

Causes and outcomes of sepsis in southeast Asia: a multinational multicentre cross-sectional study

Southeast Asia Infectious Disease Clinical Research Network*



Summary

Background Improved understanding of pathogens that cause sepsis would aid management and antimicrobial selection. In this study, we aimed to identify the causative pathogens of sepsis in southeast Asia.

Methods In this multinational multicentre cross-sectional study of community-acquired sepsis and severe sepsis, we prospectively recruited children (age ≥ 30 days and < 18 years) and adults (age ≥ 18 years) at 13 public hospitals in Indonesia (n=3), Thailand (n=4), and Vietnam (n=6). Hospitalised patients with suspected or documented community-acquired infection, with at least three diagnostic criteria for sepsis according to the Surviving Sepsis Campaign 2012, and within 24 h of admission were enrolled. Blood from every patient, and nasopharyngeal swab, urine, stool, and cerebrospinal fluid, if indicated, were collected for reference diagnostic tests to identify causative pathogens. We report causative pathogens of sepsis and 28-day mortality. We also estimate mortality associated with enrolment with severe sepsis. This study was registered with ClinicalTrials.gov, number NCT02157259.

Findings From Dec 16, 2013, to Dec 14, 2015, 4736 patients were screened and 1578 patients (763 children and 815 adults) were enrolled. Dengue viruses (n=122 [8%]), *Leptospira* spp (n=95 [6%]), rickettsial pathogens (n=96 [6%]), *Escherichia coli* (n=76 [5%]), and influenza viruses (n=65 [4%]) were commonly identified in both age groups; whereas *Plasmodium* spp (n=12 [1%]) and *Salmonella enterica* serovar Typhi (n=3 [0.2%]) were rarely observed. Emerging pathogens identified included hantaviruses (n=28 [2%]), non-typhoidal *Salmonella* spp (n=21 [1%]), *Streptococcus suis* (n=18 [1%]), *Acinetobacter* spp (n=12 [1%]), and *Burkholderia pseudomallei* (n=5 [$< 1\%$]). 28-day mortality occurred in 14 (2%) of 731 children with known statuses and 108 (13%) of 804 adults. Severe sepsis was identified on enrolment in 194 (28%) of 731 children and 546 (68%) of 804 adults, and was associated with increased mortality (adjusted odds ratio 5.3, 95% CI 2.7–10.4; $p < 0.001$).

Interpretation Sepsis in southeast Asia is caused by a wide range of known and emerging pathogens, and is associated with substantial mortality.

Funding National Cancer Institute, National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA, and Wellcome Trust, UK.

Copyright © The Author(s). Published by Elsevier Ltd. This is an Open Access article under the CC BY 4.0 license.

Introduction

Sepsis is the presence of systemic host responses to infection. Severe sepsis and septic shock are major health-care problems and kill millions of people annually worldwide.^{1–3} To reduce mortality, understanding of the causes of sepsis needs to be improved; however, this information is rarely available in tropical countries.⁴ In southeast Asia, a wide range of known and emerging pathogens could cause infections leading to sepsis and severe sepsis.⁵ Several studies have examined causes of fever^{6–10} and bacteraemia¹¹ in the region. However, none applied a predefined wide array of diagnostic tests and assessed the relative distribution of pathogenic bacterial, parasitic, and viral agents identified in patients admitted with community-acquired sepsis. Also, no single study has been conducted in multiple study sites in multiple countries.

The diagnostic criteria for sepsis and severe sepsis have been modified over time. The initial definition of sepsis was developed in 1991 through the concept of the Systemic

Inflammatory Response Syndrome (SIRS), which was characterised by two or more of: body temperature greater than 38°C or less than 36°C; heart rate more than 90 beats per min; respiratory rate of more than 20 breaths per min or a PaCO₂ of less than 32 mm Hg; and a white blood cell count of more than 12000 cells per μL or less than 4000 cells per μL .¹² Recognising limitations of the initial definitions, a 2001 task force expanded the list of criteria to diagnose sepsis to incorporate general, inflammatory, haemodynamic, organ dysfunction, and tissue perfusion variables in addition to suspected or documented infection.¹³ The expanded diagnostic criteria for sepsis have been adopted and recommended by the surviving sepsis campaign (SSC) in 2004,¹ 2008,² and 2012.³ A 2014 task force¹⁴ concluded that the term severe sepsis was redundant and recommended that the new updated definitions and clinical criteria for sepsis should replace previous definitions so that the reported incidence and observed mortality are comparable worldwide, and early recognition and timely management can be provided for

Lancet Glob Health 2017;
5: e157–67

*Members are listed at end of paper

Correspondence to:
Dr Direk Limmathurotsakul,
Faculty of Tropical Medicine,
Mahidol University,
420/6 Rajvithi Road,
Bangkok 10400, Thailand
direk@tropmedres.ac

Research in context

Evidence before this study

We searched PubMed for prospective studies that evaluated causes of fever, sepsis, and severe sepsis in southeast Asia, and were published in English between Jan 1, 1984, and Dec 31, 2014, using the following terms “sepsis” [MeSH term] AND (“Asia, Southeastern” [MeSH term]). We identified 34 studies. None of the studies applied inclusion criteria of the surviving sepsis campaign (SSC) to patients presenting with sepsis in southeast Asia. Several studies have examined causes of fever and bacteraemia in southeast Asia. However, none applied a predefined wide array of diagnostic tests and assessed the relative distribution of pathogenic bacterial, parasitic, and viral agents identified in patients admitted with community-acquired sepsis in southeast Asia.

Added value of this study

To our knowledge, this study is the first that aimed to identify potential causes of sepsis using a predefined set of diagnostic tests covering a wide range of viruses, bacteria, and parasites in southeast Asia. Typhoid fever and malaria were rarely identified in our study hospitals, which are situated in big cities in Indonesia, Thailand, and Vietnam. We observed a number of emerging pathogens including hantavirus, non-typhi *Salmonella* spp, *Streptococcus suis*, *Acinetobacter* spp, and

Burkholderia pseudomallei. We observed that clinical presentations of many tropical infectious diseases, including dengue infection, leptospirosis, and rickettsioses were not specific. We show that mortality of sepsis in developing countries in southeast Asia is substantial, and severe sepsis defined by SSC 2012 is associated with mortality. We also showed that sepsis defined by the 2014 task force (total sequential organ failure assessment score ≥ 2) was associated with mortality in adult patients. One of the strengths of our study is that the mortality for sepsis observed is robust because we contacted participants to ascertain 28-day mortality outcomes—many people in developing countries prefer to die at home, and in-hospital mortality alone could underestimate the mortality of sepsis in developing countries.

Interpretations of all the available evidence

To reduce mortality of sepsis in this region, rapid, inexpensive, and accurate multidisease diagnostic tests for tropical developing countries is urgently needed. The diagnostic criteria for sepsis and severe sepsis recommended by SSC are applicable to tropical developing countries, and could be used to determine patients who are at high risk of death from infectious diseases.

patients who are likely to have poor outcomes. In this study, we aimed to evaluate causes and mortality outcome of sepsis and severe sepsis based on the SSC 2012 definitions, and did an additional analysis using the definition of the 2014 task force.

Methods

Study design

We conducted a prospective observational study of community-acquired sepsis and severe sepsis in 13 public hospitals in Indonesia (Dr Cipto Mangunkusumo Hospital, Jakarta; Dr. Sardjito Hospital, Yogyakarta; and Dr Wahidin Soedirohusodo Hospital, Makassar), Thailand (Queen Sirikit National Institute of Child Health and Siriraj Hospital, Bangkok; Chiang Rai Prachanukroh Hospital, Chiang Rai; and Sappasithiprasong Hospital, Ubon Ratchathani), and Vietnam (National Hospital of Paediatrics and National Hospital of Tropical Diseases, Hanoi; Hue Central Hospital, Hue; Children's Hospital 1, Children's Hospital 2, and Hospital for Tropical Diseases, Ho Chi Minh City; appendix). All are tertiary public hospitals equipped with microbiology facilities and intensive care units, with a median bed number of 1200 (range 350–2200). The study protocol and related documents were approved by regional and national Ethics Committees.

Participants

We prospectively enrolled paediatric (≥ 30 days and < 18 years) and adult patients (≥ 18 years) who were admitted

with a primary diagnosis of suspected or documented infection (made by the attending physician), were within 24 h of hospital admission, and had at least three sepsis diagnostic criteria documented in the medical record. For adult patients, we used 19 variables that were consolidated from the 22 variables proposed as diagnostic criteria for sepsis by the SSC 2012,³ and included low oxygen saturation ($\text{SpO}_2 < 95\%$; panel 1). This variable was added because oxygen saturation determined by pulse oximeter is recommended by WHO guidelines for limited-resource settings.¹⁵ Altered mental status was defined as a Glasgow Coma Scale (GCS) score of less than 15, or less than 10 if intubated. For paediatric patients, in addition to three diagnostic criteria of fever or hypothermia, tachycardia, and tachypnoea, patients had to have at least one of the following symptoms: altered mental status, hypotension, hypoxaemia, and leukocytosis (panel 2). We excluded patients who were suspected of having hospital-acquired infections determined by the attending physician, had a hospital stay within 30 days before this admission, or were transferred from other hospitals with a total duration of hospitalisation of more than 72 h. Owing to concerns about the volume of blood drawn from each patient, we also excluded patients who had been enrolled into other clinical studies.

Procedures

The study was initiated on Dec 16, 2013, in Thailand, March 24, 2014, in Vietnam, and March 9, 2015, in

See Online for appendix

Indonesia. The study enrolment was closed on Dec 14, 2015, in all three countries. The study team used a standardised case-report form to record the clinical symptoms and their respective durations, known chronic conditions, laboratory tests performed by the study hospital laboratories, and primary and final diagnoses made by attending clinicians. As per protocol, every patient was evaluated by the study team, and the following rapid tests were performed immediately after enrolment: a whole blood lactate rapid diagnostic test (RDT; Lactate Pro 2, Arkray Global Business, Kyoto, Japan), a whole blood glucose RDT (ACCU-CHECK Performa, Roche Diagnostics, Mannheim, Germany), pulse oximeter (Nellcor N-65, Covidien, Dublin, Ireland), and dengue RDTs (NS1 and IgM, Standard Diagnostics, Gyeonggi-do, South Korea). All children younger than 7 years old were evaluated using an influenza RDT (QuickVue, Quidel Corporation, San Diego, CA, USA), and all patients aged 7 years or older were evaluated using a leptospirosis RDT (Leptospira IgM/IgG, Standard Diagnostics). The results of all rapid tests were reported to the attending physicians. In all cases, blood samples (4–10 mL) were collected for culture on site and for serological tests and molecular tests at reference laboratory centres of each country. Pooled nasal and throat swabs were collected for respiratory viruses if patients had respiratory symptoms. Stool samples were collected if patients had diarrhoeal symptoms. Residual cerebrospinal fluid (CSF) was collected if available. A set of reference diagnostic tests was performed for each patient according to clinical presentation (appendix). These tests included complete blood count, blood culture, and urinary analysis and urine culture in every study patient, sputum Gram smears and sputum culture if patients had respiratory symptoms, stool examination and stool culture if patients had diarrhoeal symptoms, and CSF examination and CSF culture if patients had neurological symptoms and CNS infection was suspected (appendix).

The study did not involve any clinical interventions. All treatments were provided by attending physicians and their medical teams. The rainy season was from November to March in Indonesia, July to October in Thailand, April to October in northern Vietnam, September to January in central Vietnam, and May to November in southern Vietnam. The 28-day mortality was evaluated via telephone contact if participants were no longer hospitalised and had been discharged alive.

Definitions

For each patient, the clinical presentations (in some cases, more than one) were defined based on the major presenting clinical symptoms. Acute respiratory infection was defined as manifestation of at least one respiratory symptom for no longer than 14 days. Acute diarrhoea was defined as diarrhoea for no longer than 14 days. Acute CNS infection was defined as manifestation of CNS symptoms for no longer than 14 days or presence of signs

Panel 1: Diagnostic criteria for sepsis in adult patients

Infection, documented or suspected, and some of the following:

General variables

- Fever or hypothermia (body temperature $>38.3^{\circ}\text{C}$ or $<36^{\circ}\text{C}$)*
- Heart rate >90 beats per min
- Tachypnoea (respiratory rate >20 breaths per min)
- Altered mental status (Glasgow Coma Scale <15 or <10 if intubated)†
- Significant oedema or positive fluid balance (>20 mL/kg over 24 h)
- Hyperglycaemia (plasma glucose >140 mg/dL) in the absence of diabetes

Inflammatory variables

- Leukocytosis (white blood cell count $>12\,000$ cells per μL), leucopenia (white blood cell count <4000 cells per μL), or immature forms $>10\%$ ‡
- Plasma C-reactive protein more than 2 SD above the normal value
- Plasma procalcitonin more than 2 SD above the normal value

Haemodynamic variables

- Arterial hypotension (systolic blood pressure <90 mm Hg, mean arterial pressure <70 mm Hg, or a systolic blood pressure decrease >40 mm Hg)

Organ dysfunction variables

- Low oxygen saturation determined by pulse oximetry ($\text{SpO}_2 <95\%$)§
- Arterial hypoxaemia ($\text{PaO}_2/\text{FiO}_2 <300$)
- Acute oliguria (urine output <0.5 mL/kg per h for at least 2 h)¶
- Creatinine increase >0.5 mg/dL
- Coagulation abnormalities (international normalised ratio >1.5 or activated partial thromplastin time >60 s)
- Ileus (absent bowel sounds)
- Thrombocytopenia (platelet count $<100\,000$ cells per μL)
- Hyperbilirubinaemia (plasma total bilirubin >4 mg/dL)

Tissue perfusion variables

- Hyperlactataemia (>1 mmol/L)
- Decreased capillary refill or mottling

*Variables fever and hypothermia were consolidated into a single variable. †Glasgow Coma Scale <15 or <10 if intubated were defined for the altered mental status variable. ‡Variables leukocytosis, leucopenia, and immature forms $>10\%$ were consolidated into a single variable. §Variable low oxygen saturation determined by pulse oximetry ($\text{SpO}_2 <95\%$) was added. ¶A condition of “despite adequate fluid resuscitation” is included for this criterion in the SSC 2012 diagnostic criteria for severe sepsis. Adapted from Dellinger and colleagues.³

of CNS infection on admission. Systemic infection was defined as absence of acute respiratory infection, acute diarrhoea and acute CNS infection.

To maximise consistency and minimise subjectivity, identification of the pathogens was done using a computer-based algorithm (appendix) and confirmed by

Panel 2: Diagnostic criteria for sepsis in paediatric patients

Infection, documented or suspected, with all three of the following:

- Fever or hypothermia (rectal temperature $>38.5^{\circ}\text{C}$ or $<35.0^{\circ}\text{C}$ [or equivalent])
- Tachycardia (heart rate >2 SD above the normal value for age), which could be absent in hypothermic patients
- Tachypnoea (respiratory rate >2 SD above the normal value of age)

AND at least one of the following indices

- Altered mental status (eg, drowsiness, poor quality of cry, poor reaction to parent stimuli, and poor response to social overtures)
- Systolic blood pressure <2 SD below the normal value for age, narrow pulse pressure (<20 mm Hg), or poor perfusion (capillary refill >2 s)
- Low oxygen saturation determined by pulse oximetry (SpO_2 $<95\%$)
- Leukocytosis (white blood cell count $>12\,000$ cells per μL), leukopenia (white blood cell count <5000 cells per μL), or immature forms $>10\%$

Adapted from Goldstein and colleagues.¹⁶

additional evidence of clinical diagnoses suggesting true infection (eg, endocarditis, artificial material in situ). Rickettsial pathogens evaluated were *Orientia tsutsugamushi*, *Rickettsia typhi*, and spotted fever group rickettsia (appendix).

Statistical analysis

The primary objective of this study was to determine the causes of community-acquired sepsis and severe sepsis in adult and paediatric patients. We also assessed 28-day mortality and other secondary outcomes, which will be reported elsewhere. We entered data into OpenClinica, Enterprise Edition (Waltham, MA, USA), and used STATA version 14.0 for all analyses. We calculated that 125 participants per age group per study area (three study areas per country [appendix] and a total of 750 participants per country) would provide adequate power ($>90\%$) to observe a cause of sepsis, which had a true prevalence of 2% or greater in each study age group in each study area. We assessed differences in proportions among groups using Fisher's exact test, and differences in medians with the Mann-Whitney test. We used logistic regression models stratified by study sites and age group to evaluate the factors associated with mortality. 28-day mortality was defined as the proportion of patients who died within 28 days of hospital admission. We defined severe sepsis in adult patients as described in Surviving Sepsis Campaign 2012,³ and in paediatric patients as previously described.¹⁶ We also evaluated the association between sepsis defined by the 2014 task force (total sequential organ failure assessment [SOFA] score ≥ 2) and mortality in adult patients.¹⁴ The additional analysis was restricted to only adult patients because the new sepsis definition is available only for this group.¹⁴ A sensitivity analysis was done by only including patients with a pathogen detected in the model. The study was registered with ClinicalTrials.gov, number NCT02157259. The final database and the data dictionary are publicly available online.

Role of the funding source

All study procedures, data collection, data analyses, data interpretation, and writing of the report were performed without the sponsors' involvement. Full access to data was granted to the corresponding author. All authors participated in the study design or analysis, and approved the submission of the manuscript. The SEAICRN executive committee had final responsibility for the decision to submit for publication.

Results

4736 patients (2093 children and 2643 adults) presenting at study hospitals were screened by the study team during the study period (figure 1). Common exclusion criteria were hospitalisation in the past 30 days (719 [15%] patients) and less than three sepsis diagnostic criteria

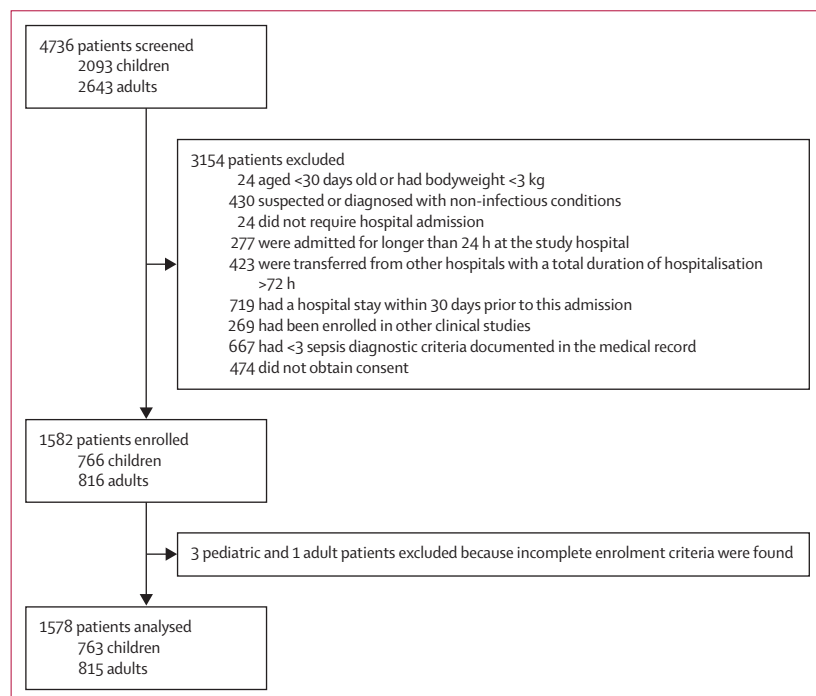


Figure 1: Study profile

For the final database and the data dictionary see <https://dx.doi.org/10.6084/m9.figshare.3486866.v1>

an adjudication committee. The blood culture result was considered contaminated if growth of coagulase-negative staphylococci, alpha-haemolytic streptococci, *Micrococcus* spp, *Propionibacterium* spp, *Corynebacterium* spp, *Burkholderia cepacia*, or *Bacillus* spp was detected without

Paediatric patients (n=763)	
Sex	
Male	433 (57%)
Female	330 (43%)
Age	
≥30 days to <1 year	171 (22%)
≥1 year to <5 years	385 (50%)
≥5 years to <18 years	207 (27%)
Country	
Indonesia	14 (2%)
Thailand	375 (49%)
Vietnam	374 (49%)
Pre-existing conditions	
Diabetes	1 (<1%)
Hypertension	0
Chronic kidney disease	0
Chronic lung disease	1 (<1%)
HIV/AIDS	2 (<1%)
28-day mortality	
Survived	717 (94%)
Died	14 (2%)
Unknown*	32 (4%)

Data are n (%). *Mortality outcome was not available in 19 children who withdrew from the study before 28 days of follow-up, and in another 13 children who could not be contacted for 28-day outcome.

Table 1: Baseline characteristics of paediatric patients with sepsis

documented in the medical record (667 [14%] patients). After giving informed consent, 1582 community-acquired sepsis patients were enrolled. The enrolment target was reached for Thailand and Vietnam (750 patients [375 children and 375 adults] per country). 82 patients (16 children and 66 adults) were enrolled in Indonesia. Enrolment in Indonesia did not reach the target sample size because of competitive enrolment with other studies during the study period. Three enrolled children and one adult were retrospectively found not to fulfill the inclusion criteria and were excluded from the analysis.

763 children (table 1) and 815 adults (table 2) were included in the analysis. Characteristics of patients by country are shown in the appendix. Convalescent blood samples were obtained from 925 patients (415 children and 510 adults), and 36853 diagnostic tests were done per protocol.

Per study protocol, 937 distinct clinical presentations were seen in 763 children (figure 2) and 927 were seen in 815 adults (figure 3). Acute respiratory infection was the most frequent clinical presentation, found in 481 (63%) children and 436 (53%) adults, followed by acute diarrhoea, acute systemic infection, and acute CNS infection in children (figure 2) and acute systemic infection, acute diarrhoea, and acute CNS infection in adults. Among those with acute respiratory infection, pneumonia was diagnosed by attending physicians in

Adult patients (n=815)	
Sex	
Male	462 (57%)
Female	353 (43%)
Age	
≥18 years to <40 years	261 (32%)
≥40 years to <60 years	270 (33%)
≥60 years	284 (35%)
Country	
Indonesia	65 (8%)
Thailand	375 (46%)
VietNam	375 (46%)
Pre-existing conditions	
Diabetes	124 (15%)
Hypertension	217 (27%)
Chronic kidney disease	50 (6%)
Chronic lung disease	36 (4%)
HIV/AIDS	2 (<1%)
28-day mortality	
Survived	696 (85%)
Died	108 (13%)
Unknown*	11 (1%)

Data are n (%). *Mortality outcome was not available in five adults who withdrew from the study before 28 days of follow-up, and in another six adults who could not be contacted for 28-day outcome.

Table 2: Baseline characteristics of adult patients with sepsis

280 (37%) of 763 children and 222 (27%) of 815 adults. Other clinical presentations were distributed as follows: 183 (24%) children and 168 (21%) adults had acute diarrhoea, 119 (16%) children and 80 (10%) adults had acute CNS infection, and 154 (20%) children and 243 (30%) adults had systemic infection. 156 (20%) children and 105 (13%) adults had multiple clinical presentations. At least one pathogen was identified in 425 (56%) children and 388 (48%) adults (figure 4), and one child (<1%) and 17 (2%) adults had a final diagnosis of non-infectious causes (appendix).

Overall, dengue virus was identified in 55 (7%) children and 67 (8%) adults (appendix). Among individuals with acute respiratory infection, diagnostic tests were positive for at least one virus in 282 (59%) of 481 children and 74 (17%) of 436 adults, including influenza viruses (44 children and 20 adults), rhinovirus (92 children and 12 adults), and respiratory syncytial virus (52 children and two adults; figures 2, 3). A virus was identified in 76 (42%) of 183 children and 20 (12%) of 168 adults with acute diarrhoea, including rotavirus (12 children and two adults), adenovirus (eight children and no adults), and norovirus (three children and one adult). Of those individuals who had clinical presentation of both acute respiratory infection and acute diarrhoea, 57 (57%) of 100 children and 12 (17%) of 70 adults had diagnostic tests

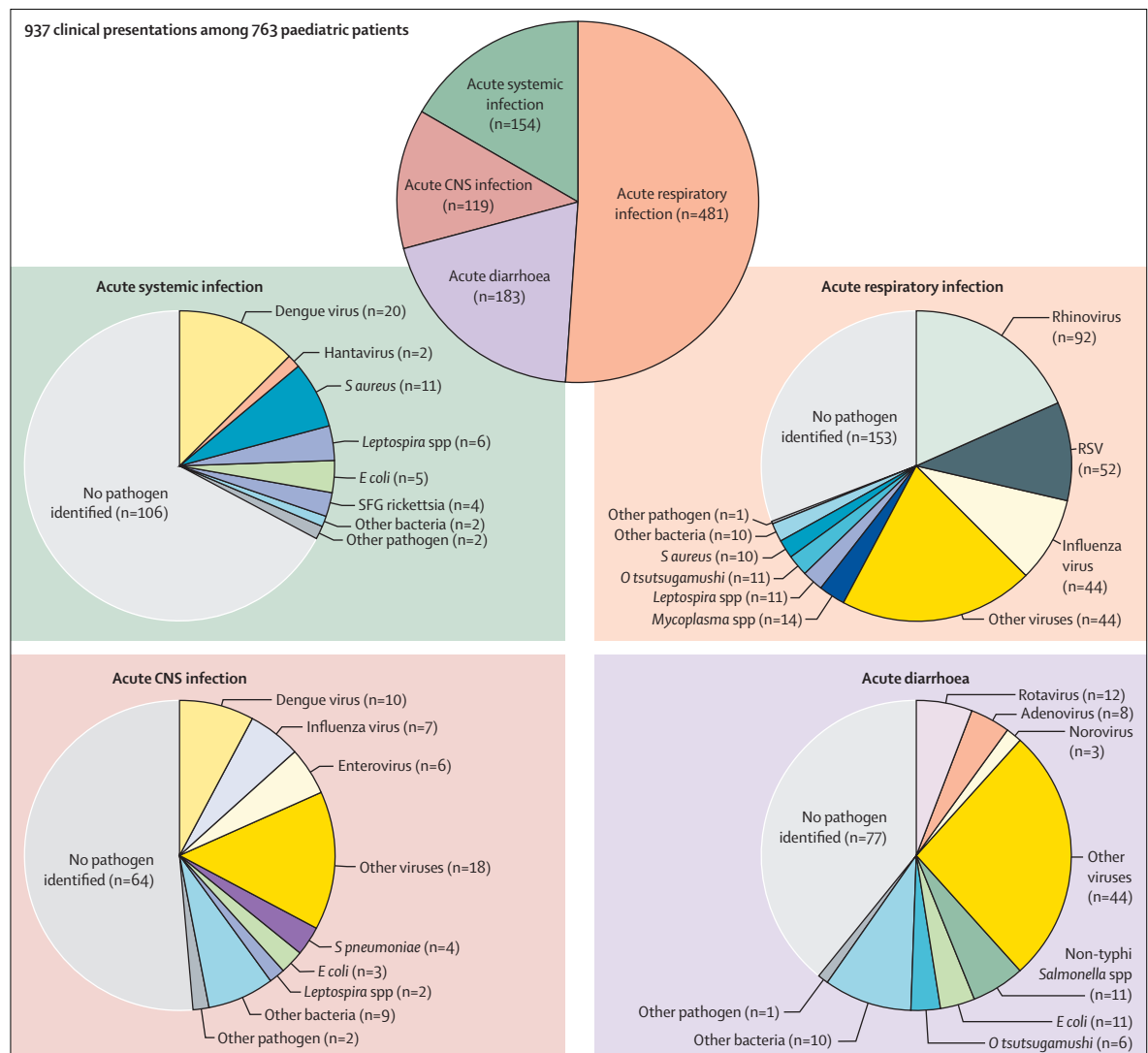


Figure 2: Distribution of clinical presentations and pathogens identified in 763 paediatric patients

Numbers in parentheses are number of clinical presentations of each pathogen identified. In some patients, more than one clinical presentation or more than one pathogen was identified. SFG=spotted fever group. RSV=respiratory syncytial virus. E Coli=Escherichia coli. S Aureus=Staphylococcus aureus. O tsutsugamushi=Orientia tsutsugamushi. S pneumoniae=Streptococcus pneumoniae. S Suis=Streptococcus suis.

positive for at least one virus; including 20 children and one adult with rhinovirus (figure 2). Viruses were identified in 40 (34%) of 119 children and ten (13%) of 80 adults who had clinical presentation of acute CNS infection, including dengue virus (ten children and eight adults), influenza virus (seven children and one adult), enterovirus (six children and no adults), and adenovirus (three children and no adults); hantavirus was identified in about 2% of children and adults (figures 2, 3).

15 (2%) of 742 children and 37 (5%) of 789 adults for whom blood culture was done, were positive for contaminant bacteria; 35 (5%) children and 96 (12%) adults were positive for pathogenic bacteria. The most frequent contaminants were coagulase-negative Staphylococcus. Overall, 12 children and 68 adults were

blood culture positive for Gram-negative bacteria, including *Escherichia coli* (n=40), *Klebsiella pneumoniae* (n=9), *Acinetobacter* spp (n=9), *Enterobacter* spp (n=6), and *Burkholderia pseudomallei* (n=3). 22 children and 24 adults were blood culture positive for Gram-positive bacteria, including *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus suis*, and beta-haemolytic *Streptococcus* spp (appendix). Among 1444 patients with negative blood cultures, blood 16S PCR was positive in 44 (3%) patients. Products of 16S PCR could be identified in 14 cases; including *S suis* (n=4), *Orientia tsutsugamushi* (n=4), *Pseudomonas* spp (n=2), *Achromobacter* spp (n=1), *S pneumoniae* (n=1), *K pneumoniae* (n=1), and Enterobacteriaceae (n=1).

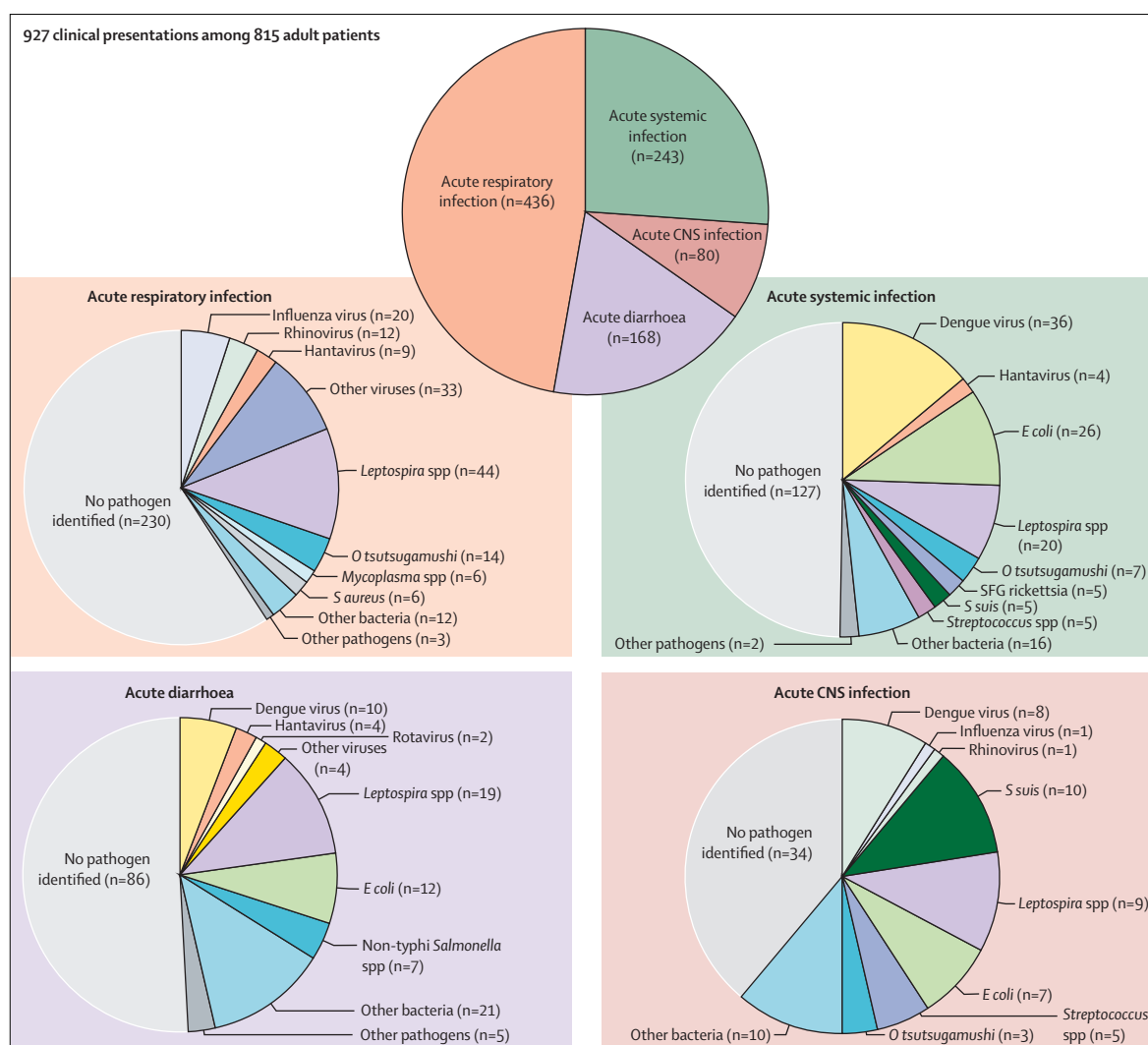


Figure 3: Distribution of clinical presentations and pathogens identified in 815 adult patients

Numbers in parentheses are number of clinical presentations of each pathogen identified. In some patients, more than one clinical presentation or more than one pathogen was identified. SFG=spotted fever group. RSV=respiratory syncytial virus. E Coli=Escherichia coli. S aureus=Staphylococcus aureus. O tsutsugamushi=Orientia tsutsugamushi. S pneumoniae=Streptococcus pneumoniae. S Suis=Streptococcus suis.

Among patients with acute respiratory infection, diagnostic tests were positive for at least one bacterium in 93 (19%) of 491 children and 147 (34%) of 436 adults. These bacteria included *S aureus* (ten children and six adults), *Mycoplasma* spp (14 children and six adults), *K pneumoniae* (no children and nine adults), *S pneumoniae* (two children and four adults), *Leptospira* spp (11 children and 44 adults), *O tsutsugamushi* (11 children and 14 adults) and *Mycobacterium tuberculosis* (two children and four adults). A bacterium was documented in 40 (22%) of 183 children and 60 (36%) of 168 adults with acute diarrhoea. These bacteria included *Salmonella enterica* serovar Typhi (no children and one adult), non-typhi *Salmonella* spp (11 children and seven adults), *E coli* (seven children and 12 adults), and *Campylobacter* spp (two children and no adults). Bacteria were identified in

17 (14%) of 119 children and 42 (53%) of 80 adults with acute CNS infection. Those included *Leptospira* spp (two children and nine adults), *E coli* (three children and seven adults), *S suis* (no children and ten adults), *S pneumoniae* (four children and one adult), spotted fever group rickettsia (two children and two adults), and *O tsutsugamushi* (no children and three adults). Overall, tropical bacterial infectious diseases were common, including leptospirosis and rickettsioses, in both children and adults.

Four children and eight adults had malaria, one child and one adult had *Entamoeba histolytica*, two adults had strongyloidiasis, and one child had cryptosporidiosis.

Pathogens identified in Indonesia, Thailand, and Vietnam were largely comparable, except non-typhi *Salmonella*, *Enterobacter* spp, *Acinetobacter* spp,

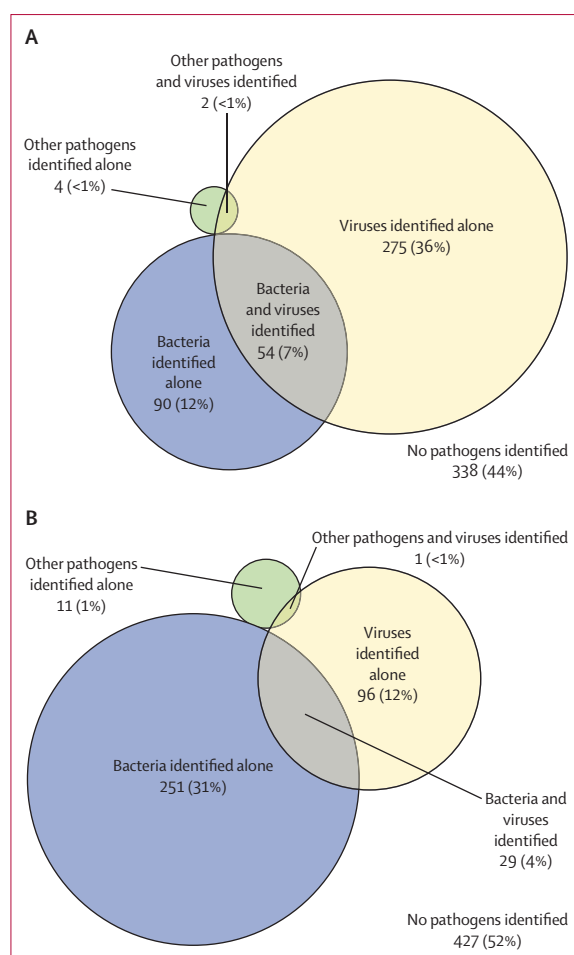


Figure 4: Overlap between pathogens identified in (A) 763 paediatric patients and (B) 815 adult patients

Numbers show number of patients not presentations.

	Outcome		Odds ratio (95% CI)*	
	Non-survivors (n=122)	Survivors (n=1413)	Univariable analysis	Multivariable analysis
Severe sepsis	110 (90%)	630 (45%)	5.1 (2.6–10.0)	5.3 (2.7–10.4)
Bacteria identified	45 (37%)	364 (26%)	1.1 (0.7–1.8)	1.0 (0.6–1.5)
Viruses identified	13 (11%)	434 (31%)	0.5 (0.3–1.0)	0.5 (0.3–0.9)

Data are n (%) unless otherwise stated. *Stratified by age groups and study sites.

Table 3: Risk factors for 28-day mortality in patients with sepsis

beta-haemolytic *Streptococcus* spp, rickettsial pathogens, dengue viruses, influenza viruses, hantaviruses, rotavirus, cytomegalovirus, and bocavirus (appendix). We found that *S suis* and respiratory syncytial virus were more commonly identified during rainy season, while influenza viruses and rotavirus more commonly identified during dry season (appendix). Emerging pathogens identified included hantaviruses (n=28 [2%]), non-typhoidal *Salmonella* spp (n=21 [1%]),

S suis (n=18 [1%]), *Acinetobacter* spp (n=12 [1%]), and *Burkholderia pseudomallei* (n=5 [<1%]; appendix).

The 28-day mortality was 14 (2%) in 731 children with known statuses (table 1) and 108 (13%) in 804 adults (table 2). Mortality outcome was not available in 19 children and five adults who withdrew from the study before 28 days of follow-up, and in another 13 children and six adults who could not be contacted for 28-day outcome. The failure to obtain 28-day mortality data was higher in Thailand (4%; 29 of 750) than in Vietnam (2%; 13 of 749) and Indonesia (1%; one of 79; appendix, $p=0.03$). Of 122 patients who died, 32 died within 2 days (26%) and 49 between day 3 and 7 (40%) of hospitalisation. In the multivariable logistic regression model, severe sepsis on admission was strongly associated with mortality (adjusted odds ratio 5.3, 95% CI 2.7–10.4; $p<0.001$; table 3). This observation was in both pediatric patients (adjusted odds ratio [OR] 8.2, 95% CI 1.9–35.5; $p=0.005$) and adult patients (adjusted OR 4.3, 95% CI 2.0–8.9; $p<0.001$). Patients with viruses identified had a lower risk of death, which was also observed when only patients with pathogens identified were included in the model (appendix). In the additional analysis, adult patients with SOFA scores ≥ 2 on admission were associated with higher 28-day mortality than adult patients with SOFA scores <2 on admission adults (99 [22%] of 454 vs 9 [3%] of 350; adjusted OR 4.1, 95% CI 1.6–10.3; $p=0.003$; appendix).

Discussion

Using a predefined set of diagnostic tests, we were able to identify the possible causative organisms of sepsis and severe sepsis in 56% of children and 48% of adults enrolled in our study. Viruses were identified in 29% of patients, bacteria in 27%, and parasites in 1%. Tropical infectious diseases were common causes of sepsis and severe sepsis in our setting. These include dengue virus, leptospirosis, and rickettsioses. Typhoid fever was rarely identified. We also observed a number of emerging pathogens including hantavirus, non-typhoidal *Salmonella* spp, *S suis*, *Acinetobacter* spp, and *B pseudomallei*. Non-typhoidal *Salmonella* infection is increasingly recognised as an important cause of sepsis and diarrhoeal illnesses in the region.^{17,18} Community-acquired *Acinetobacter* bacteraemia and pneumonia is also increasingly reported.^{19,20} *B pseudomallei* is known to be endemic but is generally underdiagnosed in the region,²¹ and little is known about hantavirus^{22,23} and *S suis*.^{24,25}

Our study emphasises the need for rapid, inexpensive, and accurate multidisease diagnostic tests for tropical developing countries. For example, a number of both paediatric and adult patients with dengue in our study might have not been diagnosed if RDTs for dengue were not used in every participating patient. This shortcoming is because diagnostic tests are usually

used for a single disease based on clinical suspicion; therefore, patients presenting with atypical symptoms are commonly misdiagnosed or undiagnosed. For example, rickettsioses, leptospirosis, and dengue are commonly found in patients presenting with acute CNS infections in southeast Asia,^{26,27} which is consistent with our results.

Our findings strongly support the recommendation of SSC to obtain blood cultures before administration of antibiotics within 3 h for patients presenting with sepsis.³ Bacteraemia was commonly observed in both age groups in our study (12% in adult patients and 5% in paediatric patients). Susceptibility testing of the bacterial agents identified from blood could be used to adjust or de-escalate antimicrobial drugs.

Our findings indicate that severe sepsis is associated with increased mortality. This strongly suggests that patients suspected of infection should be routinely screened for signs of tissue hypoperfusion or organ dysfunction, and patients with severe sepsis should immediately receive intensive care and organ support.³ We also show that adult patients with sepsis defined by the 2014 task force (SOFA score ≥ 2) are also associated with higher mortality, which supports the use of the new sepsis definition.¹⁴

In view of the mortality associated with severe sepsis, clinicians should provide the most effective empirical management even before a microbiological diagnosis is confirmed. Our results highlight the diverse causes of sepsis that should be considered in a patient's differential. For example, the clinical presentations of dengue and leptospirosis overlap, and both were common in our patient population. If a patient is managed for presumed dengue with supportive care but actually has leptospirosis, an opportunity for effective antimicrobial intervention will be missed. Awareness of the epidemiology of sepsis will help clinicians develop the most appropriate differential to guide empirical treatment before a microbiological diagnosis is known.

On the basis of our findings, the first broad-spectrum antibiotics for patients with severe sepsis in southeast Asia should cover both Gram-negative and Gram-positive bacteria, for example a combination of ceftriaxone plus doxycycline, or a combination of ceftazidime plus doxycycline. Doxycycline might need to be included as commonly used empirical antimicrobials such as beta-lactams and carbapenems are not effective against *O. tsutsugamushi*.^{28,29} Doxycycline can be used even in children younger than 8 years.³⁰ In areas where melioidosis is endemic, the third-generation cephalosporin might need to be ceftazidime as other third-generation cephalosporins are ineffective against *B. pseudomallei*.²¹

A pathogen was only identified in about half of sepsis patients. This finding is comparable with other studies in the region.^{6–10} For example, a microbiological cause of

fever was only identified in 41% of fever episodes in southern Laos,⁶ 52% in two study sites in Laos,⁷ and 47% in Cambodia.⁸ D'Acremont and colleagues⁴ reported that 88% of paediatric patients in Tanzania had microbiological evidence, and the high proportion of microbiological evidence found could be due to the different diagnostic tests used and the different setting. In our study, a proportion of patients without pathogen identified could have been infected by one of the agents we targeted because the sensitivity of collected specimens and reference diagnostic tests is not 100%.³¹ Also, many patients in our settings might have taken antibiotics before hospital admission. An alternative explanation is that patients were infected by pathogens that were not targeted by our diagnostic tests. Further studies in this group of patients are still required.

Our study has several potential limitations. First, the decision to perform some diagnostic tests in predefined subsets of patients with relevant symptoms could lead to misdiagnosis of some infectious diseases. However, this represents the variable clinical presentations of tropical infectious diseases. Second, in some patients, the pathogens identified might not be the cause of sepsis. It is possible that some pathogenic viruses and bacteria could be found in the nasopharyngeal cavity of healthy patients, and background sero-positivity for rickettsiosis and leptospirosis is also a possibility in people living in endemic areas. The presence of viruses in the nasopharyngeal cavity of healthy people in a previous study³² conducted in China was not high. Furthermore, we used conservative criteria for microbiological evidence, such as high cutoffs for serological tests for rickettsiosis and leptospirosis (appendix). Therefore, in most patients, the pathogens identified were likely to be the causes of sepsis. Further studies in healthy people in southeast Asia are also needed. Third, the study did not reach the target sample size in Indonesia. Nonetheless, we did not observe major differences between the causes and outcomes of sepsis patients in Indonesia, Thailand, and Vietnam. Fourth, the study was conducted at tertiary-care hospitals in southeast Asia. Sepsis in other settings may be caused by different distribution of pathogens.

Our study highlights the diverse causes of sepsis and their variable clinical manifestations, the need for multiplex RDTs for tropical infectious diseases, the substantial mortality of sepsis, and the use of the sepsis diagnostic criteria and the SSC recommendations in three middle-income countries in southeast Asia.

Contributors

The SEAICRN executive committee conceived and supervised the study. This committee included PS, ATA, MA, TC, WPV, KC, PHP, NVK, NVVC, ND, GT, C-YL, and DL. The SEAICRN writing committee included AKS, JT, SB, HRvD, and the SEAICRN executive committee. DL received and checked data, had full access to all materials and results, did analyses, and drafted the first report. All writing committee members helped with revisions before and after circulation to members.

Southeast Asia Infectious Disease Clinical Research Network

Pratiwi Sudarmono (Dr Cipto Mangunkusumo Hospital, Jakarta, Indonesia), Abu Tholib Aman (Dr Sardjito Hospital, Yogyakarta, Indonesia), Mansyur Arif (Dr Wahidin Sudirohusodo Hospital, Makassar, Indonesia), Armaji Kamaludi Syarif, Herman Kosasih, and Muhammad Karyana (National Institute of Health Research and Development, Jakarta, Indonesia), Tawee Chotpitayasonondh and Warunee Punpanich Vandepitte (Queen Sirikit National Institute of Child Health, Thailand), Adiratha Boonyasiri, Keswadee Lapphra, Kulkanya Chokephaibulkit, Pinyo Rattanaumpawan, and Visanu Thamlikitkul (Siriraj Hospital, Bangkok, Thailand), Achara Laongnualpanich (Chiang Rai Prachanukroh Hospital, Chiang Rai, Thailand), Prapit Teparakkul and Pramot Srisamang (Sappasithiprasong Hospital, Ubon Ratchathani, Thailand), Phan Huu Phuc and Le Thanh Hai (National Hospital of Pediatrics, Hanoi, Vietnam), Nguyen Van Kinh (National Hospital of Tropical Diseases, Hanoi, Vietnam), Bui Duc Phu (Hue Central Hospital, Hue, Vietnam), Nguyen Thanh Hung and Tang Chi Thuong (Children's Hospital 1, Ho Chi Minh City, Vietnam), Ha Manh Tuan (Children's Hospital 2, Ho Chi Minh City, Vietnam), Lam Minh Yen and Nguyen Van Vinh Chau (Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam), Direk Limmathurotsakul, Janjira Thaipadungpanit, Stuart Blacksell, and Nicholas Day (Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand), Guy Thwaites, Heiman Wertheim, Le Van Tan, Motiur Rahman, and H Rogier van Doorn (Oxford University Clinical Research Unit, Vietnam) and Chuen-Yen Lau (National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA).

Declaration of interests

We declare no competing interests.

Acknowledgments

We acknowledge the support provided by staff at all participating hospitals. This project was funded in part with federal funds from the US National Cancer Institute, National Institutes of Health (HHSN261200800001E). This research was also supported in part by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organisations imply endorsement by the US Government. DL was supported by a Wellcome Trust Public Health and Tropical Medicine Intermediate Fellowship (101103/Z/13/Z). Wellcome Trust grants supported Mahidol-Oxford Tropical Medicine Research Unit (MORU) in Thailand (106698/B/14/Z) and Oxford University Clinical Research Unit (OUCRU) in Vietnam (106680/B/14/Z).

References

- Dellinger RP, Carlet JM, Masur H, et al. Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock. *Intensive Care Med* 2004; **30**: 536–55.
- Dellinger RP, Levy MM, Carlet JM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. *Crit Care Med* 2008; **36**: 296–327.
- Dellinger RP, Levy MM, Rhodes A, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med* 2013; **39**: 165–228.
- D'Acremont V, Kilowoko M, Kyungu E, et al. Beyond malaria—causes of fever in outpatient Tanzanian children. *N Engl J Med* 2014; **370**: 809–17.
- Farrar J, Hotez P, Junghanss T, Kang G, Lalloo DG, White N. Manson's Tropical Infectious Diseases. 23 edn. Philadelphia, PA: Saunders, 2014.
- Mayxay M, Sengvilapaseuth O, Chanthongthip A, et al. Causes of fever in rural southern Laos. *Am J Trop Med Hyg* 2015; **93**: 517–20.
- Mayxay M, Castonguay-Vanier J, Chansamouth V, et al. Causes of non-malarial fever in Laos: a prospective study. *Lancet Glob Health* 2013; **1**: e46–54.
- Chheng K, Carter MJ, Emary K, et al. A prospective study of the causes of febrile illness requiring hospitalization in children in Cambodia. *PLoS One* 2013; **8**: e60634.
- McGready R, Ashley EA, Wuthiekanun V, et al. Arthropod borne disease: the leading cause of fever in pregnancy on the Thai-Burmese border. *PLoS Negl Trop Dis* 2010; **4**: e888.
- Suttinont C, Losuwanaluk K, Niwatayakul K, et al. Causes of acute, undifferentiated, febrile illness in rural Thailand: results of a prospective observational study. *Ann Trop Med Parasitol* 2006; **100**: 363–70.
- Deen J, von Seidlein L, Andersen F, Elle N, White NJ, Lubell Y. Community-acquired bacterial bloodstream infections in developing countries in south and southeast Asia: a systematic review. *Lancet Infect Dis* 2012; **12**: 480–87.
- Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992; **101**: 1644–55.
- Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med* 2003; **31**: 1250–56.
- Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016; **315**: 801–10.
- World Health Organization. IMAI District Clinician Manual: hospital care for adolescents and adults. Guidelines for the management of illnesses with limited resources. <http://www.who.int/hiv/pub/imai/imai2011/en/> (accessed Aug 1, 2016).
- Goldstein B, Giroir B, Randolph A, International Consensus Conference on Pediatric Sepsis. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. *Pediatric Crit Care Med* 2005; **6**: 2–8.
- Shahunja KM, Leung DT, Ahmed T, et al. Factors associated with non-typhoidal *Salmonella* bacteremia versus typhoidal *Salmonella* bacteremia in patients presenting for care in an urban diarrheal disease hospital in Bangladesh. *PLoS Negl Trop Dis* 2015; **9**: e0004066.
- Thompson CN, Phan VT, Le TP, et al. Epidemiological features and risk factors of *Salmonella* gastroenteritis in children resident in Ho Chi Minh City, Vietnam. *Epidemiol Infect* 2013; **141**: 1604–13.
- Ong CW, Lye DC, Khoo KL, et al. Severe community-acquired *Acinetobacter baumannii* pneumonia: an emerging highly lethal infectious disease in the Asia-Pacific. *Respirology* 2009; **14**: 1200–05.
- Kanoksil M, Jatapai A, Peacock SJ, Limmathurotsakul D. Epidemiology, microbiology and mortality associated with community-acquired bacteremia in northeast Thailand: a multicenter surveillance study. *PLoS One* 2013; **8**: e54714.
- Limmathurotsakul D, Golding N, Dance D, et al. Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis. *Nat Microbiol* 2016; **1**: 15008.
- Suharti C, van Gorp EC, Dolmans WM, et al. Hanta virus infection during dengue virus infection outbreak in Indonesia. *Acta Med Indones* 2009; **41**: 75–80.
- Pattamadilok S, Lee BH, Kumperasart S, et al. Geographical distribution of hantaviruses in Thailand and potential human health significance of Thailand virus. *Am J Trop Med Hyg* 2006; **75**: 994–1002.
- Nutravong T, Angkititrakul S, Jiwakanon N, Wongchanthong W, Dejsirilerts S, Nawa Y. Identification of major *Streptococcus suis* serotypes 2, 7, 8 and 9 isolated from pigs and humans in upper northeastern Thailand. *Southeast Asian J Trop Med Public Health* 2014; **45**: 1173–81.
- Huong VT, Hoa NT, Horby P, et al. Raw pig blood consumption and potential risk for *Streptococcus suis* infection, Vietnam. *Emerg Infect Dis* 2014; **20**: 1895–98.
- Dittrich S, Rattanavong S, Lee SJ, et al. Orientia, rickettsia, and leptospira pathogens as causes of CNS infections in Laos: a prospective study. *Lancet Glob Health* 2015; **3**: e104–12.
- Araujo F, Nogueira R, Araujo Mde S, et al. Dengue in patients with central nervous system manifestations, Brazil. *Emerg Infect Dis* 2012; **18**: 677–79.
- He S, Ge L, Jin Y, Huang A. Clinical analysis of scrub typhus-associated hemophagocytic syndrome. *Zhonghua Er Ke Za Zhi* 2014; **52**: 683–87.

-
- 29 Ono Y, Ikegami Y, Tasaki K, Abe M, Tase C. Case of scrub typhus complicated by severe disseminated intravascular coagulation and death. *Emerg Med Australas* 2012; **24**: 577–80.
- 30 Todd SR, Dahlgren FS, Traeger MS, et al. No visible dental staining in children treated with doxycycline for suspected Rocky Mountain spotted fever. *J Pediatr* 2015; **166**: 1246–51.
- 31 Limmathurotsakul D, Turner EL, Wuthiekanun V, et al. Fool's gold: why imperfect reference tests are undermining the evaluation of novel diagnostics: a reevaluation of 5 diagnostic tests for leptospirosis. *Clin Infect Dis* 2012; **55**: 322–31.
- 32 Zhang X, Wang H, Ding S, et al. Prevalence of enteroviruses in children with and without hand, foot, and mouth disease in China. *BMC Infect Dis* 2013; **13**: 606.