

Reliability of Automated Biochemical Identification of *Burkholderia pseudomallei* Is Regionally Dependent

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Misidentifications of *Burkholderia pseudomallei* as *Burkholderia cepacia* by Vitek 2 have occurred. Multidimensional scaling ordination of biochemical profiles of 217 Malaysian and Australian *B. pseudomallei* isolates found clustering of misidentified *B. pseudomallei* isolates from Malaysian Borneo. Specificity of *B. pseudomallei* identification in Vitek 2 and potentially other automated identification systems is regionally dependent.

Burkholderia pseudomallei is a saprophytic soil bacterium that causes melioidosis, a disease endemic in northern Australia and Southeast Asia affecting humans and animals (1). The clinical presentations of melioidosis range from skin infections without sepsis to disseminated infection with sepsis and high mortality. Pneumonia is present in around half of cases, and chronic infections, relapsed disease, and activation from latency are all recognized (1, 2).

Confirmation of diagnosis of melioidosis requires a positive culture of *B. pseudomallei* from clinical samples such as blood, sputum, urine, pus, joint aspirate, or swabs from throat or rectum (1). *B. pseudomallei* has been identified by combining the commercial API 20NE biochemical kit (bioMérieux) with a simple screening system involving Gram stain, oxidase reaction, typical growth characteristics, and resistance to gentamicin (3). Susceptibility to amoxicillin-clavulanate (AMC) has also been used to differentiate *B. pseudomallei* from *Burkholderia cepacia*, which is resistant to AMC (4). Unfamiliarity with *B. pseudomallei* and problems with inaccurate species identification using some automated commercial biochemical identification systems have resulted in laboratories misidentifying the bacterium as a *Pseudomonas* or other *Burkholderia* species (5–9). Confirmation of *B. pseudomallei* identity by real-time PCR of DNA extracted from cultured bacterial colonies is increasingly the standard for many laboratories (10). Various genetic targets have been published for PCR identification of *B. pseudomallei* from bacterial cultures and also for direct detection from clinical samples, with a recent review showing the type III secretion system (TTS1)-*orf2* assay to be superior in detecting *B. pseudomallei* directly from clinical specimens (11). Apart from molecular methods, *B. pseudomallei* from cultures can also be confirmed by antigen detection assays, such as latex agglutination (12). More recently, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has been adapted to identify cultured bacteria based on protein fingerprint profiles (13).

A particular problem has been the misidentification of *B. pseudomallei* as *Burkholderia cepacia* by the Vitek 2 automated biochemical system (bioMérieux) (5–8). *B. cepacia* belongs to a group of 17 phenotypically and genotypically similar species which form the *B. cepacia* complex, with *B. cepacia* specifically noted as an opportunistic pathogen infecting and causing progressive pulmo-

nary deterioration in patients with cystic fibrosis (14, 15). Other organisms that have been reportedly misidentified by the Vitek 2 system include *Candida albicans* being misidentified as Gram-negative bacilli (16) and *Candida parapsilosis* being misidentified as *Candida famata* (17).

We have compared the Vitek 2 system biochemical profiles of 68 confirmed *B. pseudomallei* clinical strains from hospitals in Sabah and Sarawak, Malaysian Borneo, with 149 *B. pseudomallei* and 18 *B. cepacia* isolates from the Royal Darwin Hospital (RDH) in Northern Territory, Australia. One isolate per patient was analyzed. All isolates were collected between September 2010 and June 2012, except for 17 isolates collected in 1994 from Sabah.

All isolates were subcultured on horse blood agar (HBA) before testing was performed on the Vitek 2 according to the manufacturer's instructions (bioMérieux). The Vitek 2 system utilizes a panel of biochemical and enzymatic tests which results in a biochemical profile that is compared against the manufacturer's bacterial taxa database. All *B. pseudomallei* isolates were confirmed by both real-time PCR targeting the well-validated *B. pseudomallei* TTS1 (10) and by a latex agglutination test (12). Of the isolates from Sarawak, 15/43 (35%) had been initially identified as *B. cepacia* by the Vitek 2 system but were subsequently confirmed as *B. pseudomallei* by both the TTS1 real-time PCR and the latex agglutination test (Table 1). These 15 patients were from hospitals from different regions in Sarawak, none had cystic fibrosis, and melioidosis was suspected clinically, with a diversity of clinical presentations, including subcutaneous infection, community-acquired pneumonia, and sepsis. Only 2/25 *B. pseudomallei* isolates from Sabah and 3/149 *B. pseudomallei* isolates from Darwin were misidentified as *B. cepacia* (Table 1).

Using Primer version 6 (Primer-E Ltd., Plymouth Marine Laboratory, United Kingdom), we performed a nonmetric multidimensional

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