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MALDI-TOF MS contribution to diagnosis of melioidosis in a nonendemic country in three French travellers

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Abstract

Melioidosis is an endemic disease in Southeast Asia and northern Australia. An increasing number of cases are being reported in nonendemic countries, making the diagnosis less obvious. We discuss the identification of *Burkholderia pseudomallei* using matrix-assisted desorption ionization–time of flight mass spectrometry on the occasion of recent cases of imported melioidosis in French travellers.

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Introduction

Burkholderia pseudomallei is the causative agent of melioidosis. It is common in Southeast Asia and northern Australia. In Thailand, 20% of all community-acquired cases of septicaemia are caused by *B. pseudomallei*, with 40% mortality. A striking rise in cases of melioidosis was observed after the December 2004 tsunami, and the disease was also more common after floods in Malaysia in 2015 (http://www.promedmail.org/direct.php?id=3218685). An augmentation of imported cases in Western countries may be strongly correlated with increased international travel (http://mkt.unwto.org/barometer) [1]. Early diagnosis of melioidosis is important considering the high mortality observed without adequate antibiotic therapy [2]. Because empirical antibiotic regimens for community-acquired pneumonia in nonendemic countries may not provide adequate treatment for melioidosis [3,4], effective tools are needed to rapidly diagnose melioidosis.

Patients and Methods

Melioidosis was diagnosed in three French travellers in December 2013 as well as in April and August 2015 at Hôpital Avicenne, Bobigny, France. Patient characteristics are listed in Table 1. Their management and outcome were strongly related to the efficiency of the diagnosis.

The first patient was admitted to the emergency department with a 3-week history of community-acquired pneumonia with treatment failure. Two days after admission, marked acute respiratory distress and multiple organ failure occurred, leading to the patient's transfer to the intensive care unit for respiratory assistance and extracorporeal circulation. Concomitant with the aggravation one blood culture drawn at admission was positive Gram-negative bacilli on Gram stain. Subculture yielded Gram-negative, oxidase-positive bacilli which could not be identified using a biochemical test strip (API20E; bioMérieux, Marcy l'Étoile, France). Using molecular biology (partial sequencing of the *16S rRNA* gene), *B. pseudomallei* was identified within 2 days. Susceptibility testing indicated a regular antimicrobial resistance pattern (Table 2).

Patient 2 was admitted shortly after his return from Borneo. At this stage, he received no antibiotics. On day 3 of hospitalization, an aerobic blood culture drawn at admission yielded Gram-negative bacilli. Rapid identification using matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS; Microflex LT; Bruker Daltonics, Leipzig, Germany) was performed directly on the pellet of the blood culture [5]. Within less than 1 hour, the analysis yielded *Burkholderia thailandensis* with a log score value of 1.83 (using the standard Bruker v4.0 database). Querying the security-relevant (SR) library, which includes bioterrorism agents (Bruker), revealed *B. pseudomallei*, with a log score value of >2.0. The identification was confirmed 5 days later by molecular biology (targeting genes *BpSCU2* and *orf11* [6]). Biochemical

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TABLE 1. Patient characteristics

Patient No.	Gender (age, years)	Clinical presentation (date)	Visited country	Risk factor	Medical imaging	Laboratory results biology	Treatment (duration)	Outcome
1	M (37)	Fever and mild cough, rapid evolution to acute respiratory distress, multiple organ failure (December 2013)	Thailand (Kho Phangan)	None; tattoo?	Chest x-ray revealed cavity-like lesion in upper lobe of right lung compatible with tuberculosis	• Leukocytes: 22.4 × 10^9 L (81% neutrophils) • HBV, HCV, HIV serologies: negative • Malaria test: negative • PCT: 0.98 µg/L	• Amoxicillin (7 days) • Amoxicillin-clavulanic acid (7 extra days) • Piperacillin-tazobactam + amikacin • Intravenous ceftazidime + cotrimoxazole (14 days) • Maintenance oral cotrimoxazole (6 months)	After several weeks, acute renal failure persisted along with reduced respiratory capacity. To date, no relapse observed.
2	M (36)	Fever, chills, alteration of general state (April 2015)	Malaysia (Borneo)	None; flood?	Computed tomography chest scan revealed upper left lobe condensation	• Leukocytes: 6.8 × 10^9 L (82% neutrophils) • HBV, HCV, HIV serologies: negative • Malaria test: negative • PCT: 6.8 µg/L	• Intravenous ceftazidime and oral cotrimoxazole (10 days) • Maintenance oral cotrimoxazole (3 months)	Favorable clinical outcome was observed rapidly despite diagnosis of secondary prostatic abscess. To date, no relapse observed.
3	F (58)	Fever, chills, acute respiratory distress, diarrhoea, vomiting (August 2015)	Cambodia	Diabetes mellitus	Chest x-ray revealed alveolar condensation lesions in lower lobe of her left lung	• Leukocytes: 19.6 G/L (88% neutrophils) • HIV serology: negative • PCT: 28.9 µg/L	• Intravenous ceftriaxone for 24 hours; after bacterial identification, intravenous ceftazidime and oral cotrimoxazole (21 days) • Maintenance oral amoxicillin–clavulanic acid (3 months)	Clinical course was rapidly favorable. Sepsis and urinary tract infection were observed 5 months after.

HBV, hepatitis B virus; HCV, hepatitis C virus; PCT, procalcitonin.

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TABLE 2. Antimicrobial susceptibility (disk diffusion test) of three *Burkholderia pseudomallei* strains

Strain	AMC	CAZ	IMP	TS	Gm	DX	CL
1	S	S	S	S	R	S	NT
2	S	S	S	S	S	S	NT
3	S	S	S	S	R	S	S

AMC, amoxicillin–clavulanic acid; CAZ, ceftazidime; CL, chloramphenicol; DX, doxycycline; Gm, gentamicin; IMP, imipenem; NT, not tested; R, resistant; S, sensitive; TS, trimethoprim–sulfamethoxazole (cotrimoxazole).

characterization (API20NE; bioMérieux) yielded *Chromobacterium violaceum*, a frequent misidentification [7]. Antimicrobial susceptibility testing by disk diffusion revealed susceptibility to compounds used in conventional treatment but an abnormal sensitivity to aminoglycosides (e.g. gentamicin) (Table 2).

Patient 3 was originally a native of Cambodia; she has resided in France for 30 years but frequently returns to her home country. Culture of aerobic blood drawn at admission yielded Gram-negative bacilli. As in patient 2, the bacilli were identified within less than an hour with MALDI-TOF MS as *B. pseudomallei*, with a log score value >2.0 using the SR library. Antimicrobial susceptibility was identical to that of the isolate of patient 1. A relapse was observed 5 months later despite good therapy compliance.

Discussion

Underlying predisposing conditions such as diabetes mellitus have been widely described (e.g. patient 3). Patients 1 and 2 had no evident risk factors, but in patient 1, skin lesions related to a tattoo may have constituted a source of exposure [2]. There was a difference in the clinical presentation of the cases: patient 1 had a history of more than 2 weeks of inappropriate therapy, which may have led to body-wide dissemination of the pathogen, possibly explaining the severity of the clinical evolution.

Culture is the reference standard for the microbiologic diagnosis of melioidosis. *B. pseudomallei* grows easily on standard media within 24 to 48 hours. Culture reading may be facilitated by using selective media, e.g. Ashdown medium, which is not widely used in nonendemic countries. Rapid diagnostic tools are also available in endemic countries, based on immunofluorescence, monoclonal antibodies or latex agglutination. In nonendemic countries, difficulties in identification stem from the lack of familiarity with the cultural characteristics and the nonuse of effective and specific diagnostic facilities. Above all, *B. pseudomallei* is usually misidentified as a culture contaminant when standard identification techniques are used (e.g. API20NE, automated Vitek) [7]. Moreover, many Malaysian strains show quite unusual biochemical characteristics and susceptibility to gentamicin, which may be misleading [7].

In the cases reported here, the microbiologic diagnosis was made in two ways. In the first case, identification of *B. pseudomallei* by phenotypic and molecular approaches took at least 4 days after the blood culture turned positive. In the two other cases, identification with MALDI-TOF MS gave a result the same day the blood culture turned positive.

MALDI-TOF MS has been shown to be an accurate and rapid procedure for identification of *B. pseudomallei* if the appropriate database is used. Because this pathogen is not represented in the Main Spectra Bruker Biotyper database, the response obtained with this database is *B. thailandensis*, with average log score values reflecting the closeness of the two species and shared peaks in their mass spectra [8]. Identification of *Burkholderia* species at the genus level using the standard Bruker database should alert the microbiologist and trigger further specific characterization. Speed in the detection of *B. pseudomallei* in blood cultures allowed us to set up effective antibiotic therapy and protective measures without delay for laboratory workers. Indeed, precautions must be taken in manipulating this pathogen, as it is considered to be a bioterrorism agent. Cultures must be handled under biosafety level 3 laboratory conditions. Incidents of exposure of laboratory workers have been reported [1].

International travel increases the potential spread of infectious diseases, and the number of travellers to endemic areas will inexorably expand. Nonendemic countries will have to face increasing rates of imported cases that must be diagnosed rapidly using efficient procedures. Difficulties in laboratory diagnosis of melioidosis may delay adequate treatment and affect disease outcome. MALDI-TOF MS can be applied successfully to the identification of *B. pseudomallei* if a suitable database is used.

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Conflict of Interest

None declared.

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