Misidentification of *Burkholderia pseudomallei* as *Burkholderia cepacia* by the VITEK 2 system

Zhiyong Zong,1,2 Xiaohui Wang,1 Yiyun Deng3 and Taoyou Zhou1

1 Center of Infectious Diseases, West China Hospital, Sichuan University, Chengdu, PR China
2 State Key Laboratory of Biotherapy, Sichuan University, Chengdu, PR China
3 Intensive Care Unit, West China Hospital, Sichuan University, Chengdu, PR China

A previously healthy Chinese male returned from working in the Malaysian jungle with a fever. A blood culture grew Gram-negative bacilli that were initially identified as *Burkholderia cepacia* by the VITEK 2 system but were subsequently found to be *Burkholderia pseudomallei* by partial sequencing of the 16S rRNA gene. The identification of *B. pseudomallei* using commercially available automated systems is problematic and clinicians in non-endemic areas should be aware of the possibility of melioidosis in patients with a relevant travel history and blood cultures growing *Burkholderia* spp.

Case report

A previously healthy Chinese male who had been working in the Malaysian jungle returned with a high fever. Blood cultures were taken on admission to hospital and grew non-fermenting Gram-negative rods that were identified as *Burkholderia cepacia* (91% probability) by the VITEK 2 compact semi-automated system using the GN (Gram-negative) card and the Advanced Expert System version VT2-R4.01 (bioMérieux). As melioidosis was suspected based on the presence of disseminated abscesses and the travel history, the 16S rRNA gene of this isolate (designated WCBP1) was amplified with the universal primers 27F and 1492R (Lane, 1991). Amplicons were purified using a commercial kit (Omega) and sequenced on an ABI 3730xl DNA Analyser (Applied Biosystems) at the Beijing Genomics Institute (Beijing, China). Similarity searches of the sequences obtained were carried out against the GenBank, EzTaxon and LeBIBI databases. The 1011 bp partial 16S rRNA gene sequence of WCBP1 was identical to *Burkholderia pseudomallei* strain Had_B73694 (GenBank accession no. FJ426359), which was recovered in a patient from Thailand (Cahn et al., 2009). WCBP1 was therefore actually *B. pseudomallei* and a diagnosis of acute melioidosis was made.

Discussion

Both *B. pseudomallei* and *B. cepacia* can cause life-threatening infections but the two species present different levels of risk to human health. *B. pseudomallei* is a Centers for Disease Control and Prevention category B agent with biological warfare potential that is classified as biosafety level 3 and can cause laboratory-acquired infections in exposed workers, while *B. cepacia* is not classified as a biological threat agent and is classified as biosafety level 2. Rapid and correct identification of both *Burkholderia* species is important for making therapeutic decisions and taking safety measures. However, commercially available automated microbiological systems that are widely used in diagnostic laboratories, VITEK 2, Phoenix (BD) and MicroScan WalkAway 96 (Siemens), do not reliably identify *B. pseudomallei*.

The misidentification of *B. pseudomallei* by VITEK 2 has been described in several reports (Kiratisin et al., 2007; Lowe et al., 2002). The earlier fluorometric-based GN bacterial identification card of the VITEK 2 was only able to correctly identify a minority (19%) of *B. pseudomallei* isolates (Lowe et al., 2002). The later colorimetric-based GN identification card has a better performance for the identification of *B. pseudomallei* but the accuracy is still suboptimal, varying from 63 to 81% (Deepak et al., 2008; Kiratisin et al., 2007; Lowe et al., 2006), depending on the media used for culture (Lowe et al., 2006). *B. pseudomallei* was not in the database of the BD Phoenix System and in a previous study most isolates (34 of 47) were misidentified as *B. cepacia* by this system (Koh et al., 2003). MicroScan WalkAway 96 exhibited 96% accuracy for identifying *B. pseudomallei* (Kiratisin et al., 2007). However, WalkAway 96 is not acceptable for the identification of *B. pseudomallei* as it gave low discrimination for *B. pseudomallei* with only 81.8% probability and had a poor specificity with 57% of *B. cepacia* isolates being incorrectly identified as *B. pseudomallei* (Kiratisin et al., 2007).

The accuracy of the widely used API 20NE manual system for the identification of *B. pseudomallei* varied significantly, from 37 to 99%, in different studies. Combining the results...
of eight published studies (Amornchai et al., 2007; Dance et al., 1989; Deepak et al., 2008; Glass & Popovic, 2005; Inglis et al., 1998, 2005; Kiratsin et al., 2007; Lowe et al., 2002), API 20NE correctly identified 1490 out of 1598 isolates, corresponding to 93% accuracy. However, API 20NE has a longer turnaround time (up to 48 h) than automated systems and some isolates are still not correctly identified by this system, so API 20NE is not also ideal for the identification of *B. pseudomallei*.

Other methods including fluorescence in situ hybridization (FISH), matrix-assisted laser desorption ionization time-of-flight MS technology (Giebel et al., 2010), latex agglutination tests and GLC have been developed to improve the identification of *B. pseudomallei*. The FISH method exhibited 100% sensitivity and 100% specificity in the identification of *B. pseudomallei* in a small-scale study but requires further validation (Hagen et al., 2011). FISH also requires fluorescence microscopy equipment and is not practical for many diagnostic laboratories. The latex agglutination test was highly sensitive (99.5% sensitivity) and specific (100%) in identifying *B. pseudomallei* isolates but requires specific antibodies that are not commonly available in non-endemic areas (Amornchai et al., 2007). GLC analysis of bacterial fatty acid methyl esters correctly identified 98% of *B. pseudomallei* isolates but requires specific equipment (Inglis et al., 2005). Correct identification of *B. pseudomallei* remains a challenge in non-endemic areas, but PCR-based methods appear to be a reliable and practical choice for the identification of *B. pseudomallei*, as described by Kiratsin et al. (2007) and shown here.

In conclusion, the identification of *B. pseudomallei* by commercially available automated systems is problematic. Clinicians and microbiologists should be aware of the possibility of misidentification of *B. pseudomallei* by automated systems. In particular, physicians in regions where melioidosis is not endemic should consider the possibility of melioidosis based on travel history and clinical manifestations when encountering isolates from blood samples that are identified as *B. cepacia*.

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References


