# AN IMPORTED CASE OF ACUTE MELIOIDOSIS CAUSED BY ST881 *BURKHOLDERIA PSEUDOMALLEI*

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**Abstract.** A previously healthy Chinese male working in Malaysia returned to China with high fever. A blood culture showed *Burkholderia pseudomallei* strain WCBP1. This isolate was sequenced, showing type, ST881, which appears to be present in Malaysia. WCP1 had unusual susceptibility to aminoglycosides and habored the *Yersinia*-like fimbrial gene cluster for virulence. The patient's condition deteriorated rapidly but he recovered after receiving meropenem and intensive care support. Melioidosis is a potential problem among Chinese imigrant workers with strains new to China being identified.

**Keywords:** *Burkholderia pseudomallei*, melioidosis, MLST

## INTRODUCTION

Melioidosis is a life-threatening disease caused by *Burkholderia pseudomallei*, a gram-negative bacillus found in water and soil (Cheng and Currie, 2005). This disease is endemic in Southeast Asia and northern Australia and has also been found in other regions (Currie *et al*, 2008). In China, melioidosis cases are currently restricted to the south (Guangxi, Guangdong, Hainan, Hong Kong, Macau and Taiwan) (Currie *et al*, 2008); this disease is also suspected to be present in Yunnan (Yang *et al*, 2002). Sichuan, in southwestern China, is not endemic for melioidosis and no cases have been identified here previously. However, Sichuan is a major location in China for exporting labor. Many workers from Sichuan are working overseas in tropical regions and can return with tropical diseases unfamiliar to physicians in their hometowns.

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## MATERIALS AND METHODS

### Case report

A 42-year-old Chinese male working in Malaysia for a dam building project returned to China with a 20 day history of high fever, dyspnea, cyanosis and then loss of consciousness for 8 hours. The fever had its onset while he was still in Malaysia and he returned to China for medical care. On admission, his white blood cell count was $8.14 \times 10^9$/l (97.9% neutrophils), his hemoglobin was 98 g/l and his platelet count was $67 \times 10^9$/l. A computed tomography (CT) scan of the abdomen showed multiple hypodense lesions in his spleen and liver (Fig 1). Blood cultures were obtained on admission and grew non-fermenting gram-negative bacilli.

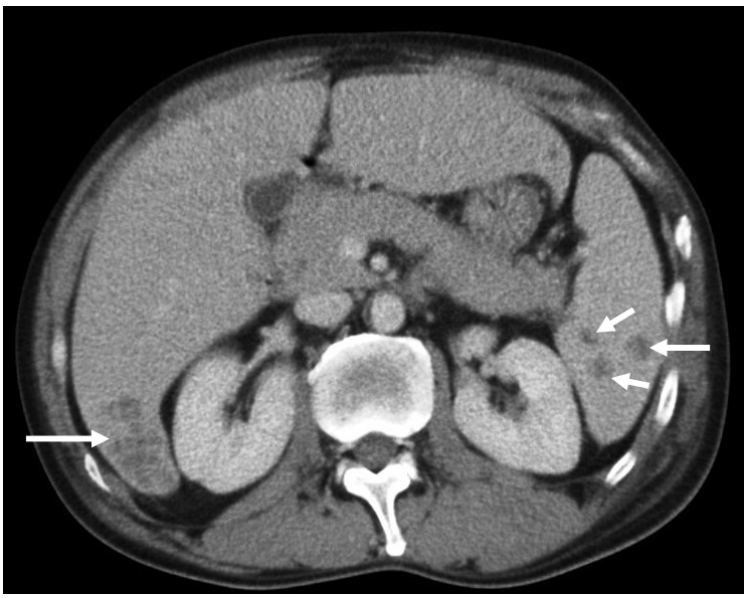

Fig 1–Multiple low-density foci in the liver and spleen shown by arrows seen on computed tomography of the abdomen.

**Strain identification**

Strain identification was performed by partially sequencing the 16S rRNA gene. The *in vitro* susceptibility tests were performed using the Vitek II automated system (BioMérieux, Durham, NC). Multilocus sequence typing (MLST) using 7 housekeeping genes (Godoy *et al*, 2003) was used to investigate whether the WCBP1 was clonally related to *B. pseudomallei* strains found elsewhere.

MLST shows *B. pseudomallei* isolates are diverse genetically (Godoy *et al*, 2003; McCombie *et al*, 2006). Novel sequence types (ST) that can cause invasive disease are identified continuously (McCombie *et al*, 2006). eBURST (version 3, http://eburst.mlst.net/) was used to assign STs to clonal groups and assess their genetic relatedness to the ST in the MLST database. A clonal group was defined as sharing identical alleles in 6 of 7 loci.

**Detection of virulence factors**

*B. pseudomallei* can harbor the *Yersinia*-like fimbrial (YLF) gene cluster or the *B. thailandensis*-like flagellum and chemotaxis (BTFC) gene cluster (Tuanyok *et al*, 2007) for virulence. Both virulence factors were screened for as described previously (Tuanyok *et al*, 2007).

## RESULTS

Melioidosis was clinically suspected based on the finding of multiple disseminated abscesses and the history of working in the Malaysian jungle. Blood cultures obtained on admission grew out *Burkholderia pseudomallei*, which was identified by partially sequencing the 16S rRNA gene (assigned WCBP1 here), confirming acute melioidosis. The patient's condition deteriorated rapidly. He developed septic shock and acute renal failure on the second day of hospitalization and was transferred to the intensive care unit. He gradually improved with meropenem, mechanical ventilation and continuous renal replace therapy. After 3 months hospitalization, receiving oral doxycycline and trimethoprim/sulphamethoxazole for 3 months, he recovered fully and was discharged home.

The isolate was susceptible to ceftazidime, cefepime, imipenem, ampicillin-sulbactam, piperacillin-tazobactam, amikacin, gentamicin, tobramycin, levofloxacin and trimethoprim/sulphamethoxazole and was intermediately susceptible to ciprofloxacin and ceftriaxone and resistant to aztreonam, ampicillin, cefotetan, ceftizoxime and nitrofurantoin.

MLST revealed the isolate belonged to a novel ST, assigned ST881 (*ace-gltB-gmhD-lepA-lipA-narK-ndh* allele profile, 4-1-6-1-8-60-1) by the *B. pseudomallei* MLST database curator. ST881 and ST997 (*ace-gltB-gmhD-lepA-lipA-narK-ndh*, 4-1-13-1-8-60-1) were different at a single locus (*gmhD*) and formed a two-member clonal group. WCBP1 had the YLF cluster but not BTFC.

## DISCUSSION

This case and other reports of imported tropical diseases (Wu and Jiang 2007; Zhang *et al*, 2007) highlight the need to be aware of uncommon imported infectious diseases among Chinese working overseas. This case and another (Cahn *et al*, 2009) show melioidosis can occur among migrant workers and travelers.

The sensitivity of this isolate to aminoglycosides is unusual, since *B. pseudomallei* is generally resistant to gentamicin (Simpson *et al*, 1999) and wild strains susceptible to aminoglycosides are rare (Simpson *et al*, 1999). The mechanism for losing resistance to aminoglycosides is unclear but could be due to the reduced activity of the AmrAB-OprA efflux system as seen *in vitro* (Moore *et al*, 1999). Further studies are needed to elaborate the mechanism for aminoglycoside susceptibility in this isolate.

Following the assigning of WCBP1 to ST881, an additional 36 human isolates of ST881 were reported recovered from Malaysia in 2011 or 2012 have been added to the *B. pseudomallei* MLST database (bpseudomallei.mlst.net) by Bart Currie and Mark Mayo from the Charles Darwin University, Australia. Except for the present imported case, all ST881 isolates have been from Malaysia. This suggests *B. pseudomallei* ST881 has been circulating in Malaysia but has not yet spread to other areas.

ST881 shares four identical alleles with ST72 (*ace-gltB-gmhD-lepA-lipA-narK-ndh*, 14-1-4-1-1-2-1) from humans in Pakistan, ST208 (1-1-6-1-1-4-1) from humans and soil in Thailand, ST321 (4-1-3-4-1-12-1) from an unknown source in Australia, ST704 (1-1-6-1-1-29-1) from a pig in China and ST834 (3-1-6-1-1-29-1) from a human in Cambodia. To better understand the phylogeny of ST881, seven allele sequences were concatenated (3,399 bp in total) and then aligned with those of 81 reference STs (ST1 to ST81) in the *B. pseudomallei* MLST database. The phylogenetic tree was established using the MEGA program (version 4) using the neighbor-joining method. This phylogenetic comparison revealed ST881 is most closely related to ST10 (1-1-13-1-1-1-1) from humans, water and soil in Thailand with 3,395 of the 3,399 bp of the concatenated sequence in common and ST9 (1-1-12-1-1-1-1) found in humans from Thailand, Kenya and Papua New Guinea with 3,394 of 3,399 bp identical. Based on the records of the *B. pseudomallei* MLST database, ST9 was firstly recovered in 1949 and ST10 was firstly found in 1965. It therefore reasonable to hypothesize that ST881 might have evolved from ST9 or ST10.

YLF was found in most isolates from Southeast Asia in one study (Tuanyok *et al*, 2007) and India in another study (Mukhopadhyay *et al*, 2011). It is more likely to be found in clinical isolates. The BTFC was common isolates from Australia (Tuanyok *et al*, 2007). WCBP1 from Southeast Asia origin had the YLF cluster only.

In summary, an imported case of melioidosis caused by *B. pseudomallei* type ST881 was seen in a Chinese migrant worker. *B. pseudomallei* type ST881 may

have originated from other STs found in neighboring countries but appears to be present only in Malaysia thus far.

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