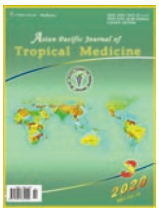

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Etiologies of tropical acute febrile illness in West Pahang, Malaysia: A prospective observational study

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ABSTRACT

Objective: To determine the etiologies of tropical acute febrile illness (TAFI) in West Pahang, Malaysia and to investigate morbidity and mortality factors in relation to TAFI.

Methods: A multicenter prospective cohort study was conducted between January and June 2016 in six district hospitals throughout the western part of Pahang State in Peninsular Malaysia. A total of 336 patients answered a standardized questionnaire and blood samples were collected for laboratory confirmation of infectious etiology. Descriptive analysis and logistic regression were performed to identify factors associated with TAFI.

Results: A total of 336 patients were included. The patients were mainly Malays (70.2%), males (61.3%), aged (44.6±17.4) years, with more than half (58.9%) presenting with gastrointestinal symptoms. The majority were diagnosed with dengue (35.7%) while malaria (4.5%) was the least frequent. The in-hospital mortality due to TAFI was 9.2%. Patients with meliodosis had five times higher mortality [Adjusted *OR*: 5.002, 95% *CI*: (1.233, 20.286)]. Patients with comorbidities such as cardiovascular symptoms (P<0.001) and renal replacement therapy initiation (P<0.001) were significantly associated with in-hospital mortality in all TAFI.

Conclusions: The etiology of TAFI in the western Pahang includes dengue, leptospirosis, malaria and melioidosis, which carry the highest risk of in-hospital mortality. The presence of cardiovascular symptoms may be used to assess the disease severity in TAFI, but more studies are needed in the future.

KEYWORDS: Comorbidities; Mortality; Tropical acute febrile illness

1. Introduction

Tropical acute febrile illnesses (TAFI) are the major causes of febrile illness in the rural areas of Southeast Asia and also involving the urban areas to certain extent. TAFI includes dengue fever, leptospirosis, malaria, scrub typhus and melioidosis have been well documented in these countries. However, limited studies had explored the determinants of these TAFI etiologies. Awareness on the etiology of TAFI will vastly improve the probability of an accurate clinical diagnosis which is important for health planners as well as clinicians in order to execute decisive management[1]. There have been few reports on the etiologies of these TAFI, recently mainly in Thailand[2], among children in Asian countries[3] and Malaysia published in 1984[4]. Since then, laboratory methods have been improved on the diagnoses thus increasing the numbers of detection cases.

According to data from the Institute for Health Metrics and Evaluation, the estimated global burden of neglected tropical diseases including Malaria was 9.42 deaths per 100 000 population

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with 815.2 disability adjusted to life-years per 100 000 population[5]. Among all these neglected tropical diseases, dengue has shown substantial increment on the prevalent cases over time with 610.9% change since 1990 globally[6]. Dengue has also contributed to more global mortality, where 1.27 million deaths reported as compared to other vector-borne diseases such as Malaria, which has shown substantial decreases in rates by 18% globally[7]. Other tropical diseases which are underestimated such as leptospirosis had caused more than 1 million severe cases and 58 900 deaths per year[8], while human melioidosis contributed to an estimated of 165 000 cases per year and 89 000 deaths[9].

All these diseases are more common in the tropics especially in Malaysia. Several studies have shown that dengue, rickettsia diseases, and leptospirosis are the main causes of febrile illness[2,4], although a large proportion had an unknown etiology with low hospital mortality, of which 0.5% mortality of all febrile patients. However, the infective causes of fever and their relative distributions may differ between district and large tertiary hospitals due to types of occupations, exposure and environment of the surrounding populations. This is highlighted by the leptospirosis outbreak in foreign competitors who participated in the Eco-Challenge competition in Sabah who had extreme environmental exposure[10]. Hence, this study aimed to identify the etiologies of tropical acute febrile illnesses in West Pahang and its related morbidity and mortality factors.

2. Materials and methods

2.1. Study site

A multicenter prospective cohort study was conducted among patients admitted for acute febrile illness at six district hospitals throughout the west part of Pahang, which is the largest state Peninsular Malaysia. Of these selected hospitals, two are tertiary hospitals, which serve as referral centers within west Pahang (Figure 1).

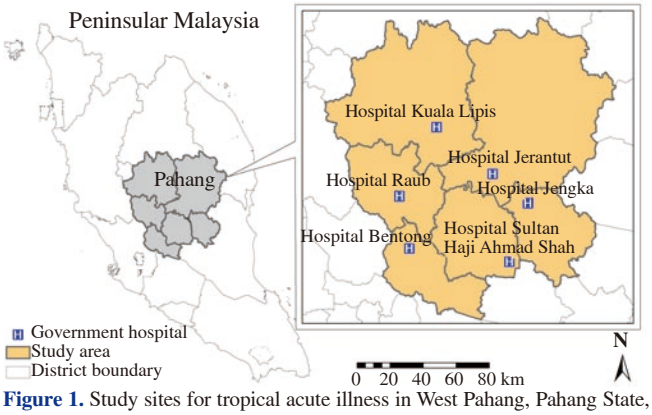

Figure 1. Study sites for tropical acute illness in West Pahang, Pahang State, Malaysia.

Study inclusion were patients aged at least 18 years old with documented fever of 38 degrees Celsius, consented to participate in this study and admitted to the study centers. The study excluded patients who are less than 18 years old, non-consensual, immunocompromised, pregnant, diagnosed with nosocomial or chronic infections as well as fever with non-infectious etiology. Each patient who fulfilled eligible criteria were given an informed consent form to participate in this study. Figure 2 shows participants selection, inclusion and exclusion in this study. Investigators collected information on the epidemiologic, clinical and biochemical data information through a standardized data collection sheet and blood samples were obtained for laboratory determination of infectious agents. Patients with acute kidney injury and acute hepatitis were called to return to hospital sites within 1 to 2 weeks for voluntary follow-up visit for repeated renal profile or liver function tests respectively.

2.2. Ethics

This study was approved by Medical Research and Ethical Committee, Ministry of Health, Malaysia with the registration number NMRR-17-2446-35529. The verbal consent was obtained prior to starting the study. Reporting of this study is following the STROBE guideline[11].

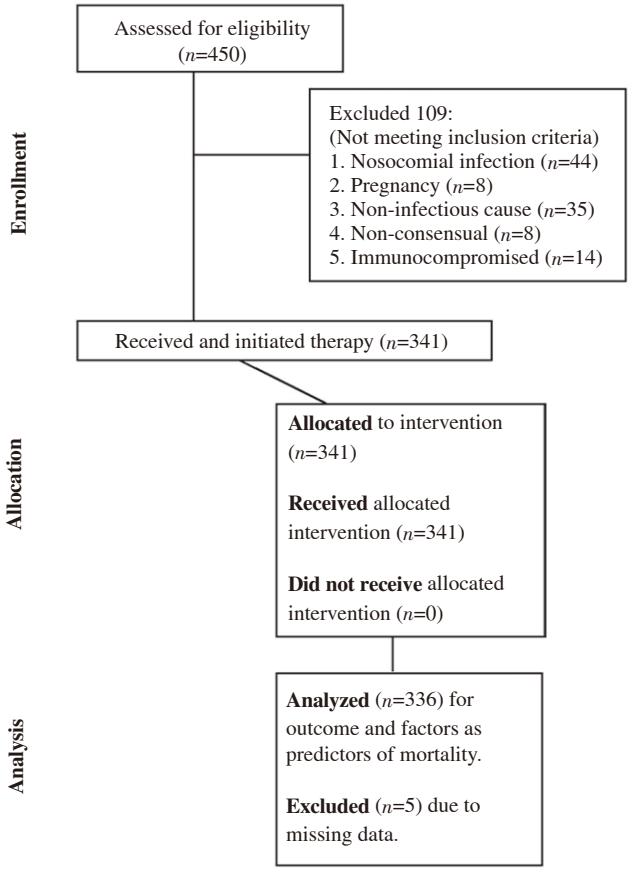

Figure 2. Diagram of participants evaluated and enrolled in this study.

2.3. Data collection

The study was carried out from January 2016 through June 2016. All patients who were admitted to any of the study hospitals and who had met the inclusion criteria(s) of the study recruitment, fulfilled case definition for acute febrile illness (AFI) and consented to participate were included in the study. A total of 341 patient were invited to participate in this study. Of these, 336 patients agreed to participate giving rise to 98.5% response rate. Patient's information of baseline blood tests was obtained such as full blood count, renal profile, liver function tests, venous blood gases and blood film for malaria parasite, including blood cultures (aerobic and anaerobic). Dengue NS1 (Non-Specific-1) antigen and dengue serology were taken on a selective basis based on strict indications of either patient resides in a dengue-prone area or patient with clinically and biochemically suggestive of viral fever residing in dengue prone area with evidence of thrombocytopaenia and leucopaenia on the blood counts.

2.4. Case definitions

Tropical acute febrile illness was defined as all acute febrile syndromes with oral temperature over 37.5 ℃ within the last 24 hours and less than two weeks in tropical and sub-tropical regions[12]. To distinguish types of diseases associated with acute febrile illness, we had established case definition for each specific disease in accordance to a guideline from the Case Definitions for Infectious Diseases in Malaysia published by the Disease Control Division, MOH[13].

Confirmed asymptomatic malaria was defined as a person with no symptoms and/or signs of malaria who showed laboratory confirmation of parasitemia. Confirmed symptomatic malaria is diagnosed in a person with symptoms of malaria: fever, headache, back pain, chills, myalgia, vomiting diarrhoea and common signs of anaemia and/or splenomegaly, with laboratory confirmation of parasitemia. Laboratory-positive malaria was any patient with malaria parasite [trophozoites of *Plasmodium* (*P.*) *falciparum*, *P. knowlesi*, *P. vivax* or mixed] visualized on thin & thick blood film smears.

A confirmed case of dengue fever is defined as a case that compatible with the clinical description of fever and either two of the following symptoms of vomiting, rash, aches, leukopenia, positive tourniquet test and laboratory confirmation. Ideally paired serum samples are required after an interval of 10 to 14 days apart. Laboratory-positive dengue case is defined by a patient with suggestive history AND one of the following: (1) negative to positive anti-DENV IgM seroconversion in paired serum specimens, or (2) a single positive anti-DENV IgM result in an acute-phase or convalescent-phase specimen, or (3) a positive DENV antigen detection by NS1 rapid test.

A confirmed case of leptospirosis is a clinical case of acute febrile illness after a history of possible water or environmental exposure contaminated with infected animal urine and with any one of the laboratory tests consisting of microscopic agglutination tests (MAT), positive culture, or positivity of two (2) different rapid diagnostic tests.

Confirmed case of melioidosis is defined as any suspected cases with positive cultures for *Burkholderia* (*B.*) *pseudomallei* or positive PCR or a four-fold rise in serological titre.

Undifferentiated acute febrile illness (UAFI) is defined as acute onset of febrile illness after complete evaluation, without a definitive diagnosis made with negative serologies test and blood culture report.

Bacteraemia is the presence of bacteria in the bloodstream and evidenced by blood culture positive and microorganism identification tests. Persons with bacteremia usually developed a symptom such as fever, nausea, vomiting, abdominal pain, diarrhea etc.

2.5. Laboratory investigation

Culture & Isolation: All blood culture samples were inoculated in blood culture bottles and incubated in automated blood culture analyzer (BD BACTEC®) at the respective hospitals. An analyzer alerted the end user via alarm system if positive blood culture was detected and proceed for further processing. Gram stain was done from positive blood culture bottle followed by sub culturing the respective samples onto agar plate, which include blood agar (BA), MacConkey agar, Chocolate agar, and Francis agar. The samples were sub cultured onto Sabouraud dextrose agar if yeast or mold were seen by gram stain. Anaerobic culture using anaerobic blood agar was done if blood culture was positive from anaerobic bottle. Initial identification was done using in house biochemical set test. Further identification was done in case of doubtful or unidentified organism based on in house biochemical set test.

Antibiotic sensitivity test was performed after adequate growth on culture plates. Antibiotic panel was tested and agar plate was used for antibiotic sensitivity test for specific organisms isolated based on the latest Clinical and Laboratory Standards Institute for antibiotic susceptibility testing.

Leptospirosis study: All patients who had blood sample taken for Leptospirosis serology were performed at the Kuala Lipis Hospital and Sultan Haji Ahmad Hospital, Temerloh. The test was performed using Leptorapide latex agglutination test by Linnodee Diagnostics®. Those who tested positive for IgM were further tested for antibody detection using the Micro agglutination test (MAT) which is a gold standard test for leptospirosis at the Institute for

Medical Research (IMR) in Kuala Lumpur. If the serum sample was positive, the serum sample reacted with live antigen and showed agglutination. More serovar used more sensitivity of test and based on IMR study, the sensitivity and specificity of MAT test were 92% and 98%. Paired sample is crucial to improve sensitivities and specificities of MAT. It is also important to note that a patient's serum IgM may be positive 5 to 10 days after onset of symptoms but not usually before this, and may remain detectable for several years. The confirmation criteria used for single serum specimen is a titre of 1: 400 and for the paired sera sample is four-fold or greater rise in titre.

Malaria study: All patients had samples taken for thick and thin blood film malarial parasites test. Giemsa stain was used to stain the smear and observed under objective microscope to determine the presence of malarial parasites. Blood sample in EDTA were sent to IMR for PCR test if parasite identified as *P. knowlesi* or any doubtful species identification.

Melioidosis study: *B. pseudomallei* is a gram-negative bacteria with bipolar or 'safety pin' appearance by gram stain appearance. Samples for blood culture, sputum, tissue, pus aspirate, and body fluid aspirate were cultured onto Francis media, which was selective and a differential culture media for *B. pseudomallei*. The organism appeared as metallic sheen colonies on MacConkey agar with yellow haze on Francis media. Some strains may appear as wrinkle colonies on blood agar. Confirmation of identification was done using Automated identification system VITEK® 2 Compact or commercial manual identification API® kits. Serology for *B. pseudomallei* was done at the IMR using in-house Enzyme-linked immunosorbent assay (ELISA) for detection of antibodies. However, serology alone should not be used for the diagnosis of melioidosis, as assessing the result is difficult due to the high background reactivity in endemic regions[14,15].

2.6. Data analysis

Data were cross checked and verified before data analysis. Data was analysed using Stata version 12 (Stata Corp). Analysis of descriptive statistics was done to describe the sociodemographic and clinical characteristics of patients with TAFI. All continuous variables were described in mean and standard deviation, while categorical variables were described in percentages and frequencies. Bivariate analysis with *Chi*-square test was done to examine any associations between clinical characteristics and types of TAFI. Preliminary bivariate analysis was done to estimate crude odds ratio for each selected variable associated with in-hospital mortality. All significant independent variables that significant or any clinically important variables were included in the final model.

Controlling for covariates, there were five multiple logistic regression models that examined dependent variable (in-hospital mortality) with each type of independent variable of TAFI. Model 1: Leptospirosis, Model 2: Dengue, Model 3: Bacteraemia, Model 4: UAFI, and Model 5: Melioidosis. Malaria was omitted from the model because there was no in-hospital mortality associated with Malaria. Interactions were assessed to ensure any scientifically relevant covariates might affect multi-collinearity. The goodness of fit test was done to ensure the fit for each of the models. Final model was created for each model that include all covariates that have significant association with in-hospital mortality. Significant associations were accepted for all adjusted odds ratio estimates, which do not include the null or P-value < 0.05.

3. Results

The majority of which were males and Malay ethnic origin. The mean age of the respondents was (44.6±17.4) years old, for alive and in hospital mortality patients, the mean age were (43.5±17.3) and (56.1±15.5) years old, respectively. As depicted in Table 1, unemployed (53.6%) and non-agricultural sector (31.9%) predominant occupation, followed by the agricultural group (14.6%). The agricultural group consists of patients mainly working in rubber and oil plantations. The unemployed group comprises of retirees, pensioners, unemployed, housewives and prisoners.

For all TAFI patients, the median for length of stay was 5 days (IQR=4), which is the same with the median of fever duration, 5 days (IQR=4). Most patients were diagnosed with dengue while malaria patients were the least. Majority of the patients did not initiate renal-replacement therapy (RRT) and developed no acute kidney injury (AKI). There were 9.2% in-hospital mortality (n=31) due to TAFI and more than half patients presented with gastrointestinal symptoms, as shown in Table 2.

Table 1. Sociodemography characteristics of tropical acute illness patients [n (%)].

Characteristics	Total (n=336)	Alive (n=305)	In-hospital mortality (n=31)
Sex			
Male	206 (61.3)	189 (91.7)	17 (8.3)
Female	130 (38.7)	116 (89.2)	14 (10.8)
Race			
Malay	236 (70.2)	216 (91.5)	20 (8.5)
Chinese	43 (12.6)	37 (86.0)	6 (14.0)
Indian	26 (7.7)	22 (84.6)	4 (15.4)
Others	13 (3.9)	12 (92.3)	1 (7.7)
Foreigners	18 (5.6)	18 (100.0)	0 (0.0)
Age, years			
<40	137 (40.8)	133 (97.1)	4 (2.9)
40-59	125 (37.2)	111 (88.8)	14 (11.2)
>60	74 (22)	61 (82.4)	13 (17.6)
Occupation			
Agriculture	49 (14.6)	47 (95.9)	2 (4.1)
Non-agriculture	107 (31.9)	98 (91.6)	9 (8.4)
Unemployed	180 (53.6))	160 (88.9)	20 (11.1)

Table 2. Clinical characteristics of tropical acute febrile illness patients (n=336).

Clinical characteristics	Total [Median (IQR)/n (%)]	Alive [Median (IQR)/n (%)]	In-hospital mortality [Median (IQR)/n (%)]	P-value
Length of stay (d)	5.0 (4.0)	4.0 (4.0)	5.0 (9.0)	0.711[#]
Duration of fever (d)	5.0 (4.0)	5.0 (4.0)	4.0 (5.0)	0.285[#]
Admission HB (g/dL)	13.8 (3.0)	13.9 (3.0)	12.1 (4.0)	<0.001[#]
Admission WCC ($\times 10^9$/L)	6.6 (9.2)	6.4 (8.6)	13.7 (14.1)	<0.001[#]
Admission platelet count ($\times 10^9$/L)	139.5 (150.0)	132.0 (140.0)	228.0 (181.0)	0.012[#]
Admission urea (mmol/L)	4.6 (3.7)	4.3 (3.2)	11.5 (7.0)	<0.001[#]
Admission creatinine (μmol/L)	94.0 (43.5)	92.0 (40.0)	179.0 (210.0)	<0.001[#]
Admission sodium (mmol/L)	134.0 (6.0)	134.0 (6.0)	133.0 (9.0)	0.449[#]
Admission ALT (U/L)	35.5 (70.5)	36 .0(69.0)	24.9 (67.0)	0.190[#]
Diagnosis				
Bacteremia	28 (8.3)	19 (67.9)	9 (32.1)	< 0.001[&]
Dengue	120 (35.7)	119 (99.2)	1 (0.8)	
Leptospirosis	76 (22.6)	74 (97.4)	2 (2.6)	
Malaria	15 (4.5)	15 (100.0)	0 (0.0)	
Meliodosis	20 (6.0)	10 (50.0)	10 (50.0)	
TAFI	77 (22.9)	68 (88.3)	9 (11.7)	
AKI				
Yes	133 (39.6)	106 (79.7)	27 (20.3)	< 0.001[&]
No	203 (60.4)	199 (98.0)	4 (2.0)	
AKI stage				
Stage 1	76 (57.1)	74 (97.4)	2 (2.6)	< 0.001[&]
Stage 2	33 (24.8)	21 (63.6)	12 (36.4)	
Stage 3	24 (18.0)	11 (45.8)	13 (54.2)	
Renal-replacement therapy				
Yes	29 (8.6)	10 (34.5)	19 (65.5)	< 0.001
No	307 (91.4)	295 (96.1)	12 (3.9)	
Signs and symptoms				
Central nervous system	76 (22.6)	69 (90.8)	7 (9.2)	0.996
Musculoskeletal	146 (43.5)	136 (93.2)	10 (6.8)	0.187
Dermatology	11 (32.7)	7 (63.6)	4 (36.4)	0.012[&]
Respiratory system	122 (36.3)	108 (88.5)	14 (11.5)	0.282
Cardiovascular system	48 (14.3)	36 (75.0)	12 (25.0)	< 0.001
Gastrointestinal system	191 (58.9)	182 (95.3)	9 (4.7)	0.001

[&]Fischer Exact Test for cell counts < 5. [#]Mann Whitney Test for non-parametric test. For length of hospital stay, duration of fever, admission HB, admission WCC, admission plate count, admission urea, admission creatinine, admission sodium, admission ALT, data were expressed as Median(IQR), for the rest, data were expressed as n (%).

Table 3. Logestic regression model estimated unadjusted and adjusted odds ratios (*OR*s) and 95% confidence intervals (*CI*s) for in-hospital moratlity and selection of tropical acute febrile illness.

Independent variables	Crude *OR*	95% *CI*	*P*-value	Adjusted *OR*[#]	95% *CI*	*P*-value
Leptopsirosis	0.215	0.050-0.924	0.039	0.217	0.036-1.298	0.094
Dengue	0.052	0.007-0.387	0.004	0.078	0.006-1.008	0.051
UAFI	1.426	0.627-3.240	0.397	1.511	0.445-5.131	0.508
Bacteremia	6.158	2.494-15.205	< 0.001	1.826	0.484-6.887	0.374
Meliodosis	14.050	5.262-37.503	< 0.001	5.002	1.233-20.286	0.024

Model: In-hospital moratlity=1, Alive=0. Notes: [#]Controlling for all covariates, age, admission HB, admission WCC, admission platelet count, cardiovascular symptom, gastrointestinal symptom, AKI, dialysis and diabetes.

Table 4. Logistic regression model estimated crude and adjusted odds ratio (*OR*s) and 95% confidence intervals (*CI*s) for in-hospital mortality and renal-replacement therapy, and cardiovascular symptoms in selected tropical acute febrile illnesses (n=336).

Independent variables	Crude *OR*	95% *CI*	*P* value	Adjusted *OR*							
				Model 1 Leptospirosis	95% *CI*	*P* value	Model 2 Dengue	95% *CI*	*P* value	Model 3 Meliodosis	95% *CI*	*P* value
Renal-replacement therapy	46.708	17.904-121.853	< 0.001	45.978	12.967-163.028	< 0.001	50.116	13.366-187.905	< 0.001	38.321	11.192-131.208	< 0.001
Cardiovascular symptoms	4.719	2.116-10.525	< 0.001	4.089	1.307-12.795	0.016	5.0124	1.607-15.633	0.005	4.825	1.594-14.603	0.005

Preliminary analysis shows that there were significant associations between in-hospital mortality with leptospirosis, dengue, bacteremia, and melioidosis (S1 Table). Dengue, bacteremia and melioidosis also showed significant associations with AKI and RRT initiation. Malaria and UAFI each showed no significant associations with all the selected clinical characteristics (S1 Table). We further tested with logistic regression to assess associations between in-hospital mortality with TAFI. Controlling for covariates, only melioidosis was associated with in-hospital mortality. The adjusted odds ratio of the in-hospital mortality was about 5 times high for a patient who had melioidosis as compared to a patient with a diagnosis other than melioidosis, as described in Table 3. Leptospirosis, dengue, bacteremia and UAFI had no significant associations with in-hospital mortality adjusted for other covariates.

Adjusted for covariates, cardiovascular symptoms and RRT initiation were significantly associated with in-hospital mortality in all TAFI. There were increased odds ratios of in-hospital mortality for TAFI patients who initiated RRT compared to TAFI patients who did not initiated RRT (Table 4). Similarly, for TAFI patients who presented with cardiovascular symptoms, the odds ratio of in-hospital mortality increased by 4 times among TAFI patients with leptospirosis, 4 times among dengue patients and 4.8 times among patients with melioidosis (Table 4).

4. Discussion

The state of Pahang, Malaysia in 1957 consisted of a population largely residing in rural areas with an urbanization rate of 22.2%, which recently has increased up to 50.5%[16]. The study as mentioned consists of 6 district hospitals, which mainly cater the rural population. This is almost similar to an earlier study performed 40 years ago in a single center located in Pahang, Malaysia by Brown *et al*.[4]. Till date there have been many other studies in the South-East Asia region mainly Thailand which also studies the etiologies of febrile illnesses[2,17]. However, the pattern of etiology in this study has changed with dengue fever being the most predominant followed by leptospirosis and melioidosis. This is consistent with the increasing incidence rate of dengue in Malaysia that had quadrupled from 44.3 cases/100 000 in 1999 to 181 cases/100 000 in 2007, and the number of reported dengue cases has increased 6.5-fold in the last decade[18]. Factors contributing were mainly associated with urbanization of the rural areas that contributed to succession and invasion of the urban mosquito such as Aedes aegypti mosquitos. Previous studies have shown scrub typhus and leptospirosis being the major caused of acute febrile illness in South-east Asia, not only among residents but also among military personnel deployed to the region[19,20]. About 10%-19% of all the investigated cases of acute undifferentiated fever in this area have been attributable to scrub typhus, with leptospirosis a slightly less common cause[1,4,17]. The shift of pattern could be due high point-of-care diagnostics to identify endemic diseases such as dengue and malaria. However, due to unavailability of appropriate diagnostic test in most hospital laboratories scrub typhus and leptospirosis have been neglected. An indifferent attitude towards febrile illness on the part of many clinicians, laboratory personnel and administrators including financial aids play an important factor towards screening for these neglected diseases. Furthermore, investigations, which are not using point-of-care testing such as leptospirosis serology and IFA, are only accessible in tertiary hospitals.

Several factors were associated with mortality included for each type of TAFI (leptospirosis, dengue, bacteraemia, UAFI and melioidosis), socio-demographic characteristics, and clinical information in this study. However, adjusting for covariates, we found patients with cardiovascular symptoms, and patients who had initiated the renal-replacement therapy significantly associated with in-hospital mortality across all TAFI. Among all the TAFI, adjusted for covariates, only melioidosis was significantly associated with mortality.

On the other hand, occupation specifically agricultural-based, did not show significance as a contributing factor for mortality. Despite a higher potential of soil contact among agriculturally based occupation than those patients who are in the non-agricultural sector, this depicts that agriculturally based occupation does not carry a higher risk for mortality in melioidosis. Some patients of non-agricultural based jobs and also unemployed perform odd jobs to some degree for generation of side income. A history of soil contact among non-agricultural workers was not included as routine information in the clerking history of patients that need to be incorporated in future to confirm this risk factor. This information is vital because *Burkholderia pseudomallei* persisted in the soil and infected human and animals could have been associated with local transmission of melioidosis infection in non-endemic areas[9].

Leucocytosis was identified in this study as a factor associated with mortality, especially amongst leptospirosis patients. In a prospective study by De Silva et al. studying the full blood count parameters in leptospirosis, the majority of study patients had normal or slightly elevated leukocyte counts, and a neutrophilic response may predict the severity of disease[21].

Melioidosis demonstrated an 5-times adjusted odds ratio of in-hospital mortality. Despite not being the most frequently diagnosed amongst the TAFI. This may partly due to its significant difference with renal complication of AKI, which leads to high mortality. The increased odds ratio in melioidosis in comparison to dengue and leptospirosis were mainly due to increased incidence in co-morbidity such as diabetes in the population. A confounding factor may be

explained by diabetes mellitus was featured as an important co-morbidity, which lead to the increase in numbers of complications and in-patient mortality. In 2005, How et al. had reported a high incidence of diabetes (74%) and concurrent high mortality of 54% among their study population citing thrombocytopaenia, fever duration and urea level among other independent associated factors for mortality[22].

There were several limitations identified in this study. Firstly, the study population consisted of hospitalized patients attended government hospital. Each respective TAFI may not represent or reflect the actual incidence of disease in West Pahang, Malaysia as we might missed AFI patients who went to private hospital, general practitioners or undiagnosed patients who did not seek treatment at the hospital[23]. Secondly, certain disease incidence may vary depending on the season of the year e.g. monsoon season hence the limited time of 6 months may not be sufficient to observe an annual pattern. Thirdly, the diagnosis of leptospirosis was not comprehensively studied i.e. convalescent samples were not performed due to the challenges in recalling patients for follow up[24]. Lastly, we did not include scrub typhus, despite having a high prevalence in previous similar studies[4] due to inadequate point of care for laboratory testing and serology in view of limitations of resources as only a single regional center offers this facility.

It is possible that the causes of TAFI to be variable within the year as well as throughout different areas in the state of Pahang given the different geographical map of the state. Hence our next step is to not only incorporate more diagnostic tests, but to extend the study duration to a longer period of time, and to expand the area of study to include other remaining hospitals in the eastern part of the state and also to map the etiologies of acute febrile illness in Pahang, Malaysia.

Further highlights should be focused on the threat of dengue and leptospirosis as vector-borne diseases, which can potentially cause significant morbidity and mortality not only in the state of Pahang but also at the national level. Rapid urbanization growth may have played a role in the shift of spectrum of tropical acute febrile illness as compared what it was reported decades ago, where the number of reported cases of dengue has increased while malaria has experienced a drop in the number of cases. Hence vector control is absolutely essential and cannot be emphasized more to prevent outbreaks during substantial urbanization in the nation's progress.

Secondly, factors associated with mortality were found among TAFI patients especially of those with confirmed melioidosis, patients with cardiovascular symptoms and patients who initiated renal-replacement therapy. Melioidosis, being a condition, which potentially causes abscesses needs to be treated adequately with long term antimicrobials and if found, drainage of abscesses which are the foci of infection. Suboptimal management can result in recurrent cases thus leading to morbidity and mortality. It is then imperative for medical practitioners to control the traditional risk factors for cardiovascular complications such as diabetes mellitus and hypertension at the primary care level. These two chronic conditions carry risks of target organ damage towards key organs such as kidney and heart, which may be accelerated during periods of acute conditions such as tropical acute febrile illnesses.

There were mortalities associated with TAFI where melioidosis was found to be significantly associated with in-hospital mortality. The etiology of TAFI in the western part of Pahang was predominant with dengue, followed by melioidosis, leptospirosis, and malaria were the least. The presence of cardiovascular symptoms and TAFI patients who initiated renal-replacement therapy will be the greatest concern for in-hospital mortality. All this information may be used to assess the disease severity in TAFI and requires more studies in the future.

Conflict of interest statement

We declare that we have no conflict of interest.

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Authors' contributions

Conception and design of work by AAMT, MHAM, WMRWAK, MRMD, MA, SSWA. SSGS, RA; data acquisition by AAMT, WMRWAK, MRMD, MA, SSWA. SSGS, RJ, RA; data analysis and interpretation by AAMT, MHAM, MRMD, MA, SSWA. SSGS, RJ, ENM, NAM; drafting the work by AAMT, WMRWAK, MHAM, ENM, RJ; revising it critically for important intellectual content by AAMT, MHAM, WMRWAK, MRMD, MA, SSWA. SSGS, RA, NAM; and final approval of the version to be published by RA, NAM.

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Supplementary Table 1. Percentages of tropical acute febrile illnesses patients by clinical characteristics (*n*=336).

	Leptospiros (%)	*P*	Dengue (%)	*P*	UAFI (%)	*P*	Bacteremia (%)	*P*	Melidosis (%)	*P*	Malaria (%)	*P*
AKI												
Yes	23.3		23.3		21.8		15		12		4.5	
		0.807		< .001		0.695		< .001		< .001		0.973
No	22.2		43.8		23.7		3.9		2		4.43	
AKI Stages												
AKI stage 1	22.4		35.5		21.1		11.8		4		5.3	
AKI stage 2	27.3	0.924	6.1	< .001	24.2	0.96	9.1	< .001	27.3	< .001	6.1	0.698
AKI stage 3	20.8		8.3		20.8		33.3		16.7		0	
Platelet count												
0 to 10	25		50		0		0		25		0	
11 to 99	11.6		62.5		8.9		2.7		5.36		8.9	
		0.003		< .001		< .001		0.002		0.053		0.043
100 to 150	35.6		42.4		15.3		3.4		0		3.4	
≥ 151	25.5		14.3		36		14.3		8.1		1.9	
RRT initiation												
Yes	17.2		10.3		13.8		27.6		31		0	
				0.003		0.221		< .001		< .001		0.223
No	23.1	0.469	38.1		23.8		6.5		3.6		4.89	
In-hospital mortality												
Yes	24.3		39		22.3		6.2		3.3		4.9	
		0.024		< .001		0.395		< .0001		< .001		0.206
No	6.5		3.23		29		29		32.3		0	

Notes: Significant at *P*-value < 0.05 ; *Chi*-square test statistics for categorical variables.