the MacConkey, blood, and chocolate agars. Colonies with the same dried appearance and a pinkish color were also observed on the BCSA. This isolate proved to be a nonfermentative gram-negative rod that was submitted to further identification using the mini-API (bioMérieux) semiautomated system with the ID32GN card. The mini-API suggested the isolate was *Burkholderia pseudomallei*, with an excellent level of identification (99%), and the results of other phenotypic tests were also consistent with *B. pseudomallei* (Table 1). Species identification was also confirmed by PCR amplification of the 16S rRNA gene using primers 27FB (9) and 1492RB (5). The amplicon (1,200 bp) was directly sequenced, and a good-quality sequence of 800 bp was analyzed. The sequence was used for a BLAST search against the GenBank database, and the best match obtained was with *B. pseudomallei* (gi 33286699), with 99% identity. The clinical condition of the patient improved after a 3-week course of piperacillin-tazobactam and tobramycin.

In April 2006, the patient was readmitted for an episode of respiratory exacerbation and *B. pseudomallei* was again isolated from her sputum. After a 3-week course of meropenem, ceftazidime, amikacin, and trimethoprim-sulfamethoxazole her clinical and radiological status improved.

A 2-year follow-up showed that *B. pseudomallei* was repeatedly recovered from her sputum, the patient having had frequent pulmonary exacerbations requiring hospital admissions and showing gradual deterioration of lung function (FEV1 fell from 102% of predicted to 60% of predicted).

The serum of the patient was tested by enzyme-linked immunosorbent assay against *B. pseudomallei* by the Health Protection Agency, Colindale, United Kingdom, on two occasions, and im-

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TABLE 1. Characteristics of *Burkholderia pseudomallei* isolated from a Brazilian CF patient

Characteristic or test	Result	Test compatible with *B. pseudomallei*[a]
Gram stain	Gram-negative rod with bipolar staining	Yes
Oxidase	+	Yes
Motility	+	Yes
Oxidation of:		
Glucose	+	Yes
Xylose	+	Yes
Maltose	+	Yes
Adonitol	+	Yes
Sucrose	+	Yes
Lactose	+	Yes
Mannose	+	ND[b]
Arabinose	−	Yes
Arginine dihydrolase	+	Yes
Lysine decarboxylase	−	Yes
Ornithine decarboxylase	−	Yes
Beta-hemolysis	+	ND
Indole	−	ND
Hydrolysis of:		
Gelatin	+	Yes
Esculin	+	Yes
DNA	−	ND
Urea	+	Yes
ONPG[c]	−	Yes
PYR[d]	−	ND
Growth on:		
BCSA	+	Yes
MacConkey	+	Yes
42°C	+	Yes
Citrate agar	+	Yes
NaCl (6.5%)	−	ND
NaCl (0%)	+	ND
Nitrate reduction	+	Yes
Gas from nitrate	+	Yes
Polymyxin (300 U) resistance	+	Yes
Penicillin (10 U) resistance	+	Yes
Gentamicin (30 μg) resistance	+	Yes

[a] Adapted from references 13 and 16.
[b] ND, not described.
[c] ONPG, *O*-nitrophenyl-β-D-galactopyranoside.
[d] PYR, pyrrolidonyl-α-naphthylamide.

munoglobulin G titers were 2,000 (August 2005) and 1,000 (April 2006).

CF is the most common life-threatening genetic disorder among Caucasians. The main cause of morbidity and mortality in CF is respiratory disease associated with chronic bacterial infection, and the most prevalent bacterial pathogens associated with this are *S. aureus*, *P. aeruginosa*, and the *Burkholderia cepacia* complex (11).

The aerobic, nonfermentative, gram-negative, soil-dwelling rod *B. pseudomallei* is the causative agent of melioidosis, a severe disease endemic in areas of Southeast Asia and northern Australia (2). However, sporadic cases have been reported in west and east Africa, the Caribbean, Central and South America, and the Middle East (2). The first reported case of meliodosis in Brazil was in 2003 in the northeast state of Ceará (12). Afterwards, a few other cases, in non-CF patients, were described in this region (15).

B. pseudomallei has only rarely been described in CF patients, most cases occurring after traveling to a region of endemicity (Table 2). In this article, we have described a case of *B. pseudomallei* respiratory infection acquired by a CF patient in Brazil. To our knowledge, this is the first description of *B. pseudomallei* in a CF patient acquired outside the known regions of endemicity. Diabetes mellitus, a recognized risk factor for melioidosis (2), was also present in our patient, and this might have contributed to *B. pseudomallei* acquisition.

Melioidosis usually presents as a febrile illness, ranging from an acute fulminant sepsis to a chronic debilitating localized infection (2). Most CF patients with *B. pseudomallei* present with pulmonary exacerbations (Table 2), which are indistinguishable from those due to other common CF organisms. Only one patient was reported to have developed severe pulmonary sepsis and died (14), but another five patients presented with acute pulmonary infections and recovered clinically (6, 14, 18). Most of the described patients have also experienced progressive clinical deterioration after the isolation of *B. pseudomallei*, although a few have presented no clinical change in the course of the disease (6, 14). In this case report, *B. pseudomallei* was detected during an episode of clinical exacerbation of the respiratory disease, although one could speculate that this organism might have already been present in the patient's airways. *B. pseudomallei* has been repeatedly recovered from our patient's sputum over a period of 2 years.

Due to the difficulty in identifying *B. pseudomallei*, it is likely that reported cases of this organism in CF patients could represent an underestimation of the real incidence of this infection since many laboratories have no experience in recognizing this microorganism (3, 7). Considering that the isolation of *B. pseudomallei* from CF patients may have significant therapeutic and prognostic implications, as well as considerable safety precautions, it is important to precisely identify unusual nonfermentative gram-negative organisms repeatedly recovered from these patients, even from regions where the organisms are nonendemic. Some biochemical reactions are useful to differentiate *B. pseudomallei* from similar nonfermentative rods, like *Pseudomonas stutzeri* and the *B. cepacia* complex isolates. Whereas *B. pseudomallei* produces gas from nitrate and is arginine dihydrolase positive, most isolates of the *B. cepacia* complex are negative for both characteristics. *P. stutzeri* is negative for arginine dihydrolase, glucose oxidation, and gelatin hydrolysis (13). However, it has been shown (8, 10) that the accuracy for the identification of *B. pseudomallei* by commercial methods is system dependent. While a few systems are able to identify *B. pseudomallei* with very high accuracy, other systems present very low accuracy or do not have *B. pseudomallei* in their database. The latter systems misidentified *B. pseudomallei* as *B. cepacia* with a high confidence value (95 to 99%). Therefore, we understand that an isolate outside the areas of endemicity suspected of being *B. pseudomallei* according to any phenotypic method should be also confirmed by genetic techniques (e.g., 16S rRNA sequencing).

TABLE 2. *Burkholderia pseudomallei* in cystic fibrosis patients reported in the literature

Reference	Age (yr), sex	Origin	Clinical presentation	Treatment[a]	Outcome and follow-up
4	20, male	Malaysia	ND[b]	ND	ND
17	38, male	Thailand	Multiple lung infections	i.v. CAZ, PIP, and TZP with or without aminoglycosides or CIP for 2 wk	Multiple exacerbations, at least 7 yr of documented colonization
18	25, female	Thailand	Acute lung infection	Continuous i.v. CAZ + SXT for 6 wk + oral SXT, DOX, and CHL for 30 wk	Clinical recovery, eradication of *B. pseudomallei*, 1 yr of follow-up
6	9, male	Australia	Acute lung infection and bacteremia	i.v. CAZ + TOB, CAZ + MEM, SXT, and MEM; duration of treatment ND	Clinical recovery after prolonged therapy, development of resistance to multiple drugs
6	7.5, female	Australia[c]	ND	Oral DOX	Clinically healthy, *B. pseudomallei* in sputum for a time, follow-up ND
6	10, male	Australia	Colonization, mild infection	i.v. CAZ + SXT for 2 wk + SXT for 3 mo	Healthy, eradication of *B. pseudomallei*, 18 mo of follow-up
6	38, male	Australia	Acute lung infection	i.v. CAZ + TOB, duration ND	*B. pseudomallei* recovered 3 mo after completion of therapy
14	23, male	Australia	Pulmonary exacerbations; severe respiratory infection	Oral SXT + AMC; i.v. CAZ + MEM + oral SXT with or without oral TET and CIP for 8 wk	Death, persistent recovery of *B. pseudomallei* from sputum, follow-up ND
12	36, male	Australia	Frequent pulmonary exacerbations	i.v. CAZ, MEM, and oral SXT either alone or in combination; oral TET or SXT between exacerbations	Progressive deterioration, persistent recovery of *B. pseudomallei* from sputum, follow-up ND
14	15, male	Australia	Asymptomatic	None	Follow-up ND
14	24, female	Australia[c]	Two severe exacerbations	i.v. CAZ + MEM + TOB for 3 wk	Clinically successful treatments, microbiologic outcome ND
1	17, male	Malaysia	Chronic worsening respiratory symptoms and deteriorating lung function	i.v. SXT + CAZ for 2 mo followed by oral DOX + SXT for 4 mo	Lung function recovery, negative sputum cultures, 5 mo of follow-up

[a] i.v., intravenous; AMC, amoxicillin-clavulanic acid; CAZ, ceftazidime; CIP, ciprofloxacin; DOX, doxycycline; MEM, meropenem.
[b] ND, not described.
[c] Subtropical region.

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