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 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.
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 \( B. \text{pseudomallei} \) isolates from Sarawak that were misidentified as \( B. \text{cepacia} \) (Fig. 1A). The nMDS ordination also revealed a tight clustering of the correctly identified \( B. \text{pseudomallei} \) isolates regardless of country of origin, while the \( B. \text{cepacia} \) 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 \( B. \text{pseudomallei} \) isolates were distinct from correctly identified \( B. \text{pseudomallei} \) (\( R \) 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 \( \beta\)-N-acetyl-glucosaminidase (BNAG) and \( \beta\)-N-acetyl-galactosaminidase (NAGA), which hydrolyze polysaccharides, were distinct between correctly and misidentified \( B. \text{pseudomallei} \) isolates. A total of 88% of correctly identified \( B. \text{pseudomallei} \) isolates contained BNAG substrates resulting in a positive test as opposed to 13% of misidentified isolates.

### TABLE 1 Number of isolates tested with the Vitek 2 system

<table>
<thead>
<tr>
<th>Sample origin</th>
<th>Total no. of ( B. \text{pseudomallei} ) isolates(^a)</th>
<th>Total no. of ( B. \text{cepacia} ) isolates tested</th>
<th>No. of ( B. \text{pseudomallei} ) isolates correctly identified as ( B. \text{pseudomallei} )(^b)</th>
<th>No. of isolates with low discrimination(^c)</th>
<th>No. of ( B. \text{pseudomallei} ) isolates misidentified as ( B. \text{cepacia} )(^d)</th>
<th>No. of ( B. \text{cepacia} ) isolates correctly identified(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabah, Malaysian Borneo</td>
<td>25</td>
<td>Not done</td>
<td>22 (88)</td>
<td>1 (4)</td>
<td>2 (8)</td>
<td>Not done</td>
</tr>
<tr>
<td>Sarawak, Malaysian Borneo</td>
<td>43</td>
<td>Not done</td>
<td>23 (53)</td>
<td>5 (12)</td>
<td>15 (35)</td>
<td>Not done</td>
</tr>
<tr>
<td>Darwin, Australia</td>
<td>149</td>
<td>18</td>
<td>146 (98)</td>
<td>0</td>
<td>3 (2)</td>
<td>18 (100)</td>
</tr>
</tbody>
</table>

\(^a\) Positive by TTS1 and agglutination tested.

\(^b\) With a 90 to 99% probability of being \( B. \text{pseudomallei} \).

\(^c\) Low discrimination between \( B. \text{cepacia} \) and \( B. \text{pseudomallei} \).

\(^d\) With a 90 to 99% probability of being \( B. \text{cepacia} \).

\(^e\) Numbers in parentheses refer to the percentages of total isolates of the same state/country origin.

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For Table 1, the number of isolates tested with the Vitek 2 system from different origins are listed, including the total number of each species, the number correctly identified, and those with low discrimination or misidentified.

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**FIG 1** Nonmetric multidimensional scaling (nMDS) ordination on the Euclidean distance resemblance matrix of the Vitek 2 biochemical profile of 235 \( B. \text{pseudomallei} \) and \( B. \text{cepacia} \) isolates from Australia and Malaysian Borneo. (A) Samples were identified as either \( B. \text{pseudomallei} \), \( B. \text{cepacia} \), \( B. \text{pseudomallei} \) misidentified as \( B. \text{cepacia} \), 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, \( B. \text{pseudomallei} \); Bcep, \( B. \text{cepacia} \); Bcep misID, \( B. \text{pseudomallei} \) misidentified as \( B. \text{cepacia} \); BNAG, \( \beta\)-\( N\)-acetyl-glucosaminidase; NAGA, \( \beta\)-\( N\)-acetyl-galactosaminidase.
also evident in Fig. 1B and D. The exopolysaccharide (EPS) poly-
\( \beta -(1-6)-N \)-acetylgalactosamine (PNG) is a substrate of the en-
zyme BNAG and is produced by *Burkholderia* spp. (18). PNG has
been reported to be an important component in biofilm forma-
tion 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 uncer-
tain, but it has been suggested that the amount of capsular poly-
saccharide in *B. pseudomallei* compared to that in other *Burkhold-
eria* 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 vari-
ably based on geographical location. That there was 98% accuracy
for the recent Australian strains tested in this study shows substan-
tial improvement since prior studies (5, 6). The Sarawak data are
supported by the recent report from China of the same misiden-
tification 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 devel-
oped and utilized for patients infected in diverse geographical lo-
cations. PCR using validated targets (11) and ultimately whole-
genome sequencing can confirm correct identification of species.
Alternatively, for laboratories with limited resources, a combina-
tion of latex agglutination and AMC susceptibility testing assists in
distinguishing *B. pseudomallei* from *B. cepacia* (4).

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**REFERENCES**

367:1035–1044.
2007.)
of *Pseudomonas pseudomallei* in clinical practice: use of simple screening
parison of routine bench and molecular diagnostic methods in identifica-
nonautomated systems for identification of *Burkholderia pseudomallei*. J.
media to evaluate the new VITEK 2 colorimetric GN card for identifica-
2003. Automated identification systems and *Burkholderia pseudomallei*.
eria pseudomallei* as *Burkholderia cepacia* by the VITEK 2 system. J. Med.
Microbiol. 61:1483–1484.
*Burkholderia pseudomallei* misidentified by automated system. Emerg.
2006. Development and evaluation of a real-time PCR assay targeting the
44:85–90.
Mayo M, Kelley E, Seymour ML, Sarovich DS, Pearson T, Engelhaler
DM, Wagner DM, Keim PS, Schupp JM, Currie BJ. 2012. Comparison of
TaqMan PCR assays for detection of the melioidosis agent *Burkholderia
Sirisinha S. 2000. Monoclonal antibody-based rapid identifica-
tion of *Burkholderia pseudomallei* in blood culture fluid from patients
Performances of the Vitek MS matrix-assisted laser desorption ioniza-
tion-time of flight mass spectrometry system for rapid identification of bacteria
14. Coenye T, Vandamme P, LiPuma JJ, Govan JR, Mahenthiralingam E.
2003. Updated version of the *Burkholderia cepacia* complex experimental
Microbiol. 34:87–95.
Gram-negative bacilli in the clinical laboratory. Scand. J. Infect. Dis. 39:
907–910.
silosis* as *C. famata* in a clinical case of vertebral osteomyelitis. Am. J. Med.
18. Yakandawala N, Gawande PV, LoVetri K, Cardona ST, Romeo T, Nitz
M, Madhyastha S. 2011. Characterization of the poly-\( \beta -(1-6)-N \)-
acetylgalactosamine polysaccharide component of *Burkholderia* biofilms.
capsular polysaccharide of *Burkholderia* (*Pseudomonas*) *pseudomallei*
polysaccharide of *Burkholderia* (*Pseudomonas*) *pseudomallei*. J.