Reliability of automated biochemical identification of Burkholderia pseudomallei is regionally dependent (Short-Form Paper - Journal of Clinical Microbiology)

Running Title: Malaysian B. pseudomallei misidentification by VITEK 2

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Abstract

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.

Manuscript

*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 recognised (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-clavunate (AMC) has also been used to differentiate *B.

*pseudomallei* from *B. cepacia* which is resistant to AMC (4). Unfamiliarity with *B.

*pseudomallei* and problems with inaccurate speciation using some automated commercial biochemical identification systems have resulted in laboratories misidentifying the bacterium as a *Pseudomonas* or other *Burkholderia* species (5,6,7,8,9). Confirmation of *B. pseudomallei* identity by real-time polymerase chain reaction (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 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) (5,6,7,8). *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 *C. parapsilosis* being misidentified as *C. 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 the 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 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* Type III Secretion System (TTS1) (10) and by 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 from Sabah and 3/149 *B. pseudomallei* from Darwin were misidentified as *B. cepacia* (Table 1).

Using Primer v6 (Primer-E Ltd, Plymouth Marine Laboratory, United Kingdom), we performed a nonmetric multidimensional scaling (nMDS) ordination on the Euclidean distance resemblance matrix of the VITEK 2 biochemical profiles of these 235 isolates. The nMDS (stress value 0.19) showed a distinct clustering of the 15 *B. pseudomallei* isolates from Sarawak that were misidentified as *B. cepacia* (Figure 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 (Figure 1A, 1C). 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 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. 88% of correctly identified *B. pseudomallei* isolates contained BNAG substrates resulting in a positive test as opposed to 13% of misidentified isolates. This is also evident in Figures 1B and 1D. The exopolysaccharide (EPS) poly-β-(1-6)-N-acetyl-glucosamine (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-acetylglactosamine 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* when compared to 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 harbour a vast intra-species 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 speciation. Alternatively, for laboratories with limited resources a combination of latex agglutination and AMC susceptibility testing assists in distinguishing *B. pseudomallei* from *B. cepacia* (4).

**Acknowledgments**

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**References**


Table 1. Number of isolates tested with VITEK 2 system

<table>
<thead>
<tr>
<th>Sample origin</th>
<th>Total no. B. pseudomallei isolates§</th>
<th>Total no. B. cepacia isolates tested</th>
<th>No. of B. pseudomallei isolates correctly identified as B. pseudomallei¶</th>
<th>No. of isolates with low discrimination*</th>
<th>No. of B. pseudomallei isolates misidentified as B. cepacia**</th>
<th>No. of B. cepacia isolates correctly identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabah, Malaysian Borneo</td>
<td>25 not done</td>
<td>22 (88%)</td>
<td>1 (4%)</td>
<td>2 (8%)</td>
<td>not done</td>
<td></td>
</tr>
<tr>
<td>Sarawak, Malaysian Borneo</td>
<td>43 not done</td>
<td>23 (53%)</td>
<td>5 (12%)</td>
<td>15 (35%)</td>
<td>not done</td>
<td></td>
</tr>
<tr>
<td>Darwin, Australia</td>
<td>149 18</td>
<td>146 (98%)</td>
<td>0</td>
<td>3 (2%)</td>
<td>18 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

§ Positive by TTS1 and agglutination tested
¶ 90-99% probability B. pseudomallei
* Low discrimination between B. cepacia and B. pseudomallei
** 90-99% probability B. cepacia
(%) refers to % of total isolates of the same state/country origin
Figure 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, N-acetylgalactosamine.