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Research article

Etiology of febrile illness among patients seeking care at a district hospital in Manyara, Tanzania

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Abstract

Managing febrile illness is complicated due to the increasing involvement of non-malarial pathogens. This hospital-based cross-sectional study was conducted in Kiteto District Hospital in Northern Western, Tanzania, between January and February 2019. Out-patients with a temperature ≥38˚C were consecutively enrolled until the sample size was reached. Whole blood samples were collected aseptically and tested for 36 pathogens using a multiplex PCR. Out of the 426 febrile patients, 184 (43.2%) had pathogens detected in their bloodstream. *Brucella spp.* was isolated in 61 (14.3%) patients, followed by Dengue virus 51 (12.0%), *Salmonella* Typhi 39 (9.2%), *Plasmodium falciparum* 37(8.7%), *Coxiella burnetii* 23 (5.4%) and *Leptospira spp.,* 17 (4.0%). Forty patients (21.7%) had more than one pathogen, whereby co-infection with *Brucella spp.* and *C. burnetii* was frequent among study participants. Dengue virus was the most frequent in children aged below 14 years, whereas *Brucella spp.* was the dominant agent in adults. The cause of fever was not identified in 242 (56.8%) of these patients. Houses surrounded by long grasses were risk factors for febrile illness caused by *P. falciparum*, consuming untreated milk and consuming sour milk were risk factors for febrile illness caused by bacteria zoonoses while not washing hands after visiting the toilet was a risk factor for febrile illness by *S.* Typhi. Regarding management, 143 (34.8%) were given anti-malaria despite malaria rapid diagnostic test (MRDT) negative results, while 26.5% were given both anti-malaria and antibiotics for febrile illness. In this study, the majority of febrile illness cases were due to non-malarial pathogens, with *P. falciparum* accounting for only 8.7% of the cases. We found that about one-third of the cases were wrongly treated for malaria, and more than a quarter were wrongly given antibiotics. A review of the management of febrile illnesses is warranted.

Keywords: Febrile illness, Non-malarial pathogens, Multiplex PCR, Bacterial zoonoses.

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Introduction

Febrile illness, which may be caused by a large variety of infectious agents, remains one of the most difficult conditions to diagnose and treat and is a significant cause of morbidity and mortality in Tanzania (Debora et al., 2016; Mahende et al., 2014; Lengeler et al. 2014). More than 200 infectious etiologies of fever have been identified worldwide, including viruses, bacteria, parasites, fungi, and fungi (Stoler et al., 2016). Some of these agents include *Brucella* spp., *Rickettsia* spp., *Borrelia* spp., *Scrub typhus*, *Leptospira spp.*, and arboviruses such as dengue, chikungunya, and Zika virus (Moreira et al., 2018; Wongsrichanalai et al., 2003).

The spectrum of pathogens varies by geographic, environmental conditions, socioeconomic factors, and time (Moreira et al., 2018; Alavi et al., 2013). Furthermore, new agents of febrile illness continue to emerge and re-emerge (Chow et al., 2011). In malaria-endemic areas like Tanzania, fever has almost always been associated with *Plasmodium falciparum,* and febrile patients have been managed as malaria cases. However recent studies conducted in the Northern part of Tanzania have shown that

a large proportion of febrile cases were not due to malaria (Crump et al., 2013; Liu et al., 2016).

These studies reported that most cases of fever were due to bacteria zoonoses such as leptospirosis, spotted fever rickettsiosis, brucellosis, and viruses such as chikungunya (Crump et al., 2013; Liu et al., 2016). Kiteto district, which is located in a malaria hypo-endemic area with malaria prevalence ≤1%, has been experiencing increased cases of febrile illness. According to the 2018 integrated disease surveillance report (IDSR), Kiteto District Hospital (KDH) reported 24,018 fever cases, of which only 2,624 (11%) were confirmed as malaria. The etiology of the remaining 89% remained unknown due to a lack of appropriate laboratory diagnostic techniques.

Thus, we conducted this study in the Kiteto district to determine infectious agents causing non-malarial febrile illness, which is pivotal for the proper management of patients. We aimed to bring to the attention the repertoire of infectious agents causing febrile illness in their catchment area and factors associated with their occurrence for planning appropriate interventions.

Material and Methods

Study design, duration, and setting

From January to February 2019, a hospital-based cross-sectional study was conducted at Kiteto District Hospital (KDH), located in the Northwest part of the Manyara region, Tanzania. KDH has 120 beds, and according to the 2012 census, it served an estimated population of 244,669. Currently, as per the 2022 census, it serves an estimated population of 352,305.

Study population

This study included patients of all ages seeking care for febrile illness at KDH. The patient's eligibility was based on the following case definition: i) patient of any age seen at the outpatient department with body temperature ≥38.0˚C; ii) history of fever that had persisted for 2-7 days without identified cause. Enrolment was subject to obtaining an ascent for children aged ≤12 years and informed consent for adults. We excluded all patients who were unable to communicate, those with known malignancy, and those who had undergone any invasive procedure or surgery in the last three months.

Data collection, Sample collection, and transportation

A pretested structured questionnaire was used to gather information on social demographic characteristics such as age, sex, marital status, occupation, clinical information, and risk factors for zoonotic infections. A trained phlebotomist aseptically collected 2.0 mL of blood in EDTA tubes from each enrolled patient. Samples were transported in a cold chain to Kilimanjaro Christian Research Institute (KCRI) and were stored at -20˚C until the day of processing.

Laboratory procedure by TaqMan Array Card-Based Real-Time Polymerase Chain Reaction (RT-PCR)

TaqMan Array Card (TAC) RT-PCR test was used for the simultaneous detection of 26 organisms: 14 viruses, 8 bacteria, and 3 protozoa of relevance to Sub-Sahara Africa. These include 15 viruses; Chikungunya virus, Crimean-Congo haemorrhagic fever virus, Dengue, Ebola virus, Bundibugyo virus, Sudan virus, Hantaviruses (HTN and SEO), Hepatitis E, Marburg, Nipah virus, O'nyong virus, Rift Valley fever virus, West Nile virus, Yellow fever virus), eight bacteria: *Bartonella spp., Brucella spp., C. burnetii, Leptospira spp., Rickettsia spp., Salmonella enterica* and *Salmonella enterica serovar* Typhi, *Yersinia pestis*, and three protozoa: *Leishmania spp., Plasmodium spp.,* and *Trypanosoma brucei* (Liu et al., 2016).

Total nucleic acid extraction

Total nucleic acid (TNA) purification was done from blood samples using a high-purity viral nucleic acid large-volume kit (Roche Life Sciences; Basel, Switzerland). For each blood sample, a working solution was prepared by mixing 2.5 mL of binding buffer, Poly (A) 15 µL, MS2 1.0 µL, and PhHV 1.0 µL, then mixed in a tube before use. Phocine Herpesvirus1 (PhHV) and bacteriophage (MS2) were included as internal controls to monitor and validate the extraction process. 2.5 mL of the prepared lysate mix was mixed with 2.5 mL of the blood sample and 250 µL of Proteinase K and mixed well following incubation at 70˚C for 15 minutes in a heat block. Immediately after incubation, 1.25 mL of the binding buffer was added, mixed, and transferred to the spin column with a high-purity extender assembly, then centrifuged at 4,000 g for 5 minutes. Wash buffer was then applied to the spin column for purification and removal of inhibitors, and finally, 150 µL of elution buffer was used to elute total nucleic acid into a new Lobind Eppendorf tube. The extract was stored at -80 ˚C for further downstream processing.

TaqMan Multiplex qPCR detection

A ready TaqMan Fast virus one-step multiplex master mix for qPCR (Life Technologies; California, USA) 25.0 µL was mixed with 75.0 µL of each total nucleic acid extracted, and one negative control sample (nuclease-free water was used) into each PCR tube, respectively, making a total reaction mix of 100 µL per PCR reaction and mixed well. A total of 100 µL of each PCR reaction mix was transferred in TaqMan array card ports, respectively, and centrifuged at 1200 rpm for two consecutive minutes in a one-minute spin interval to allow even distribution of the fluid in

the TAC reaction wells. The card was sealed on the lab bench and set to the ViiA™ 7 real-time PCR system (Life Technologies; California, USA) to the following PCR cycling conditions: Reverse Transcription (RT) at 50˚C for 5 minutes, RT initial denaturation at 95˚C for 20 seconds, following (denaturation at 95˚C for 3 seconds and annealing/extension at 95˚C for 30 sec for 45 cycles). The amplification curves of the control samples were predefined to cross the threshold value (CT values). All positive samples were defined parallel to positive control samples and amplification curve values, respectively. All samples with CT values <40 were considered weak negative samples.

Data handling and analysis

Data were coded, entered, cleaned, and analyzed using Epi info version 3.5 Statistical software and STATA 13.1. Descriptive analysis was done by running frequencies and calculating means, standard deviations, and quartiles of study variables. The odds ratio was used to measure the strength of the association between outcome and exposure. Factors with p<0.05 were considered as having a significant association with the etiology of febrile illness. All factors with p≤0.2 at bivariate analysis were entered into the unconditional logistic regression model to generate independent factors associated with the development of a particular etiology of febrile illness.

Results

Social demographic distribution of study respondents of the Kiteto District Hospital (KDH) at Manyara.

A total of 426 febrile patients were recruited in the study. The median age was 28 years, with an interquartile range (IQR) of 20 to 38 years. The majority of the study participants were female (261/426; 61.3%) (Table 1).

Etiology of febrile illness among patients presenting with fever at Kiteto District Hospital in Manyara region

Out of 426 febrile patients, 184 (43.2%) had pathogens detected in their bloodstream. *Brucella spp.* was isolated in 61 (14.3%) patients, followed by dengue virus 51 (12.0%), *S.* Typhi 39 (9.2%), *P. falciparum* 37 (8.7%), *C. burnetii* 23 (5.4%) and *Leptospira* 17 (4.0%) (Table 2). Dengue virus was the most frequently detected agent in children aged below 14 years, whereas *Brucella spp.* was the dominant agent in adults. No patient had chikungunya, Crimean-Congo haemorrhagic fever virus, ebola virus, bundibugyo virus, Sudan virus, Hantaviruses (HTN and SEO), Hepatitis E, Marburg, Nipah virus, O'nyong virus, Rift Valley fever virus, West Nile virus, Yellow fever virus, *Rickettsia spp.*, *Yersinia pestis*, *Trypanosoma brucei* detected in their bloodstream (Table 2).

The occurrence of co-infections among patients presenting with fever at Kiteto District Hospital in Manyara region

The combinations of isolation of infectious agents are shown in Table 3. Out of 184 detected pathogens, 40 (21.7%) had more than one pathogen. One hundred and forty-four (33.8%) participants had a single agent detected, 37 (8.7%) were infected with 2 agents, and 3 (0.7%) were infected with 3 agents.

Proportion of febrile illness

The proportion of febrile illness caused by Dengue virus was significant among patients who had a travel history to risk areas compared to those who did not travel [12.5%, (40/319) vs 10.3%, (11/107)] *p=0.025.* Among patients with febrile illness caused by zoonotic bacteria, the proportion of febrile illness was significant among livestock keepers [21.8%, (37/131) vs 19.3% (57/295)], *p=0.042*. Patients whose houses were not separated from animal houses had significant febrile illness compared to those whose houses were separated from animals [34.4%, (31/90) vs 14.6%, (6/41)], *p=0.02* (Table 4).

Factors associated with febrile illness

In univariate analysis, factors that were found to be significantly associated with febrile illness include houses surrounded by long grasses, history of travel to risk areas, animal houses separate from people, drinking untreated milk, drinking sour milk, and consuming sick animals. Houses surrounded by grasses had 1.43 odds of having febrile illness caused by *P. falciparum* than houses without long grasses (cOR=1.43, 95% CI 0.19-2.95, *p<0.035*). The odds of having febrile illness among travelers was 1.25 than non-travelers (cOR=1.25, 95% CI 0.62-2.54, *p<0.025*). For febrile illness caused by bacterial zoonoses, patients whose houses were not separated from their livestock had three times the odds of having febrile illness compared to those whose house was separate from the animal house (cOR=3.06, 95% CI 1.16-8.08, *p<0.023*), while those who consumed untreated milk had 1.9 odds of having febrile illness compared to those who consumed treated milk (cOR=1.90, 95% CI 1.19-3.03, *p<0.007*) and patients who consumed sick animals had 1.8 odds of having febrile illness (cOR=1.88, 95% CI 1.18-2.99, *p < 0.008*).

Table 1: Social demographic distribution of study participants

Variable	Children 1-4 years (n=23), (%)	Older children 5-14 years (n=45), (%)	Adult ≥15 years (n=358), (%)	Total n=426 (%)
Age (years)				
Median	3	10	30	28
Gender				
Male	10 (43.5)	34 (75.6)	121 (33.8)	165 (38.7)
Female	13 (56.5)	11 (24.4)	237 (66.2)	261 (61.3)
Education level				
Informal	22 (95.7)	30 (66.7)	164 (45.8)	216 (50.7)
Primary	1 (4.3)	12 (26.7)	131 (36.6)	144 (33.8)
Secondary	0	3 (6.7)	42 (11.7)	45 (10.6)
College	0	0	21 (5.9)	21 (4.9)
Marital status				
Single	23 (100.0)	45 (100.0)	31 (8.7)	99 (23.2)
Married	0	0	238 (66.5)	238 (55.9)
Cohabit	0	0	67 (18.7)	67 (15.7)
Widow	0	0	22 (6.1)	22 (5.2)
Occupation				
Formal employment	0	0	28 (7.8)	28 (6.6)
Children/Students	15 (65.2)	30 (66.7)	11 (3.1)	56 (13.0)

Table 2: Isolation frequency of the causative agents of febrile illness among patients presenting with fever at Kiteto District Hospital in Manyara region by age group

Isolate	Children (1-5 years) No. (%)	Older children (5-14 years) no. (%)	Adult ≥15 years no. (%)	All age no. (%)
Brucella spp.	2 (8.7)	7 (15.6)	52 (14.5)	61 (14.3)
Dengue virus	5 (21.7)	8 (17.8)	38 (10.6)	51 (12.0)
C. burnetii	0 (0.0)	1 (2.2)	22 (6.1)	23 (5.4)
Leptospira spp.	0 (0.0)	1 (2.2)	16 (4.5)	17 (4.0)
P. falciparum	3 (13.0)	6 (13.3)	28 (7.8)	37 (8.7)
S. Typhi	2 (8.7)	4 (8.9)	33 (9.2)	39 (9.2)

Table 3: The proportion of co-infection among patients presenting with fever at Kiteto District Hospital in Manyara region

Organism isolated	Number of patients (%)
Single infection	144 (33.8)
Co-infections	
Brucella spp. and *P. falciparum*	4 (0.9)
Brucella spp. and *S.* Typhi	4 (0.9)
Brucella spp. and Dengue virus	5 (1.2)
P. falciparum and Dengue virus	4 (0.9)
Brucella spp. and *C. burnetii*	6 (1.4)
C. burnetii and Dengue virus	1 (0.2)
S. Typhi and Dengue	4 (0.9)
S. Typhi and *P. falciparum*	3 (0.7)
S. Typhi and *C. burnetii*	4 (0.9)
Leptospira spp. and *P. falciparum*	2 (0.5)
Brucella spp., Dengue and *P. falciparum*	2 (0.5)
Brucella spp., C. burnetii and Dengue virus	1 (0.2)

Table 4: Proportion of febrile illness among study participants

Variable	Positive n (%)	Negative n (%)	*p-value*
	P. falciparum		
Age group			
1-4	3 (13.0)	20 (87.0)	0.97
5-14	6 (13.3)	39 (86.7)	
≥15	28 (7.8)	330 (92.3)	
Sex			
Male	18 (10.9)	147 (89.1)	0.195
Female	19 (7.3)	242 (92.7)	
Using mosquito net			
Yes	10 (8.1)	113 (91.9)	0.79
No	27 (8.9)	276 (91.1)	
Window with wire mesh			
Yes	12 (11.4)	93 (88.6)	0.25
No	25 (7.8)	296 (92.2)	
House surrounded by long grasses			
Yes	27 (7.5)	335 (92.5)	0.03
No	10 (15.6)	54 (84.4)	
	Dengue virus		
Age group			
1-4	5 (21.7)	18 (78.3)	0.69
5 -14	8 (17.8)	37 (82.2)	
≥15	38 (10.6)	320 (89.4)	
Sex (n= 426)			
Male	25 (15.1)	140 (84.9)	0.11
Female	26 (10.0)	235 (90.0)	
History of travel to risk area			
Yes	40 (12.5)	279 (87.5)	0.025
No	11 (10.3)	96 (89.7)	
	S. Typhi		
Age group			
1-4	2 (8.70)	21 (91.30)	0.933
5-14	4 (8.9)	41 (91.1)	
≥15	33 (9.2)	325 (90.8)	
Sex			
Male	14 (35.9)	151 (39.02)	0.703
Female	25 (64.1)	236 (60.98)	
Water treatment			
Yes	11 (10.6)	93 (89.4)	0.56
No	28 (8.7)	294 (91.3)	
Mode of water treatment			
Boiling	8 (9.0)	81 (91.0)	0.199
Filtration	3 (20.0)	12 (80.0)	
None	28 (8.7)	294 (91.3)	
Hand washing after toilet			
Yes	7 (5.9)	112 (94.1)	0.11
No	23 (11.2)	183 (88.8)	
	Bacterial zoonotic infections		
Age-group			
1-4	2 (8.7))	21 (91.3)	0.1
5-14	9 (20.0)	36 (80.0)	
≥15	83 (23.2)	275 (76.8)	
Sex			
Male	36 (21.8)	129 (78.2)	0.922
Female	58 (22.2)	203 (77.8)	
Livestock keeper			
Yes	37 (28.2)	94 (71.8)	0.042
No	57 (19.3)	238 (80.7)	
Animal vaccinated			
Yes	26 (26.3)	73 (73.7)	0.376
No	11 (34.4)	21 (65.6)	
Animal house separate from people			
Yes	6 (14.6)	35 (85.4)	0.02

No	31 (34.4)	59 (65.6)	
Consume treated milk			
Yes	38 (16.9)	187 (83.1)	0.007
No	56 (27.9)	145 (72.1)	
Mode of milk treatment			
None	56 (27.9)	145 (72.1)	0.000
Boiling	38 (16.9)	187 (83.1)	
Using sour milk			
Yes	56 (19.2)	235 (80.8)	0.04
No	38 (28.2)	97 (71.8)	
Consume sick animal			
Yes	45 (29.2)	109 (70.8)	0.007
No	49 (18.0)	223 (82.0)	

In multivariate analysis, houses surrounded by long grasses had twice the odds of having febrile illness caused by *P. falciparum* (aOR=2.3, 95% CI 1.05-5.01, *p<0.037*. Consuming untreated milk had 5 odds of having a febrile illness (aOR=5.26, 95% CI 1.89-14.64, *p<0.001),* and consuming sour milk had 1.5 odds of having a febrile illness (aOR=1.5, 95% CI 1.15-3.70, *p<0.035)* by bacteria zoonoses. Patients who did not wash their hands after visiting the toilet had twice the odds of having febrile illness by *S.* Typhi (aOR=2.15, 95% CI 0.89-5.07, *p<0.089* (Table 5).

The prescription pattern and management of a patient presenting with fever among patients presenting with fever at Kiteto District Hospital in Manyara region.

The management of febrile patients was done according to the malaria rapid diagnostic test (MRDT) result, which is only a provisional diagnostic test. Out of 376 total prescriptions, 143 (34.8%) were given anti-malaria drugs despite MRDT negative results, while 26.5% were given both anti-malaria drugs and antibiotics for febrile illness (Table 6).

Table 5: Multivariate regression model for risk factor associated with febrile illness

Risk factors	**cOR (95%CI)**	***p-value***	**aOR(95%CI)**	***p-value***
		P. falciparum		
House surrounded by long grasses				
Yes	1.43 (0.19-2.95)	0.035	2.3 (1.05-5.01)	0.037
No	Ref			
		Dengue		
History travel to risk area				
Yes	1.25 (0.62-2.54)	0.025	-	-
No				
		Bacterial Zoonotic infections (n=94)		
Animal house separate from people				
Yes	Ref			
No	3.06 (1.16-8.08)	0.023	1.67 (0.51-5.43)	0.40
Treated milk				
Yes	Ref			
No	1.90 (1.19-3.03)	0.007	5.26 (1.89-14.64)	0.001
Using sour milk				
Yes	1.64 (1.05-5.01)	0.040	1.50 (1.15-3.70)	0.005
No	Ref			
Consume sick animal				
Yes	1.88 (1.18-2.99)	0.008	0.52 (0.09-3.00)	0.464
No	Ref			
		S. Typhi		
Hand washing after visiting the toilet				
No	1.04 (0.21-1.19)	0.11	2.15 (0.89-5.07)	0.089
Yes	Ref			

*Ref: Reference

Table 6: Appropriateness of prescriptions given to patients presenting with fever

*MRDT results	Antimicrobial agents			
	Anti-malaria (%)	Antibiotics (%)	Antimalaria and Antibiotic (%)	Not treated (%)
MRDT positive	13 (54.2)	1 (4.2)	10 (41.7)	0 (0.00)
MRDT negative	143 (34.8)	96 (24.6)	113 (26.5)	50 (12.4)

*MRDT: Malaria rapid diagnostic test

Discussion

In Sub-Saharan Africa, the burden of febrile illness is high, with fever being a frequent complaint among patients seeking medical care. However, the identification of microbes causing fever is still a challenge (Prasad *et al.,* 2015). Using multiplex PCR, we identified a range of microbial patterns associated with febrile illness in our study. The majority of febrile illness (22.1%) was caused by zoonotic bacteria, i.e., 14.3% *Brucella spp.,* 5.4% *C. burnetii,* and 4.0% *Leptospira*, followed by arbovirus 12%, dengue and *S.* Typhi 9.2%. *P. falciparum*, the causative agent of malaria, was found only in 8.7% of the patients. Our findings are in agreement with previous studies conducted in Northern Tanzania, which found bacterial zoonosis to be the commonest etiology of febrile illness (26.2%), followed by viral (7.9%) and parasitic infections (1.6%) (Crump *et al.*, 2013; Hercik *et al.*, 2017).

There were age differences in the occurrence of the pathogens. Dengue virus was the most frequently detected agent in children under 14 years, and this could be due to incompetent immunity among children compared to adults. Children are unable to thoroughly protect themselves from mosquitoes that carry the virus (Verhagen et al.,2014). In contrast, *Brucella* was the dominant agent in adults, which may be attributed to occupation whereby most farmers and livestock keepers (Assenga *et al.*, 2015). Additionally, febrile illness caused by *P. falciparum* was more common among males compared to females, an observation that has been associated with night outdoor stay (Mwanziva *et al.*, 2008). We also found out that people living in houses surrounded by grasses were more likely to have febrile illness caused by *P. falciparum*, similar to findings in other studies due provision of breeding grounds for mosquitoes, vectors responsible for transmission of malaria pathogens, including *P. falciparum* (Kibuuka *et al.*, 2015).

In this study, multiple infections were detected in 9.4% of cases. Co-infection with two pathogens was found in about 10% of the cases, in which most co-infections were caused by *Brucella spp.* and *C. burnetii.* These findings are similar to other studies in Tanzania, which found that febrile ill patients were infected with more than one pathogen, thus posing a challenge in the thorough treatment of these patients (Mahende *et al.*, 2014; Hercik *et al.,* 2014; Chipwaza *et al.*, 2015).

Despite the decreasing incidence of malaria in Tanzania due to effective control measures by the World Health Organization (WHO) and the Ministry of Health (MoH), patients in resource-limited settings are still treated for malaria as the major cause of febrile illness among patients (Gallay *et al.,* 2018). In our study, we found that, out of 88.3% of total prescriptions given to patients with febrile illness, 34.8% of patients received anti-malaria despite having MRDT negative results, and 24.6% were given antibiotics while 26.5% received both antimalaria and antibiotics. Inappropriate prescription of antimicrobials predisposes to antimicrobial resistance (AMR) due to developing resistance by these microbes (Hasan *et al.,* 2021), and this calls for implementing diagnostic stewardship as well as antimicrobial stewardship within the hospital to reduce the burden of AMR.

It was notable in our study that people living in houses surrounded by long grasses, which are breeding grounds for mosquitoes causing malaria, were at high risk of having a febrile illness. Long grasses may be attributed to the agricultural practices in the region characterized mainly by livestock keeping in which grasses are the main source of food for these animals, in which removal or cutting down of grasses will affect the food provision for the livestock while keeping the grasses will affect the health of farmers and the entire population due to increased risk of acquiring parasitic febrile illness by *P. falciparum* and hence posing a challenge in intervention and complete eradication of malaria (Nonga *et al.,* 2011). This, therefore, calls for appropriate approaches in designing interventions for the complete elimination of malaria by destroying these breeding places without affecting the livestock or lifestyle in this

community. Additionally, we found consuming untreated milk and sour milk to be another risk factor for febrile illness by zoonotic bacteria, and this may be due to poor processing and storage of milk and milk products leading to milk-borne infections (Dhanashekar *et al.*, 2012). One of the main economic activities among people from the Northern part of Tanzania, including the Manyara region, is livestock keeping, whereby livestock keeping has been a source of food and income among Northerners in Tanzania. However, poor hygiene practices during milking, storage, transporting, and processing of the milk may introduce these pathogenic bacteria into the milk thus causing milk-borne infections. Pathogens such as *Brucella spp., Bacillus cereus, Coxiella burnetii, Listeria monocytogenes, Salmonella spp., and Yersinia enterolitica* have been implicated in causing milk-borne infections among milk consumers and hence contributing to febrile illness (Corbel M., 2006). In our study, handwashing was significantly associated with febrile illness caused by *S.* Typhi. Similar findings have been made with other studies done in Sub-Sharan Africa, whereby ineffective hand washing and poor hand hygiene practices, especially before food preparation and after going to the lavatory, have been implicated in causing febrile illness (Adane *et al.*, 2018; Kigozi *et al.*, 2023).

This study has particular strength in that we used a robust diagnostic technique: TAC, RT-PCR test, a multiplex polymerase chain reaction capable of simultaneously detecting 26 pathogens, 15 viruses, 8 bacteria, and 3 protozoa of particular relevance in our setting. Most of these pathogens were previously not detected during the investigation of febrile illness due to the high cost of diagnostics, hence, most cases with fever are treated as malaria with antimalarials or in combination with antibiotics. Thus, this study offers insight into the challenges in the empiric management of febrile illness based on MRDT alone, which is the case in the majority of Sub-Saharan African settings. Further, we could not determine the cause of fever in 242 (56.8%) of the patients, and this could be due to the fact that the febrile illness was caused by pathogens other than the 26 detectable by multiplex PCR that was used or the febrile illness could be caused, non-infectious agents.

Conclusion

The etiology of febrile illness was mainly bacterial zoonosis with *Brucella spp.*, *C. burnetii*, and *Leptospira spp.*, accounting for most pathogens causing febrile illness, followed by the dengue virus and *S.* Typhi. The majority of patients were treated for malaria, and some received both anti-malarial and antibiotics, which shows the need for enhanced diagnostic stewardship to assist clinicians in correctly diagnosing etiological agents of febrile illness and thus promoting antimicrobial stewardship through appropriate prescription of antimicrobials, hence reducing the burden of AMR.

Article Information

Ethics approval and consent to participate. Approval and clearance were obtained from the Muhimbili University of Health and Allied Sciences (MUHAS) Senate Research and Publications Committee. Informed consent was pre-requisite for enrolment for all participants. Patient information was treated with confidentiality.

Conflict of Interest. The authors declare to have no competing interests.

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Authors' contributions. MEA and MIM conceived the study, and MEA and BW conducted the lab work. MEA, FM, BW, EMN, and MIM prepared the manuscript. All authors participated in preparing and approving the submitted manuscript.

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