Influence of Biochemical Features of *Burkholderia pseudomallei* Strains on Identification Reliability by Vitek 2 System

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Abstract

Background: *Burkholderia pseudomallei* is a Gram-negative saprophytic soil bacterium that causes melioidosis, a potentially fatal disease endemic in wet tropical areas. The currently available biochemical identification systems can misidentify some strains of *B. pseudomallei*. The aim of the present study was to identify the biochemical features of *B. pseudomallei*, which can affect its correct identification by Vitek 2 system. **Materials and Methods:** The biochemical patterns of 40 *B. pseudomallei* strains were obtained using Vitek 2 GN cards. The average contribution of biochemical tests in overall dissimilarities between correctly and incorrectly identified strains was assessed using nonmetric multidimensional scaling. **Results:** It was found (R statistic of 0.836, $P = 0.001$) that a combination of negative N-acetyl galactosaminidase, β-N-acetyl glucosaminidase, phosphatase, and positive D-cellobiase (dCEL), tyrosine arylamidase (TyrA), and L-proline arylamidase (ProA) tests leads to low discrimination of *B. pseudomallei,* whereas a set of positive dCEL and negative N-acetyl galactosaminidase, TyrA, and ProA determines the wrong identification of *B. pseudomallei* as *Burkholderia cepacia* complex. **Conclusion:** The further expansion of the Vitek 2 identification keys is needed for correct identification of atypical or regionally distributed biochemical profiles of *B. pseudomallei*.

Keywords: Biochemical identification, *Burkholderia cepacia*, *Burkholderia pseudomallei*, nonmetric multidimensional scaling, Vitek 2

INTRODUCTION

Burkholderia pseudomallei is a Gram-negative saprophytic soil β-proteobacterium that causes melioidosis, a severe disease of humans and animals. Melioidosis is endemic in a number of areas of Southeast Asia, Northern Australia, the Pacific, Central and South America, and the Caribbean.[1] According to recent estimates, the global burden of melioidosis is about 165,000 human cases per year, and an infection is probably endemic in at least 79 countries.[2]

Melioidosis is acquired more often through contact with contaminated soil and water by percutaneous inoculation. The human cases are often spatially and temporally clustered, following the severe weather events such as heavy rains and flooding.[3] Clinical manifestation of melioidosis varies widely from asymptomatic disease to rapidly progressive pneumonia and sepsis. The symptoms often are very similar to other both infectious and non-infectious diseases-tuberculosis, mycosis, sarcoidosis, cancer, which makes the differential clinical diagnosis of melioidosis quite a challenge.[4,5] Treatment

of melioidosis is hampered by an intrinsic resistance of *B. pseudomallei* to various antimicrobials.[3,6]

The correct laboratory diagnosis of melioidosis is essential for a favorable outcome but can be difficult. *B. pseudomallei* is usually poorly isolated from clinical specimens and may not be correctly identified when isolated.[5,7] Routinely used serological tests generally are not sufficiently specific or sensitive. Although laboratory diagnosis of melioidosis has advanced using of more sensitive and rapid tests such as polymerase chain reaction (PCR), DNA sequencing, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry,[8] the correct identification of *B. pseudomallei* isolates through conventional bacteriologic scheme

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is very important, especially in clinical microbiology laboratories in nonendemic areas where clinical suspicion is insufficient.

Due to duration and complexity of routine bacteriological identification of *B. pseudomallei*,[5,6] the suitability of various commercial biochemical tests has been studied for a long time. As has been noted repeatedly, the currently available biochemical systems can misidentify some strains of *B. pseudomallei* as *Chromobacterium violaceum* or *Burkholderia cepacia* complex.[7,9-12] A particular problem is associated with misidentification of *B. pseudomallei* as *B. cepacia* by Vitek 2 analyzer (bioMérieux) widely used in clinical microbiology laboratories.[12,13]

The aim of this study was to identify the biochemical features, which can cause an incorrect identification of *B. pseudomallei* by Vitek 2 system.

MATERIALS AND METHODS

Strains, culture condition, and identification on Vitek 2 platform

Forty strains of *B. pseudomallei* studied in this work are listed in Table 1. All strains were confirmed by specific PCR, DNA sequencing, and conventional bacteriologic method. The strains were subcultured on LB agar (HiMedia Laboratories Pvt. Ltd, India) for 24 hours at 37°C before testing on Vitek 2. The GN cards (bioMérieux) were used for analysis according to the manufacturer's instructions.

Comparative analysis of biochemical profiles

The comparative analysis of binary biochemical profiles (0-lack of the feature, 1-positive) was carried using PRIMER package version 7.0.10 (Primer-E Ltd., Plymouth Marine Laboratory, United Kingdom). The clustering of the strains was performed using nonmetric multidimensional scaling (nMDS) on Euclidean distances similarity matrix of biochemical profiles. The reliability of biochemical patterns difference between properly and incorrectly identified strains was assessed using nonparametric analysis of similarity (ANOSIM). The ANOSIM percentage was used to evaluate an average contribution of each biochemical test in overall dissimilarity between clusters.

RESULTS

Biochemical identification

The most of studied *B. pseudomallei* strains (77.5% of the total) were identified correctly with the reliability of 90%–99% [Table 1]. Five strains (12.5%) were identified at low discrimination as *B. cepacia* complex/*B. pseudomallei* with the recommendation to use additional differentiating tests. Four strains (10%) were identified as *B. cepacia* complex with the conclusion "very good identification" (1 strain) and "excellent identification" (3 strains).

Nonmetric multidimensional scaling analysis

The nMDS allowed to group the strains of *B. pseudomallei* into 8 clusters [Figure 1]. The clusters A, F, E, and G included one

Strain	Identified as	Identification reliability	Cluster
B. pseudomallei 107	*B. cepacia* complex	Excellent	A
B. pseudomallei 2	*B. pseudomallei*	97%	B
B. pseudomallei 130	*B. cepacia* complex	Excellent	B
B. pseudomallei 138	*B. cepacia* complex	Very good	B
B. pseudomallei 60839	*B. cepacia* complex	Excellent	B
B. pseudomallei 1	*B. pseudomallei*	98%	C
B. pseudomallei 97	*B. pseudomallei*	99%	C
B. pseudomallei 112	*B. pseudomallei*	99%	C
B. pseudomallei 115	*B. pseudomallei*	96%	C
B. pseudomallei 117	*B. pseudomallei*	98%	C
B. pseudomallei 128	*B. pseudomallei*	99%	C
B. pseudomallei 133	*B. pseudomallei*	97%	C
B. pseudomallei 136	*B. pseudomallei*	99%	C
B. pseudomallei 56770	*B. pseudomallei*	99%	C
B. pseudomallei 56812	*B. pseudomallei*	99%	C
B. pseudomallei 56830	*B. pseudomallei*	98%	C
B. pseudomallei 57582	*B. pseudomallei*	97%	C
B. pseudomallei 59437	*B. pseudomallei*	99%	C
B. pseudomallei 98	*B. pseudomallei*	93%	D
B. pseudomallei 100	*B. cepacia/B. pseudomallei*	Low discrimination	D
B. pseudomallei 102	*B. cepacia/B. pseudomallei*	Low discrimination	D
B. pseudomallei 103	*B. cepacia/B. pseudomallei*	Low discrimination	D
B. pseudomallei C-141	*B. pseudomallei*	95%	E
B. pseudomallei 139	*B. pseudomallei*	96%	F
B. pseudomallei 108	*B. pseudomallei*	99%	G
B. pseudomallei 99	*B. cepacia/B. pseudomallei*	Low discrimination	H
B. pseudomallei 109	*B. pseudomallei*	98%	H
B. pseudomallei 110	*B. pseudomallei*	99%	H
B. pseudomallei 111	*B. pseudomallei*	90%	H
B. pseudomallei 113	*B. pseudomallei*	99%	H
B. pseudomallei 114	*B. pseudomallei*	98%	H
B. pseudomallei 116	*B. pseudomallei*	98%	H
B. pseudomallei 131	*B. pseudomallei*	99%	H
B. pseudomallei 132	*B. pseudomallei*	99%	H
B. pseudomallei 134	*B. pseudomallei*	98%	H
B. pseudomallei 135	*B. cepacia/B. pseudomallei*	Low discrimination	H
B. pseudomallei 137	*B. pseudomallei*	98%	H
B. pseudomallei 60631	*B. pseudomallei*	96%	H
B. pseudomallei 60806	*B. pseudomallei*	99%	H
B. pseudomallei 611083	*B. pseudomallei*	98%	H

B. pseudomallei: *Burkholderia pseudomallei*, *B. cepacia*: *Burkholderia cepacia*

strain each. Three strains identified incorrectly as *B. cepacia* and one correctly identified strain ("excellent identification") were

included in cluster B. Thirteen strains with high probability of identification (97%–99%) were combined in cluster C. The cluster H proved to be the most extensive, which included 11 strains with "excellent identification," 2 strains with "good identification," and 2 strains at low discrimination. The cluster D includes 4 strains, of which 3 are at low discrimination and 1-with a high probability of identification (93%) [Table 1].

The contribution of individual biochemical tests in misidentification of *Burkholderia pseudomallei*

The ANOSIM showed statistically significant differences in biochemical patterns of correctly and incorrectly identified strains (*R* statistic of 0.836, *P* = 0.001). The results estimate the average contribution of biochemical tests in overall dissimilarity between biochemical profile clusters are shown in Table 2.

It seems clear that the reliability of identification decreased in case of absence of phosphatase (PHOS), β-N-acetyl glucosaminidase (BNAG), N-acetyl galactosaminidase (NAGA) and the presence of D-cellobiase (dCEL), L-proline arylamidase (ProA), and tyrosine arylamidase (TyrA) activities. Thus, in strains identified at low discrimination, the dCEL and TyrA tests were positive in 100% of cases, and ProA in 80%

Figure 1: Nonmetric multidimensional scaling ordination on the euclidean distance similarity matrix of the Vitek 2 biochemical profile of 40 *Burkholderia pseudomallei* strains identified as *Burkholderia pseudomallei* and misidentified as *Burkholderia cepacia*, or strains at low discrimination

of cases. At the same time, the NAGA (80% of cases), BNAG, and PHOS (60% of cases) tests were negative [Table 2].

The comparison of biochemical profiles included in cluster B (*B. pseudomallei* 2 identified with the reliability of 97%, and *B. pseudomallei* 130, 138, and 60839 misidentified as *B. cepacia*) showed positive dCEL and negative PHOS in all cases. The BNAG test was negative in only one (*B. pseudomallei* 138) of the three wrongly identified strains [Table 3]. The glutamyl arylamidase Pna, D-maltose, D-mannitol (dMAN), D-sorbitol (dSOR), citrate, and malonate utilization tests also varied, but any consistent patterns affecting identification have not been revealed in these cases. The key tests in this cluster were ProA and TyrA, which were positive in correctly identified strain, similar to 58% and 64.5% of all correctly identified strains, respectively, whereas all misidentified strains were negative for these biochemical features [Table 2].

DISCUSSION

The troubles on the correct identification of *B. pseudomallei* by various biochemical systems have been described repeatedly. According to different data, the reliability of API 20NE (bioMérieux) manual system for identification of *B. pseudomallei* varied significantly,[7,9,10,14] that, combined with a long operating time, not allows to consider this system as the best option for *B. pseudomallei* identification.

A part of various automated systems does not include a representative set of biochemical profiles for *B. pseudomallei*,[15] whereas others give mainly low discrimination for *B. pseudomallei* or misidentification as *B. cepacia*.[9]

Widely used Vitek 2 system gives the most stable identification results, especially with the use of colorimetric-based GN cards.[16] Nevertheless, the identification accuracy still varies considerably for *B. pseudomallei* strains of different geographical origin.[11] An important task is to establish exactly what biochemical tests due to its variability lead to misidentification or low discrimination of *B. pseudomallei*.

As revealed by Lowe *et al.*, the uncertain results of several biochemical tests (D-cellobiose, D-glucose, dMAN, dSOR, gamma-glutamyltransferase, L-lysine arylamidase,
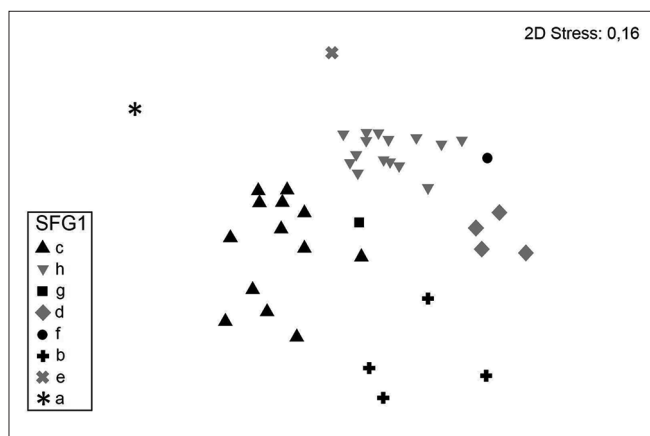
Table 2: The average contribution of selected biochemical tests in overall dissimilarity between clusters of the strains

Test	Contribution to overall dissimilarity between clusters (%)					Number of strains (%) with positive test		
	C–H	C–D	H–D	C–B	H–B	Correct identification	Low discrimination	Misidentification
PHOS	3.44	7.40	9.76	8.75	6.77	25 (80.6)	2 (40)	1 (25)
dCEL	4.16	7.89	6.83	10.73	5.86	10 (32.3)	5 (100)	3 (75)
NAGA	4.32	5.82	7.08	7.86	5.94	24 (77.4)	1 (20)	0
BNAG	<3	9.25	9.76	4.35	<3	28 (90.3)	2 (40)	3 (75)
ProA	7.94	8.49	<5	<4	5.44	18 (58)	4 (80)	0
TyrA	7.42	7.84	<5	<4	5.44	20 (64.5)	5 (100)	0

PHOS: Phosphatase, dCEL: D-cellobiase, NAGA: N-acetyl galactosaminidase, BNAG: β-N-acetyl glucosaminidase, ProA: L-proline arylamidase, TyrA: Tyrosine arylamidase

Table 3: The biochemical tests varied in strains constituting cluster B										
Strain	dCEL	NAGA	BNAG	AGLTp	dMAL	dMAN	ProA	TyrA	dSOR	CIT	MNT
B. pseudomallei 2	+	-	+	-	+	+	+	+	+	-	-
B. pseudomallei 138	+	-	-	+	-	-	-	-	-	+	-
B. pseudomallei 130	+	-	+	-	-	-	-	-	-	-	-
B. pseudomallei 60839	+	-	+	-	+	+	-	-	+	-	+

dCEL: D-cellobiase, NAGA: N-acetyl galactosaminidase, BNAG: β-N-acetyl glucosaminidase, AGLTp: Glutamyl arylamidase Pna,
dMAL: D-maltose, dMAN: D-mannitol, ProA: L-proline arylamidase, TyrA: Tyrosine arylamidase, dSOR: D-sorbitol, CIT: Citrate, MNT: Malonate,
B. pseudomallei: *Burkholderia pseudomallei*, +: positive test, -: negative test

and phosphatase) would result in a reduction of Vitek 2 discriminating ability for *B. pseudomallei*.[10] More recently, Podin *et al.* noted that in particular, two enzymatic tests, BNAG and NAGA, were distinct between correctly and misidentified *B. pseudomallei* isolates.[11]

The results of the present study showed that a set of negative NAGA, BNAG, PHOS and positive dCEL, TyrA, ProA tests lead to low discrimination of *B. pseudomallei*, and the presence of dCEL activity combined with negative tests for NAGA, TyrA, and ProA would result in misidentification of *B. pseudomallei* as *B. cepacia* complex.

CONCLUSION

The obtained results demonstrate the effect of the variability of several biochemical features on the accuracy of *B. pseudomallei* identification. The data mentioned above demonstrate the need of further expansion of the Vitek 2 identification keys for atypical or regionally distributed biochemical profiles of *B. pseudomallei*. Also important, that clinicians and diagnostic laboratories stuff should be aware on the possibility of misidentification of *B. pseudomallei* as *B. cepacia* using automated biochemical identification systems.

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Conflicts of interest
There are no conflicts of interest.

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