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Melioidosis: Overview of seropositivity in Malaysia

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Abstract. Introduction: Melioidosis is endemic in Malaysia but prevalence data is limited. Methodology: In this cross-sectional study, sera suspected for melioidosis were received from local hospitals from year 2013 to 2014. These data were grouped into different age groups with more focus in children aged <15 years old. Results: Children <15 years old were more exposed to *Burkholderia pseudomallei* [Adjusted Odds ratio (AOR)=4.71, 95% Confidence Interval (CI): 4.04, 5.50) compared to the other age groups. Females, instead of males, had a slightly higher risk for melioidosis. There were more seropositive cases against melioidosis in eastern coast states (Kelantan, Terengganu, Pahang) and Sarawak. Sabah natives and non-Malaysians had higher risk of exposure to *B. pseudomallei*. Conclusion: Age group and region were independent risk factors for exposure against *B. pseudomallei*. Paediatric melioidosis is of concern and a marker against intensity of exposure.

INTRODUCTION

Melioidosis is an infectious disease caused by soil bacteria, *Burkholderia pseudomallei*. The disease is endemic in Southeast Asia including Thailand and Malaysia and also in northern Australia (Cheng & Currie, 2005; Puthucheary, 2009). Melioidosis was brought to attention due to the risk as a bioterrorism agent by Centers for Disease Control and Prevention, USA. There were also sporadic cases reported worldwide such as in Brazil and Africa [Cheng & Currie, 2005; Rolim *et al.*, 2005). To date, there is yet any vaccine available against melioidosis. Despite being an endemic region, compared to the neighbouring countries such as Thailand and Singapore, melioidosis is not categorised as notifiable disease in Malaysia. Therefore, the data regarding melioidosis in Malaysia are not well-established. In this report, the distribution of seropositive cases for melioidosis by age, gender, ethnicity, residential region in Malaysia was studied.

METHODOLOGY

Institute for Medical Research (IMR) is the reference laboratory for the serodiagnosis of melioidosis in Malaysia. Sera of all suspected cases from local hospitals were sent to IMR for laboratory diagnosis. The cross-sectional study was done within two years from year 2013 to year 2014. Sera received was tested for Immunoglobulin M (IgM) against *Burkholderia pseudomallei* by the Indirect Immunofluorescent Antibody Technique (IFAT) as described by Ashdown, 1981, using whole cell of antigen prepared from local *Burkholderia pseudomallei* isolate. The serum was defined as positive for the titre ≥1:160. In this study, only serum samples with completed demographic data (gender, age range, ethnicity and residential region) were included in our final data analysis. The age of the patients were categorised into eight categories from <15 to 75 years old. The ethnics portrayed in the data include Malaysian citizen which are Malay, Chinese,

Indian, Orang Asli, Sabah and Sarawak natives as well as non-Malaysian. Bumiputeras refer to Malay, Orang Asli, Sabah and Sarawak natives (Department of Statistics Malaysia). The analysis of seropositivity in different region in Malaysia was also included in the study. There were central (Kuala Lumpur, Selangor, Putrajaya, Negeri Sembilan); northern (Perlis, Kedah Perak, Penang); southern (Melaka, Johor), eastern coast (Kelantan, Terengganu, Pahang), Sabah and Sarawak inclusive of Labuan. The estimated incidence rate was calculated based on the population in Malaysia according to age, gender, ethnicity and region for year 2013 and 2014 (Department of Statistics Malaysia).

Statistical Analysis

All statistical analysis were performed using SPSS ver.19.0 for Windows (SPSS Inc., Chicago, IL, USA). Multivariable logistic regression analysis was conducted to determine the effect of each selected demographic factor (gender, ethnicity, age group, residential region) on the risk of seropositivity among the patients with suspected melioidosis infection. Variable selection was done by using enter method. Any potential two-way interactions were examined among the demographic in the final model. Hosmer Lemeshow test was applied to assess the goodness-of-fit of the final model. The extended Mantel Haenszel Chi Square for linear trend analysis was performed to investigate whether there was any dose-response relationship between age and risk seropositivity among children aged <15 years old.

RESULTS

During the study period, a total of 17,234 samples were received which consisted of 11,018 (63.9%) samples from males and 6,216 (36.1%) from females. One thousand eight hundred and nine samples (10.5%) were serologically positive based on the specified cut-off value at 1:160. Among the males, 1,117 (10.1%) whilst 692 (11.1%) of the females were seropositive. In Table 1, the estimated incidence of melioidosis per 100,000 population was higher in males at 4.8 (2013) and 2.4 (2014) as compared to females at 3.0 (2013) and 1.7 (2014). According to the age group, for both males and females, those classified under 45 to 74 years old had higher incidence rate than the other age groups. Bumiputeras constitute the largest share followed by Indian, non-Malaysian and Chinese. State of Sarawak was observed to be the region with the most numbers of melioidosis seropositive cases while the least cases were from southern region.

As shown in Table 2, age was the most significant risk factor especially children <15 years old (AOR=4.71, 95% CI:4.04, 5.50) for melioidosis seropositivity among the other demographic characteristics included in this study. There was a statistically significant association between region and risk of seropositive melioidosis. Eastern coast (AOR=1.47, 95% CI:1.27, 1.70) and

Table 1. Estimated Incidence of Melioidosis (per 100,000 population)* in 2013 and 2014

Demographic Characteristics	2013	2014
Gender		
Male	4.8	2.4
Female	3.0	1.7
Age group		
<15	3.7	2.0
15-24	2.2	1.2
25-34	3.0	1.1
35-44	4.6	2.0
45-54	5.9	3.8
55-64	6.3	3.7
65-74	6.4	3.3
>75	3.4	2.9
Ethnicity		
Bumiputeras[a]	5.0	2.5
Chinese	0.9	0.9
Indian	3.4	3.0
Non-Malaysian	3.9	1.2
Region		
Northern	2.7	1.9
Central	2.2	1.7
Southern	1.6	0.7
Eastern Coast	8.3	4.5
Sabah	1.6	0.8
Sarawak	11.9	4.1

[a]Includes Malay, Orang Asli, Sabahan and Sarawakian (Department of Statistics Malaysia, 2013-2014).

Table 2. Association of selected demographic factors with melioidosis seropositivity among suspected melioidosis infection patients

Demographic characteristics	Adjusted* OR	95%CI	*p* value
Gender			
Male	reference		
Female	1.12	1.01, 1.25	0.027
Age group			
<15	4.71	4.04, 5.50	<0.001
15-24	2.82	2.32, 3.42	<0.001
25-34	2.21	1.85, 2.64	<0.001
35-44	2.05	1.73, 2.43	<0.001
45-54	1.62	1.39, 1.90	<0.001
≥55	reference		
Ethnicity			
Malay	0.92	0.75, 1.13	0.446
Chinese	reference		
Indians	1.20	0.92, 1.57	0.184
Orang Asli	1.17	0.83, 1.66	0.373
Sabah native	1.61	1.12, 2.33	0.011
Sarawak native	1.27	0.98, 1.65	0.075
Non-Malaysian	1.37	1.06, 1.78	0.016
Region			
Central	reference		
Northern	1.32	1.12, 1.56	0.010
Southern	1.28	1.01, 1.62	0.045
Eastern Coast	1.47	1.27, 1.70	<0.001
Sabah	0.90	0.66, 1.24	0.520
Sarawak	1.46	1.19, 1.80	<0.001

Multiple variables logistic regression was performed. There were no significant two-way interactions among the independent variables in the final model. Hosmer- Lemeshow goodness of fit test indicated that model was fits (p=0.458). *adjusted for other variables.

Sarawak (AOR=1.46, 95% CI:1.19, 1.80) portrayed the highest risk association (p<0.001) with melioidosis compared to the other regions. Females were observed to be more exposed to the risk for melioidosis compared to males (AOR=1.12, 95% CI:1.01, 1.25). Melioidosis was also found to be common in Sabah natives (AOR=1.61, 95% CI:1.12, 2.33) and non-Malaysian group (AOR=1.37, 95% CI:1.06, 1.78). The Chi-squared test for linear trend showed that the increased in age was significantly associated with elevation in risk of melioidosis seropositivity (p=0.002).

DISCUSSION

Puthucheary's review had shown that most of the melioidosis patients have history of soil contact and underlying diseases such as diabetes type II (>60%); heavy alcoholism; renal diseases and others. The route of transmission is through inoculation, inhalation and ingestion (Puthucheary, 2009). However, paediatric melioidosis is of concern especially in endemic countries which includes Malaysia, Northern Thailand and Australia. Our study showed that children <15 years old was the most vulnerable group against melioidosis and this is in agreement with Wuthiekanun *et al.*, 2006. Our study was in concordance with fellow investigators which showed that there was an increasing trend of antibody titre towards *B. pseudomallei* antigen in children living in endemic region (Cheng *et al.*, 2008; Wuthiekanun *et al.*, 2006). We observed a sharp increase in cases after the age of three and the seropositivity of the children remained high at OR>2, p=0.002 followed by a descending trend after the age of eleven.

The reason could be due to the immature immune system in children and therefore easily infected by opportunistic pathogen, *Burkholderia pseudomallei* (Puthucheary *et al.*, 2009). In Malaysia, flash flood occurs every now and then especially in the early and end of the year. In Singapore, Liu and co-workers had reported that after a flood, the non-ambulated and elderly were the most diagnosed with melioidosis (Liu *et al.*, 2015). Our study showed that age group <15 years old remained the high percentile group indeed but the elderly did not exhibit similar trend. However, all the samples received were inclusive of wet and dry season.

Based on calculation for Malaysia from year 2013 to 2014, the average incidence rate per 100,000 people for melioidosis when compared to Thailand were as follows: <15 years old (Malaysia:2.85; Thailand:4.80); 15-24 years old (Malaysia:1.70; Thailand:2.30); 25-34 years old (Malaysia:2.05; Thailand: 5.70); 35-44 years old (Malaysia:3.30; Thailand:14.70); 45-54 years old (Malaysia: 4.85; Thailand:29.10); 55-64 years old (Malaysia:5.00; Thailand:45.4); 65-74 years old (Malaysia:4.85; Thailand:36.1); and >75 years old (Malaysia:3.15; Thailand:19.8) (Limmathurotsakul *et al.*, 2006). Generally, the average incidence rate in Malaysia is low compared to northeast Thailand where melioidosis is endemic but higher than Singapore (Limmathurotsakul *et al.*, 2006; Cheng *et al.*, 2008; Liu *et al.* 2015). In Malaysia, most of the seropositive cases were from eastern coast and Sarawak, most probably due to the active agricultural activity such as oil palm plantation and timber in both areas (Puthucheary, 2009). The interesting point is, despite low incidence rate, the younger generations were found to be more susceptible to melioidosis using multiple variables logistic regression, $p<0.001$. In adults, the peak age-specific incidence occurred in 55-64 years old, similar to Thailand (Limmathurotsakul *et al.*, 2010) and as reported by Puthucheary, 41-59 years old but differed from Singapore: >65 years old (Puthucheary, 2009).

We observed that females had higher exposure to *B. pseudomallei* compared to males. This is different when compared to previous reports from Northeast Thailand, Singapore and even local data from Kedah, Pahang and Kuala Lumpur that observed more positive cases in males [Limmathurotsakul *et al.*, 2006; Liu *et al.*, 2015; Puthucheary, 2009; Hassan *et al.*, 2010; How *et al.*, 2009; Pagalavan, 2005). However, gender was not a crucial factor as the AOR of females against males was minimal at 1.12, $p<0.05$. Since the natural reservoir of *B. pseudomallei* is soil, both males and females are generally exposed to the environment (soil) due to the outdoor activity such as gardening and farming.

As from ethnicity aspect, the average incidence rate per 100,000 people was high in Bumiputeras and the least in Chinese. The occurrence of this incidence may be due to the ethnic distribution in Malaysia (Hassan *et al.*, 2010; How *et al.*, 2009; JPM, 2015) whereby the Bumiputeras are the dominant population in Malaysia. Analysis using multiple variables logistic regression showed that Sabah natives (AOR=1.61, CI=1.12, 2.33, $p<0.05$) and non-Malaysian (AOR=1.37, CI=1.06, 1.78, $p<0.05$) were found to have significantly higher risk compared to the other ethnics. The reason may be due to the occupational activity. Most of the non-Malaysians were recruited in agricultural and construction sectors.

The interpretated data in this study was based on serodiagnosis of the suspected patients' sera. The risk association between clinical, occupational factors and melioidosis will warrant a further study. Since the clinical signs and symptoms of *B. pseudomallei* infected patients are similar with other diseases such as tuberculosis (Puthucheary, 2009), there is a high possibility of underestimation of the true scenario or undetected cases. Besides, culture still remains the gold standard for confirmation of melioidosis (Cheng & Currie, 2005; Puthucheary, 2009). However, serology provides as supporting diagnostic test for the true incidence of melioidosis (Ashdown, 1981). Misdiagnosis will lead to a fatal outcome especially in septicaemic cases because of the long duration oral therapy for melioidosis patients (Puthucheary, 2009). Inappropriate and inadequate period of

treatment will lead to relapse or recurrence of melioidosis episodes (How *et al.*, 2009).

CONCLUSION

In Malaysia, age and region were significant demographic risk factors against melioidosis. Children below 15 years old and those resided in eastern coast as well as Sarawak were observed to have the highest risk of exposure against melioidosis.

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Conflict of interests
No conflict of interest is declared.

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