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Assessing the national antibiotic surveillance data to identify burden for melioidosis in Malaysia

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ABSTRACT

Objectives: A leading cause of morbidity and mortality in Southeast Asia, the epidemiological data on melioidosis disease occurrence and mortality in Malaysia is not comprehensive. The aim of this study is to determine the burden of melioidosis and assess the National Surveillance for Antibiotic Resistance (NSAR) data as a potential tool melioidosis surveillance in Malaysia.
Methods: We performed a retrospective analysis on the *B. pseudomallei* reposited data submitted to the NSAR network between January 2014 and December 2020. The data were screened for information on patient demographics and specimen types. Additional patient comorbidities and outcomes were drawn from parallel surveillance for bacteremic melioidosis.
Results: The average annual incidence rate of melioidosis between 2014-2020 was 3.41 per 100,000 population and was significantly different between states (P <0.001). The highest incidence was observed in Pahang at 11.33 per 100,000 population. Individuals of Malay ethnicity, from the states of Pahang, Johor, Perak, and Negeri Sembilan aged 40-49, who were diabetic and working in agriculture-related sectors had a higher risk of succumbing to the infection.
Conclusion: Assessing the NSAR data proved to be a useful tool for the determination of the incidence and socio-demographic risk factors attributed to melioidosis in Malaysia.

Introduction

Melioidosis is a growing concern worldwide and is predominantly endemic in Southeast Asia, northern Australia, the Indian sub-continent, Africa, and South America [1]. The disease is caused by the environmental soil saprophytic Gram-negative bacterium *Burkholderia pseudomallei,* and infections usually occur via skin or inhalation [2,3].

In recent years, melioidosis has emerged as an important cause of morbidity and mortality, especially fatal community-acquired bacteremic pneumonia [4]. Environmental conditions, particularly the hot and humid weather of the tropics and agricultural activities increase the risk of infection [2]. Manifestation of this disease depends heavily on the patient's immune status with many of them suffering from pre-existing illnesses such as type II diabetes mellitus and chronic renal failure [2,5], or patients undergoing immunosuppressive treatments [6]. Clinical diagnosis of melioidosis is complicated and can lead to mixed interpretations as the symptoms are similar to other diseases such as tuberculosis, leptospirosis, dengue, and cancer [4,5,7]. A missed diagnosis may result in serious consequences especially among patients who develop sepsis where mortality rates can be as high as 50% [5,6,8].

Malaysia has a population of 32.37 million [9] spread over 13 states and two Federal Territories (Figure 1). The Malaysian health service encompasses multiple levels of health facilities which include polyclinics, specialist, and district hospitals as well as major and state hospitals (tertiary hospitals) [10]. The Ministry of Health Malaysia (MOH) has mandated the reporting of selected infectious diseases to assist in prevention, control, and identification of risk factors; however, this list does not include melioidosis. As such, the real national burden and prevalence of the disease are not known [5]. As melioidosis is not classified as a notifiable disease in Malaysia, the true number of cases is anticipated to be high, despite only a small number appearing in the medical literature. Any attempts to undertake a retrospective surveillance of melioidosis incidence in Malaysia are therefore significantly hampered by the lack of data. One approach to circumvent this limitation is to utilize the National Surveillance for Antibiotic Resistance (NSAR) program initiated by MOH to monitor and analyze antibiotic resistance of several

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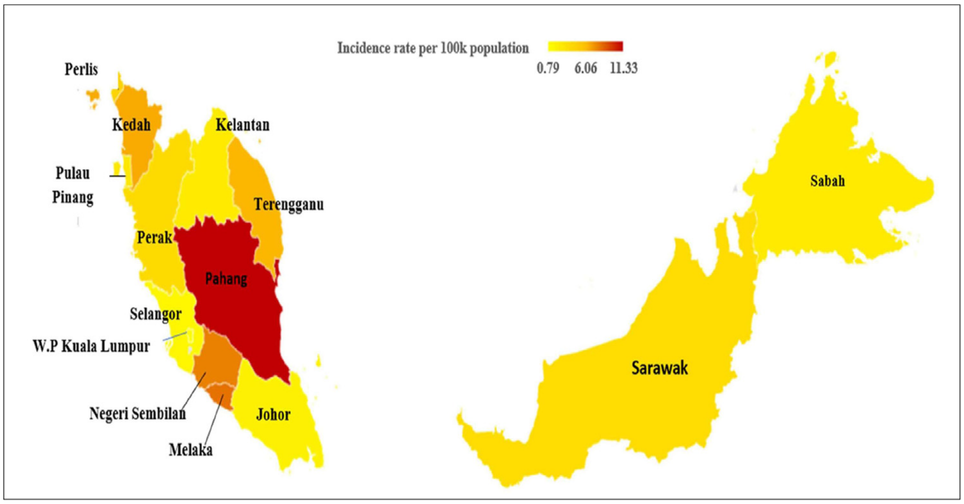

Figure 1. Average annual distribution of melioidosis patients per 100,000 population in Malaysia.

bacterial isolates in Malaysia [11]. Although there are 154 government hospitals throughout Malaysia, the NSAR program involves a network of microbiology laboratories from 43 hospitals across the 13 states of Malaysia including Sabah and Sarawak in Malaysian Borneo. These 43 hospitals are mainly tertiary health care institution, which receives and assist other primary and secondary health facilities (hospitals from district level) equipped with advanced medical laboratories (accredited by national/local EQA programmes), and infectious disease physicians that play a crucial role in the diagnosis and treatment of melioidosis. The NSAR data is routinely collected, monitored, and analyzed by the Bacteriology Unit, Institute for Medical Research, Malaysia.

B. pseudomallei has been reported to be inherently resistant to many antibacterial agents; hence, the microbiology laboratories in local hospitals contribute these resistance data to the NSAR programme database. Taking advantage of this resource of *B. pseudomallei* resistance data as an indicator of melioidosis cases, the present study was performed to evaluate the use of the NSAR resource for melioidosis surveillance in Malaysia. Using the data, we determined the incidence, sociodemographic and patient outcomes of *B. pseudomallei* culture-positive cases as reported by the Malaysian clinical microbiology laboratories from 2014 to 2020.

Methods

A retrospective and cross-sectional analysis was conducted using the NSAR database (https://imr.nih.gov.my/MyOHAR/index.php/site/archive_rpt). The database was screened for *B. pseudomallei* isolates submitted via the WHONET system (whonet.org) [12] between January 2014 and December 2020. The WHONET is a free desktop Windows application for the management and analysis of microbiology laboratory data with a particular focus on antimicrobial resistance surveillance developed and supported by the WHO Collaborating Centre for Antimicrobial Resistance Surveillance and Research (https://www.who.int/initiatives/glass/network) [13]. *B. pseudomallei* isolates were obtained from clinical samples of patients admitted to the local hospitals between 2014 and 2020. For each individual isolate, information was screened for patient demographics (gender, age and location) as well as source of specimen. Antibiotic susceptibility patterns were not analyzed due to variability in interpretation by the different participating hospitals during reporting. Data on race, occupation, patient comorbidities and outcome within the NSAR network was determined to be incomplete.

To fill these gaps in the surveillance data, isolates from melioidosis patients with bacteremia obtained by the Institute for Medical Research Malaysia (IMR) between 2019 and 2020 from all hospitals in the country, were referred. Patient information for 84 isolates was analyzed and correlated with sequence types (ST) [14]. This additional data was included to complement the NSAR surveillance data.

Categorical variables were analyzed and presented as frequencies (n) and percentages (%). The descriptive analysis was presented as mean and median. The chi-square test was used to compare percentages, and a P-value of <0.05 was considered statistically significant. For association statistical analysis, Principal Component Analysis was performed using the following variables: age, gender, ethnicity, location, patient comorbidities and patient outcome. The representative variables of the principle component were chosen based on their factor loadings.

Results

In total, 43 of 154 government hospitals in Malaysia routinely participate in the NSAR program (Supplementary Data 1). The 43 participating hospitals and laboratories were located in all the states of both Peninsular Malaysia (11 states and one Federal Territory) and Malaysian Borneo (Sabah and Sarawak). Culture-confirmed melioidosis was defined as ONE patient whose clinical specimen(s) displayed a positive *B. pseudomallei* culture.

NSAR data analysis (2014-2020)

A total of 17,840 culture-confirmed melioidosis cases were reported from 2014 to 2020 via the WHONET network software for NSAR reporting. The total number of patients reported was 9206 resulting in an approximately 1:2 (patient: isolate) ratio. The average annual melioidosis incidence rate during the 7-year study period was 3.41 per 100,000 population and was significantly different between states (P <0.001). However, we did not observe any clear trend throughout the years surveyed. The average incidence rate for melioidosis in states within Peninsular Malaysia was 3.39 per 100,000 population whereas in Sabah and Sarawak, it was 3.52 per 100,000 population. The highest incidence was observed in the state of Pahang at 11.33 per 100,000 population, followed by Melaka, Negeri Sembilan, Kedah and Terengganu (8.12 to 6.27 per 100,000 population). Lower incidence was noted in Selangor and Kuala Lumpur (Figure 1).

Out of the 9206 melioidosis patients from the NSAR database, 7365 (80.0%) were male with a median age of 50 (45-54) and this was the age group with the highest melioidosis incidence rate (Table 1). The most common clinical specimens that were culture positive for *B. pseudomallei* were blood (70.5%), followed by respiratory specimens (11.2%), pus (8.4%) and other specimens such as tissue, urine, conjunctival swab and ear swab (9.9%) (Figure 2).

Table 1
Data retrieved from the National Surveillance for Antibiotic Resistance surveillance database for culture-confirmed melioidosis cases reported by routine clinical microbiology laboratories in Malaysia.

Factors		Year of isolation						
		2014	2015	2016	2017	2018	2019	2020
Number of Isolates (n = 17840)		1594	1960	2090	3630	2738	2650	3178
Gender ratio (Male: Female) (7365:1841)		1:5	1:4	1:3	1:4	1:3	1:4	1:3
Age group (n = 4477)								
<14 (n = 171)		33 (7.5%)	16 (3.3%)	16 (2.9%)	25 (3.1%)	23 (3.2%)	22 (3.2%)	36 (4.6%)
15-24 (n = 177)		14 (3.2%)	24 (4.9%)	19 (3.5%)	31 (3.8%)	27 (3.8%)	26 (3.8%)	36 (4.6%)
25-34 (n = 344)		35 (7.9%)	40 (8.2%)	46 (8.4%)	53 (6.5%)	57 (7.9%)	57 (8.2%)	56 (7.1%)
35-44 (n = 735)		62 (14.1%)	73 (15.1%)	106 (19.4%)	118 (14.5%)	121 (16.9%)	106 (15.3%)	149 (18.9%)
45-54 (n = 1152)		113 (27.7%)	125 (25.8%)	137 (25.1%)	206 (25.4%)	205 (28.7%)	184 (26.6%)	182 (23.0%)
55-64 (n = 1155)		113 (25.7%)	123 (25.4%)	146 (26.8%)	222 (27.4%)	167 (23.4%)	190 (27.4%)	194 (24.6%)
>65 (n = 743)		69 (15.7%)	83 (17.1%)	75 (13.8%)	156 (19.2%)	115 (16.1%)	108 (15.6%)	137 (17.3%)
Number of patients by location								
Peninsular Malaysia	Perlis	6	12	10	16	18	13	11
(n = 6302)	Kedah	113	122	60	218	170	171	175
	Pulau Pinang	45	36	33	77	57	59	61
	Perak	58	98	125	155	81	77	87
	Selangor	87	76	94	115	115	108	112
	Kuala Lumpur	21	16	18	14	5	7	11
	Negeri Sembilan	38	79	72	111	128	116	109
	Melaka	0	0	39	55	52	57	54
	Johor	40	50	67	75	75	69	71
	Pahang	157	159	141	263	170	140	159
	Terengganu	58	62	61	77	71	64	67
	Kelantan	49	44	60	53	43	39	43
Borneo (n = 1509)	Sabah	55	102	144	97	75	95	93
	Sarawak	64	114	101	101	96	137	151

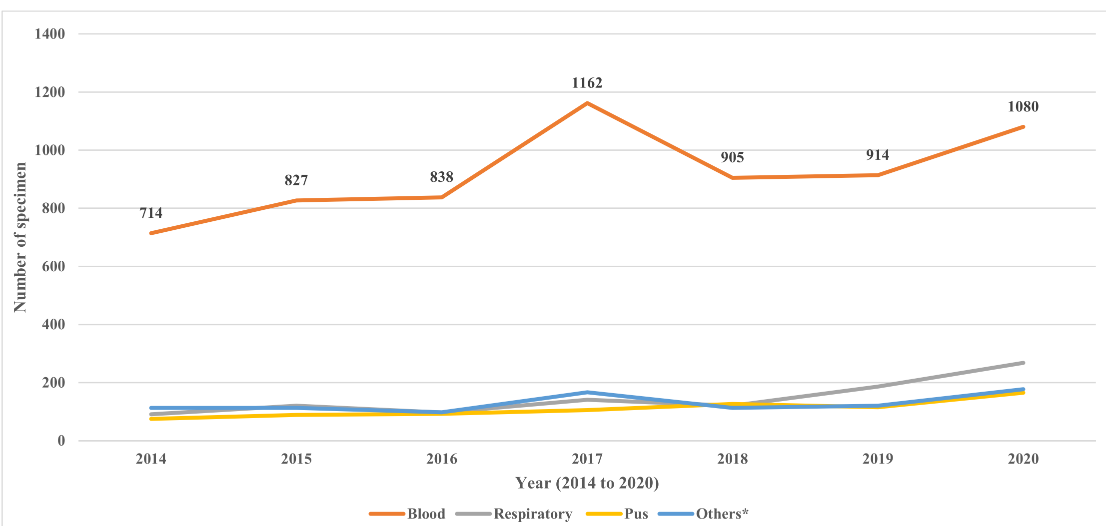

Figure 2. Distribution of *B. pseudomallei* reported in the National Surveillance for Antibiotic Resistance database isolated from different specimen types.

Sociodemographic analysis of bacteremic melioidosis patients

The sociodemographic data on the 84 melioidosis patients with bacteremia sourced from IMR demonstrated similar trends with the NSAR data whereby there was a preponderance of males (77.4%) with a mod age of 52 and median of 49-50 years old (Table 2). The Malay community had a higher risk of infection at 57% in comparison to Chinese, Indians and other ethnicities in Malaysia. Bacteremia was common among patients whose occupation was associated with the agriculture sector (Table 2) and the Malay community was more commonly related to occupations in this sector.

Principal component analysis was conducted to determine a cluster of patients with a higher incidence of bacteremic melioidosis in Malaysia. Seven variables were identified based on the patients' sociodemographic data and were correlated to the vectors (states in Malaysia) to determine the risk distribution pattern (Figure 3). Based on the analysis, bacteremic melioidosis patients were grouped into three clusters. The first cluster comprised of patients from the states of Kedah, Pahang, Johor, Perak, Negeri Sembilan and Terengganu. The variables indicated that the patients from these states are mainly Malay men aged 40-59 years old, active in agriculture-based activities/occupations, are diabetic and succumbed to melioidosis. The second cluster comprised of patients from the state of Melaka and Kuala Lumpur, Federal Territory as well as Sabah and Sarawak. The patients from Peninsular Malaysia from this cluster are not involved in agriculture-related activities but suffer from hypertension, a condition that coexists with other comorbid factors such as type II diabetes and dyslipidemia, also associated with melioidosis. Nonetheless, these patients recovered after treatment. The other groups in this cluster were patients from Sabah and Sarawak comprised of members of the Iban, Bidayuh, Ulu, Kadazan, Murut and

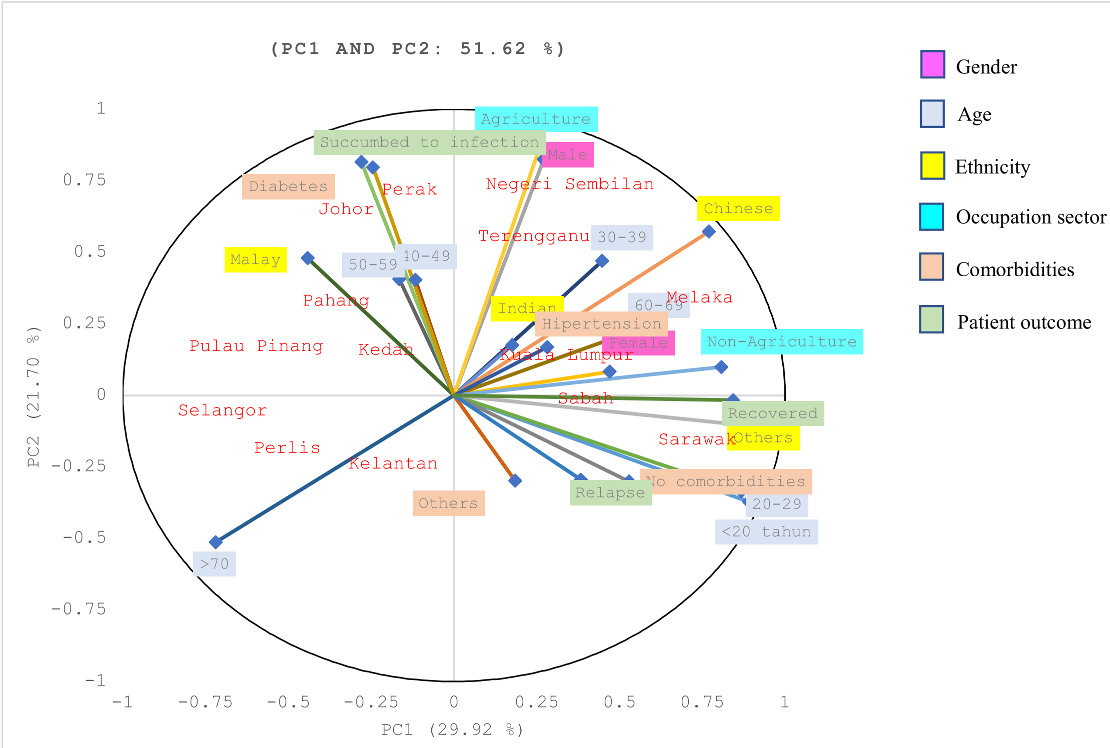

Figure 3. Correlation between demographic factors using principal component analysis. PC1 and PC2 of the principal component analysis show the correlation between the socio-demographic and clinical factors of melioidosis patients. Vectors (states in Malaysia) are correlated to variables (socio-demographic/clinical factors) based on the distribution angle. Variables that are distributed within the vector angle of >90° have higher correlation and variables with vector with angle of <90° are negatively correlated.

Bajau indigenous communities (categorized as others in the PCA plot). These patients were less than 29 years old, with no underlying disease with two exceptions of patients with urosepsis and colorectal cancer. The patients from cluster two of the PCA analysis either recovered after treatment or relapsed. The third cluster based on the PCA analysis comprised of the states of Pulau Pinang (Penang), Selangor, Perlis and Kelantan. The patients in this cluster mainly fell into the age group of 40-59 years old or >70 years old. The patients from this cluster were also at risk of underlying diseases such as diabetes and other diseases.

Discussion

Melioidosis was first documented in Malaysia during an outbreak in 1913 [15] and is now recognized as being endemic in this country. Despite this, the MOH has not designated melioidosis as a notifiable disease in Malaysia and the true incidence of the disease nationwide is unknown. The NSAR is a surveillance program to monitor and analyze antibiotic resistance of major clinical bacterial isolates in Malaysia. We took advantage of the NSAR data to complete this first retrospective analysis on the nationwide burden of melioidosis. To circumvent limitations in the information and data available within the NSAR, we incorporated the data from an additional 84 bacteremic melioidosis cases that were collected independently by the Institute for Medical Research, Malaysia. Together the collective data provided a more comprehensive picture of the incidence of melioidosis in Malaysia over the period of 7 years spanning 2014-2020, as well as enabled a correlation of the sociodemographic data with patient comorbidities and outcomes.

Melioidosis, as an infectious disease, is a challenge to diagnose but trained personnel in the clinical microbiology laboratories at the representative hospitals successfully reported 17,840 cases during the 7 years (2014-2020) or an average of ~1315 cases per year. This trend is similar to that of culture-confirmed melioidosis cases reported from Thailand, which borders northern Malaysia [16]. However, *B. pseudomallei* is often misidentified or disregarded as a contaminant [17] and this may indicate underreporting of the actual number of isolates. Laboratory isolation and identification of *B. pseudomallei* can take from 2-7 days and the use of selective culture media such as Ashdown or Francis media is limited [18]. Laboratory technicians need to be trained to make accurate observations and to identify *B. pseudomallei* growing on common media such as blood agar precisely. We predict that the estimated disease burden may be higher in Malaysia than reported in the NSAR network. Similarly, the predicted global distribution of *B. pseudomallei* suggests that melioidosis is severely underreported in other endemic countries [1].

Evaluating the incidence of a communicable disease among different geographical locations could highlight areas where diagnosis or monitoring requires additional attention [16,19,20]. Based on the reports extracted from the NSAR network, the average incidence rate of melioidosis during the 7-year study period was 3.41 per 100,000 population per year, in Malaysia. Similarly, analysis of 7126 *B. pseudomallei* (2012 to 2015) in Thailand also had an average incidence rate of 3.95 per 100 000 population per year during the 4-year study period [16]. The incidence rate in Malaysia was higher in the state of Pahang (11.33 per 100,000 population) than any of the other states in Malaysia. Other states with relatively high incidence were Melaka, Negeri Sembilan, Kedah and Terengganu. Previous studies have calculated annual incidences for melioidosis ranging from 4.3 per 100,000 in the state of Pahang [21,22] to as high as 16.35 per 100,000 in the state of Kedah [23]. The high incidence level in these areas was proposed to be linked to ecological factors as well as social factors, predominantly the agriculture-based occupation of the infected individuals.

The 2020 Malaysian census [9] reports an equal proportion of males and females, however our analysis shows that males have a higher risk of contracting melioidosis. This is not surprising as males are more often

Table 2
Demographics of bacteremic melioidosis patients collected by Institute for Medical Research Malaysia from 2019 to 2020.

Factors	Number of patients (n)	Percentage distribution (%)
Age distribution (n = 59)		
<30 years old	4	6.8
30-39 years old	6	10.2
40-49 years old	14	23.7
50-59 years old	16	27.1
60-69 years old	12	20.3
>70 years old	7	11.9
Gender (n = 84)		
Male	65	77.4
Female	19	22.6
Race (n = 84)		
Malay	48	57.1
Chinese	13	15.5
Indian	9	10.7
Other races	14	16.7
Occupational sector(n = 48)		
Agriculture	25	52.1
Non agriculture	23	47.9
Health status (n = 84)		
Diabetes	60	71.4
Hypertension	3	3.6
Other comorbidities	8	9.5
No comorbidities	17	20.2
Treatment outcome (n = 84)		
Treated/ Alive	25	29.8
Relapse	15	17.9
Succumbed to infection	44	53.4

associated with agriculture-based work activities such as farming in the corresponding states with high incidences, for example Kedah (paddy farming) and Pahang (oil palm and rubber plantations). More middle-aged (40-60 years old) individuals are at risk of *B. pseudomallei* infection particularly diabetics. Type II diabetes is a prominent risk factor for melioidosis and a significant number (981,398) of Malaysians age are registered in the National Diabetes Register [24]. Furthermore, the registry also reports that the ethnic group that makes up the highest proportion of type II diabetics are Malays [24] which correlates well with our findings of higher cases of melioidosis reported among middle-aged Malays. The analysis of the NSAR shows that 70.5% of the melioidosis patients were blood culture positive indicating bacteremia. Further interrogation into the bacteremic cases data indicated that, 71.4% of the bacteremic melioidosis patients are diabetic. A previous study in Perak, Malaysia [25], had also reported that 71.1% of its melioidosis patients were diabetic. This finding suggests diabetes mellitus as a significant risk factor for melioidosis with a probability, three times more likely to be infected by melioidosis than non-diabetic patients [26].

In addition, a study on seropositivity of melioidosis patients by Hadi et al. [27], which analyzed more than 26,000 sera samples (collected from 2015 to 2019), found that the majority of the seropositive patients were aged over 55 years old with the highest number of cases reported from Sarawak (15.1%), followed by Pahang (13.9%) and Kuala Lumpur (11.3%). Previous reports from Malaysia had similar findings as well with higher melioidosis incidence among male patients, aged 40 and 60 years of age from the Malay ethnicity. Similar finding was also reported from Thailand with 68% of the melioidosis patients being male and aged 44.5-63 years old [16]. Socioeconomic, ethnicity and geographical factors play an important role in the distribution and impact of melioidosis. It was reasoned that there is a higher potential for males from the Malay ethnicity to be involved in soil-related occupations and activities facilitating exposure especially among the Malay ethnicity who are rice paddy farmers and agriculture employees predominant in certain states such as Pahang, Kedah and Kelantan [4,5,28]. A study from Australia, reports socioeconomic disadvantages as a risk for melioidosis infections among aboriginals and Torres Strait Islanders [29].

Our analysis on the data available within the NSAR database suggests that younger patients, particularly children, are less susceptible to a *B. pseudomallei* infection. However, a recent study on melioidosis in Malaysian children estimated an average annual incidence of culture-confirmed melioidosis at ~ 5.5 per 100,000 children in Sarawak [30]. The 7-year study on pediatric patients in Sarawak also reported that although only 17% of children had culture-confirmed melioidosis, the other pediatric patients had spleen abscesses, typically with a Swiss cheese appearance or small dispersed lesions, highly suggestive of melioidosis [30]. There is a clear disease manifestation variation among children from rural regions especially from Sarawak, and while the reason for this variation may remain unclear [5], socioeconomic backgrounds may play an important role. It has been reported that in Sarawak, 80% of children with melioidosis reside in traditional longhouses in rural areas. In these areas, gravity-fed water systems supply homes with untreated water from streams and some low-income families generally present with poor nutritional status [30]. Further to these findings in Sarawak, reports from Sabah [31] and Pahang [22] indicate that most of the pediatric melioidosis patients have underlying medical illnesses such as diabetes mellitus, chronic renal failure and thalassemia major.

The 7-year analysis of the NSAR data spanning 2014 to 2020 shows that *B. pseudomallei* is most commonly isolated from blood. This is an indication of bacteremic melioidosis whereby septic shock is known to occur in one-fifth of melioidosis cases and a mortality of 100% [8]. Our two-year surveillance on bacteremic melioidosis from 2019 to 2020 supported this finding whereby almost 50% of the patients succumbed to infection. This may be due to the fact that almost 79.8% of the patients had an underlying disease, with ~ 60% diabetic. Overall, middle-aged Malay men from the states of Pahang, Johor, Perak and Negeri Sembilan have a higher risk of dying from bacteremic melioidosis. The high mortality in Malaysia reflects delays in timely and accurate diagnosis since melioidosis mimics other tropical diseases, lack of laboratory resources such as point-of-care test in rural areas, and delays in patients especially in rural area to seek treatment. In our previous study, we have characterized these isolates based on their ST which revealed a low diversity. The common STs identified were ST84, ST54, ST46, ST51 and ST289 with a close association to Southeast Asian isolates [14].

Despite its incompleteness, the NSAR database was able to provide sufficient information to determine the incidence and sociodemographic distribution of melioidosis in Malaysia. The analysis highlighted the overall incidence at both country and state level with a number of states being deemed as localities of concern. There were some limitations of this study: The NSAR data was only based on the data reported by 43 hospitals out of 154 hospitals throughout the country as well as reports from private health care institutions that may lead to sampling error. Full participation of all government and private hospitals in the country is important to identify the true burden of melioidosis in Malaysia. Another limitation of the NSAR will be that the input data is based on annual reporting which hampered associating the month when the pathogen was isolated with the corresponding weather at the time. Nevertheless, until mandatory notification for melioidosis is decreed, the true incidence of melioidosis is undoubtedly higher than the actual reported case incidences and the findings from our study may only be an indicator of the tip of the iceberg.

Conclusion

This study highlighted the incidence and sociodemographic factors for melioidosis in Malaysia using the NSAR data, which proved to be a useful tool to determine the incidence and sociodemographic distribution of melioidosis in Malaysia. Although mandatory notification would be useful, it is hoped that the findings from this study contribute to creating awareness among healthcare personnel and the general public on the incidence and burden of melioidosis in the country.

Declarations of competing interests

The authors have no competing interests to declare.

CRediT authorship contribution statement

Revathy Arushothy: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **Mohammad Ridhuan Mohd Ali:** Conceptualization, Data curation, Formal analysis, Writing – review & editing. **Hana Farizah Zambri:** Project administration, Data curation, Resources. **Rohaidah Hashim:** Project administration, Resources, Conceptualization, Funding acquisition, Supervision. **Sylvia Chieng:** Supervision, Validation, Visualization, Writing – review & editing. **Sheila Nathan:** Conceptualization, Data curation, Formal analysis, Supervision, Validation, Visualization, Writing – review & editing.

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Ethical approval

The Malaysian Research Ethics Committee and National Medical Research Registry approved this study under the protocol number NMRR-18-805-41423.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2023.11.014.

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