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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 pulmonary 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 multidi-

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TABLE 1 Number of isolates tested with the Vitek 2 system

Sample origin	Total no. of *B. pseudomallei* isolates[a]	Total no. of *B. cepacia* isolates tested	No. of *B. pseudomallei* isolates correctly identified as *B. pseudomallei*[b,e]	No. of isolates with low discrimination[c,e]	No. of *B. pseudomallei* isolates misidentified as *B. cepacia*[d,e]	No. of *B. cepacia* isolates correctly identified[e]
Sabah, Malaysian Borneo	25	Not done	22 (88)	1 (4)	2 (8)	Not done
Sarawak, Malaysian Borneo	43	Not done	23 (53)	5 (12)	15 (35)	Not done
Darwin, Australia	149	18	146 (98)	0	3 (2)	18 (100)

[a] Positive by TTS1 and agglutination tested.
[b] With a 90 to 99% probability of being *B. pseudomallei*.
[c] Low discrimination between *B. cepacia* and *B. pseudomallei*.
[d] With a 90 to 99% probability of being *B. cepacia*.
[e] Numbers in parentheses refer to the percentages of total isolates of the same state/country origin.

mensional scaling (nMDS) ordination on the Euclidean distance resemblance matrix of the Vitek 2 biochemical profiles of these 235 isolates. The nMDS (stress value of 0.19) showed a distinct clustering of the 15 *B. pseudomallei* isolates from Sarawak that were misidentified as *B. cepacia* (Fig. 1A). The nMDS ordination also revealed a tight clustering of the correctly identified *B. pseudomallei* isolates regardless of country of origin, while the *B. cepacia* isolates were more diverse (Fig. 1A and C). A permutation-based, nonparametric analysis of similarities (ANOSIM) confirmed this finding, with strong evidence that the biochemical profiles of the misidentified *B. pseudomallei* isolates were distinct from correctly identified *B. pseudomallei* (*R* statistic of 0.345, $P < 0.001$).

An analysis of similarity percentages (SIMPER) calculating the average contribution of each biochemical test to the overall observed dissimilarity between clusters revealed that, in particular, two enzymatic tests, the β-*N*-acetyl-glucosaminidase (BNAG) and β-*N*-acetyl-galactosaminidase (NAGA), which hydrolyze polysaccharides, were distinct between correctly and misidentified *B. pseudomallei* isolates. A total of 88% of correctly identified *B. pseudomallei* isolates contained BNAG substrates resulting in a positive test as opposed to 13% of misidentified isolates. This is

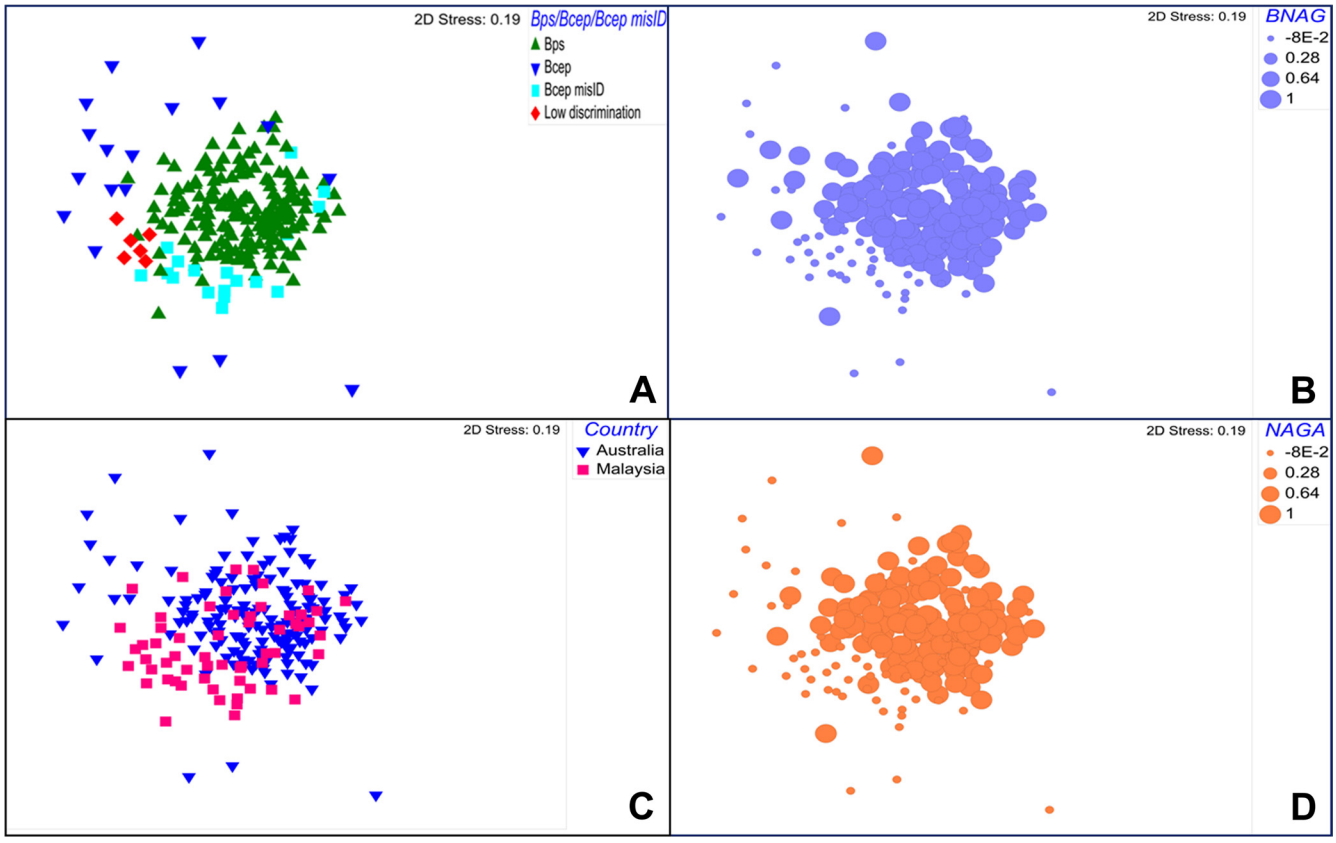

FIG 1 Nonmetric multidimensional scaling (nMDS) ordination on the Euclidean distance resemblance matrix of the Vitek 2 biochemical profile of 235 *B. pseudomallei* and *B. cepacia* isolates from Australia and Malaysian Borneo. (A) Samples were identified as either *B. pseudomallei*, *B. cepacia*, *B. pseudomallei* misidentified as *B. cepacia*, or isolates with low discrimination; (B) the bubble size reflects the presence (large) or absence (small) of BNAG substrate in an isolate; (C) analysis based on isolates from both countries, Australia and Malaysia; (D) the bubble size reflects the presence (large) or absence (small) of NAGA substrate in an isolate. Abbreviations: Bps, *B. pseudomallei*; Bcep, *B. cepacia*; Bcep misID, *B. pseudomallei* misidentified as *B. cepacia*; BNAG, β-*N*-acetyl-glucosaminidase; NAGA, β-*N*-acetyl-galactosaminidase.

also evident in Fig. 1B and D. The exopolysaccharide (EPS) poly-β-(1-6)-*N*-acetylglucosamine (PNAG) is a substrate of the enzyme BNAG and is produced by *Burkholderia* spp. (18). PNAG has been reported to be an important component in biofilm formation in *Burkholderia* species, potentially contributing to multidrug resistance (18). *N*-Acetylgalactosamine, a derivative of NAGA, has also been documented as one of the basic components for EPS of *B. pseudomallei* (19). The implications for virulence and immune response of these different biochemical profiles remains uncertain, but it has been suggested that the amount of capsular polysaccharide in *B. pseudomallei* compared to that in other *Burkholderia* species may well contribute to its relative virulence (20).

As an environmental bacterium adapted to a diverse range of tropical and subtropical habitats globally, *B. pseudomallei* is known to harbor a vast intraspecies genomic diversity as a result of high recombination frequency (21). It is therefore not surprising that the biochemical database of the Vitek 2 system performs variably based on geographical location. That there was 98% accuracy for the recent Australian strains tested in this study shows substantial improvement since prior studies (5, 6). The Sarawak data are supported by the recent report from China of the same misidentification in a case of melioidosis imported from Malaysia (8).

In conclusion, clinicians and laboratory scientists need to be aware of continuing potential misidentification of *B. pseudomallei* as *B. cepacia* by the Vitek 2 automated biochemical identification system, especially in patients with suspected melioidosis acquired in exotic locations, such as Malaysian Borneo. Similar difficulties are likely to be encountered with other automated identification systems, such as MALDI-TOF MS, as they are increasingly developed and utilized for patients infected in diverse geographical locations. PCR using validated targets (11) and ultimately whole-genome sequencing can confirm correct identification of species. Alternatively, for laboratories with limited resources, a combination of latex agglutination and AMC susceptibility testing assists in distinguishing *B. pseudomallei* from *B. cepacia* (4).

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