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Case Report: Imported Melioidosis from Goa, India to Israel, 2018

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Abstract. A previously healthy young man presented with a chronic cavitary pulmonary infection that began while in Goa, India. *Burkholderia pseudomallei* was cultured from sputum samples. The infection fully resolved after prolonged antibiotic treatment. Other than traveling during the monsoon season, extensive use of well-water for water-pipe smoking of cannabis was identified as a possible risk factor for infection. This is one of the first reports of travel-associated melioidosis from India. Genomic and immunological characterization suggested that the *B. pseudomallei* isolate collected from the reported case exhibited limited similarity to other *B. pseudomallei* strains.

INTRODUCTION

Melioidosis is caused by *Burkholderia pseudomallei*, a Gram-negative, oxidase-positive, aerobic, soil-dwelling bacillus that is endemic to some areas of Southeast Asia and Northern Australia.[1] Cases and case series were sporadically reported from India,[2–6] but travel-associated cases originating in India are extremely rare.[7,8] Furthermore, despite frequent intercontinental recreational circulation between Israel and endemic areas, melioidosis cases are extremely rare possibly because most of the travelers do not belong to high-risk groups. Several risk factors for developing melioidosis have been described, including diabetes, excessive alcohol consumption, and chronic renal disease. Humans and animals are infected by percutaneous inoculation, inhalation, or ingestion. Disease outbreaks are commonly associated with heavy rain and floods. Person-to-person spread and zoonotic infection are uncommon. Melioidosis covers a wide spectrum of clinical presentations and severities and may affect almost any organ in the body. Pneumonia, the most common clinical presentation of melioidosis, can manifest with acute fulminant sepsis, or a chronic indolent course, mimicking tuberculosis.[1]

PATIENT DESCRIPTION

The patient was a healthy 29-year-old man, who traveled to Goa, a state within the coastal region of Western India, during the months August–October 2017. His entire stay was at the beach area, where he reported episodes of heavy rain (monsoon). He also reported extensive recreational cannabis use using a water pipe that was frequently filled with local well-water. The patient reported fever, chills, night sweats, weight loss of about 5 kg, cough, and hemoptysis that began near the end of his stay in India and persisted for 5 months, until his presentation to the hospital at the end of March 2018. Physical examination did not show any significant findings, and laboratory results included mild leukocytosis, mild microcytic anemia, elevated C-reactive protein (80 mg/L), and hyperglobulinemia. The patient tested negative for HIV. Chest X-RAY and computerized tomography (CT) scan (Figure 1A and B) showed an infiltrate with cavitation on the right upper lung. Abdominopelvic CT scan was normal. Sputum was negative for *Mycobacterium tuberculosis* by polymerase chain reaction (PCR) (GeneXpert Ultra, Cepheid, Sunnyvale, CA) and culture. Direct Gram stain of the sputum showed Gram-negative rods, and culture subsequently yielded a non-fermenting oxidase-positive Gram-negative bacterium that was identified as *B. pseudomallei* by matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) mass spectrometer (VITEK MS, Biomerieux, Marcy-l'Étoile, France) and as *B. thailandensis* by an automated identification system (VITEK XL 2, Biomerieux). Subsequently, the identity of the isolate as *B. pseudomallei* was corroborated by real-time (RT)-PCR using previously described *B. pseudomallei* primers.[9] Blood cultures were sterile. The *B. pseudomallei* isolate is referred as *B. pseudomallei* MAA2018 (acronym of Melioidosis case from Assuta hospital, Ashdod, 2018). Antimicrobial susceptibility testing by Etest (Biomerieux) showed the isolate to be susceptible to trimethoprim/sulfamethoxazole, doxycycline, ceftazidime, imipenem, meropenem, ciprofloxacin, and chloramphenicol, and resistant to amoxicillin/clavulanate, streptomycin, gentamicin, clarithromycin, and rifampicin.

The patient was treated with ceftazidime 2gr qh8 for 14 days, with defervescense after 12 days, followed by oral trimethoprim/sulfamethoxazole for 3 months. At the end of his treatment, the patient fully recovered and had an almost complete resolution of the lesion in the chest CT scan (Figure 1C). Follow-up until 8 months after the end of treatment showed no sign of relapse. Analysis of serum collected from the patient 2.5 months after treatment onset (more than 6 months after disease onset), by a specific anti–*B. pseudomallei* ELISA, showed a high antibody level of more than 1:2,000,000 reciprocal-dilution titers, whereas naive human sera had less than 1:100 titers (see Materials and Methods).

Written consent for publication was given by the patient.

MATERIALS AND METHODS

Strains. *Burkholderia pseudomallei* strains used in this study are as follows: MAA2018, BP1, and BP2. The genomes of all three strains were sequenced by high throughput sequencing using the Illumina MiSeq machine (San Diego, CA), 150 nt paired end, following Nextera library preparation, and analyzed using the SPAdes software (Center for Algorithmic Biotechnology, St. Petersburg, Russia). MAA2018 is extensively detailed in the report. BP1 and BP2 are clinical isolates of *B. pseudomallei* from melioidosis cases previously

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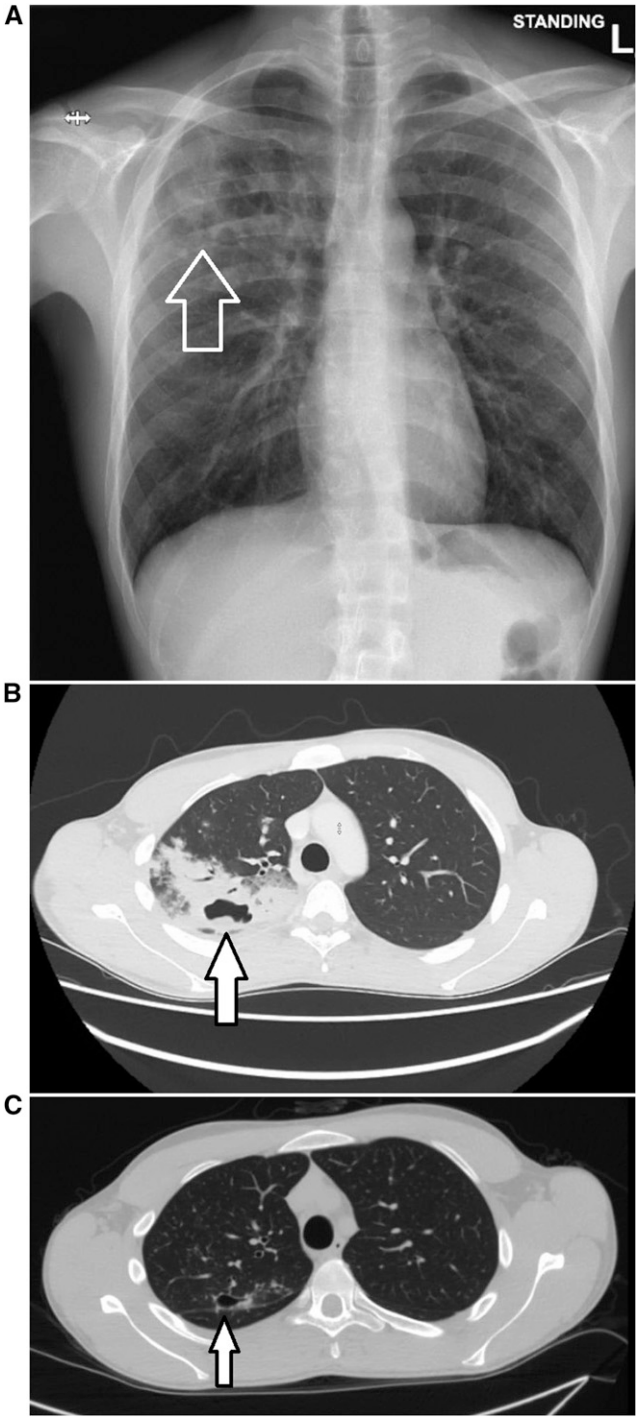

FIGURE 1. (**A**) Chest X-ray showing infiltration and cavitation in the right upper lobe, (**B**) chest CT scan showing cavitary lesion at the right upper lobe, and (**C**) CT scan at the end of treatment showing near resolution of findings. Arrows point major radiographic findings (**A–C**).

diagnosed in Israel. Both strains originated from patients traveling from Thailand. The BP1 clinical isolate[10] exhibits some homology to the 1710b strain (NCBI accession numbers NC_007434 and NC_007435), yet it differs significantly from this strain by 11,259 and 10,865 single nucleotide polymorphisms (SNPs) on chromosome 1 and 2, respectively (ribosomal RNA sequences of the BP1 strain are available in the NCBI database as strain Had_B73694, accession number FJ426359.1). BP2 exhibits homology to the 1106a strain (NCBI accession numbers NC_009076 and NC_009078), yet it differs from this strain by 1,568 and 1,436 SNPs on chromosomes 1 and 2, respectively. To the best of our knowledge, this clinical isolate was not published nor is available in the database. Handling of live *B. pseudomallei* was under BL3 laboratory safety conditions. Handling of inactivated bacterial fractions was under BL2 laboratory safety conditions, following at least 8 days of observation of inoculated LB-agar plates for sterility confirmation.

PCR analysis. Diagnostic RT-PCR analysis was carried out as described.[9] Two sets of primers were used; set 1: cgcaaaactttctggggtagt and cctgatcgctcttcg, set 2: aataaatcat aagaccgacatcacgcacagc and aataaatcataaacgtgaggccgg agatgt.

ELISA for quantitative seroconversion evaluation. MAA2018 cells were grown overnight in Luria broth (LB) to an optical density of 10 OD (equivalent of 5×10^9 CFU/mL), washed extensively in phospate buffer saline (PBS, Biological Industries, Bet Haemek, Israel), concentrated by centrifugation to 5×10^{10} CFU/mL PBS, and heat-inactivated at 80°C for 5 hours with occasional shaking. Heat-inactivated bacteria (10^8 CFU equivalents/mL in carbonate-bicarbonate buffer (Sigma, Israel) were used to coat 96-wells ELISA microtiter plates. Serial serum dilutions were prepared in PBT (PBS supplemented with 2% w/v bovine serum albumin (BSA), 0.5% w/v Tween 20, and 0.5% w/v sodium azide) and incubated for 1 hour in the coated plate at 37°C, washed extensively and developed (1 hour, 37°C) with antihuman alkaline phosphatase-conjugated secondary antibody (Jackson Immunoresearch, West Grove, PA) according to the manufacturer instructions, and quantified in Versamax microplate reader (Molecular Devices, Sunnyvale, NE), following incubation with SigmaFast pNpp (Sigma) for 10 minutes.

Western blot analysis. Bacterial pellets or bacterial secreted proteins collected from *B. pseudomallei* LB cultures (20 hours postinoculation with an overnight starter, at an initial optical density of 0.05 OD) were analyzed by SDS-PAGE and Western blotting. The final concentration of the culture was typically 10 OD (approx. 5×10^9 CFU/mL) in LB media. The equivalent of 2×10^8 CFU (for analysis of the cell-associated fraction) or proteins secreted by 2×10^8 CFU (for analysis of the secreted material) was loaded per gel lane after 10 minutes boiling in SDS-load buffer. Sodium DodecylSulfate-PolyAcrilamide Gel Electrophoresis (SDS-PAGE) was carried out on 4% to 12% NuPage Bis-Tris gels (Invitrogen, Carlsbad, CA) using Precision Plus Molecular weight markers (Bio-Rad, Hercules, CA). Western blots were generated using the Nitrocellulose Western iBlot Gel transfer Semi-dry system (Invitrogen). The membranes were blocked in LiCor blocking buffer (Lincoln, NE) for 1 hour at room temperature and probed with primary antibody overnight at 4°C. The membranes were washed three times for 10 min in PBST (PBS containing 0.05% Tween), probed with secondary antibody for 1 hour at room temperature, and washed twice. The blots were scanned using the LiCor laser-based image detection method, following development with IRDye® 800 CW-conjugated anti-mouse or antihuman. Primary and secondary antibodies were used at 1:1,000 and 1:20,000 dilutions, respectively.

Animal experimentation. BALB/c (20-25 g, Charles River, Kent, UK) or C57BL/6J (20-25 g, Jackson) mice were infected for virulence evaluation. For infection, bacterial cultures were

set in LB media by inoculation with an overnight starter at an initial density of 0.05 × OD units. Bacteria were grown for 3–4 hours at 37°C, to mid-logarithmic phase (approx. 2 × OD units), centrifuged, and resuspended in PBS at the desired concentration (1 × OD unit = 5×10^8 CFU) such that mice were infected intranasally (IN) with 40 µL or subcutaneously (SC) with 100 µL bacterial suspension and serial 10-fold dilutions. A total of five mice per dose were used (range: 10–10^6 for IN infection and 10^3–10^8 for SC infection). The remaining bacterial dose suspensions were plated for total viable counts (CFU mL^{-1}) to confirm the dose administered to the animals. The animals were observed daily for 30 days. The lethal dose required to kill 50% (LD_{50}) of the animals was calculated by nonlinear fit regression using the GraphPad Prism (version 5.0) statistical analysis software (San Diego, CA). Animal experiments were approved by the Israeli Institute for Biological Research (IIBR) committee for animal research, protocol number M-41-18. The experimental animals were handled according to the National Research Council 1996 Guide for the Care and Use of Laboratory Animals.

MICROBIOLOGICAL EVALUATION

Whole genome sequencing revealed that MAA2018 exhibits some similarity to strain 2008724758 in the NCBI database (GenBank accession no. CP018382), a clinical isolate collected in 2010 in California from a case with unknown travel history.[11] Comparison of MAA2018 genome to that of strain 2008724758 evidenced 8708 and 8166 genetic differences in chromosomes 1 and 2, respectively. No similarity could be detected to other *B. pseudomallei* strains in the database. The draft genomic sequence of the isolate is available now in the NCBI database as SLUE01000001–SLUE01000183.[12] The isolate exhibited high pathogenicity in an inhalation murine model of melioidosis and medium pathogenicity by subcutaneous exposure to mice. The LD_{50} by IN exposure was

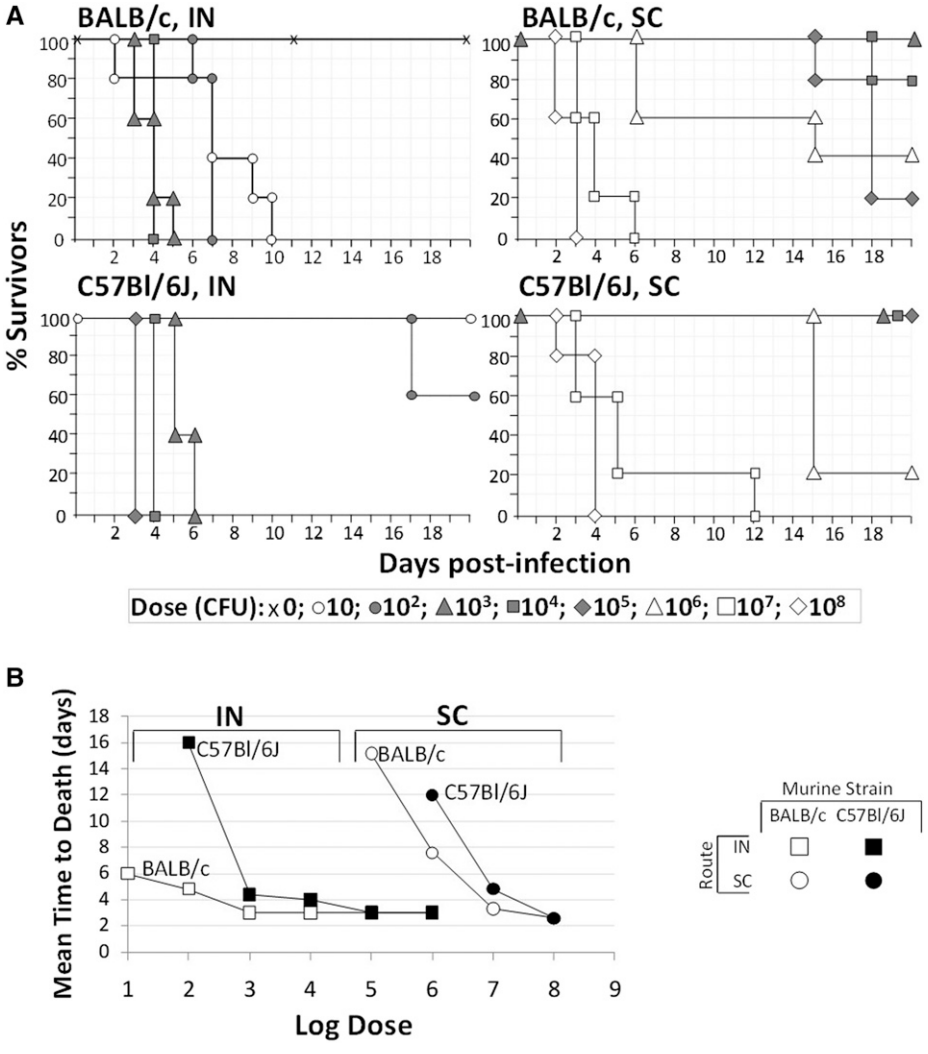

FIGURE 2. (**A**) Kaplan–Meier survival profiles and (**B**) mean time to death (MTD) of mice (BALB/c or C57Bl/6J, as indicated) infected with *Burkholderia pseudomallei* MAA2018 by intranasal (IN) or subcutaneous (SC) exposure. Mice (5 animals/experimental group) were infected with increasing doses of bacteria. The significances of the symbols in (**A**) (increasing doses) and (**B**) (strains of mice and routes of exposure) are detailed in the lower part of (**A**) and the right part of (**B**), respectively. As expected, the virulence of the strain by IN administration is significantly higher than that by SC administration both in terms of a low Lethal Dose 50% and a shorter MTD. Note that the MTD values are significantly higher in C57Bl/6J mice-infected IN with low doses, in line with the notion that this strain of mice represents a better model for the chronic disease.[13]

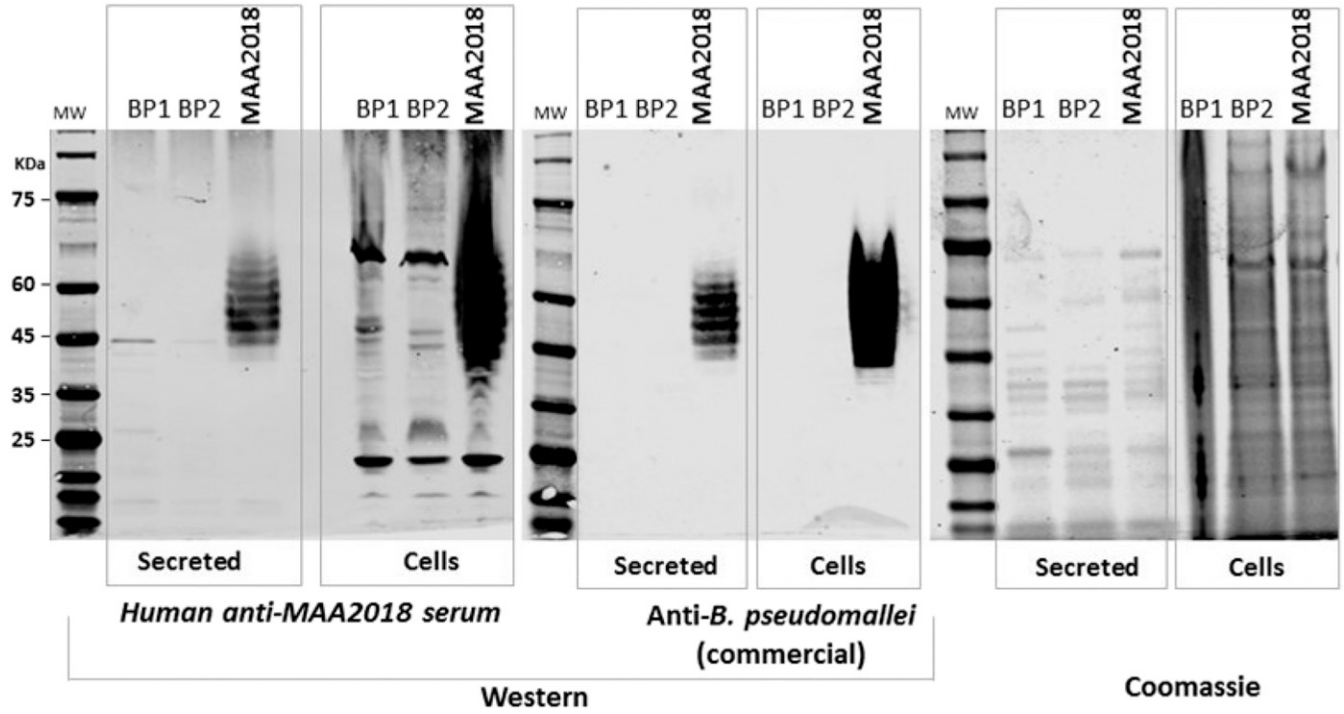

FIGURE 3. Western blot analysis of the *B. pseudomallei* MAA2018 isolate. Cellular and secreted fractions of *B. pseudomallei* MAA2018 isolate as well as of two additional clinical isolates of *B. pseudomallei* (referred to as BP1 and BP2, see "Materials and Methods") were probed with human convalescent sera collected from the melioidosis case documented in this report (left panels) and a murine monoclonal commercial anti–*B. pseudomallei* anti-sera (middle panels, catalog orb 179838, Biorbyt Ltd, Cambridge, UK), as indicated. The total protein stain (marked Coomassie) of the gels is depicted in the right panels.KDa-kilodaltons; MW-molecular weight.

calculated to be as low as 6 CFU and 30 CFU in BALB/c and C57BL/6J mice, respectively. By SC infection, the LD_{50} exhibited by the MAA2018 isolate was 10^6 CFU in both murine strains. The Kaplan–Meier survival profiles and mean time to death data are depicted in Figure 2. These data suggest that MAA2018 belongs to a class of highly virulent *B. pseudomallei* strains.[13] The antigenic specificity of the isolate was evaluated by Western blot analysis using sera collected from the patient, in comparison to the two other isolates, BP1 and BP2: both originated in Thailand (see Materials and Methods). The data depicted in Figure 3 clearly indicate that MAA2018 exhibits little cross-reactivity to the BP1 and BP2 isolates, in line with the notion that *B. pseudomallei* strains isolated from diverse geographical locations are antigenically distinct. This conclusion was substantiated by Western blot analysis using a commercial monoclonal antibody. Based on the signal pattern observed on the Western blots, it is conceivable that the antigenic specificity of the MAA2018 isolate mainly resides in its surface lipopolysaccharide (LPS), such as described by others.[14,15]

DISCUSSION

Although rare, melioidosis should be considered a possible infection in returning travelers with appropriate symptoms. Suspicion should arise in travelers returning from endemic areas such as Southeast Asia and Northern Australia, after exposure to heavy rain and floods and in patients with clinical risk factors such as diabetes mellitus or immunosuppression. Melioidosis is not endemic in Israel and was previously reported in a Thai immigrant and an Eritrean immigrant in Israel.[10,16] Melioidosis was reported sporadically from different states in India, with more than 10 cases each from Karnataka, Tamil Nadu, Telangana, Kerala, Pondicherry, and West Bengal.[2–6] Only five cases were reported from the state of Goa,[6] and only two travel-associated imported cases from India were reported,[7,8] one of which was not definitely acquired in India.[8] The MAA2018 *B. pseudomallei* isolate collected from our case appears to be distinct genetically and antigenically from strains isolated from other cases in Israel originating from Thailand, and from global strains with deposited genotypes. As India is a popular destination for travelers from Israel and other countries, this report serves as a warning sign for the possible acquisition of this disease in India. The presented patient had no medical risk factors for the development of melioidosis. A possible exposure to *B. pseudomallei* could be the use of well-water for water-pipe smoking of cannabis, producing aerosols of water for direct inhalation, supported by the localized nature of the patient's disease. Nevertheless, his travel during the monsoon season could serve as a sufficient exposure. Pitfalls in the diagnosis, as are shown in this report, are the similarity of pulmonary melioidosis to tuberculosis and misidentification of *B. pseudomallei* as other environmental *Burkholderia* spp. by automated systems.

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