Invited Review

Challenges in the Etiology and Diagnosis of Acute Febrile Illness in Children in Low- and Middle-Income Countries

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Received September 20, 2015; accepted March 4, 2016; electronically published April 7, 2016.

Acute febrile illness is a common cause of hospital admission, and its associated infectious causes contribute to substantial morbidity and death among children worldwide, especially in low- and middle-income countries. Declining transmission of malaria in many regions, combined with the increasing use of rapid diagnostic tests for malaria, has led to the increasing recognition of leptospirosis, rickettsioses, respiratory viruses, and arboviruses as etiologic agents of fevers. However, clinical discrimination between these etiologies can be difficult. Overtreatment with antimalarial drugs is common, even in the setting of a negative test result, as is overtreatment with empiric antibacterial drugs. Viral etiologies remain underrecognized and poorly investigated. More-sensitive diagnostics have led to additional dilemmas in discriminating whether a positive test result reflects a causative pathogen. Here, we review and summarize the current epidemiology and focus particularly on children and the challenges for future research.

Key words. antimicrobial stewardship; clinical algorithm; diagnostics; epidemiology; etiology; management; molecular; resource-limited settings; serology; undifferentiated fever.

Acute febrile illness is a common cause of hospital admission, and although it is not recognized as a disease state by the World Health Organization (WHO), its associated infectious causes contribute to substantial morbidity and death among children worldwide [1]. Studies among adults with febrile illness who required hospital admission documented case fatality ratios that ranged from 5% to 24% [2, 3]. In 2010, global estimates of causes of child death determined that almost two-thirds of deaths in children younger than 5 years were attributable to infectious causes, of which malaria was considered 1 of the top 3 causes [1]. In low- and middle-income countries (LMICs), where limited resources hinder diagnostic capacity, clinical management is infrequently supported by knowledge of the predominant local and regional etiologic pathogens. As a result, particularly in sub-Saharan Africa, acute febrile illness is commonly diagnosed as malaria. However, since the introduction of rapid diagnostics for malaria and implementation of malaria-control strategies in several countries, the number of diagnosed malaria cases has declined substantially [4–9]. As a consequence, the overdiagnosis of malaria and overtreatment in febrile illness has been increasingly recognized [10–13] and necessitates an improved understanding of nonmalarial causes of acute febrile illness [14].

This review provides an overview of the epidemiology of acute febrile illness in children in LMICs, assesses the epidemiologic, diagnostic, and consequent management challenges in these settings, and provides brief recommendations for future directions.

EPIDEMIOLOGY

In high-income countries where *Haemophilus influenzae* type b and *Streptococcus pneumoniae* immunization programs have been widely implemented and led to substantial reductions in invasive disease [15, 16], systemic bacterial infections are uncommon. In these regions, localized bacterial infections, such as urinary tract infection and pneumonia, are the predominant bacterial causes of fevers in the

Journal of the Pediatric Infectious Diseases Society, Vol. 5, No. 2, pp. 190–205, 2016. DOI:10.1093/jpids/piw016

pediatric population [17–19]; however, viruses constitute up to 76% of etiologies of fever without an apparent source [20].

In LMICs, where there is ongoing but not yet widespread introduction of *H influenzae* type b and pneumococcal vaccines in most regions and where typhoidal and nontyphoidal *Salmonella* remain important causes of invasive bacterial disease but the currently available typhoid vaccine has suboptimal efficacy, there is scant literature on the burden of disease attributable to febrile illness among children. Researchers tend to investigate particular syndromes such as pneumonia [21] or specific pathogens such as malaria [22] rather than broadly evaluate the causes of febrile illness in their population. We reviewed the literature dating back to the 1970s for studies that evaluated the etiologies of acute febrile illness in both inpatient and outpatient settings that involved or focused on a pediatric group (Table 1) and associated clinical syndromes (Table 2). We identified pertinent articles in PubMed/Medline by using the search terms "acute febrile illness," "undifferentiated fever," "low- and middle-income countries," and "developing countries" and the names of continents and regions (eg, South Asia). Additional studies were identified by searching bibliographies and the Worldwide Antimalarial Resistance Network [23]. To assess the proportion of patients with febrile illness who had an identifiable pathogen, we included only prospective studies in which acute febrile illness was a primary enrollment criterion or, in the case of seroepidemiology studies, a primary indication for blood culturing. Patients of all ages were included in our search, although we focused our data collection on studies with children <18 years of age. We excluded studies that examined specific risk populations, those that used subjective or poorly defined inclusion criteria, and those that had inconsistencies in the methodology or the data. Data were extracted from the articles by using predefined data fields.

Thirty-seven studies that evaluated children exclusively were identified, and an additional 16 studies evaluated both children and adults (Appendix Table 1). Twenty-three studies were performed in an outpatient setting. Few neonatal studies were included, because fever is not as common a symptom as lethargy and feeding intolerance in cases of neonatal sepsis [24]; hence, acute febrile illness was not the primary enrollment criterion in most of these studies. In addition, neonates are less likely to be febrile than older children [25]; 1 prospective cross-sectional study among neonates noted that lack of fever (<36°C) was more likely to be associated with a positive blood culture result than a temperature of >37.5°C ($P = .008$) [26], which illustrates the challenges in evaluating acute febrile illness in this population. The following sections describe findings from different geographic regions.

Africa

In the 1990s, when diagnostic capacity was much more limited, WHO recommendations were to empirically treat all cases of fever in children presumptively as malaria [27]. As culture-based methods improved and various serologic assays and rapid diagnostics for malaria were made available, researchers began recognizing nonmalarial causes of fever and identified a much wider spectrum of etiologies. Among children, studies of acute febrile illness that used a combination of microbiologic methods identified malaria in 1.3% to 64.4% [11, 28], *Leptospira* spp. in 0.4% to 7.7% [11, 29], *Rickettsia* spp. in 0.4% to 7.4% [11, 29], *Coxiella* spp. in 0.1% to 2.6% [11, 29], and *Brucella* spp. in up to 2.0% [11]. Respiratory viruses, with adenovirus comprising 30%, accounted for >50% of the fevers in an outpatient pediatric study conducted in Tanzania [29]. Other pathogens identified to be causative agents of acute febrile illness in patients with bloodstream infections include *Mycobacterium avium* complex, which causes 1% of cases [30], *Salmonella* spp. in 0.1% to 19% of cases [29, 31], *S pneumoniae* in 0.1% to 6.5% of cases [29, 32], *Escherichia coli* in 0.1% to 7.2% [33, 34], and *Staphylococcus aureus* in 0.2% to 22.4% [35, 36]. Among adults, other fungal etiologies of bloodstream infections, such as *Cryptococcus neoformans* (1.8% to 9% [3, 37]) and *Histoplasma capsulatum* (1.0% [38]), have been reported.

Asia

Typhoid, rickettsioses, dengue, and leptospirosis have been prevailing concerns in different regions of this continent. In Bangladesh, acute febrile illness is closely associated with typhoid, and the relative risk for preschool-aged children has been calculated to range from 8.9 to 12 compared with that of older persons [39, 40]. Children younger than 2 years have an incidence rate of 443/100 000 child-years, which is higher than the incidence rate of 405/100 000 child-years for those younger than 5 years [41].

In south Asia, dengue, typhoid, and paratyphoid have been the most commonly detected pathogens (25% and 23.2%, respectively) among febrile patients [42, 43]. Other pathogens that were studied in smaller numbers included *Rickettsia* spp. (positive in 17% of all tested isolates) [43], West Nile virus (positive in 5%), and Hantavirus (positive in 2%) [43]. More studies have been conducted in southeast Asia, where the predominant pathogens have been identified as scrub typhus (in 1%–19.3% of febrile cases) [44, 45], typhoid (1.8%–23%) [46, 47], dengue (5.4%–43.1%) [46, 48], Japanese encephalitis virus (3.4%–5.8%) [46, 49], chikungunya (1.2%–28.4%) [50, 51], *Burkholderia*

Table 1. Etiologies of Acute Febrile Illness in Low- and Middle-Income Countries According to Region[a]

	Etiology (%) [Reference]					
Organism	Southeast Asia	South Asia	East Africa	West Africa	Southern Africa	Latin America
Gram positive						
Staphylococcus aureus	0.1 [49] to 5.0 [143]		0.2 [134] to 1.6 [122]	1 [31] to 22.4 [35]	0.3 [25] to 7.1 [32]	
Streptococcus pneumoniae	0.5 [49] to 4.8 [143]		0.1 [29] to 7 [133]	0.8 [131] to 6.4 [130]	2.8 [25] to 6.5 [32]	
Streptococcus pyogenes	0.2 [46] to 1.5 [120]		2.2 [123] to 2.3 [13]		0.3 [25]	
Streptococcus agalactiae	0.1 [120] to 0.5 [143]				0.6 [25]	
Enterococcus spp.	0.2 [120] to 2 [47]		2.3 [33]			
Gram negative						
S entirica serovar Typhi	1.8 [46] to 23 [47]	23.2 [43][b]	0.4 [33] to 10.5 [124]	0.3 [35] to 2.3 [131]	0.7 [25] to 1 [30]	
Non-Typhi *Salmonella*			0.1 [29] to 4.4 [124]	0.7 [35] to 19 [31]	3.2 [32] to 8 [30]	
Shigella			0.1 [29] to 0.3 [122]	1 [31]		
Escherichia coli	0.2 [49] to 7 [47]		0.2 [29, 130] to 7.2 [33]	0.5 [131] to 14 [128]	0.1 [34] to 3 [30]	
Klebsiella spp.	0.1 [49] to 3 [126]		0.2 [123] to 0.4 [33]	0.6 [131] to 4 [128]	1.3 [32]	
Pseudomonas spp.	0.1 [46] to 5 [47]		0.1 [29] to 1.3 [122]	0.2 [131] to 3.7 [35]	0.3 [32]	
Citrobacter spp.			4.2 [33]			
Enterobacter spp.	0.1 [120] to 0.5 [52]			0.2 [131]	0.1 [34]	
Acinetobacter spp.	0.2 [46] to 1.0 [52]		0.2 [29] to 1.6 [122]	1.3 [131]	1 [30]	
Serratia marcescens			0.2 [123]			
Proteus mirabilis	1 [126]		0.4 [33]	0.4 [35]		
Neisseria meningitidis	0.2 [46]		1.3 [124]	0.8 [130]	0.1 [34]	
Haemophilus spp.	0.2 [52, 120] to 9.8 [143]		0.1 [29, 134] to 3.2 [28]	0.2 [131] to 2.5 [130]	0.3 [34] to 1.0 [25, 32]	
Moraxella catarrhalis	10.3 [118]					
Bartonella spp.	9.9 [139]					
Leptospira spp.	1.2 [58] to 27 [45]		0 [133] to 7.7 [11]			15.9 [55]
Brucella spp.			2.0 [11]			1.5 [55]
Coxiella burnetti			0.1 [29] to 2.6 [11]			46 [55]
Borrelia spp.		1 [43]	2 [133]			
Burkholderia cepacia				0.1 [124]		
Burkholderia pseudomallei	0.2 [49] to 1.5 [44]					
Mycoplasma pneumoniae	7.1 [143] to 25 [138]					
Chlamydia pneumoniae	0.5 [143] to 15 [138]					
Rickettsioses	8.8 [51] to 20.1 [44]		7.4 [11]			7.5 [55]
Rickettsia typhi	0.5 [44, 49] to 8.0 [58]	17 [43]	0.6 [29]			
Rickettsia spp.	0.5 [49] to3 .5 [50]	2 [43]	0.4 [29] to 3 [133]			
Orientia tsutsugamushi	1 [45] to 19.3 [44]					
Viruses						
Influenza	1.0 [52] to 38.8 [49]		19.1 [29] to 20.3 [13]			
Parainfluenza	2.3 [52] to 7.7 [46]		3.4 [29] to 10.1 [13]			
Respiratory syncytial virus	1.1 [46] to 29.2 [143]		0.3 [123] to 5.7 [29]			
Human metapneumovirus			1.9 [29] to 3.2 [13]			
Human rhinovirus	8.6 [52] to 22.0 [125]		21.1 [29]			
Coronavirus			10.3 [29]			
Bocavirus			11.0 [29]			
Adenovirus	0.2 [119] to 1.8 [143]		10.5 [13] to 26.6 [29]			
Rotavirus						
Enterovirus	19.1 [52]		0.1 [29]			
Parvovirus B19			1.3 [29]			
Cytomegalovirus			0.8 [29]			
Epstein-Barr virus			0.2 [29]			
Human herpes virus 6			7.9 [29]			

Continued

Table 1. Continued

Organism	Etiology (%) [Reference]					
	Southeast Asia	South Asia	East Africa	West Africa	Southern Africa	Latin America
Yellow fever						2.4 [56] to 3.3 [55]
Dengue	5.4 [46] to 43.1 [48]	25 [42]	0 [29]			5.6 [55] to 26 [56]
Japanese encephalitis	3.4 [46] to 5.8 [49]					
Chikungunya	1.2 [58] to 28.4 [50]		0 [29] to 10.2 [11]			
West Nile virus		5 [42]	0 [29]			
Rift Valley			0 [29]			
Venezuelan equine encephalitis						0.3 [58] to 2.1 [59]
Mayaro						
Ilhéus						0 [55] to 0.9 [56]
Oropouche						27 [55]
Guaroa						0.3 [55] to 1.1 [56]
St Louis encephalitis						0.1 [56]
Hepatitis A	1.4 [50]		0.1 [29]			9 [55]
Hepatitis E			0 [29]			
Measles	0.2 [120]					
Hantavirus		2 [43]				
Parasites						
Plasmodium spp.	0 [60] to 52.1 [138]		1.3 [11] to 64.4 [28]		19 [30] to 40 [25]	5.9 [56] to 12.8 [55]
Entamoeba histolytica			0.2 [29]			
Toxoplasma gondii			0.2 [29]			
Mycobacteria						
Mycobacteria tuberculosis	0.5 [46]					
Mycobacteria avium complex					1 [30]	
Fungi						
Yeast/*Candida* spp.	0.1 [46]		0.4 [33]	0.2 [131]		
Cryptococcus neoformans			1.8 [37] to 9 [3][c]			
Histoplasma capsulatum			1.0 [38][c]			

[a] Multicountry studies were grouped according to the region in which the majority of the participants were enrolled.
[b] Typhoid/paratyphoid.
[c] Adults.

Table 2. Clinical Syndromes of Acute Febrile Illness in LMICs

Location, Year [Reference]	Age	No. of Cases	Clinical Syndrome (%)
Asia			
Malaysia, 1975–1979 [44]	All ages (median, 20 to 29 y)	1629	URTI (7.8) LRTI (4.8) UTI (2.1) Diarrhea (1.0) Meningitis (0.5) Septicemia (0.4) Soft-tissue infections (0.3)
Papua New Guinea, 1991–1993 [120]	<3 mo	2168	URTI (48.1) LRTI (33) Sepsis (2.4) Meningitis (0.8)
Indonesia, 1997–2000 [77]	1 to 80 y	236	LRTI (19.1) UTI (8.5) Typhoid fever (7.2) Meningitis/encephalitis (6.4) Malaria (4.4) URTI (2.5) Sepsis (1.7)
Pakistan, 1999–2001 [141]	<16 y	4196	Typhoid (51) Malaria (5) UTI (2.4) Septicemia (0.8)
Papua New Guinea, 2003 [126]	<3 y (median, 17 mo)	98	LRTI (11) UTI (9) Malaria (7) Meningitis (4) Bacteremia (1)
Cambodia, 2008–2010 [87]	7 to 49 y		URTI (76.4)[a] Enteric fever (16.9)[a] Malaria (6)[a] LRTI (0.6)[a]
Cambodia, 2009–2010 [46]	<16 y (median, 2 y)		LRTI (38.3) Undifferentiated fever (25.5) Diarrheal disease (19.5) CNS infection (3.1)
Africa			
Ghana, Kenya, 1987–1992 [122]	>8 y	639	LRTI (10.2) Gastrointestinal infection (8.3) Urogenital tract infection (7.4) Musculoskeletal infection (6.1) CNS infection (1.4) Cardiac infection (1.6) Postsurgical infection (10.5)
Zimbabwe, 1993–1994 [32]	<8 y	309	Pneumonia (81.9) Septicemia (12.0) Diarrhea/dysentery (11.7) Meningitis (5.8)
Malawi, 1996–1997 [25]	≤15 y	338	Definite focus of infection in only 41/338 episodes (12.1) Meningitis (68) Pneumonia (20) Septic arthritis (5) Septic wound (5) UTI (2)
Kenya, 2001 [33]	3 mo to 12 y (mean, 2 y 8 mo)	264	Malaria (59.8) Bacteremia (12.1) UTI (14.4)
Gabon, 2008 [97]	<18 y (median, 2 y)	418	LRTI (33.9) Malaria (23) Gastroenteritis (16.7) Meningitis (3.3) UTI (2.4)

Continued

Table 2. Continued

Location, Year [Reference]	Age	No. of Cases	Clinical Syndrome (%)
Tanzania, 2008 [29]	2 m to 10 y (median, 12 m to <36 m)	1005	URTI (36) LRTI (16) Systemic infection (11) Nasopharyngeal viral infection (10) Malaria (9) Gastroenteritis (8) UTI (5) Typhoid fever (3) Skin/mucosal infection (1) Meningitis (0.2)
Kenya, 2011–2012 [13]	1 to 12 y	554	ARI (41) Malaria and ARI (39)

Abbreviations: ARI, acute respiratory illness; CNS, central nervous system; LMIC, low- and middle-income countries; LRTI, lower respiratory tract infection; URTI, upper respiratory tract infection; UTI, urinary tract infection.
[a] Clinical diagnoses in malaria rapid diagnostic test–negative cases.

pseudomallei (0.2%–1.5%) [44, 49], and influenza (1.0%–38.8%) [49, 52].

Latin America

Dengue and leptospirosis have been predominant infectious causes of fever in this region, although it is challenging to discriminate between them. A postmortem study of patients with suspected dengue-related fatality revealed that 83% of them actually had leptospirosis [53]. Similarly, Venezuelan equine encephalitis virus (VEEV) can be difficult to distinguish from dengue, and coinfection can be common. This fact was highlighted in a serologic study conducted in Venezuela in which 55.2% of the samples randomly drawn from a population during a VEEV outbreak tested positive for VEEV; however, 41.9% also tested positive for dengue [54], which suggests that the epidemic may be have been caused by both pathogens.

Studies that used a broader range of diagnostic tests have led to the detection of *Leptospira*, *Rickettsia*, *Coxiella*, and *Brucella* spp. from the Amazon Basin [55] and of arboviruses in relation to >30% of febrile episodes analyzed in a multicountry study [56]. Two studies that evaluated febrile illness in this region identified the most common etiologies as dengue (5.6%–26%), malaria (5.9%–12.8%), yellow fever (2.4%–3.3%), and other arboviruses (1.3%) [55, 56]. In addition, *Coxiella* spp. were identified in 46% of a subgroup of tested specimens, *Leptospira* spp. in 15.9%, *Rickettsia* spp. in 7.5%, and *Brucella* spp. in 1.5% [55, 56].

CHALLENGES IN ACUTE FEBRILE ILLNESS

Epidemiologic Challenges

Knowledge of epidemiology in a region is crucial for informing public health strategies, allocating resources, and monitoring the effects of interventions. However, substantial challenges hinder the compilation of this knowledge, and the primary handicap is the lack of data from many LMICs. A systematic review of severe febrile illness in LMICs identified only 45 studies conducted in 22 locations, of which there were no eligible studies identified from southern or middle Africa, eastern Asia, Oceania, or Latin America or from Caribbean regions or the European region [57]. However, that review excluded studies conducted in outpatient settings, which amounted to >120 studies [57]. Because patients may not commonly present to a healthcare facility for care during a febrile episode, a substantial number of cases may be missed, leading to an underestimation of the true burden. The limits of passive surveillance were highlighted in 1 study in which febrile patients were all referred to a community clinic, but only 30% of them subsequently reported for evaluation [56].

Another limitation is that many studies that evaluate febrile illness are conducted over only a few months and do not span a full calendar year. Therefore, these studies would not adequately account for seasonal variations in disease and illness, the occurrence of which has been extensively documented [3, 37, 49, 58].

Diagnostic Challenges

Clinical Diagnosis. Clinical history and examination are the mainstays of diagnosis in many resource-limited settings, and clinical algorithms have been developed for diagnosing febrile disease. Following the WHO recommendations of that time [27], a study that involved <1100 children found that a mother's report of fever in her child was 93% sensitive and 21% specific for the detection of malaria parasitemia on a blood smear [59]. However, in general, clinical algorithms have poor precision and cannot sufficiently discriminate among the etiologies of fever [60–63].

Culture-Based Methods. Bacterial cultures remain the gold standard for diagnosis, but they are limited by being

Table 3. Major Etiologies of Acute Febrile Illness and Associated Testing

Organism	Preferred Diagnostic Method(s)	Comments
Gram positive		
Staphylococcus aureus	Blood culture	Blood culture is insensitive; MALDI-TOF and PCR can significantly decrease time to identification on positive cultures, and PCR can provide antimicrobial-susceptibility data
Streptococcus pneumoniae	Blood culture, blood PCR	PCR of *S pneumoniae* can be positive in healthy asymptomatic controls; pneumococcal urine antigen in children can reflect nasopharyngeal carriage
Streptococcus pyogenes	Throat PCR, antigen and culture	Sensitivity of test highly depends on the quality of the throat-swab specimen
Gram negative		
Salmonella	Bone marrow culture has higher sensitivity than blood culture; stool, urine, bile cultures	Serologic assays (eg, Widal test) are insensitive
Escherichia coli	Blood culture	
Klebsiella pneumoniae	Blood culture	
Enterobacter spp.	Blood culture	
Leptospira spp.	Blood, CSF, urine cultures; blood and urine PCR; fourfold increase in acute and convalescent serologies (MAT is gold standard)	Specialized culture media required, may require incubation for up to 16 wk; low sensitivity
Brucella spp.	Blood, bone marrow, tissue cultures; fourfold increase in acute and convalescent serologies (serum agglutination test is gold standard)	Specialized culture media required, may require incubation for minimum 4 wk; low IgM titers may persist for years after initial infection; serologic assay may cross-react with *Y enterocolitica*, *F tularensis*, and *V cholerae*
Coxiella	Fourfold increase in acute and convalescent serologies (IFA is gold standard); blood PCR	A negative blood PCR result will not rule out infection
Burkholderia pseudomallei	Blood, sputum, throat, rectum, skin lesion cultures; blood PCR	Blood PCR is less sensitive than culture; serologic assays inadequate in areas of endemicity because of high background seropositivity
Rickettsia typhi	Blood culture; serology (IIFT has relative sensitivity and specificity), immunohistochemical staining and PCR of skin eschar or rash	Specialized culture media required and not available in routine clinical laboratories; Weil-Felix test is insensitive and nonspecific
Orientia tsutsugamushi	Blood PCR	
Viruses		
Influenza	Nasopharyngeal PCR	Shell vial and viral culture are helpful, but results may take several days
Parainfluenza	Nasopharyngeal PCR	
Respiratory syncytial virus	Nasopharyngeal PCR	
Human metapneumovirus	Nasopharyngeal PCR	Acute and convalescent serum for rise in titers is used in research settings to confirm initial infection
Human rhinovirus	Nasopharyngeal PCR	PCR is the only way to detect species C virus; PCR may be detected in asymptomatic patients
Human coronavirus, non-SARS, non-MERS	Nasopharyngeal, respiratory PCR	Upper and lower respiratory tract specimens are most appropriate for detection
Human coronavirus, SARS/MERS	Nasopharyngeal, respiratory, stool PCR; serology	Lower respiratory tract specimens have higher yield, serology is useful for diagnosis, and most likely positive in first week of illness
Bocavirus	Nasopharyngeal, blood PCR; serology	Blood PCR is required for diagnosis, because positive nasopharyngeal secretions are too nonspecific to be useful
Adenovirus	Nasopharyngeal PCR, blood PCR, antigen detection, cell culture	Enteric adenovirus types 40 and 41 usually cannot be isolated in standard cell culture Persistent and intermittent shedding after acute infection can complicate clinical interpretation
Enterovirus	CSF, nasopharyngeal, blood, stool, rectal, throat, conjunctival, tracheal aspirate, vesicle fluid, urine or tissue PCR	Standard PCR is more sensitive than cell culture and can detect all enteroviruses (except new group C enteroviruses), including those difficult to cultivate in culture
Parechovirus	Stool, respiratory, blood, CSF PCR.	Grows poorly in culture, and culture typing reagents not widely available for types 3–14
Parvovirus B19	Serology; blood PCR	Positive IgM indicates infection probably occurred within the previous 2–3 mo
Cytomegalovirus	Blood PCR; urine, fluid, tissue cell culture	Standard virus cultures must be maintained for >28 days; shell vial culture and IFA stain provides results within days
Human herpes virus 6	Blood PCR	Blood detection has high specificity
Human herpes virus 7	Blood PCR, serology	IgM not always detectable in children with primary infection yet may be present in asymptomatic previously infected people.

Continued

Table 3. Continued

Organism	Preferred Diagnostic Method(s)	Comments
Yellow fever	Blood, CSF, or tissue viral culture or NAAT, fourfold increase in acute and convalescent serologies	Serological cross-reaction with related arboviruses from same viral family can occur;
Dengue	Blood PCR; blood IgM; fourfold increase in acute and convalescent serologies; blood, CSF, or tissue viral culture or NAAT	
Japanese encephalitis	Serology, blood, CSF PCR; CSF IgM; immunohistochemical staining of tissues	
Chikungunya	Blood PCR; blood IgM; fourfold increase in acute and convalescent serologies; blood, CSF, or tissue viral cultures or NAAT	IgM can persist for ≥90 days
Rift Valley	Blood PCR	
West Nile virus	CSF, blood IgM; fourfold increase in acute and convalescent serologies	May more likely be detected early in illness using culture or NAATs; PCR positive early in illness
Venezuelan equine encephalitis	Fourfold increase in acute and convalescent serologies	
Hantavirus	Blood PCR; blood IgM; fourfold increase in acute and convalescent serologies (IIFT)	
Hepatitis A	Serology; blood PCR	PCR positive early in illness; viral RNA not detected readily in BAL fluid; viral culture is not useful
Hepatitis E	Serology; blood, stool PCR	IgM detected >1 y after infection
Parasites		
Plasmodium spp.	Microscopy of thick and thin blood films, blood PCR, blood antigen detection	Antigen detection has poor sensitivity for *P vivax*
Mycobacteria		
Mycobacteria tuberculosis	Sputum, blood, gastric aspirate, bronchial washing, fluid, urine, or tissue culture	NAATs have various sensitivities and specificities for sputum, gastric aspirate, CSF, and tissue specimens
Fungi		
Cryptococcus neoformans	Serum antigen, blood, or fluid culture; fungal stain and culture of pulmonary or skin lesions	Antigen detection can be falsely negative when antigen concentrations are low or very high (prozone effect), if infection is caused by unencapsulated strains, or if the patient is immunocompromised

Abbreviations: BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; IFA, immunofluorescence antibody; IgM, immunoglobulin M; IIFT, indirect immunofluorescence test; MAT, microscopic agglutination assay; MALDI-TOF, matrix-assisted laser desorption ionization time of flight; MERS, Middle Eastern respiratory syndrome; NAAT, nucleic acid amplification test; PCR, polymerase chain reaction; SARS, severe acute respiratory syndrome.

resource intensive and slow (Table 3). In addition, their sensitivity varies according to the fluid and volume collected [64], host factors such as age and comorbidities [65], previous antibacterial agent use [66], and the stage of illness [67]. For example, blood cultures are 40% to 80% sensitive for the detection of invasive *Salmonella* spp. and are even more sensitive in the first week of illness when the bacterial concentration is high compared with that in subsequent weeks [68, 69]. Similarly, bone marrow cultures have approximately 90% sensitivity for the detection of *Salmonella* spp. and are relatively unaffected by antibiotics, whereas stool cultures and rectal swabs have <40% sensitivity [68]. Certain bacteria, such as *Rickettsia* and *Leptospira* spp., require cell-based systems or enriched culture media for cultivation and may present hazards to laboratory workers, which prevents their implementation in routine diagnostic laboratories. Respiratory tract diseases account for a large proportion of febrile illness; however, in patients with community-acquired pneumonia, blood culture results are positive in only a fraction of cases [70].

Serology. A broad range of pathogens can be detected by serology, but this approach is limited by the need for acute and convalescent sera to establish seroconversion. This limitation is reflected in a study in which acute serum antibody testing detected only 52.8% and 40.8% of subsequently confirmed dengue and leptospirosis infections, respectively [45]. For *Leptospira*, the microscopic agglutination test has a sensitivity of 41% during the first week, which rises to 96% beyond the fourth week of illness [71]; however, this requirement for separate blood draws 4 weeks apart has limited practicality. Single samples have been used for presumptive diagnosis, but cutoff values vary among sites, which limits the ability for diagnostic confirmation and makes it difficult to distinguish whether a positive result reflects acute disease. The limited sensitivity and specificity of a single agglutination test have also been noted in other serologic assays, such as the Widal test, particularly early in the course of an illness and in settings of endemicity [72, 73]. Reliance on the detection of immunoglobulin M antibodies may also lack sensitivity, especially if blood samples are drawn too early in the course of illness.

In addition, serologic assays may have too little or too much cross-reactivity with other related species. The Widal test poorly identifies *Salmonella enterica* serovar Typhi and *S enterica* serovar Enteritidis, despite the similarity between the O antigens of the subspecies [74], whereas serologic assays for flaviviruses are hampered by extensive cross-reactivity among the species [75]. Enzyme-linked immunosorbent assays have been used to study the normal antibody response during illness, although for some disease processes, the results are still limited by a lack of specificity. A study of febrile illness in Laos that used predominantly serologic methods identified 7% of patients who had apparent multiple infections [76].

The use of newer serologic assays led to the identification of pathogens that had not been detected previously in the Amazon Basin, such as *Leptospira* spp., *Rickettsia* spp., *Coxiella* spp., Ilheus virus, and *Brucella* spp. [55], and of hantavirus in Nepal [43]. In addition, acute and convalescent sera led to the recognition that dengue infections were clinically underdiagnosed, with 16% of acute febrile episodes not clinically diagnosed as dengue that were later supported by positive dengue serology and 5.3% that were confirmed by NS1 antigen testing [50]. However, because of the issue of cross-reactivity, many patients have several positive serologic results, negative bacteriologic results, and unremarkable nonspecific clinical presentations consistent with several possible diagnoses [77], which makes interpretation challenging. A study conducted in Thailand in the 1990s, which used different serologic assays for *Leptospira* spp., *B pseudomallei*, *Salmonella* spp., influenza, Japanese encephalitis virus, dengue, and chikungunya, still failed to detect an etiology in >60% of the cases [58].

Molecular Diagnostics. Molecular diagnostics has led to increased detection of pathogens; however, detection rates are pathogen and organ specific. Molecular diagnosis of *Rickettsia* spp. by polymerase chain reaction (PCR) has a sensitivity of 90% when skin biopsy samples are used but falls to 50% when blood is used [78]. Similarly, PCR can be poorly sensitive for the detection of invasive *Salmonella* spp. because of the low burden of organisms in bodily fluids [79].

Newer molecular diagnostics have also broadened the spectrum of bacteria and viruses that can be investigated and identified [80–82] and have particularly expanded the investigation of respiratory viruses [83–85]. New molecular approaches to defining the viruses that can be found in humans, otherwise known as the human virome, will lead to a better understanding of how microbes affect human health and disease and may lead to new treatments for patients with a variety of clinical syndromes [86]. However, this research is not yet being conducted in LMICs.

Causative Pathogens Versus Colonization. It can be difficult to determine whether positive test results indicate a causative pathogen or whether these pathogens are merely innocent bystanders, particularly for pathogens isolated from sites that are not normally sterile. A study of >900 children in rural Tanzania examined blood smears for

malaria and performed testing for the *lytA* gene for *S pneumoniae* and the *pal* gene for *H influenzae* in dried blood spots collected from febrile children [28]. However, although the afebrile children were not controls and had still presented with a history of fever, the detection of both *lytA* and *pal* genes in febrile and afebrile children made the interpretation of positive results problematic [28].

Case-control studies of febrile illness have consistently diagnosed pathogens in controls and in cases. One adult study found that 9.5% of febrile patients had malaria parasitemia, but there was no significant difference compared with the afebrile control group (8%) [2]. A prospective study conducted in rural Cambodia enrolled patients with acute febrile illness and a parallel group of nonfebrile accompanying people as controls. Although malaria, most *Leptospira* spp., influenza, and dengue were identified more frequently among the febrile patients, the detection of these same pathogens in the afebrile control group at a nontrivial rate was striking [87]. It is surprising that *Leptospira interrogans* was detected by PCR at a significantly higher frequency in the afebrile controls than in the febrile patients (8.5% vs 5.1%, respectively; $P = .04$) [87], which highlights the caution required when interpreting positive results.

Point-of-Care Tests. Rapid point-of-care tests are becoming more widely available. For patients who presented with febrile illness to an Ebola treatment unit in Sierra Leone, rapid diagnostic tests (RDTs) were useful in confirming a wide range of diagnoses other than Ebola virus disease and reducing patient lengths of stay [88]. Although they may represent some improvement over older serologic assays, they may still lack sensitivity and specificity as a result of the cross-reactivity of the selected antigens, and they can also be limited by the lack of a reference standard. These limitations were seen in the case of 2 RDTs for *S enterica* serovar Typhi, for which the sensitivity ranged from 56% to 84% and the specificity ranged from 72% to 95% [89]. However, although malaria RDTs have sensitivities of up to 95% [90] and are consequently useful for targeting treatment in resource-limited settings [91, 92], clinic-based diagnoses of malaria RDT-negative cases are poorly predictive for the pathogen, have not yet improved health outcomes for patients compared to clinical diagnosis, and do not provide guidance for appropriate management [87, 93].

Management Challenges

Overtreatment With Antimalarial Agents. Despite extensive data documenting a decline in malaria transmission in sub-Saharan Africa [4, 7–9, 94, 95], antimalarial therapy remains commonly prescribed even in the absence of positive test results. In an adult study in Malawi, malaria was the presumptive clinical diagnosis in 48% of the patients, and 56% were presumptively treated for malaria despite negative smear results [3]. Another adult study in Tanzania noted that malaria was the admission diagnosis in 74.7% of the cases, but 4% of those without an admission diagnosis of malaria were still prescribed an empiric antimalarial agent [12]. A Tanzanian region that was previously endemic for malaria later documented low transmission intensity; however, malaria continued to be diagnosed at a high rate, and 99.6% of those who were negative according to microscopy were still treated with antimalarial agents [96]. In a pediatric study that enrolled 467 infants and children, malaria was the clinical diagnosis for 60.4% of them but was the actual cause of fever in only 1.3% [11]. Another pediatric study conducted in Gabon found that 46% of the children who had negative blood smear results received antimalarial therapy [97]. One patient who had neither a clinical nor a biologic malaria diagnosis was also treated with an antimalarial agent [97]. In a Kenyan study, 80% of all children who presented with acute febrile illness, including 72% of children with a negative malaria smear result, were prescribed antimalarial agents [13]. In a study performed in India, 33.8% of >1600 febrile patients received antimalarial drugs, 75% of whom had ≥1 negative malaria test result [98]. A study of 250 febrile Ugandan children treated with antimalarial agents despite a negative malaria test result subsequently identified 45 of them to be cases of bacteremia instead [99]. Inappropriate use of antimalarial agents and misdiagnosis of malaria has consequences in terms of delayed diagnosis, inappropriate treatment, and unnecessary exposure to the adverse effects of antimalarial agents [100].

Overtreatment With Antibacterial Agents. Given the difficulty in discriminating the etiology of a fever based on clinical features alone, overtreatment with antibacterial drugs is also a common occurrence. In Laos, of the 1095 patients with data for hospital antimicrobial use, 56% received an antimicrobial drug, and 12% received more than 1 [49]. On the basis of final diagnosis, only 7% of these patients were regarded as having been treated appropriately [49]. Although malaria was the admission diagnosis for 148 adults in Tanzania, 44.8% of them were still prescribed empiric antibacterial agents [12]. Of those without an admission diagnosis of malaria, 59% were prescribed an antimicrobial agent [12]. A Rwandan study on malaria hospitalization after implementation of a malaria-control program documented a significant decrease in the risk of high parasitemia after the intervention (80.4% laboratory-confirmed cases in the preintervention period and

48.1% in the postintervention period) [95]. Antibacterial agents, however, were prescribed to 70.7% of all the children with clinical malaria throughout the study period [95]. The majority of these studies were conducted in an inpatient setting, which reflects the differing etiologic agents and competing management priorities of outpatient versus inpatient care, where empiric antibacterial treatment for hospitalized patients is justifiable. Rates of multidrug-resistant *S enterica* serovar Typhi of up to 80% in southeast Asia [101] and 70% in east Africa [102], and substantial antimicrobial resistance of non-Typhi *Salmonella* [103, 104], can be attributed partly to the unnecessary overuse of antimicrobial drugs. This is further complicated by the substandard medicines in circulation in several LMICs, which risks patient safety and may be promote antimicrobial resistance [105–107], and it may affect the relevance of the WHO's Integrated Management of Childhood Illness guidelines in treating the causes of acute febrile illness.

Delayed Diagnosis and Treatment With Antibacterial Agents. In regions where dengue fever and leptospirosis are endemic, the differentiation between the two is essential, because effective treatment for leptospirosis with antibacterial drugs is available. During a concurrent epidemic of dengue and leptospirosis in an urban center in Brazil in the mid-1990s, patients with leptospirosis who were initially misdiagnosed as having dengue were more likely than other patients to have required admission to the intensive care unit and to have died during hospitalization [108]. In rural Laos, where dengue accounted for >30% of febrile cases, only 17% of the patients with leptospirosis and 14% with rickettsial infection were treated appropriately with doxycycline [76]. Dengue was suspected and clinically diagnosed in only 20% of the 81 confirmed dengue cases [76]. A study that evaluated causes of fever in children in Thailand noted that nonclassical presentations of scrub and murine typhus were often missed or diagnosed as enteric fever [51], which highlights the importance of recognizing the disease because it affects antimicrobial management. Hence, in resource-limited settings, it is imperative to strike a balance between empirically treating when there are few available diagnostics and noting the need for judicious antibacterial prescribing.

Underrecognized Burden of Viral Etiology of Fevers. An outpatient pediatric study in Tanzania found that upper respiratory tract infection accounted for 36% of fevers, of which more than half were caused by respiratory viruses and 10% were nasopharyngeal viral infections [29]. Influenza has also been poorly investigated as a cause of acute fevers, and although studies have documented its prevalence to range from 1.0% to 38.8% in southeast Asia [49, 52], and 19.1% to 20.3% in east Africa [13, 29], studies on febrile illness in other regions did not incorporate influenza testing. Reflected by detection rates of up to 5.8% in 1 study [49], Japanese encephalitis virus is an underappreciated cause of acute undifferentiated fever in Asia [109]. In Africa, the endemicity regions of Lassa fever virus have expanded and become more widely distributed throughout west Africa [110], which adds an etiologic consideration of acute febrile illness in that region. Among pediatric studies, up to 64% of acute febrile illness pathogens were not diagnosed [11], indicating that much remains to be elucidated about the wide spectrum of viral etiologies of fever in LMICs.

Development of New Diagnostic Aids. Biomarkers have been evaluated extensively with regard to their ability to predict the evolution, invasiveness, and severity of infection in febrile patients. Studies that examined a variety of biomarkers, such as C-reactive protein, procalcitonin, midregional adrenomedullin, midregional pro-atrial natriuretic peptide, and copeptin, among others, have not conclusively identified biomarkers that reliably and consistently assist in identifying high-risk microbial infection [111, 112]. However, newer biomarkers, such as myovirus resistance protein A (MxA), have shown promise in differentiating viral from bacterial infections [113].

In addition to identifying high-risk microbial infections, an approach to differentiating between asymptomatic colonization and active disease has been to incorporate host immune response profiles into the interpretation of patient data. Assessing host gene-expression profiling as a correlate of host response to infection can not only act as an aid in the diagnosis of pathogens detected by molecular techniques that are often associated with asymptomatic carriage or colonization but also provide insight into disease progression and treatment response [114]. The feasibility of host RNA transcription profiling in the practice setting is being explored in a multicenter study of young febrile infants in emergency departments in the United States [115] and was validated and shown in Kenyan children to be more sensitive in detecting active tuberculosis disease than the currently used Xpert MTB/RIF real-time PCR assay, even for patients with inconclusive culture results [116]. This remains a fecund area for further research.

CONCLUSIONS

Acute febrile illness in children is emerging as a research priority now that we are seeing the success of malaria-control efforts among LMICs in reducing malaria transmission, although it has yet to be recognized by the

WHO or other international health bodies as its own disease state and health metric [1]. Acute febrile illness is associated with major case fatality ratios; however, the lack of distinguishing clinical features of these infections and obstacles to making the correct diagnoses limit appropriate care. Diagnostic capacity in many LMICs remains limited, and the etiologies in many recent studies were unknown, which impedes our ability to develop effective clinical algorithms or to make informed clinical decisions. In addition, diagnostics remain imperfect tools, as is the case when one cannot obtain both acute and convalescent serologies on a patient and when interpretation is challenging, such as when molecular diagnostics detect pathogens even in asymptomatic controls. In addition, the prevalence of viral etiologies in acute febrile illness is only now being recognized slowly, although it remains poorly documented in many LMICs. As a result of the paucity of data, antimalarial agents are overused (administered in up to 99% of patients with negative smear results in 1 study [96]), and overtreatment with empiric antibacterial agents also occurs (administered in up to 60% of patients without an admission diagnosis of malaria [12]).

Despite advances in our understanding of acute febrile illness in children, there remains a need to further develop diagnostics for enhanced surveillance in LMICs and to rapidly and comprehensively assess etiology. Similar to research being conducted on pneumonia and diarrhea [21, 117], comprehensive, standardized, multicenter etiology research is needed to understand the causes of acute febrile illness. An improved understanding of the etiologies of acute febrile illness is critical for developing management algorithms for the appropriate use of antimalarial and antibacterial drugs and essential for monitoring the impact on antimicrobial resistance.

Supplementary Data

Supplementary materials are available at the *Journal of the Pediatric Infectious Diseases Society* online (http://jpids.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

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