



A Prospective Study of Etiological Agents Among Febrile Patients in Sierra Leone

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ABSTRACT

Introduction: Sierra Leone has one of the highest burdens of febrile illnesses in the world. As the incidence of malaria diminishes, a better understanding of the spectrum of etiological agents was important for accurate diagnosis and empirical treatment of febrile illness.

Methods: Blood, nasopharyngeal, and fecal specimens were collected from febrile patients for serological, molecular detection, and

microbiologic culture to identify potential pathogens.

Results: For this prospective study, 142 febrile patients were enrolled. The prevalence of malaria was higher in children aged 5–15 years old ($P = 0.185$) and adults ($P = 0.018$). Acute respiratory infection (ARI) presented more commonly in the under 5 years old group ($P = 0.009$). For diarrhea, all children groups ($P = 0.024$) were predominant. A total of 22.5% of the febrile patients had malaria infection, 19.7% had typhoid infection, and 2.8% were coinfecting with malaria and typhoid. ARI was the most common causes of fever, accounting for 31.7% of patients, influenza A virus, *Mycoplasma pneumoniae*, and five other respiratory pathogens were found. Diarrhea accounted for 16.2%, and seven kinds of diarrhea bacteria were isolated. Hepatitis B accounted for 8.5%,

Han Wang and Jing Zhao contributed equally to this work.

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including five cases of spontaneous bacterial peritonitis, and ascites smear staining were both Gram-negative bacteria. Tuberculous encephalitis, parasitic diseases (ascaris and filariasis), and skin infection caused by *Staphylococcus aureus* accounted for 0.7%, 2.1%, and 0.7%, respectively.

Conclusions: Evidence of a wide spectrum of febrile etiological agents other than malaria was identified. The spread of malaria rapid diagnostic tests (RDTs) out of hospital and establishment of a national standard for Widal test will reduce the misdiagnosis of febrile diseases. Antibiotics against Gram-negative bacteria are helpful for empirical treatment.

PLAIN LANGUAGE SUMMARY

Sierra Leone has one of the highest burdens of febrile illnesses in the world. Evidence of a wide spectrum of febrile pathogens other than malaria has been proven in this study. We considered that the etiology of febrile patients was closely related to local geography, heredity, immune features, economic industry, living habits, air pollution, medical and health conditions, and this was fully analyzed and discussed. The screening process used in this study can further simplify and identify the etiological agents of fever in more than 70% of the study population. This laid the foundation for the establishment of a more simplified and efficient

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diagnosis and treatment process in the local area. We also found the characteristics of age distribution of different febrile diseases. Children were an important susceptible population to fever. This study indicated the importance of reliable diagnostic tests for febrile pathogens and provided the necessary information for RDT requirements. The spread of malaria RDTs out of hospital and establishment of a national standard for Widal test will reduce the misdiagnosis of febrile diseases. For empirical treatment, antimalarial treatment was still targeted at falciparum malaria in Sierra Leone. Antibiotics against Gram-negative bacteria contributed to the empirical treatment of febrile diseases. For patients with acute respiratory tract infection, Gram-positive coccal antibiotics could be candidates for treatment. In addition, systematic and professional treatment of liver diseases should be promoted to reduce infection complications.

Keywords: Fevers; Malaria; Typhoid; Acute respiratory infection; Diarrhea

Key Summary Points

Why carry out this study?

Sierra Leone has one of the highest burdens of febrile illnesses in the world. As the incidence of malaria diminishes, a better understanding of the spectrum of etiological agents was important for accurate diagnosis and empirical treatment of febrile illness.

What was learned from the study?

Evidence of a wide spectrum of febrile pathogens other than malaria has been proven in Sierra Leone. Children were an important susceptible population to fever.

The etiology of febrile patients was closely related to local geography, heredity, immune features, economic industry, living habits, air pollution, medical and health conditions.

The implementation of malaria RDTs out of hospital and establishment of a national standard for Widal test will reduce the misdiagnosis of febrile diseases.

Antibiotics against Gram-negative bacteria contributed to the empirical treatment of febrile diseases.

DIGITAL FEATURES

This article is published with digital features, including a summary slide and plain language summary, to facilitate understanding of the article. To view digital features for this article go to <https://doi.org/10.6084/m9.figshare.14680608>.

INTRODUCTION

Fever is the most common symptom of infectious diseases in tropical countries. Western Africa has one of the highest burdens of febrile illnesses, such as malaria, in the world. In Sierra Leone, malaria is the reason behind nearly four in ten hospital consultations [1], with its entire population of over 8 million people at risk of the disease. Substantial progress has been made in fighting malaria. From 2000 to 2015, the incidence rate of malaria cases was reduced by 20–40% in Sierra Leone [1]. The malaria mortality rates was reduced by more than 40% between 2010 and 2015, the highest in West Africa [2]. Most notably, in 2013, there were 2,576,550 suspected malaria cases, with a confirmed rate of 66%. In 2014, there were 2,647,375 suspected malaria cases, with a confirmed rate of 52%. In 2020, 31.1% of suspected malaria-infected patients were diagnosed with malaria by microscopy and rapid diagnostic tests (RDTs), so the disease in the remaining 68.9% of patients was caused by other fever-related pathogens [3, 4]. With the gradual application of malaria RDT in Africa, the proportion of non-malarial febrile illnesses has been found to be increasing, which was particularly noteworthy in malaria endemic areas of Africa [5].

A variety of pathogens were known to cause malaria-like fever, while some viral and bacterial diseases have been identified in febrile patients in Africa [6, 7]. However, it was still difficult to diagnose non-malarial febrile illness in areas with insufficient health resources because of the lack of affordable diagnostic tests and the prevalence of non-malarial febrile illness. These factors lead to misdiagnosis of malaria and delay the optimal treatment of other febrile diseases.

A systematic review of the etiology of febrile illness in low- and middle-income countries shows that only 4.9% of the case studies come from West Africa [8]. Health workers in Africa believe additional training and point-of-care tests would improve their ability to diagnose and manage non-malarial fevers [9]. Educating caregivers on signs and symptoms of endemic febrile illness, as well as the introduction of additional tests to identify, would be important steps to get patients to appropriate treatment earlier. We therefore did a prospective investigation of the spectrum of etiological agents among febrile patients to improve the understanding of the relative distribution of pathogens such as bacteria, parasites, and viruses, thereby facilitating diagnosis and management decisions for malaria and other febrile diseases.

METHODS

Study Design and Ethics

For this prospective study, we recruited consecutive febrile patients seeking healthcare at the 34 Military Hospital of Republic of Sierra Leone Armed Forces and Center for Tropical Infectious Disease Control and Prevention (CTDC) in Freetown from August 2018 through March 2019. The 34 Military Hospital was also the Headquarters of the Joint Medical Unit of Sierra Leone Armed Forces. CTDC was jointly carried out by the Chinese Military Medical Expert Group and the Sierra Leone Military doctors from the 34 Military Hospital. Freetown is the capital and largest city of Sierra Leone, located in the Western Area of the country. The population of Sierra Leone and

Freetown were 8.05 million and 1.20 million, respectively. Malaria was endemic in the whole of Sierra Leone, and the country's entire population, including Freetown, was at risk of the disease [3].

Patients with continuous fever (axillary temperature 38 °C or higher), of both sexes, met the following inclusion criteria: first visit due to fever, disease duration of 1 week or less, and not taking antimalarial drugs or antibiotics during the preceding week were included [6]. Infants younger than 2 months of age were excluded, since infectious agents and thus therapeutic approaches can differ substantially according to World Health Organization (WHO) guidelines [10]. The age range of the participants for inclusion in the study was 2–65 years old. Details of the study were discussed with the parents or guardians of children 2–15 years of age; the parents or guardians provided written informed consent. The patients with HIV infection and severe disease symptoms (i.e., neurological diseases, heart disease, shock, trauma, bleeding, etc.) were also excluded from this study. The study was approved by the regional ethics committee in the 34 Military Hospital, Republic of Sierra Leone Armed Forces (Deliberation JMU/1600). The 34 Military Hospital approved this study to carry out studies of etiological agents on human tissues, secretions, body fluids, and blood samples among febrile patients. The study was done according to the Helsinki Declaration. Informed consent was obtained from individuals (or their parents/guardians for children) for participation in this study.

Clinical Assessment and Processing

For all participants who met the inclusion criteria at the CTDC facility, a baseline questionnaire was administered. Data included demographic data, signs and symptoms, type of medication taken prior to health facility visit, and past infection history. A systematic set of investigations was performed according to the clinical diagnosis and treatment process of fever [11] for all patients. The process was adjusted to three steps to suit the local working conditions:

1. All patients were tested for malaria and typhoid to exclude the two main febrile pathogens. Thus 4 ml of whole blood was collected, 2 ml of which was used for malaria RDT and preparation of blood films for microscopic examination. The rest was used to separate plasma for Widal test.
2. The second step will be to identify those febrile illnesses that are reliably diagnosed on clinical grounds, such as throat pain/tonsillitis, typical skin symptoms including rash/(non-)vesicular rash/blister/herpes/skin irritation and ulceration/ swelling of the lower leg, meningeal irritation, diarrhea etc.

An acute respiratory infection (ARI) was defined as any acute infection (at most 1 week in duration) that manifested as at least one respiratory sign or symptom and was localized to the upper or lower respiratory tract [12–15]. Upper respiratory infections (URI) were infections of the mouth, nose, throat, larynx, and trachea. URI included nasopharyngitis, sinusitis, pharyngitis, laryngitis, tonsillitis, laryngotracheitis, and so on [16]. Lower respiratory tract infection was a respiratory disease caused by pathogens invading the lower respiratory tract and alveoli, including bronchiolitis and pneumonia [17]. For patients with throat pain/tonsillitis, nasal and throat swabs were collected and immediately placed in tubes containing Universal Transport Medium (Copan, Brescia, Italy). Real-time reverse transcription polymerase chain reaction (PCR) assays for the detection of influenza A and B, respiratory syncytial virus, and parainfluenza virus were performed as described previously [18, 19]. Patients with key symptoms or signs (if coughing or with an abnormal chest auscultation) that should lead to specific laboratory investigations will undergo chest X-ray (XH-MD6-32 mobile digital X-ray camera, Shandong, Xinhua Medical Instrument Stock Company Ltd, China). Sputum culture, serologies (IgM) for *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* were also performed.

Patients with skin symptoms suspected of

herpes simplex virus, mumps, rubella, measles, varicella zoster virus, dengue fever, and filariasis will undergo venous puncture (4 ml) for serological IgM detection. Skin secretion samples need to be identified by microbiological culture. Cerebrospinal fluid (CSF) test was performed in patients with meningeal irritation syndrome.

Patients with typical diarrhea symptoms need to collect stool samples for identification by microbial culture and direct microscopic examination.

3. Patients with history or suspected liver disease, abnormal alanine amino transferase (ALT) or ascites were serological screened for hepatitis A, B, C, and E. If accompanied by renal dysfunction, hantavirus was also screened for. Ascites routine test was performed in all patients with ascites.

Laboratory Analyses

Malaria

Positive malaria RDT [malaria Ag *Plasmodium falciparum* (*P. falciparum*)/*Plasmodium vivax* (*P. vivax*), SD BIOLINE, USA] OR positive blood slide for malaria, regardless of the presence of another cause of fever. Malaria microscopy: Thick films stained with 10% Giemsa were used for definitive parasite counts; 200 high power fields were screened before a slide was declared negative.

Typhoid

Positive typhoid RDT OR positive stool culture *Salmonella typhi* (*S. typhi*).

Serological diagnosis was carried out using Widal agglutination test (Tianrun biopharmaceutical Co., Ltd, Ningbo, China) to determine the antibody titers of the sera against *Salmonella* H (flagella) and O (somatic) antigens. The serological testing was done in accordance with manufacturers' guidelines, an antibody titer of at least 1:80 for both typhi O and H was taken as a cutoff value to indicate recent infection of typhoid fever.

Microbiologic Investigations

Stool samples were collected in clean wide-mouthed plastic containers from 23 patients with diarrhea. All stool samples were macroscopically observed for areas that contain blood and/or mucus/parasite. A loopful of each stool sample was inoculated onto *Salmonella-Shigella* agar (SSA) and MacConkey agar (MAC, all from ProSpecT, Remel, Lenexa, Kansas, USA). The inoculated plates were then incubated at 37 °C for 24 h. Isolates identified as *S. typhi* were differentiated from other *Salmonella* spp. using Triple Sugar Iron (TSI) agar (Becton Dickinson, Franklin Lakes, USA) [20]. Cultural and morphological characteristics of the isolates were studied thereafter and the isolates were duly identified following standard microbiological methods using VITEK 2 compact automatic bacteria analyzer (bioMérieux Company, France) [21–23]

Sputum specimens were treated with equal volume of sputasol, vortexed for 1 min, and then held for 30 min at room temperature; 10 µL of digested sample was dispensed onto each of three different plates and streaked in a three-quadrant pattern with an inoculation loop. The media used in this study were Columbia agar with 5% sheep blood (blood agar, Oxoid, England), chocolate agar (Choc agar, Oxoid, England), and China blue agar (a strong selective medium which is similar to MacConkey agar plate; CB agar, Beiruite Biotechnology Co., Ltd, Zhengzhou, China). Skin secretion samples were dispensed onto each of those three different plates. Sputasol (Oxoid, England) was used to digest the sputum specimens [24].

Serological Test

Serologies were performed using the following commercial kits: IgM for mumps, varicella zoster virus, and hantavirus: Beijing Beier Bioengineering Co., Ltd, Beijing, China; IgM for herpes simplex virus 1 and 2, measles, rubella, *M. pneumoniae*, *C. pneumoniae*, dengue fever: Oumeng medical diagnosis Co., Ltd, Beijing, China; IgM for HAV: WANTAI BioPharm Co., Ltd, Beijing, China. Hepatitis B markers (HBsAg,

anti-HBS, HBeAg, anti-HBE, anti-HBC) and IgG for *Mycobacterium tuberculosis* (TB): Aibo biomedical Co., Ltd, Hangzhou, China; IgM for HCV: InTec PRODUCTS, INC. Xiamen, China; IgM/IgG for HEV: Beijing modern gold biology technology Co., Ltd, Beijing, China; IgM for filariasis: BinaxNOW Filariasis, Inverness Medical, Portland, ME, USA, respectively. The above operations and results are judged according to the manufacturers' instructions.

Ascitic Fluid and Cerebrospinal Fluid (CSF) Test

(1) Routine test [25, 26] and (2) biochemistry test were carried out by an automatic biochemical analyzer (TC6030L, TECOM, Jiang Xi, China). Ascitic fluid infection was classified according to the criteria described by Wiest et al. [25]. Meningitis was defined as previously described [26]. Ascites and cerebrospinal fluid were centrifuged and precipitated immediately after extraction. The precipitates were smeared and stained with Gram stain.

Statistical Analyses

Demographic characteristics were summarized in terms of means and standard deviation or percentages. The distribution of clinical diagnosis of 142 patients with fever was represented by pie chart. We compared the distribution of different age groups of patients with major diseases, using the chi-square test. *P* values of less than 0.05 were considered statistically significant. The SPSS 17.0 software package (SPSS, Chicago, IL, USA) was used for statistical analysis.

RESULTS

Clinical and Household Characteristics of Study Participants

A total of 158 febrile patients were initially enrolled; 16 were subsequently excluded from the study (2 were diagnosed with liver cancer, 12 with HIV, 1 with humeral fracture, and 1

with suspected hereditary disease due to abnormal hemoglobin electrophoresis). Of the 142 patients enrolled in the study, the average temperature at enrollment was 38.4 ± 1.3 °C. Women accounted for 51.2% of the total. Among the major diseases, the female infection rate was the highest in malaria (62.5%), followed by typhoid (53.6%), diarrhea (47.8%), and ARI (46.7%). For all patients combined, the duration of fever before admission was 5.7 (mean) and 1.8 (median) days. Of the symptoms commonly found in febrile patients, chill (41.5%), fatigue (27.5%), headache (26.1%),

Table 1 Clinical characteristics of the study participants at enrollment

Variable	Value ^a N (%)
Age, years (range)	24.5 (2–65)
< 5	31 (21.8%)
5–15	34 (23.9%)
15–55	55 (38.7%)
> 55	22 (15.5%)
Sex	
Number of male patients	68 (47.9%)
Number of female patients	74 (52.1%)
Chief symptom	
Mean temperature (°C ± SD)	38.4 (± 1.3)
Chill	59 (41.5%)
Fatigue	39 (27.5%)
Headache	37 (26.1%)
Abdominal pain	28 (19.7%)
Cough	25 (17.6%)
Diarrhoea/dysentery	23 (16.2%)
Nausea	21 (14.8%)
Stomach pain	15 (10.6%)
Vomiting	14 (9.9%)

^a Data for all 142 patients were included in the calculations

and abdominal pain (19.7%) were common (Table 1).

Among the patients, the average age was 24.5 ± 24.8 years. The patients were divided into four groups according to age: < 5 years old, 5–15 years old, 15–55 years old (adult), and > 55 years old (elderly group) (Table 1). Of the 142 patients, 31 (21.8%) were < 5 years old, 34 (23.9%) were 5–15 years old, 55 (38.7%) were 15–55 years old, and 22 (15.5%) were > 55 years old [27]. The differences in the distribution of the age group were statistically significant (χ^2 values = 16.732, $P = 0.021$). Malaria, typhoid fever, diarrhea, and ARI were the major diseases found in this study, accounting for more than 90% of the total. The prevalence of malaria was higher in children aged 5–15 years old ($\chi^2 = 9.549$, $P = 0.185$) and adults ($\chi^2 = 18.852$, $P = 0.018$), as expected in malaria endemic areas [28]. This epidemic trend was basically similar

to typhoid fever and diarrhea, but not exactly the same as diarrhea. Diarrhea was predominant in children ($\chi^2 = 15.358$, $P = 0.024$), with a total proportion of 60% higher than adults and the elderly. ARI occurred in all age groups, but presented more commonly in < 5 years old ($\chi^2 = 22.165$, $P = 0.009$). Children (< 15 years old) are the important population with febrile diseases (45.8%) (Figs. 1, 2).

Diagnoses

The distribution of clinical diagnoses of all 142 febrile patients is shown in Table 2. Figure 2 shows a flowchart of investigations applied.

A total of 22.5% of the febrile patients had malaria infection, 19.7% had typhoid infection, 31.7% had ARI, 16.2% had diarrhea, 8.5% had hepatitis B infection, 2.8% had typical skin symptom, 2.10% had parasitic diseases, and

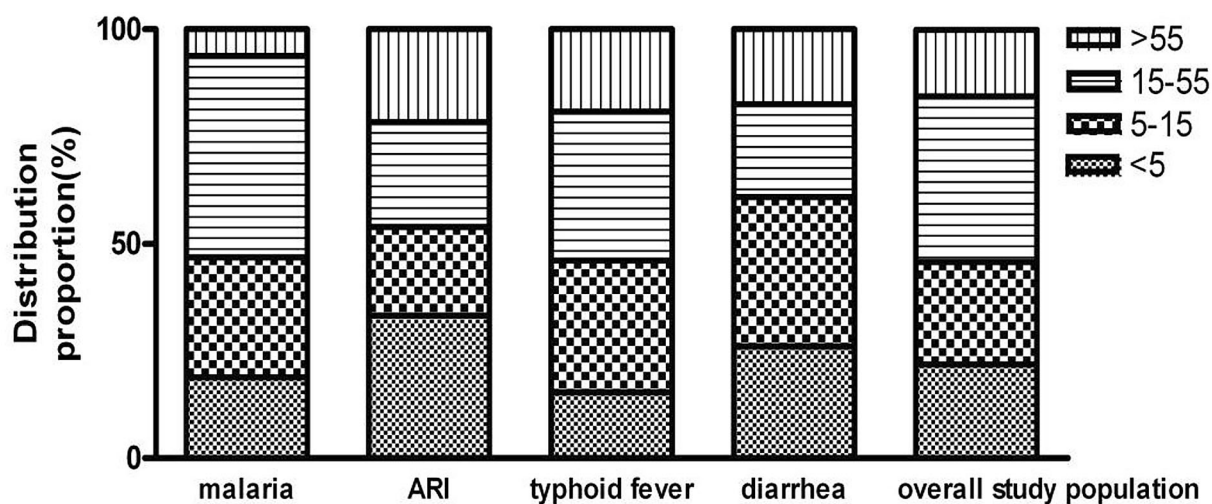
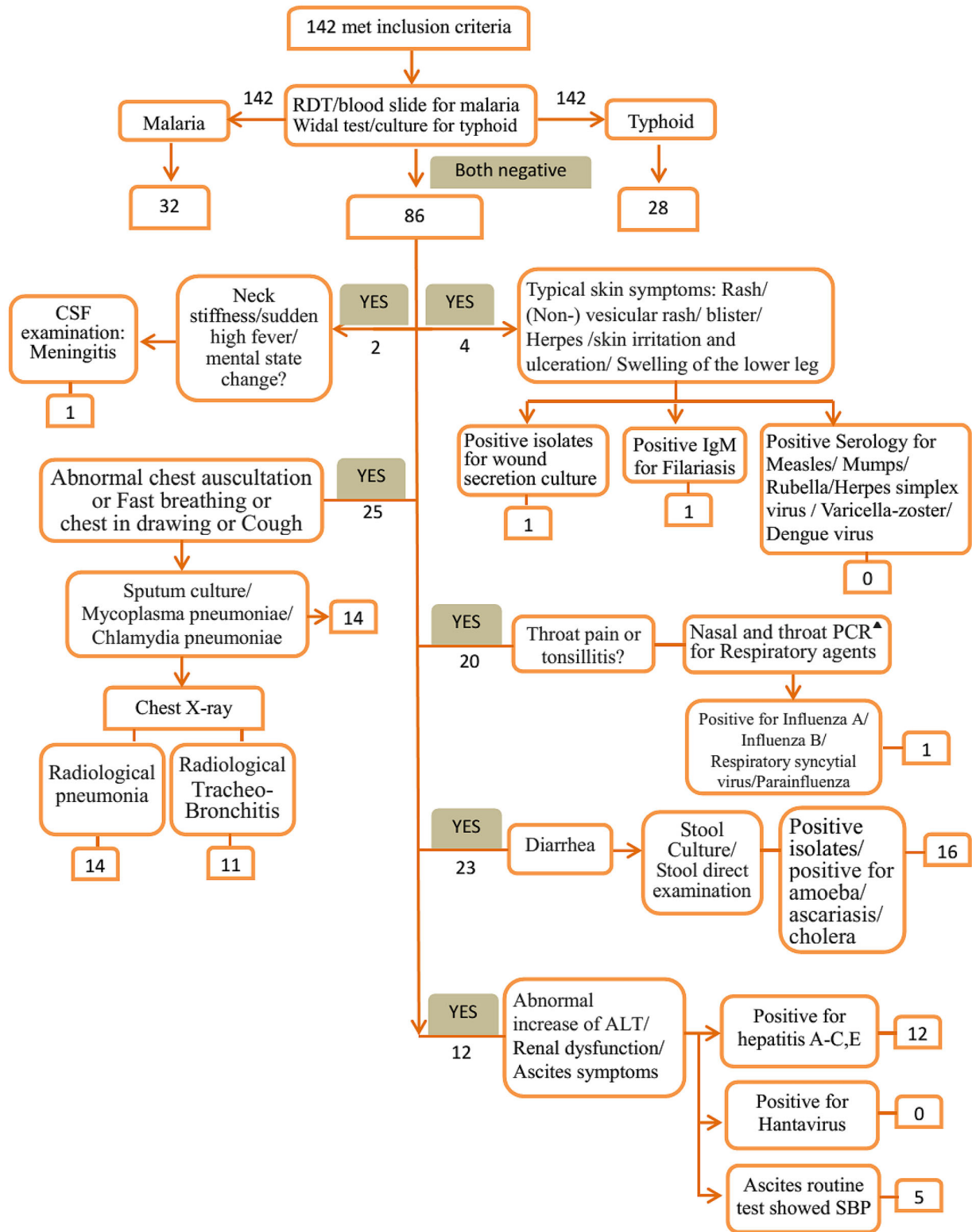


Fig. 1 The distribution of major diseases according to age group. The patients with major diseases were divided into four groups: < 5 years old, 5–15 years old, 15–55 years old (adult), and > 55 years old (elderly group) [27]. Of the 32 malaria patients, 6 (18.8%) were < 5 years old, 9 (28.1%) were 5–15 years old, 15 (46.9%) were 15–55 years old, and 2 cases (6.3%) were > 55 years old. 45 cases of acute respiratory infection, the distribution of the above four age groups: 15 cases (33.3%), 9 cases (20.0%), 11 cases (24.4%), and 10 cases (22.2%). The distribution of 28 cases of typhoid fever: 4 cases (15.4%), 8 cases (30.8%), 9 cases (34.6%), and 5 cases (19.2%). The distribution of 23 cases

of diarrhea: 6 cases (26.1%), 8 cases (34.8%), 5 cases (21.7%), and 4 cases (17.4%), respectively. The chi-square test was used to compare the distribution of the age index. The differences in the distribution of the age group were statistically significant ($\chi^2 = 16.732$, $P = 0.021$). Briefly, malaria was predominant in children aged 5–15 years old ($\chi^2 = 9.549$, $P = 0.185$) and adults ($\chi^2 = 18.852$, $P = 0.018$). Diarrhea was predominant in children ($\chi^2 = 15.358$, $P = 0.024$), with a total proportion of 60% higher than adults and the elderly. Acute respiratory infection presented more commonly in < 5 years old ($\chi^2 = 22.165$, $P = 0.009$)



◀**Fig. 2** Flowchart of investigations performed in 142 febrile patients. *PCR* polymerase chain reaction, *RDT* rapid diagnostic test, *CSF* cerebrospinal fluid, *ALT* alanine aminotransferase, *Ab* antibody, *SBP* spontaneous bacterial peritonitis. ▲Nasal and throat PCR for respiratory agents: influenza A and B, respiratory syncytial virus, and parainfluenza

1.40% had meningismus (Fig. 3). Each individual pathogen is listed in Table 2.

Prevalence of Malaria and Typhoid Fever

All patients were tested and screened for malaria and typhoid. Thirty-two patients (22.5%) were diagnosed with malaria infection. Of them, 30 cases (21.1%) were positive for *P. falciparum*, 1 case (0.7%) for *P. vivax*, and 1 (0.7%) for both *P. falciparum* and *P. vivax*. Twenty-eight (19.7%) patients had antibody titers of at least 1:80 for both O and H antigens; of them, 20 (14.1%) had at least 1:320 titers (Supplementary Table 1). We collected 14 stool samples from patients with typhoid fever, but only two strains of *S. typhi* were isolated (Table 2). The Widal test result showed that the prevalence of coinfection was 4 (2.8%). All four of them (100%) were coinfecting with *P. falciparum*; no cases were with *P. vivax*. Using stool culture, the prevalence of coinfection was only 1 (0.7%).

Prevalence of Diarrhea

Stool culture was performed with samples from 23 patients (16.2%) with diarrhea. A total of 55 stool samples were collected, and 14 positive isolates (9.9%) were obtained. Non-typhoidal serovars of *Salmonella enterica* (NTS) were the most common isolate with four positive cases (28.6%). In addition to NTS, a variety of diarrhea bacteria were isolated, including *Shigella flexneri* in three cases (21.4%), enteropathogenic *Escherichia coli* in two cases (14.3%), *Shigella sonnei* in one case (7.1%), and *Staphylococcus aureus* in one case (7.1%). Two cases (14.3%) of *Vibrio parahaemolyticus* and one case (7.1%) of *Aeromonas hydrophila* were also isolated from feces. Stool routine examination showed that two patients were infected with *Ascaris lumbricoides*; no *Amoeba histolytica* was found.

Prevalence of Acute Respiratory Infection

Acute respiratory infection was the most common cause of fever, accounting for 45 of 142 patients (31.7%) in the study. Infections were localized to the upper respiratory tract in 20 patients (including 9 cases of rhinitis, 7 cases of tonsillitis, and 4 cases of pharyngitis) and to the lower respiratory tract in 25 patients (11 with bronchiolitis and 14 with radiologically confirmed pneumonia). Twenty patients with upper respiratory tract were screened for influenza virus A, influenza B, parainfluenza, and respiratory syncytial virus by reverse transcription PCR. We found one patient was positive for influenza A virus, while the other three viruses were all negative in patients with upper respiratory tract infection. Sputum samples were collected from 18 patients with lower respiratory tract infection. Sputum culture showed that there were four cases of *Pseudomonas aeruginosa*, three cases of *Klebsiella pneumoniae*, three cases of *E. coli*, two cases of *S. aureus*, and one case of *S. pneumoniae*. Gram-negative rods accounted for 76.9% of the total. In addition, IgM antibody of *M. pneumoniae* was positive in one patient.

Prevalence of Other Common Communicable Diseases

A total of 12 patients with fever were diagnosed as hepatitis B infection, including nine cases (75%) of cirrhosis with ascites. Ascites test showed that five cases were spontaneous bacterial peritonitis (SBP), and all of them were Gram-negative bacteria by smear staining. The mean value of white blood cell count in ascites of patients with SBP was 4.7×10^9 cells/L, and the mean value of polymorphonuclear leukocyte count was 3.8×10^9 cells/L. CSF examination was performed in one patient with tuberculosis, and the results were consistent with the characteristics of tuberculous encephalitis [26]. Parasitic diseases were still prevalent. Except for malaria and *A. lumbricoides* found in feces, serological test showed that one patient was positive for filariasis IgM antibody. One case had obvious suppurative infection of

Table 2 Number and proportion of the study participants at enrollment by each individual pathogen

Pathogen	Specimen tested	Method of detection	Number (%) of infected Total <i>n</i> = 142
Bacteria			
<i>Pseudomonas aeruginosa</i>	Sputum	Culture	4 (2.8%)
<i>Klebsiella pneumoniae</i>	Sputum	Culture	3 (2.1%)
<i>Escherichia coli</i>	Sputum	Culture	3 (2.1%)
<i>Staphylococcus aureus</i>	Sputum	Culture	2 (1.4%)
<i>Streptococcus pneumoniae</i>	Sputum	Culture	1 (0.7%)
<i>Mycoplasma pneumoniae</i>	Serum	Serology	1 (0.7%)
<i>Chlamydia pneumoniae</i>	Serum	Serology	0 (0.0%)
<i>Staphylococcus aureus</i>	Wound secretion	Culture	1 (0.7%)
<i>Salmonella enterica</i>	Stool	Culture	4 (2.8%)
<i>Shigella flexneri</i>	Stool	Culture	3 (2.1%)
Enteropathogenic <i>E. coli</i>	Stool	Culture	2 (1.4%)
<i>Shigella sonnei</i>	Stool	Culture	1 (0.7%)
<i>Vibrio parahaemolyticus</i>	Stool	Culture	2 (1.4%)
<i>Aeromonas hydrophila</i>	Stool	Culture	1 (0.7%)
<i>Staphylococcus aureus</i>	Stool	Culture	1 (0.7%)
<i>Cholera O1</i>	Stool	Microscopy	0 (0.0%)
<i>Cholera O139</i>	Stool	Microscopy	0 (0.0%)
Viruses			
Influenza A	NP	RT-PCR	1 (0.7%)
Influenza B	NP	RT-PCR	0 (0.0%)
Respiratory syncytial virus	NP	RT-PCR	0 (0.0%)
Parainfluenza	NP	RT-PCR	0 (0.0%)
Herpes simplex virus	Serum	Serology	0 (0.0%)
Mumps	Serum	Serology	0 (0.0%)
Measles	Serum	Serology	0 (0.0%)
Varicella zoster virus	Serum	Serology	0 (0.0%)
Rubella	Serum	Serology	0 (0.0%)
Dengue	Serum	Serology	0 (0.0%)
Hepatitis A	Serum	Serology	0 (0.0%)
Hepatitis B	Serum	Serology	12 (8.5%)
Hepatitis C	Serum	Serology	0 (0.0%)

Table 2 continued

Pathogen	Specimen tested	Method of detection	Number (%) of infected Total <i>n</i> = 142
Hepatitis E	Serum	Serology	0 (0.0%)
Hantavirus	Serum	Serology	0 (0.0%)
Parasites			
<i>Plasmodium falciparum</i>	Blood	Antigen detection and microscopy	30 (21.1%)
<i>Plasmodium vivax</i>	Blood	Antigen detection and microscopy	1 (0.7%)
<i>P. falciparum</i> and <i>P. vivax</i>	Blood	Antigen detection and microscopy	1 (0.7%)
Typhoid	Blood	Serology	28 (19.7%)
Ascariasis	Stool	Microscopy	2 (1.4%)
Filariasis	Blood	Serology	1 (0.7%)
<i>Amoeba histolytica</i>	Stool	Microscopy	0 (0.0%)
Others			
G ⁺ /G ⁻ induced peritonitis	Ascites	Microscopy	5 (3.5%)
<i>Tubercle bacillus</i>	CSF	Microscopy	1 (0.7%)

NP nasopharynx, *RT-PCR* reverse transcription polymerase chain reaction, *G* Gram-positive bacteria, *CSF* cerebrospinal fluid

skin and mucosa. *S. aureus* was isolated from the skin secretion of the patient.

No *Cholera* O1/O139, *A. histolytica*, herpes simplex virus, mumps, measles, rubella, varicella zoster virus, dengue, hepatitis A, hepatitis C, hepatitis E, or hantavirus was found in the 142 febrile patients by means of the applied detection methods (Table 2).

DISCUSSION

In this study, the screening process used was able to identify more than 70% of the fever pathogens. More pathogens will be found if respiratory viruses are screened more widely. This process laid the foundation for the establishment of a more simple and feasible clinical evaluation process of febrile disease in Sierra Leone. Our findings also indicated that

although malaria and typhoid fever were still serious (39.4% in total), ARI and diarrhea have become the leading causes of febrile illness, reaching 47.9%. In addition, hepatitis B and parasite were also important etiological agents that can not be ignored.

Sierra Leone continued to have high child mortality rates, with the United Nations estimating that under 5 mortality rate (U5MR) was 114‰ [29]. This study confirmed that not only children under 5 years old but also children under 15-year-old children were important susceptible populations to fever. The incidence rate of malaria was related to the frequency of malaria exposure, while younger children may have longer protection in tents. Therefore, malaria was predominant in children aged 5–15 years and adults, and its prevalence generally increased with age. The risk of diarrheal pathogens such as NTS was higher in children

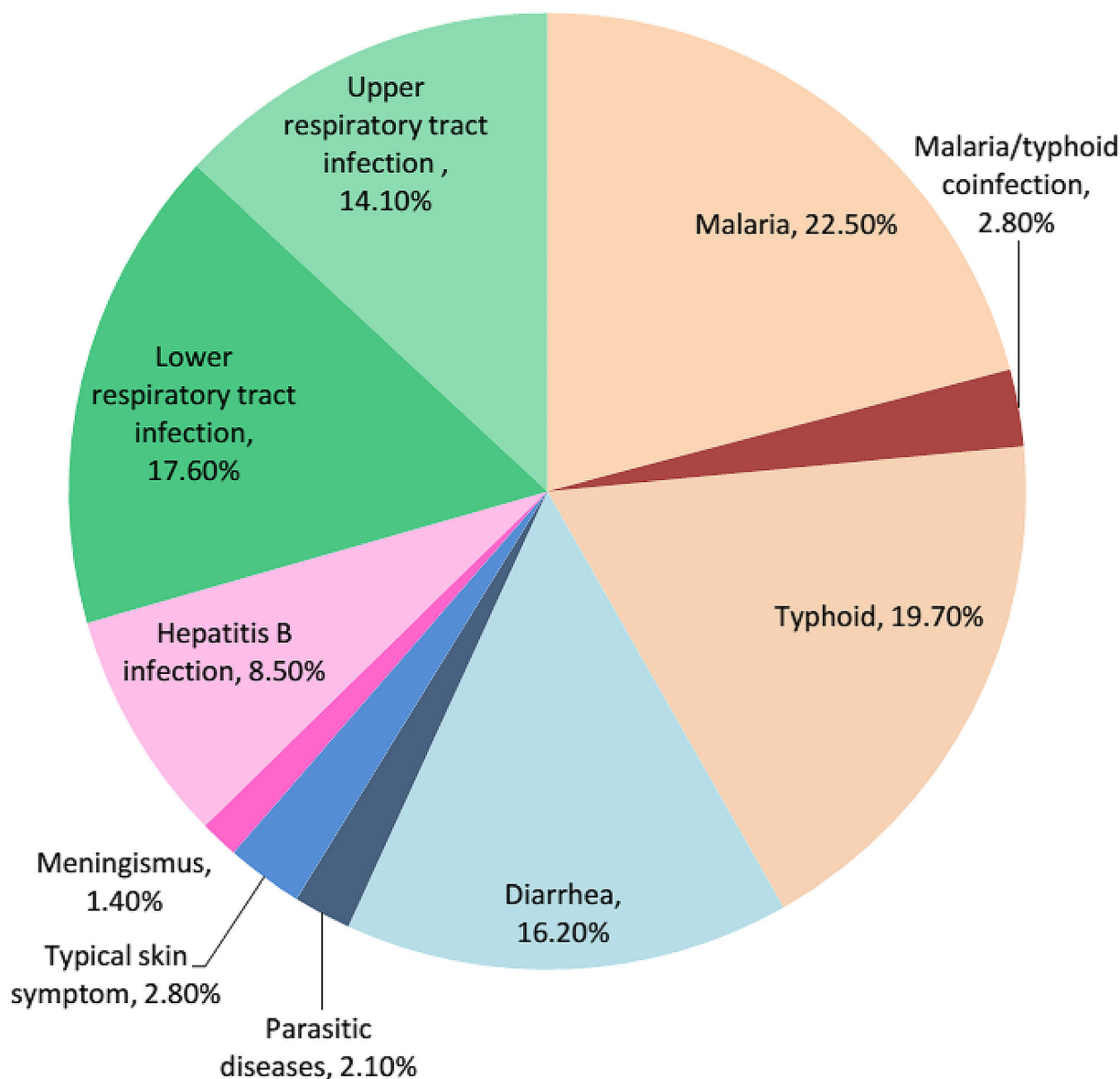


Fig. 3 Distribution of clinical diagnoses among 142 febrile patients. Numbers are percentages of all diagnoses. The distribution of clinical diagnoses of febrile illness was as follows: 32 cases of malaria, 28 of typhoid, 23 of diarrhea, 20 of upper respiratory tract infection, 25 of lower respiratory tract infection, 12 of hepatitis B infection, 4 of

typical skin symptom, 3 of parasitic diseases, and 2 of meningismus. The parasitic diseases cases were from 2 patients with diarrhea and 1 patient with typical skin symptoms due to swelling of lower leg. Percentages may not sum to 100 because of coinfection and rounding

[30, 31], and the risk factors involved were malaria, malnutrition, sickle cell disease, HIV, and anemia [28]. Children accounted for more than 60% of the diarrhea group in the current study. ARI was a global problem and was generally susceptible in all age groups, but it

seemed to be more common in younger children because of weak immunity. In terms of children accounting for 45.8% of febrile patients, early antimalarial and antibiotic treatment was helpful to reduce USMR and improve children's health.

In this study, 22.5% of the febrile patients had malaria infection, which was consistent with a recent malaria detection study [32]. Malaria in the country once reached 40% of outpatient morbidity [1]. Owing to the implementation of the National Malaria Control Programme, the malaria infection rate has shown a downward trend, and people's awareness of timely antimalaria drugs for infection has been significantly improved. In some African countries, in the case of treatment, buying medicine from pharmacy shops was common (48%). This was followed by the use of herbal remedies (31%), while appreciable numbers adopted self-treatment methods (18%) [33]. Furthermore, in endemic settings, less than half of patients carried malaria parasite in their blood without showing any symptoms. This asymptomatic parasitemia could be attributed to the level of immunity they have developed because of constant mosquito bites and frequent attack of malaria infection [33]. These factors reduced the number of patients with malaria who have access to health facilities. In addition, women seemed to be more vulnerable to malaria infection (62.5%). Our hospital provided important medical services for residents in the capital and the surrounding rural areas. In Sierra Leone, malaria prevalence in rural areas was significantly higher than that in urban areas [1]. When we visited rural areas, we found that most men left rural areas to work in the capital city, which was also the earliest and most frequent area in the country where malaria programs were implemented [1]. As a result, women may have more opportunities to be infected with malaria when they were farming. Owing to the high sensitivity of malaria RDT, it was suitable for screening. Therefore, the spread of malaria RDTs outside the hospital, such as pharmacy and chief tribe, could help reduce the misdiagnosis rate.

P. falciparum was the dominant species in the epidemic area, accounting for 93.8%, while *P. vivax* only accounted for 6.2%. Unlike earlier reports, we did not find *Plasmodium ovale* [34]. *P. vivax* had traditionally shown a small prevalence in West and Central Africa, attributed to the high prevalence of Duffy-negative people [Fy(a-b-)] who were described as being resistant

to *P. vivax* infection [35]. The prevalence of *P. vivax* was significantly lower than that in Ethiopia, East Africa, where the infection rate of vivax malaria could reach 56.2% [36]. Furthermore, earlier studies have identified *Anopheles gambiae*, *Anopheles funestus*, *Anopheles melas*, and *Anopheles nili* as efficient local malaria vectors, with *P. falciparum* being the dominant parasite species [37, 38]. Therefore, antimalarial treatment was still aimed at falciparum malaria.

The Widal test was the main method for typhoid detection available in most medical facilities in Sierra Leone, microbiologic culture was still relatively lacking [39]. The prevalence of typhoid fever by Widal test was 19.7%, which was much lower than the report of 31.4% in Bo, another city in Sierra Leone [40]. It was also lower than a more recent study wherein 44.9% of the cases in Freetown had antibody titer of at least 1:80 for serotype O and H [41]. Similarly, we noted that in Nigeria, the prevalence of typhoid varies significantly from 10.3% to 73.9% in different cities and regions [36]. Repeated exposure to *S. typhi* in endemic areas made it difficult to establish a stable baseline titer for the population. A cutoff titer of O agglutinin of 1:40, 80, 100, 160, and 200, while that of H agglutinin was 1:40, 80, 160, and 200 respectively, was the optimal indicator of typhoid fever in different regions [42–46]. Through the preliminary study on the titer of the Widal test in local healthy physical examination populations, we found that 1:160 was more suitable as a positive threshold, and at most 1:80 may be the baseline titer of the population, similar to the study of Egypt in Africa (Supplementary Table 1) [45]. We found that most patients (71.4%) had high titer (at least 1:320) of Widal test. There were only three patients with typhoid fever whose titer was 1:80. Typhoid was the only cause of fever found in these three patients and we immediately treated them with antibiotics. Although from the perspective of therapeutic diagnosis, the rehabilitation of patients could be used as the basis for the diagnosis of typhoid infection, it still could not rule out the possibility of other bacterial or even viral infections. In addition, according to a survey, 32% of patients would delay 4–6 days or more before seeking treatment

until the symptoms were obvious [33]. Toward the end of the first week of illness, titers of either O or H antibody may rise to as high as 1:160 [45, 47, 48]. Accordingly, the current study also indicated that as a high epidemic area of typhoid fever, appropriately increasing the cutoff value to 1:160 may be a better choice, which could narrow the scope of gray area and reduce the false positive results. It difficult to provide reliable diagnostic results with the Widal test was because of its variability and cross-reactivity [49]. The baseline of antibody titer and the standardization were the key issues affecting the Widal test. Therefore, national standards can be established to improve the detection accuracy and effectively reduce the detection variation between regions.

Even in a high titer in Widal test, the causative organism may be due to other species of *Salmonella*, rather than *S. typhi*. Sansone and Hennekens published a case report where the Widal reaction to typhoid O antigen on admission for an unexposed patient was 1:320, with an increase in titer to 1:20,480 by the fourth day [50]. In this study, the result of malaria and typhoid coinfection was 2.8%, lower than the previous 6.5% reported in Ethiopia [36], and comparable with the 2.5% reported in Cameroon [51]. Some coinfection rates could reach 17% or higher, which was overestimated because of the coexistence of *S. typhimurium* [52]. Definitive diagnosis of typhoid required isolation of *S. typhi* from blood or stool [53]. However, only two cases of *S. typhi* were isolated from feces. It was reported that the positive rate of *S. typhi* in blood/feces culture was only 2.6–8.9% [54]. High-quality microbiologic diagnostics may not always be available. In addition, it was difficult to obtain fecal samples from every Widal test-positive outpatient. However, if a simple manual microbial identification method can be used to identify diarrhea bacteria, such as *S. typhimurium*, it can play an important role in the differential diagnosis.

Diarrhea was an important cause of fever. Among the 14 positive isolates, NTS was the most common isolate (28.6%). This was consistent with the previous report that NTS predominated in all African regions, especially sub-

Saharan Africa [28]. In malaria endemic areas, the rate of NTS increased during the high transmission/rainy season, which was related to the antibody production induced by malaria, mucosal barrier dysfunction, hemolysis, and so on [28]. The rainy season also had a direct impact on diarrhea, while in Sierra Leone it accounted for half of the year. Improper treatment of human excreta would lead to the rapid spread of diarrhea bacteria. In addition to NTS, a variety of diarrhea bacteria were isolated, including *S. flexneri*, *S. sonnei*, enteropathogenic *E. coli*, and *S. aureus*, which were similar to the diarrhea spectrum in South Africa [55].

Sierra Leone is adjacent to the Atlantic Ocean and has rich fishery resources. About 95% of people in Sierra Leone depend on crude fuels, mainly wood and charcoal [56, 57]. Inadequate combustion leads to seafood not being fully cooked and causes diarrhea. Diarrhea pathogens associated with fishery and aquatic products, including *V. parahaemolyticus* and *A. hydrophila*, were isolated. In 2012, the country had an outbreak of cholera [58]. Therefore, considering the sample size of the study, these isolated strains were present as relatively minor but potentially significant pathogens. Antibiotics against Gram-negative bacteria contributed to the empirical treatment of diarrhea. Patients should be reminded to fully cook their food and vinegar can be added to seafood.

Acute respiratory infection was the most common cause of fever, accounting for 31.7%. Although only one of our patients tested positive for influenza A virus, the other three viruses were all negative in patients with upper respiratory tract. Therefore, it may suggest that upper respiratory infection is mainly caused by rhinovirus, adenovirus, or coronavirus [6, 16]. More than 75% of the pathogens causing lower respiratory tract infection were Gram-negative rods, including *P. aeruginosa*, *K. pneumoniae*, and *E. coli*. We also isolated *Streptococcus pneumoniae* and *S. aureus*, which may well be one of the major triggers for the development of severe pneumonia [6]. This spectrum of isolates was similar to that of Gambia in West Africa [59]. Antibiotics for Gram-negative bacilli and Gram-

positive cocci can be used as prior empirical treatment for patients with ARI.

Risk factors for ARI include crowded living environment, malnutrition, lack of immunity, and exposure to tobacco smoke or indoor air pollutants, which may be adverse factors contributing to the occurrence of ARI in West Africa [60]. Exposure to air pollutants might act to increase the severity of respiratory infections and thereby increase the proportion of illnesses considered clinically to involve the lower respiratory tract, and even to increase morbidity and mortality. A number of air pollutants have been shown to adversely affect components of the defense mechanisms against infectious organisms [61]. The number of severe pneumonia cases in Sierra Leone was estimated to exceed 25,000 per year [62]. In the world, at least 2 billion people were exposed to the toxic effects of biomass fuel consumption, 1 billion were exposed to outdoor air pollution, and 1 billion were exposed to tobacco smoke [63]. In Sierra Leone, ARI prevalence was higher for children in homes with wood stoves compared with homes with charcoal stoves, but ARI prevalence for both types of fuels was higher compared with reported prevalence elsewhere [56]. Although the local government has been committed to garbage disposal, the environmental pollution caused by Garbage Mountain and long-term incineration of garbage cannot be ignored. In low-income countries, strengthening children's immunization programs and increasing the availability of pneumonia vaccines must be a priority.

A number of other common communicable diseases continued to affect the people of Sierra Leone [64]; 75% of patients with hepatitis B showed decompensated cirrhosis in the current study, and Gram negative bacteria were still the main pathogens of SBP. Cirrhosis with ascites was a chronic and severe disease, which was different from acute infectious diseases such as malaria, typhoid fever, diarrhea, and ARI. The ascites of liver cirrhosis was particularly noteworthy because patients with liver cirrhosis were prone to severe complications of ascites infection, causing fever, and acute progression leading to adverse consequences. In addition, even though the prevalence of hepatitis virus

was rarely reported, a considerable number of patients in our hospital facility were patients with liver cirrhosis, and there was a lack of specialist hepatologists in the local area. Early diagnosis of bacterial infections in patients with hepatitis, especially those with decompensated cirrhosis, requires particular attention. Sierra Leone had one of the highest TB burdens per capita in the world, and its national HIV/AIDS prevalence rate was about 1.7% [65]. HIV infection increased risk for a number of febrile illnesses [66], and tuberculous encephalitis was found in the study. In order to respect the privacy of our patients, the research on HIV and TB infection was not included. National neglected tropical disease (NTD) is considered endemic in the country [29]. Sporadic cases including *A. lumbricoides* 1 (1.4%) and filariasis 1 (0.7%) were found. However, after the National NTD Master Plan 2016–2020 was officially launched, NTD has been significantly decreased in Sierra Leone [29].

This study indicated the importance of reliable diagnostic tests for febrile pathogens and provided the necessary information for RDT requirements. Malaria still threatens Sierra Leone; 22.5% were diagnosed with malaria infection. Limited by economic conditions, a considerable number of febrile patients in Africa bought medicine from pharmacies and adopted self-treatment methods; furthermore, asymptomatic patients could also lead to missed diagnosis. The implementation of malaria RDTs outside the hospital, such as pharmacy and chief tribe, could help reduce the misdiagnosis rate. For the diagnosis of typhoid, the baseline of antibody titer and the standardization were the key issues affecting the Widal test. Therefore, national standards could be established to improve the detection accuracy and effectively reduce the detection variation between regions. The etiology of patients with fever in Sierra Leone was closely related to the local geography, heredity, immune features, economic industry, air pollution, living habits, medical and health conditions. The local geography was suitable for the growth of *Anopheles*, which was the efficient local vector of *P. falciparum*. The genetic characteristics of Duffy negative blood group in the West African population were also

related to the high prevalence of falciparum malaria. In endemic areas, frequent exposure to malaria and typhoid antigens leads to the immune features of asymptomatic parasitemia and increased baseline antibody titers. As a result of Sierra Leone being adjacent to the Atlantic Ocean, diarrhea pathogens were related to economic industries—fishery and aquatic products. Improper handling of human excreta could lead to rapid spread of diarrhea bacteria. In Sierra Leone, about 95% of people depend on crude fuels, and the environmental pollution caused by incineration of garbage cannot be ignored and contributes to the occurrence of ARI. Strategies to improve medical and health conditions should also pay attention to some common chronic infectious diseases that cause fever, such as liver disease, tuberculosis, and so on. For empirical treatment, antimalarial treatment was still targeted at falciparum malaria in Sierra Leone. Furthermore, in this study, we found that Gram-negative bacteria, including *S. typhi*, *S. flexneri*, a variety of diarrhea bacteria and respiratory pathogens, were important pathogens of febrile diseases. In addition to 19.7% of patients infected with *S. typhi*, Gram-negative bacteria accounted for 92.9% of fecal culture-positive bacteria, 76.9% of sputum-culture positive bacteria, and all pathogens of spontaneous bacterial peritonitis. Therefore, antibiotics against Gram-negative bacteria contributed to the empirical treatment of febrile diseases.

This study has some limitations. First, we could not identify the etiological agents of fever in approximately 28.9% of the study population. A large number of data have shown that viral infection was an important pathogen in patients with ARI. Therefore, we did not carry out a large-scale screening of respiratory tract-related viruses in this study [6, 67, 68]. In addition, considering the local conditions and the feasibility of popularization and application, we did not add blood and urine culture and drug sensitivity tests, which we believe will also improve the positive rate of etiological agents. Another febrile disease study conducted in Cameroon showed that the causative

pathogen could not be identified in 30% of patients, and the spectra of febrile pathogens were significantly different from those in this study [69]. Secondly, there were insufficient data to assess the characteristics of coinfection in this study. As we known, the presence of viral–bacterial coinfection may well be a major trigger for the development of severe pneumonia [70]. Multiple infections were likely to lead to pathogen interaction and dynamics changes, which also suggested the need to expand the research object and carry out multicenter research.

CONCLUSIONS

The etiology of patients with fever in Sierra Leone was closely related to the local geography, heredity, immune features, economic industry, air pollution, living habits, medical and health conditions. Evidence of a wide spectrum of febrile pathogens emphasized the importance of a reliable diagnostic test for febrile pathogens and the necessary information for RDT requirements were provided from the study. The fever screening process of this study and its further simplification will help to carry out more effective diagnosis and empirical treatment of febrile illness in resource-limited areas in Sierra Leone. In addition, systematic and professional treatment of liver diseases should be promoted to reduce infection complications.

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Authorship Contributions. HW: study design, funding acquisition, patient recruitment, specimen collection, data collection, statistical analysis and result interpretation, manuscript writing. QL: study design, statistical analysis, result interpretation, manuscript writing. WW, SS, MM, SC, KK and DIB: patient recruitment, specimen collection, data collection. JZ, NX, XH, YL, GN, YZ, GW, XL, YC, YY, SB: result interpretation, data arrangement, statistical analysis, manuscript writing. RQ: study design, specimen collection, result interpretation. All authors reviewed the manuscript, agreed to submission for publication, and approved the final version.

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Compliance with Ethics Guidelines. The study was approved by the regional ethics committee in 34 Military Hospital, Republic of Sierra Leone Armed Forces (Deliberation JMU/1600). 34 Military Hospital approved this study to carry out etiological agents studies on human tissues, secretions, body fluids, and blood

samples among febrile patients. The study was done according to the Helsinki Declaration. Informed consent was obtained from individuals (or their parents/guardians for children) for participation in this study.

Conflict of Interest. We have no competing interests to declare.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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