ORIGINAL ARTICLE

Clinical information associated with bacterial culture proven *Burkholderia pseudomallei* among melioiodosis patients in Buriram province

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Received: 13 January 2023 **Revised:** 29 May 2023 **Accepted:** 30 May 2023 **Available online:** September 2023
DOI: 10.55131/jphd/2023/210303

ABSTRACT

Indirect hemagglutination assay (IHA) is a general rapid diagnosis procedure in melioidosis patients combined with clinical information. The Burkholderia pseudomallei culture technique requires prolonged time before getting culture results. Related factors of bacterial culture proven melioidosis and the appropriate cut-off point for IHA are unfashionable and need to be updated. A cross-sectional study was conducted among melioidosis patients using data reported in the medical records. Univariable analysis was performed by Chi-square test and Student T-test as appropriate. Multivariable logistic regression was finally used to identify the contributing factors to bacterial culture proven cases. The results were shown as adjusted odds ratio (AdjOR) and 95%CI. Validity domains, sensitivity, specificity, predictive values, and likelihood ratios at each cut-off point for IHA were demonstrated. The results revealed that after adjustment for abdominal pain symptoms, diabetes mellitus (DM)(p-value < 0.001) and dyspnea symptoms (p-value $= 0.025$) were the associated factors of bacterial culture proven cases. The IHA titer > 1: 10240 provided the highest positive and negative predictive values, 30% and 69% respectively. Both positive and negative likelihood ratios of this cut-off point were also accounted for as 0.95 and 1.003, respectively. Health personnel should perform melioidosis diagnosis by the use of clinical information and an appropriate cut-off point for IHA, especially in diabetic patients who have dyspnea.

Key words:
IHA; bacterial culture proven; DM

Citation:
Worrayot Darasawang. Clinical information associated with bacterial culture proven *Burkholderia pseudomallei* among melioiodosis patients in Buriram province. J Public Hlth Dev. 2023;21(3):31-42 (https://doi.org/10.55131 jphd/2023/210303)

INTRODUCTION

Melioidosis is a communicable disease caused by *Burkholderia pseudomallei,* which is mainly found in the soil of tropical regions[1]. Patients with melioidosis commonly have various clinical manifestations[2]. Sepsis and septic shock are the major complications of melioidosis, accounting for 99% of global years of life lost due to this disease[3,4]. The Southeast Asian region has also reported melioidosis mortality[5]. The mortality rate of melioidosis in Thailand was estimated to be 10-35% in public and university hospitals[6]. Among Thai melioidosis patients, sepsis was also found to be the major complication, affecting 78% of cases[7].

Burkholderia pseudomallei is generally cultivated in the blood agar, McConkey agar, and triple iron agar[8,9]. It grows at 42 ^{o}C[9,10]. Bacterial culture is the gold standard for melioidosis diagnosis; however, the culture result takes several days[11]. Indirect hemagglutination assay (IHA) is an alternative laboratory investigation for rapid diagnosis in melioidosis which is used worldwide[11]. IHA detects *Burkholderia pseudomallei* antibodies by using several antigens, such as melioidin, extracellular protein, and lipopolysaccharide. IHA result is reported as titer level[12].

Appropriate cut-off point for IHA in melioidosis diagnosis can vary depending on the geographical area[13]. Several previous studies have determined the appropriate cut-off point for IHA. The study carried out in Ubon Ratchathani province revealed the sensitivity and specificity in each cut-off point and recommended that IHA titer $\geq$ 1:160 provided the highest sensitivity and specificity, 70% and 67% respectively. Moreover, it provided 80% of positive predictive value (PPV) and 55% of negative predictive value (NPV)[14]. Leelarasamee A.'s study[15] reported the case management recommendation for each cut-off point of IHA. The titer of IHA less than 1:80 demonstrated the absence of *Burkholderia pseudomallei* infection, whereas the titer greater than 1:320 revealed the likelihood of getting an infection. IHA test accuracy was implemented in Srinagarind Hospital and the sensitivity and specificity of the test were 50% and 72% respectively[16].

Diagnosing melioidosis can be quite difficult and requires significant clinical skill due to its various clinical presentations, and the cut-off point for IHA is controversial. Moreover, the recommended cut-off point for IHA from previous literature is out of date. Updating the appropriate cut-off point for IHA and identifying contributing factors to *Burkholderia pseudomallei* detection can improve diagnosis and early proper management. Buriram province recently reported 36.4% of melioidosis mortality[17] and was selected as the study area. The present study aimed to determine the associated factors of bacterial culture-proven melioidosis and identify the appropriate cut-off point for IHA.

METHODS

A cross-sectional study was conducted in Buriram hospital, the representative area of Buriram province that was reported as the endemic area of melioidosis. The population was the melioidosis patients who were reported in the medical records by the use of International Classification of Diseases 10 (ICD 10) as A24 (Glander of melioidosis)[18] at Buriram hospital. All of them were extracted between 1st January and 31st December 2021. Inclusion criteria were the medical records of melioidosis patients who were 40 years or over and hospitalized during the study period. The medical records without bacterial culture investigation were not excluded.

Outcome and covariate definitions

Bacterial culture proven was defined as a medical record that reported evidence of *Burkholderia pseudomallei*. IHA titer was defined as the titer results provided by the laboratory center of Buriram hospital at the same period of bacterial culture investigation. The titers were shown as follows: 1:160, 1:320, 1:640, 1:1280, 1:2560, 1:5120, 1:10240, 1:20480 or higher. IHA validity was measured by 4 domains, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Likelihood ratios, both positive and negative, were also described to determine the test performance.

Data collection and preparation

Some variables from the electronic hospital database, hospital number (HN) and ICD 10 code, were first captured. Medical records were reviewed by the researcher and reported in the case record form. All case record forms were coded and entered into the Epi Data version 3.1. Double data entry was performed at different time periods. Missing values were managed by pairwise deletion.

Statistical analysis

Categorical variables were demonstrated as frequency and percentage. Comparison between a specific covariate and bacterial culture proven was portrayed as prevalence ratio (PR), 95% CI, and p-value. P-value was calculated by chi-square or Fisher's exact test as appropriate. Mean and standard deviation (SD) were described in normally distributed continuous data. Comparison of two-group means was revealed as the difference of means (MD) and p-value from the student T-test. The significance level was indicated at 0.05. All significance factors were adjusted and analyzed by multivariable logistic regression. The results were demonstrated as adjusted odds ratio (AdjOR) and 95%CI.

Ethical consideration

Ethical approval to implement this study was approved by the Ethics Review Committee for Research Involving Human Research Subjects, Buriram hospital. The approval number is BR 0033.102.1/26 and the date of approval was 18 July 2022.

RESULTS

General information of melioidosis patients

All 346 melioidosis patients were extracted from the electronic hospital database. Five patients (1.45%) were excluded because they did not perform the bacterial culture. Finally, 341 melioidosis patients were included in the analysis (Figure 1).

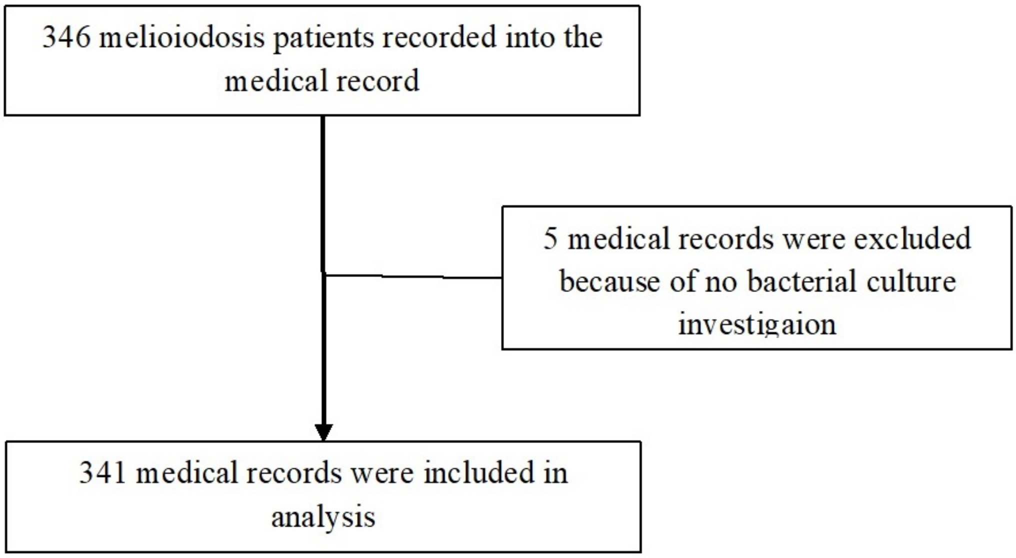

Figure 1 Flow chart of melioidosis general information

Characteristics of melioidosis patients

Most of them were male, 77.4%. The average age was 59.81 years, whereas the average body mass index (BMI) was 21.64 kg/m^2. Agriculture was the major reported occupation (52.8%). Most of them, 90.3%, use universal health coverage as their health insurance.

Fever symptom was reported by more than three-quarters of them (77.4%). Dyspnea and cough symptoms were mentioned by a similar proportion of them, 48.1% and 41.3% respectively. Fatigue was reported among 37.5% of them. Loss of appetite, abdominal pain, skin and soft tissue pain symptoms were reported by less than half of them. Diabetes mellitus (DM) was mostly recorded underlying disease among melioidosis patients, accounting for 47.8% of cases.

Burkholderia pseudomallei was predominantly detected in blood specimens (78.2%). Only 5 patients showed evidence of *Burkholderia pseudomallei* detection in both sputum and blood specimens (Table 1). The time interval between the investigation of the bacterial culture and the detection of positive *Burkholderia pseudomallei* was 3-5 days. The value of IHA titer was from <1:160 to ≥ 1:20480. The titer ≥ 1:20480 was reported in 2 patients.

Table 1. Characteristic of melioidosis patients (N = 341)

Characteristics	Number	Percentage
Sex		
Female	77	22.6
Male	264	77.4
Age (years)		
Mean (SD)	59.81 (10.70)	
Median (Min : Max)	60 (40 : 89)	
BMI		
Mean (SD)	21.64 (3.65)	
Median (Min : Max)	21.33 (12.48 : 21.87)	

Characteristics	Number	Percentage
Occupation		
Agriculture	163	52.8
Labour	62	20.1
Government officer	9	2.9
Merchant	6	1.9
Unemployed	69	22.3
Health insurance		
Universal health coverage	308	90.3
Social security scheme	9	2.6
Government	23	6.7
Own payment	1	0.3
Clinical presentation*		
Fever		
Yes	264	77.4
No	77	22.6
Cough		
Yes	141	41.3
No	200	58.7
Dyspnea		
Yes	164	48.1
No	177	51.9
Loss of appetite		
Yes	26	7.6
No	315	92.4
Fatigue		
Yes	128	37.5
No	213	62.5
Abdominal pain		
Yes	40	11.7
No	301	88.3
Skin and soft tissue pain		
Yes	22	6.5
No	319	93.5
Underlying diseases*		
DM	162	47.8
HT	104	30.6
CKD	36	10.6
HIV	5	1.5
Liver cirrhosis	27	7.9
Cancer	15	4.4
Thalassemia	6	1.8

Characteristics	Number	Percentage
Bacterial culture		
Burkholderia pseudomallei	110	32.3
Non- *Burkholderia pseudomallei*	30	8.8
Not found	201	58.9
Specimen detected *Burkholderia pseudomallei*		
Sputum	12	10.9
Blood	86	78.2
Body fluid	7	6.4
Both sputum and blood	5	4.5

**Multiple responses*

Univariable and multivariable analysis

The proportion of bacterial culture proven cases among males was 31.4%, which is similar to the proportion among females (35.1%), p-value = 0.54. The MD of age among bacterial culture proven and non-bacterial culture proven groups was 0.47 years (p-value = 0.702). MD of BMI between the two groups was similar (MD = 0.14 kg/m^2, p-value = 0.26). The prevalence of bacterial culture proven cases among individuals involved in agriculture was 33.7%, higher than non-agriculture group (29.5) without statistical significance, p-value = 0.41.

Fever symptom was not a contributing factor to bacterial culture proven cases (PR = 1.04 (0.71 – 1.52), p-value = 0.81). The prevalence of bacterial culture among melioidosis patients with dyspnea symptoms was 1.56 times higher than those without dyspnea symptoms with statistical significance (p-value = 0.005). Abdominal pain was also a contributing factor to bacterial culture proven cases with statistical significance (p-value = 0.033). Fatigue and loss of appetite were not associated with bacterial culture proven cases.

The proportion of bacterial culture proven cases was similar among the CKD and non-CKD groups, whereas the proportion among patients with liver cirrhosis was lower than among those without. Thalassemia did not demonstrate an association, however DM showed significance association with bacterial culture proven cases (PR = 1.84 (1.33 – 2.53), p-value <0.001) (Table 2).

Table 2 Univariable analysis of factors associated with bacterial culture proven (N= 341)

Factors	Number of sample	Bacterial culture proven n	(%)	PR	95% CI	p-value
Overall	341	110	(32.3)	N/A	N/A	N/A
Sex						0.54
Male	264	83	(31.4)	0.89	0.63 – 1.27	
Female	77	27	(35.1)	Ref		
Age	341	N/A	N/A	0.47*	N/A	0.702
BMI	277	N/A	N/A	0.14*	N/A	0.26

Factors	Number of sample	Bacterial culture proven n	(%)	PR	95% CI	p-value
Occupation						0.41
Agriculture	163	55	(33.7)	1.14	0.82 – 1.59	
Non-agriculture	146	43	(29.5)	Ref		
Fever						0.81
Yes	264	86	(32.6)	1.04	0.71 – 1.52	
No	77	24	(31.2)	Ref		
Cough						0.19
Yes	141	51	(36.2)	1.22	0.90 – 1.67	
No	200	59	(29.5)	Ref		
Dyspnea						0.005
Yes	164	65	(39.6)	1.56	1.13 – 2.13	
No	177	45	(25.4)	Ref		
Fatigue						0.86
Yes	128	42	(32.8)	1.03	0.74 – 1.41	
No	213	68	(31.9)	Ref		
Loss of appetite						0.48
Yes	26	10	(38.5)	1.21	0.72 – 2.02	
No	315	100	(31.7)	Ref		
Abdominal pain						0.033**
Yes	40	7	(17.5)	0.51	0.25 – 1.02	
No	301	103	(34.2)	Ref		
Skin and soft tissue pains						0.67
Yes	22	8	(36.4)	1.13	0.63 – 2.02	
No	319	102	(32)	Ref		
DM						<0.001
Yes	162	69	(42.6)	1.84	1.33 – 2.53	
No	177	41	(23.2)	Ref		
HT						0.35
Yes	104	37	(35.6)	1.17	0.84 – 1.71	
No	236	72	(30.5)	Ref		
CKD						0.59
Yes	36	13	(36.1)	1.13	0.71 – 1.81	
No	303	96	(31.7)	Ref		
Liver cirrhosis						0.47
Yes	27	7	(25.9)	0.79	0.41 – 1.53	
No	313	102	(32.6)	Ref		
Cancer						0.64
Yes	4	15	(26.7)	0.82	0.35 – 1.93	
No	105	325	(32.3)	Ref		

**MD*
***Irrelevance result between confidence interval and p-value was further analyzed by multivariable logistic regression*
****Fisher Exact Test*
*****Thalassemia and HIV weren't analyzed because of they were not enough records.*

All significant predictors due to univariable analysis were adjusted by multivariable logistic regression. After adjustment for abdominal pain symptoms, DM (AdjOR = 2.35 (1.46 – 3.78), p-value < 0.001) and dyspnea symptom (AdjOR = 1.73 (1.07 – 2.82), p-value = 0.025) revealed the significant effect on bacterial culture proven cases (Table 3).

Table 3 Multivariable analysis of factors associated with bacterial culture proven using multivariable logistic regression

Factors	Adjusted OR	95% CI	P-value
Dyspnea symptom	1.73	1.07 – 2.82	0.025
DM	2.35	1.46 – 3.78	<0.001

**Adjusted for abdominal pain symptom*

Validity of the IHA in different cut-off point

Of 335 patients who had both IHA and bacterial culture results, 104 patients who had bacterial culture proven, were used to describe the validity of the IHA. The IHA titer ≥ 1:160 revealed the highest sensitivity, at 86.5%. Titer ≥ 1:10240 revealed the highest specificity, at 93.9%. PPV and NPV accounted for 30% and 69% respectively. Positive likelihood ratios also were the highest reported, 0.95, for IHA titer ≥1:10240 (Table 4).

Table 4. Sensitivity, specificity, PPV, NPV, likelihood ratios of IHA in each cut-off point

IHA titer cut-off point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
≥1: 160	86.5	N/A *	28	N/A *	N/A*	N/A*
≥1: 320	74	3.5	25.7	22.9	0.76	7.42
≥1: 640	57.7	12.1	22.8	38.9	0.64	3.49
≥1: 1280	37.5	33.8	20.3	54.5	0.65	1.84
≥1: 2560	18.3	63.6	18.4	63.4	0.50	1.28
≥1: 5120	12.5	79.2	21.3	66.8	0.60	1.104
≥1: 10240	5.8	93.9	30	69	0.95	1.003

**No record cases for calculation*
*** The cut-off point > 1: 20480 wasn't determined due to not enough records.*
****LR: likelihood ratio*

DISCUSSION

Melioidosis is an important infectious disease worldwide. The incidence of melioidosis was frequently reported among individuals aged 40-60 years[19]. Males have shown higher incidence of the disease than females[20]. Around 81% of melioidosis patients were involved in agriculture and farming[21], however all demographic profiles were not associated with bacterial culture proven melioidosis in our study.

The multivariable analysis showed that contributing factors to bacterial culture proven cases were DM and dyspnea symptoms. The odds of having bacterial culture proven cases among the DM group were 2.35 times higher than those in the non-DM group. Association between DM and bacterial culture proven cases was mentioned in several previous studies[21,22,23,24]. These studies revealed that DM was a major underlying disease found in melioidosis cases because it impairs the host's defense mechanism and predisposes individuals to melioidosis. Thalassemia[25] was reported as a contributing factor to melioidosis in a previous study; however, it was not consistent with our study.

Patients with dyspnea symptoms had 73% higher odds of bacterial culture proven than those in the non-dyspnea group. Dyspnea patients with DM can have several diseases diagnosis such as pulmonary disease or diabetes ketoacidosis[26,27]. According to the study results, it means that melioidosis needs to be taken into account as a potential differential diagnosis among dyspnea patients with DM.

The validity of IHA was determined worldwide in both endemic and non-endemic areas. Matthew O'Brien[28] demonstrated the overall validity of IHA in Australia, an endemic area of melioidosis. The results revealed that the sensitivity, specificity, PPV, and NPV were 90%, 91.3%, 40.9%, and 99.3%, respectively. Another study[29] was also carried out in Australia, which found that the sensitivity of the IHA was 56% during the hospitalization, in a cut-off point titer of 1:40. The sensitivity is lowered when they increase the cut-off point of the titer. This finding was synchronous with our study.

The appropriate cut-off point for IHA was recommended in several previous literatures. A study implemented in Tanzania examined the relationship between bacterial culture proven cases and reciprocal antibody titer of IHA. It recommended that the appropriate cut-off point for IHA was 1:40[30].

The principle of screening test selection was mentioned previously[31]. High PPV and NPV are the indicators of good and ideal screening test. Higher PPV and NPV imply lower false positive and false negative rates. Lower false positive and negative rates represent the better likelihood ratios for the test[32]. Minimizing false positive and false negative rates is an important characteristic of good screening test. For the present study, the appropriate cut-off point for IHA should be $\geq$ 1: 10,240, as it demonstrates the highest PPV, NPV, and positive likelihood ratio.

IHA alone for melioidosis diagnosis has some consideration issues. Positive IHA is detected in children and healthy people in endemic areas, including Thailand[33,34]. Seropositive IHA accounted for 28% of cases in a previous study[35]. High false positive diagnosis of IHA was extensively reported, because healthy people, without clinical illness, living in endemic areas have melioidosis antibodies[36]. The seropositive IHA indicates exposure to other species of *Burkholderia*. Cross-reactivity between *Burkholderia mallei* and antibodies against *Burkholderia pseudomallei* has been previously observed due to their similar genomic sequences and multilocus sequence typing[37]. Furthermore, the IHA also detects seropositivity for *Burkholderia thailandensis* in healthy individuals residing in melioidosis-endemic regions. Approximately 44% of seropositive healthy individuals had isolates of *Burkholderia thailandensis*[38]. Combination of patient's clinical information, demographic profile, and appropriate laboratory test is a good strategy to improve better diagnosis and early initial antibiotic treatment.

The present study has both strengths and limitations. For strengths, this study tried to identify contributing factors for bacterial culture proven diagnosis and determined the appropriate cut-off point for IHA. However, there is a limitation to secondary data collection, as it may be susceptible to errors that can occur during the medical recording process.

RECOMMENDATIONS

Combination of IHA titer ≥ 1: 10,240 and a patient's clinical information should be considered and recommended in melioidosis clinical practice guidelines. Melioidosis diagnosis should be a concern for diabetic patients who have dyspnea.

The future study should be carried out by the use of primary data collection and focus on the household environmental factors possibly related to bacterial culture proven cases.

CONCLUSIONS

The melioidosis diagnosis consisted of several components i.e. clinical and laboratory components. Diabetic patients who have dyspnea symptoms may undergo testing for melioidosis infection. Consideration of the appropriate IHA cut-off point can help for better diagnosis and early antibiotic treatment.

ACKNOWLEDGMENTS

The author would like to acknowledge the public health officers who provided the prior information about the patients, computers, and supported devices for data collection.

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