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Comparison of Automated and Nonautomated Systems for Identification of *Burkholderia pseudomallei*

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The identification of *Burkholderia pseudomallei*, the causative agent of melioidosis, is usually not difficult in laboratories in areas where it is endemic. With the increase in international travel and the threat of bioterrorism, it has become more likely that laboratories in areas where it is not endemic could encounter this organism. The increase in the use of and dependence upon automated identification systems makes accurate identification of uncommonly encountered organisms such as *B. pseudomallei* critically important. This study compares the manual API 20NE and 20E identification systems with the automated Vitek 1 and 2 systems. A total of 103 *B. pseudomallei* isolates were tested and correctly identified in 98%, 99%, 99%, and 19% of cases, respectively. The failure of the Vitek 2 to correctly identify *B. pseudomallei* was largely due to differences in the biochemical reactions achieved compared to expected values in the database. It is suggested that this deficiency in the Vitek 2 may be due to the large number of uncertain results reported for these isolates. These results reduce the discriminating ability of the instrument to distinguish between uncommonly encountered isolates such as those of *B. pseudomallei*.

Burkholderia pseudomallei, the causative agent of melioidosis, is endemic in regions of southeast Asia and northern Australia (8, 10, 13). Within Australia, disease is particularly prevalent in north Queensland and the Northern Territory, with peaks of disease usually associated with high levels of rainfall in the wet season. (2, 11, 14, 15). Three forms of the disease are recognized, acute, subacute, and chronic. Infection occurs via ingestion, inoculation, or inhalation. Acute melioidosis commonly presents as a fulminant septicemia, often resulting in death within a few days of exposure. Mortality rates for acute septicemic melioidosis remain high despite antibiotic therapy (6, 18; J. Warner, D. Learoyd, B. Pelowa, J. Koehler, and R. G. Hirst, Abstr. Ann. Sci. Meet. Med. Soc. Papua New Guinea, p. 38, 1998). Chronic infection often remains asymptomatic and may persist for years (5, 13, 14). Reactivation of chronic infection to an acute form can occur when the host is immunocompromised (13, 14, 16).

The laboratory diagnosis of melioidosis is best made with a culture of the appropriate clinical material. Serology has a role in the diagnosis of this condition, particularly in patients from areas where it is not endemic who travel to an area where it is endemic and develop a suggestive clinical condition. Serum samples from patients resident in areas where it is endemic can be positive in the absence of clinical disease. This would represent previous exposure and does not necessarily imply current disease. Other diagnostic methods that have been used include latex agglutination for detection of antigen in urine (17) and molecular detection (3).

With the increase in international travel, melioidosis is increasingly likely to show up in regions where it is not normally endemic (7). For the routine diagnostic laboratory, however, limited experience with this organism may result in misidentification. One previous publication has reported the use of the API 20NE and 20E (bioMerieux, Paris, France) in the identification of *B. pseudomallei* and compared it with the use of simple screening tests in areas where it is endemic (9). The authors advocated the use of the API 20NE in areas where it is not endemic for the identification of *B. pseudomallei*. The advent of automated methods of bacterial identification has led to a replacement of these manual methods by systems such as Vitek 1 and Vitek 2 (bioMerieux). The ability of these systems to identify *B. pseudomallei* has not been reported previously. The possible misidentification of *B. pseudomallei* as *Burkholderia cepacia* has been described previously (9). The ability of automated methods to reliably distinguish between these organisms becomes particularly critical in laboratories not familiar with *B. pseudomallei*.

This study compares the ability of two automated systems, Vitek 1 and Vitek 2, with both the API 20NE and 20E to identify *B. pseudomallei*.

MATERIALS AND METHODS

Organisms. One hundred and three clinical isolates of *B. pseudomallei* were subcultured onto Columbia horse blood agar from storage at −70°C. These isolates were obtained from clinical cases of melioidosis, of which 64 were bacteremic. Included among these isolates were the NCTC 13178 and NCTC 13179 type strains of *B. pseudomallei*. All these isolates had been previously identified with the API 20NE system (bioMerieux).

API 20NE and 20E identification. Fresh subcultures of the organisms from Columbia horse blood agar were used in both API systems as per the manufacturer's instructions. The inoculum was prepared as per the manufacturer's instructions. For the API 20E, the cupules were slightly overfilled. The strips were incubated at 35°C for 48 h. Profiles were determined with the API 20E Analytical Profile Index, third edition. The APILAB PLUS software program version 3.3.3 does not include *B. pseudomallei* in the database. For the API 20NE, the inoculum was prepared as per the manufacturer's instructions, and inoculated strips were incubated at 35°C for 48 h. Profiles were determined with APILAB PLUS software program version 3.3.3.

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TABLE 1. Profile numbers

Profile no.	No. of isolates identified
API 20E profiles	
2006725	63
2006625	19
2002725	9
2002625	8
2006727	1
2006604	1
2006004	1
0006725	1
API 20NE profiles	
1156577	47
1156575	17
1156576	21
1156574	7
1036577	4
0156576	1
1156556	1
1154576	1
1150514	1
1150554	1
0156577	1
1156557	1

Vitek 1 and 2. Both Vitek 1 and Vitek 2 cards were set up as per the manufacturer's instructions. All isolates were tested from MacConkey agar following an overnight incubation. Software versions for the Vitek 2 were AES-R02.00N, AIX-R04.00, BCI-R06.01, DB2-R05.00, HHP-R01.02, LSN-R06.01, SYS-R06.01, TRC-ON, UNI-R05.00, and VT2-R02.03.

RESULTS

The API 20NE and 20E correctly identified 98% and 99% of *B. pseudomallei* isolates, respectively. The most common misidentification was *Chromobacterium violaceum*. The API 20NE and 20E profile numbers obtained are shown in Table 1. Vitek 1 and 2 identified 99% and 19% of isolates correctly, respectively.

The most common identification obtained with the Vitek 2 (37%) was that of various nonfermenting gram-negative bacilli. This is a grouping for *Acinetobacter haemolyticus, A. johnsonii, A. junii*, and *A. lwoffii*; *Alcaligenes faecalis*, *A. denitrificans*, and *A. xylosoxidans*; *Bordetella avium* and *B. bronchiseptica*; Centers for Disease Control group IV C-2; *Comamonas acidovorans* and *C. testosteroni*; *Moraxella lacunata*, *M. nonliquefaciens*, and *M. osloensis*; *Oligella ureolytica* and *O. urethralis*; *Pseudomonas alcaligenes*, *P. fluorescens*, *P. mendocina*, *P. pseudoalcaligenes*, *P. putida*, and *P. stutzeri*; and *Psychrobacter phenylpyruvicus.* The percent identifications for each of the test systems are shown in Table 2.

DISCUSSION

The identification of *B. pseudomallei* is generally not a problem for laboratories that are accustomed to seeing this organism in areas where it is endemic. A few basic microbiological tests are usually sufficient to allow a presumptive identification to be made, and in regions with limited resources, this is usually adequate. These simple screening tests are oxidase positivity, bipolar staining of gram-negative rods, resistance to gentamicin and colistin, characteristic metallic sheen, and colony characteristics on Ashdown's agar (9). The use of other systems has been reported. These include the API 20E (bioMerieux) (1) and the Microbact 24E (MedVet, Adelaide, Australia) (19).

As mentioned earlier, the API 20NE (bioMerieux) has been shown to identify 97.5% of isolates (9). Another study, however, showed that up to 20% of *B. pseudomallei* isolates could be misidentified with the API 20NE in comparison with the Microbact 24E. *Chromobacterium violaceum* was the most common misidentification in this study (12). This might account for reported cases of apparent *C. violaceum* sepsis in areas where melioidosis is endemic.

Two isolates which had previously been reported as *B. pseudomallei* were not identified as such with the API 20NE in this study. These isolates were morphologically similar to *B. pseudomallei* and had characteristic antibiograms. One of these was identified as *B. pseudomallei* with the Vitek 1 and API 20E. The other discrepant isolate was not identified by any of the systems tested. A 16S rRNA profile of this isolate confirmed that it was likely to be *B. pseudomallei*.

Our study did not examine *Burkholderia thailandensis*, a new species, which has recently been described as an environmental arabinose-positive variant of *B. pseudomallei* (4). This organism has not yet been incorporated into the databases of the identification systems used in this study.

All isolates used in this study came from a wide regional area of northern Australia. These include the two NCTC strains used, which originated from this region. The possibility of variation in biochemical profiles related to geographic source has been raised (12). We did not examine isolates from outside our region. Future studies of a wider geographical source of isolates may reveal biochemical variations.

The advent of automated identification systems has brought new challenges to the diagnostic laboratory. Direct interfaces between the automated identification system and the laboratory computerized reporting system could result in an incorrectly identified organism being reported without question. This would be more likely in laboratories not accustomed to seeing organisms like *B. pseudomallei*. The importance of cor-

TABLE 2. Identifications

System	Identification	% of strains
API 20E	*Burkholderia pseudomallei*	99
	Chromobacterium violaceum	1
API 20NE	*Burkholderia pseudomallei*	98
	Chrombacterium violaceum	2
Vitek 1	*Burkholderia pseudomallei*	99
	Unidentified organism	1
Vitek 2	Various nonfermenting gram-negative bacilli	37
	Burkholderia cepacia	24
	Burkholderia pseudomallei	19
	Myroides spp.	8
	Inconclusive identification	7
	Chromobacterium violaceum	2
	Unidentified organism	2
	Pseudomonas aeruginosa	1

rect identification of *B. pseudomallei* is highlighted by the need for long-term suppressive therapy in the treatment of melioidosis.

This study confirms the utility of both the API 20NE and API 20E systems in the identification of *B. pseudomallei*. Most medium to large diagnostic laboratories would have an automated identification system such as the Vitek 1 or its upgrade, the Vitek 2. Surprisingly, in this study, the Vitek 2 performed quite unsatisfactorily in the identification of *B. pseudomallei*.

The API 20NE, API 20E, and Vitek 1 systems identify an unknown isolate based on the probability that the reactions achieved match those of an organism in the database. The Vitek 2 determines the identification of an isolate by evaluating each result and assigning a value to that result, i.e., a typicity value. A value of 1.0 is assigned to the most typical result. In the case of an uncertain result, a value of 1.0 is also assigned because this test could not contribute to distinguishing between species. A value between 0 and 1.0 is assigned for atypical results. The typicity index is generated by the aggregation of all typicity values. A perfect result for all values would give a typicity index of 1.0. The software then uses decision steps based on preset threshold values to determine the final identification of the unknown isolate.

The frequency of positivity of *B. pseudomallei* isolates in the current Vitek 2 database has been reported as 83% for cellobiose, 80% for glucose, and 99% for sorbitol (BioMerieux USA). In this study, 52% of isolates were cellobiose positive, 35% were flagged as uncertain, and 13% returned negative values. The Vitek 2 would therefore treat 87% of the isolates as having a positive reaction for cellobiose. In the subset of results for which *B. pseudomallei* was either the first or second choice (34 isolates), 100% were positive for cellobiose. The figures for the glucose results for all isolates were 33% positive, 16% uncertain, and 51% negative, leading to the software's treating 49% of the results as positive. For the subset of isolates, 70% were positive, 3% were uncertain, and 27% were negative. The sorbitol results for all isolates were 16% positive, 19% uncertain, and 65% negative, leading to the software's treating 35% of the results as positive. In the subset of results, 38% were positive, 35% were uncertain, and 27% were negative; in effect, 73% were positive.

With only 35% of all results for sorbitol returning a positive (i.e., typical) result, it would seem either that the expected frequency is incorrect (possible strain variation of Australian isolates) or that the algorithms for sorbitol and *B. pseudomallei* in the software are incorrect. The high percentage of uncertain results would result in a reduction of the discriminating ability of the Vitek 2 identification system. In all, seven tests yielded >10% uncertain results for the isolates (D-cellobiose, 35%, D-glucose, 16%; D-mannitol, 25%; D-sorbitol, 19%; γ-glutamyltransferase, 29%; L-lysine arylamidase, 11%; and phosphatase, 30%). Again, the discriminating ability of the software would be compromised if isolates have multiple uncertain results.

This study has demonstrated a limitation in the ability of the Vitek 2 to discriminate between *B. pseudomallei* and various nonfermenting gram-negative bacilli or *Bcepacia*. It is likely that this is a factor related to the software rather than specific microbiological features of the panel. Addressing this may allow the microbiological diagnosis of this organism to be made with confidence with the Vitek 2. Accurate laboratory diagnosis of this important condition will lead to the institution of appropriate therapy and management.

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