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Comparison of Routine Bench and Molecular Diagnostic Methods in Identification of *Burkholderia pseudomallei*[▽]

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This study compared the identification of *Burkholderia pseudomallei* with that of related organisms. Bench tests and latex agglutination were compared with molecular identification. Using bench tests and latex agglutination alone, 100% (30/30) of *B. pseudomallei* isolates were correctly identified. Amoxicillin-clavulanate susceptibility testing was also a good and simple discriminatory test.

Melioidosis is an infectious disease caused by *Burkholderia pseudomallei*, which is endemic in Southeast Asia and northern Australia. Cases occur mainly during periods of heavy rain (13). It is a clinically diverse infection affecting many organ systems and commonly presents as a fulminant septicemia (3, 4).

There has been controversy as to the optimal identification system for *B. pseudomallei* (2, 9, 10, 11, 12, 19). The reliability of the API 20NE and the Vitek 1 systems (bioMérieux, Marcy L'Etoile, France) has been questioned and molecular confirmation suggested (14). The reliability of presumptive tests (oxidase, Gram staining, resistance to gentamicin and polymyxin) in the identification of this organism has previously been described as 100% accurate (6). It should be noted that neither system will distinguish related species such as *Burkholderia thailandensis* from *B. pseudomallei*.

The commonest misidentification of *B. pseudomallei* when using identification systems is with *Burkholderia cepacia*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, and *Chromobacterium* spp. (1). A recent study compared the API 20NE system and a latex agglutination assay and found that the API 20NE system identified 99% of *B. pseudomallei* isolates correctly. It did not however distinguish between *B. thailandensis* and *B. pseudomallei*. The addition of the latex agglutination test correctly identified 99.5% of isolates and was negative for 98% of the *B. thailandensis* isolates and other oxidase-positive gram-negative bacilli (1). Molecular identification of the organism has been described, using a number of genomic targets (14, 17, 18).

A previous study compared basic bench diagnostic presumptive tests with *B. pseudomallei* slide agglutination using a monoclonal antibody, API 20NE (bioMérieux, Marcy L'Etoile, France), cellular fatty acid analysis, and molecular detection (10). This showed that the PCR alone had a sensitivity and specificity of 100%. API 20NE performed poorly in this study, with a sensitivity of 37% and a specificity of 92% (10). The agglutination test used had a sensitivity of 94% and a specificity of 83%. Although fatty acid analysis had a sensitivity of 98% and a specificity of 83%, it was acknowledged that this technology was not widely available. Interestingly, the presumptive tests (oxidase, Gram staining, resistance to gentamicin and polymyxin) did not distinguish between *B. pseudomallei*, *B. cepacia*, and *B. thailandensis*. The aim of this study was to compare the diagnostic efficacies of standard presumptive identification methods (oxidase, gentamicin resistance, and amoxicillin-clavulanate susceptibility), including a specific latex agglutination assay, with specific molecular detection in the identification of *B. pseudomallei* to determine whether low-cost nonmolecular techniques may still be useful in resource-poor areas for the diagnosis of melioidosis.

Of the total of 43 bacterial isolates used, 30 were *B. pseudomallei*, three were *B. cepacia*, five were *B. thailandensis*, one was *Chromobacterium violaceum* (nonpigmented), and four were *P. aeruginosa*. All isolates were clinical isolates except for the *B. thailandensis* isolates, which were of environmental origin. *Burkholderia mallei*, a closely related species, was not used as a comparator because it is not misidentified as *B. pseudomallei* or vice versa with identification systems. It is also susceptible to gentamicin. All *B. pseudomallei* isolates investigated were from North Queensland. The identity of all isolates was confirmed using the Vitek 1 and API 20NE systems, and the isolates were stored at −70°C. These isolates had been validated in a previous study (12). The sequenced *B. pseudomallei* K96243 isolate was used as a control for real-time PCR. All isolates were subcultured onto Columbia horse blood agar (bioMérieux, Australia), incubated in air at 37°C for 24 h, and checked for purity. Single colonies were inoculated into Mueller-Hinton broth (bioMérieux, Australia) and incubated at 37°C for 24 h. Mueller-Hinton agar (bioMérieux, Australia) was used for susceptibility testing. All isolates were coded to ensure that the operator performing the identification was unaware of the identity of the isolate. Oxidase tests were performed by a standard oxidase reagent-impregnated strip method with appropriate controls. Susceptibility testing was carried out using a standard method with discs containing 20/10 μg amoxicillin-clavulanate and 10 μg gentamicin (5). The plates were incubated in air at 37°C for 24 h. As there are no CLSI zone diameter standards for *B. pseudomallei*, the standards for *P. aeruginosa* and *Enterobacteriaceae* were used. Zones of inhibition to gentamicin of ≥15 mm and to

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TABLE 1. Isolates presumptively and definitively identified as *B. pseudomallei*

Species	No. of isolates tested	No. (%) of isolates identified as *B. pseudomallei* with[a]:		
		Oxidase and susceptibility profile testing	Latex agglutination	qRT-PCR
B. pseudomallei	30	29 (97)	30 (100)	30 (100)
B. thailandensis	5	4 (80)	0 (0)	0 (0)
B. cepacia	3	0 (0)	0 (0)	0 (0)
P. aeruginosa	4	0 (0)	0 (0)	0 (0)
C. violaceum	1	0 (0)	0 (0)	0 (0)

[a] Isolates tested by oxidase and susceptibility profile testing were presumptively identified, while isolates tested by latex agglutination and quantitative real-time PCR (qRT-PCR) were definitively identified. When presumptive identification was compared with definitive identification, presumptive identification had a sensitivity of 97%, a specificity of 69%, a positive predictive value of 88%, and a negative predictive value of 90% ($P < 0.0001$; Fisher's exact test).

amoxicillin-clavulanate of ≥18 mm were considered susceptible (5). The latex reagent and the techniques used have been reported in detail in a previous study (1). PCR amplification was performed as previously described, with similar primers and probes (17), using Rotor-Gene 3000 (Corbett Life Science, Australia) with minor modifications. Bovine serum albumin was not added to the master mix. ImmoMix *Taq* (Bioline) was used with deoxynucleoside triphosphate (200 μM) at a final $MgCl_2$ concentration of 2.5 mM.

The following primers and probes were used: primer BPSS1187/BURPS1710b_A0179 (*B. pseudomallei*-unique sequence) (forward, ATCGAATCAGGGCGTTCAAG; reverse, CATTCGGTGACGACACGACC) and probe 6-carboxyfluorescein-CGCCGCAAGACGCCATCGTTCAT-6-carboxytetramethylrhodamine. The probe is labeled with a reporter dye, 6-carboxyfluorescein, and a quencher dye, 6-carboxytetramethylrhodamine.

A total of 33 isolates were presumptively identified as *B. pseudomallei* on the basis of a positive oxidase test, resistance to gentamicin, and susceptibility to amoxicillin-clavulanate. These included four of the five *B. thailandensis* isolates and 29 of the 30 *B. pseudomallei* isolates (Table 1). One of the *B. thailandensis* isolates was not presumptively identified as *B. pseudomallei* as expected, due to a reduced zone of inhibition to amoxicillin-clavulanate. One of the *B. pseudomallei* isolates failed to be presumptively identified as *B. pseudomallei*, as it had a zone of inhibition to gentamicin of 22 mm. Nevertheless, it was confirmed with both latex agglutination and quantitative real-time PCR. *B. pseudomallei* is intrinsically resistant to gentamicin, although rare isolates which are susceptible to gentamicin have been described (16). When presumptive identification was compared with definitive identification (Table 1), presumptive identification had a sensitivity of 97%, a specificity of 69%, a positive predictive value of 88%, and a negative predictive value of 90% ($P < 0.0001$; Fisher's exact test). If *B. thailandensis* isolates were excluded, presumptive identification would have a specificity of 100% and a sensitivity of 97%.

We used amoxicillin-clavulanate susceptibility rather than colistin resistance to distinguish between *B. cepacia* (resistant) and *B. pseudomallei* (sensitive). When tested against amoxicillin-clavulanate, 93.6% (278/297) of *B. cepacia* isolates tested over a 10-year period were resistant (Antibiogram, Pathology Queensland; unpublished data). All isolates of *B. pseudomallei*, in this study, tested susceptible to amoxicillin-clavulanate. A previous study also demonstrated that 100% (69/69) of *B. pseudomallei* isolates were susceptible to amoxicillin-clavulanate (15). Colistin, on the other hand, does not reliably distinguish *B. cepacia* from *B. pseudomallei*, as both are almost invariably resistant (7).

It is acknowledged that the number of isolates tested in this study is small and that the results need to be interpreted with caution. Nevertheless, this study has demonstrated that presumptive tests are highly predictive in the identification of *B. pseudomallei*. While presumptive identification will misidentify *B. thailandensis* as *B. pseudomallei*, this is unlikely to be of clinical significance, as *B. thailandensis* is rarely recovered from clinical specimens (8). The use of amoxicillin-clavulanate susceptibility testing for presumptive identification of *B. pseudomallei* has not been described previously. Combined with a latex agglutination assay, it would further validate the identification of *B. pseudomallei*. Therefore, we conclude that these tests lend themselves to be used in regions where kit identification methods are costly and where sustainable molecular detection techniques are unrealistic.

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