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Case Report

Burkholderia pseudomallei: First case of melioidosis in Portugal

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ABSTRACT

Burkholderia pseudomallei is a Gram-negative bacillus and the causative agent of melioidosis, a serious infection associated with high mortality rate in humans. It can be naturally found as an environmental saprophyte in soil or stagnant water, and rice paddies that predominate in regions of endemicity such as Northeast Thailand. *B. pseudomallei* is a Biosafety Level 3 organism due to risks of aerosolization and severe disease and is now included in formal emergency preparedness plans and guidelines issued by various authorities in the United States and Europe. Here, we report the first case of imported melioidosis in Portugal. *B. pseudomallei* was isolated from the patient's blood as well as from a left gluteal abscess pus. The isolate strain showed the unusual resistance profile to first-line eradication therapy trimethroprim/sulfamethoxazole. Whole genome sequencing revealed its similarity with isolates from Southeast Asia, suggesting the Thai origin of this Portuguese isolate, which is in agreement with a recent patient's travel to Thailand.

Introduction

Burkholderia pseudomallei is a Gram-negative bacillus that can be naturally found as an environmental saprophyte in soil or stagnant water [1]. It has been classified as a biothreat agent due to risks of aerosolization, low infectious dose and high mortality rate even with antimicrobial therapy [2,3].

B. pseudomallei is the etiologic agent of melioidosis, a serious infection acquired by ingestion, inhalation or inoculation [4], and is characterized by a wide range of clinical manifestations, including pneumonia and sepsis [3]. According to the recently published [5] estimated burden of melioidosis in 2015, the population at risk, as well as the number of melioidosis cases and deaths are specially relevant in South Asia, East Asia, Pacific and Sub-Saharan Africa but are also important in Latina American, Caribbean, Middle East and North Africa. In fact in 2015 it is estimated at 1525 million people are at risk to acquired melioidosis in South Asia. Of note, Florida in the USA has been predicted to be environmental suitable for B. pseudomallei persistence [5]. To date, no human case or isolation of *B. pseudomallei* have been described in Portugal. Here, we report the first case of imported melioidosis in Portugal and the characterization of the isolated strain.

Case report

In January 2011, a 62-year-old female patient was admitted to the Hospital da Luz, Lisbon, Portugal, complaining of lower back pain and a left lumbar mass for two months with subsequent development of skin lesions and bilateral knee arthritis for two weeks that worsened over four days, with night sweats episodes without quantified fever. The patient had traveled to Thailand on October 2010, during a heavy rain period. One month after arriving home, the patient developed a left sciatic pain resistant to analgesic therapy with a normal lumbar spine CT scan. Three weeks before admission to Hospital da Luz, the patient noticed a left lumbar mass and pustular lesion in the chin. One week later, the patient went to a dermatology consultation, after noticing ulcerated lesions on the outer side of the right arm and leg, and was treated with amoxicillin/clavulanic acid and mupirocin for five

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days, with partial resolution of the ulcerated lesions. Four days before admission, the patient started feeling pain in both knee and ankle joints, with edema and inflammatory signs. With continuing complaints of left sciatic pain and left lumbar palpable mass, the patient went to the hospital Emergency Service (in Hospital Luz) in January 2011. On examination the patient had a left palpable lumbar mass with 15 cm in greater diameter, arthritis of both knees and two skin lesions on the right leg suggestive of erythema nodosum.

Laboratory results showed a mild anemia and elevated inflammatory parameters. Abdominopelvic CT scan showed a left gluteal abscess (14×11 cm) with left ilium bone osteomyelitis and a smaller contralateral gluteal abscess (5 cm). *B. pseudomallei* was isolated from the patient's blood as well as from the left gluteal abscess. Identification was made in Vitek 2 system (bioMérieux) and species identity was subsequently confirmed by a probe-based real time PCR assay [6]. The patient was treated with intravenous meropenem for 14 days, as recommended for treatment of patients with complications during the acute phase [7], and regression of the knee and ankle arthritis and skin lesions.

Considering the finding of resistance of the patient's *B. pseudomallei* strain (here designated as PtBps01) to the first-line eradication-phase therapy trimethroprim/sulfamethoxazole (SXT), which is infrequently described [8–10], the patient was discharged with the recommended [7] alternate oral antimicrobial therapy with amoxicillin/clavulanic acid. Right gluteal abscess was drained after two months of treatment and eradication therapy was maintained for a total period of seven months, until full resolution of the infectious process.

Genome sequencing

To perform the full genome characterization, the strain PtBps01 was subject to whole genome sequencing on a MiSeq Ilumina platform (Illumina Inc., San Diego, CA, USA), and comparative genomics against fully-sequenced *B. pseudomallei* strains available in GenBank representative of several genome groups was performed. Globally, the draft genome of PtBps01 harbors 7,117,230 bp with an average GC-content of 68.2%. Both core- and pan-genome phylogenetic analyses grouped PtBps01 with isolates from Southeast Asia, suggesting the Thai origin of this Portuguese isolate. It appears to be most genetic relatedness to the previously characterized Thai 1106a isolate, exhibiting >99% of similarity in the core genome. PtBps01 accessory genome revealed two putative intact prophages of 40.7Kb (%GC 64.6) and 25.1Kb (%GC 66.0), both comprising 30 predicted CDSs revealing homology with other *Burkholderia* phage sequences. No putative plasmids were found in PtBps01.

The putative genetic basis for the observed unusual STX resistance was also investigated, in particular those involving the BpeEF-OprC efflux pump as it was already shown to confer resistance to STX [11]. However, only three synonymous changes (one in *bpe*E at 1014 bp and two in *bpe*F at 120 bp and 153 bp) in PtBs01 isolate were found, suggesting that antimicrobial resistance is governed by unidentified regulatory mechanism(s).

Full-genome sequence was also used to extract loci sequences for MLST analysis, revealing the allelic profile 1-4-2-3-8-4-3, which corresponds to sequence type (ST) 376. Based on public *B. pseudomallei* MLST databases, no European isolate reported so far displayed such ST profile. The ST376 seems to be restricted to Southeast Asia, as it was previously observed in water samples collected in the 1960s in Thailand [12], as well as in human isolates obtained in 2012 from a Laotian melioidosis patient with suppurative parotitis and two Malay patients with bacteremia.

Conclusion

Altogether, the travel history of the Portuguese infected woman and the genetic data of the isolated strain clearly indicate that this first Portuguese case of melioidosis is an importation from Thailand. Considering that the signs of melioidosis are nonspecific (resembling tuberculosis), this case reinforces the need for an appropriate screening for the disease etiology in people traveling from endemic areas.

Nucleotide sequence accession numbers. The complete genome sequence of *B. pseudomallei* strain (PtBps01) has been assigned the GenBank accession no. JNUQ00000000.

Consent section

Written informed consent was obtained from the patient for publication of this case report. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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