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DIAGNOSIS OF MALARIA AND TYPHOID FEVERS USING BASIC TOOLS: A COMPARATIVE ANALYSIS OF A RETROSPECTIVE DATA WITH A PROSPECTIVE EVALUATION IN AN ENDEMIC SETTING

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ABSTRACT
Malaria and typhoid fever are among important and endemic diseases in the tropical countries such as Nigeria. Diagnosis of most cases of malaria/typhoid co-infections are based on clinical suspicion alone or unreliable diagnostic tools leading to poor or misdiagnosis. A retrospective analysis was conducted on the positivity rate for malaria parasite and typhoid fever among patients who attended the Bayero University, Kano (BUK) Health Clinic from January to December 2013. A prospective study was also carried out on 200 febrile patients and 80 apparently healthy subjects (controls) with the view to determine the diagnostic profiles of malaria and /or typhoid fever co-infection using standard protocols (microscopy, rapid diagnostic test (RDT) for malaria parasite (MP), Widal test, stool and blood cultures for typhoid fever). Both test and control subjects were evaluated for the presence or otherwise of the malaria and /or typhoid aetiologic agents. Results obtained from the retrospective study for the period January to December 2013 indicated that, of the 2362 tests conducted, 318 (13.5%) were positive for MP using RDT and 722 (30.6%) were positive for typhoid fever using Widal test. Co-infection rate obtained was 89 (3.8%). Of the 200 subjects evaluated for the prospective study however, 42 (21%) and 34 (17%) were positive for MP using microscopic technique and RDT respectively. Microscopy was established to be more sensitive than RDT. Eighty nine (45%) were Widal positive. The culture method for typhoid fever diagnosis was negative. Co-infection rate stood at 17 (8.5%). No association however was found between the two disease conditions (p=0.6032). Significant proportion of the patients 86 (43%) were neither positive for malaria nor for typhoid fevers. Three isolates of non-typhoid salmonella specie and three other bacterial isolates were recovered from the stool and blood samples of the patients respectively. Baseline titre value for Widal test at BUK community was established as 160 and above for O antibodies to S. typhi, hence the criterion employed in the prospective evaluation. Laboratory evidence of malaria and typhoid co-infection rate at a titre of ≥160 was 8.5% using microscopy and 7% using RDT. The use of RDT is simple and easy, but has less sensitivity, thus may leave some patients with malaria un-detected. Based on the results of these findings, vis a vis the proportion of individuals negative for both malaria and typhoid fevers, clinicians should revisit causes of febrile illnesses other than malaria or typhoid and hence the need to include other tests for the detection of other causes.
Keywords: Malaria diagnosis, Typhoid fever, RDT, Widal test, Co-infections, Nigeria.

INTRODUCTION

Malaria is one of the important public health problems in many countries, especially in tropical areas (Parry *et al.*, 2002). Malaria is caused by a parasite of the genus Plasmodium. The disease is caused by five species of plasmodium, but *Plasmodium falciparum* causes serious morbidity and extensive mortality (Vollaard *et al.*, 2005). According to the World Health Organization (WHO) about 1.2 billion people are at high risk of symptomatic malaria in 2013 (WHO, 2014).It has been noted that about 110 million people in Nigeria have malaria, and 11% of deaths among pregnant women and 35% of death among infants are malaria related (Jonathan *et al.*, 2011).Typhoid fever too is widely recognized as a major public health problem in most developing tropical countries with an estimated 12 to 33 million cases occurring annually (Uneke, 2008). It is a systemic infectious disease caused by Salmonella enteric sub-specie enteric serotype Typhi (Uneke, 2008).

Both typhoid and malaria share social circumstances which are imperative to their transmission, therefore, a person living in such an environment is at risk of contracting both these diseases, (Brian and Sulaiman, 2006) and such coinfection was named *typhomalarial* fever (Prasanna, 2011 and Hosamani, 2013).

Mbuh *et al*, (2003) reported a co-infection rate of Malaria and Salmonella typhi as 10.1% and 0.5% using Widal test and blood culture respectively in Zaria, Nigeria. A co-infection rate of 10.33% and 1.33% using Widal test and blood culture respectively was also reported in Sokoto, Nigeria (Alhassan *et al.*, 2012).

MATERIALS AND METHODS

The present study was conducted at the Bayero University, Kano Health Clinic located within the Old Campus of the university located on latitudes 100 33N to 110 15N and longitudes 340CE to 8^0 20CE. Prior to the commencement of the work ethical approval was obtained from the ethical committee of university and all subjects recruited for the study were duly informed and consented about the study prior to collection of specimens from them.Sample size used in this work was determined using the relationship put forward by Sarmukaddam and Gard, (2006), using a prevalence from a previous (Alhassan *et al.*, 2012). This was determined as 143 which we rounded up to 200. The study was designed to be a retrospective and prospective case control study involving patients with febrile illnesses suspected of having malaria and typhoid fever co-infection and 80 apparently healthy individuals were recruited as controls.

Sample Collection and Handling

Five millilitres (5ml) of blood was aseptically collected by venepuncture technique from the subjects following standard procedures (Chessbrough, 2004). Two millilitres (2ml) each of the 5 Millitres samples were directly dispensed into blood culture bottles containing 18mls thioglycolate broth immediately for the isolation of *Salmonella* sp if any from such sample. These were incubated at 37°C with subculture onto Deoxycolate citrate agar (DCA) made after every two days for 7 days. Bacterial colonies isolated were identified by microscopic technique and confirmed biochemical methods as described by Ochei and Kalhatkar, (2008). Widal test (slide titration method) was performed on all the samples using commercial antigen suspension following the manufacturer's instruction for the somatic (O) and flagella (H) antigens (Chrololab diagnostic kit.) (Raphael and Spencer, 1983).

Rapid Diagnostic Test (RDT) for Malaria parasite

Rapid Diagnostic Test for Malaria Parasite test was performed on all blood samples using commercial prepared kit SD BIO LINE Malaria Antigen P.f by standard diagnostics, Inc, Korea. Using a 5µl disposable specimen loop provided, the circular end of the loop was dipped into the anticoagulated blood sample, and carefully it was placed into the round sample well, then four drops of the assay diluents were vertically added into the square assay diluents well. The results were read within 30 minutes according to manufacturer's instructions.

Malaria Parasite Microscopy

Thick blood films were made on a clean grease free glass slide from an anti-coagulated blood samples, air dried and stained using the field's staining technique as described by Ochei and Kalhatkar, (2008). The dried smear was examined on at least 100 fields using X100 objective. These were done for both the tests and the controls samples. The results were recorded.

Stool sample

Stool samples were cultured on selenite F and incubated at 37°c overnight. Subculture from the selenite F were made onto a solid media (DCA) and incubated at 37°c overnight. Culture that yielded non-lactose fermenting colonies were subjected to biochemical tests for proper identification as describe by (Ochei and Kalhatkar, 2008).

Statistical Analysis

The data generated in this study was analysed for statistical significance difference Chi-squire (X^2) test and Fisher's exact test depending on the size of the data, using a statistical software GraphPadinstat Version 3.05.

RESULTS

Retrospective data on the positivity rate of malaria parasite among patients suspected of having malaria and/or typhoid fever showed that 318 representing 13.5% were positive for malaria parasite while the remaining 2044 representing 86.5% were negative for the Malaria parasite using RDT (Table 1).

Table1: Retrospective data (Jan 2013-Dec 2013) for Malaria parasite positive rate using Rapid Diagnostic Test (RDT) among patients suspected of Malaria/typhoid fever co-infection (N=2362

SUBJECTS	NUMBER	PER CENT
Positive	318	13.50%
Negative	2044	86.50%
Total	2362	100%

Retrospective data on *S. typhi* O and H antibodies among 2362 patients suspected of malaria and/or typhoid fever indicated that, at a titre <80 1423 (60.2%) of the suspects were positive for the O antibodies to *S. typhi* when the titre is increased to ≥80 the positivity rate decreases to 39.8%, while at ≥160, the proportion was 30.6%. The proportion was lower (5.6%) at a titre of 320 and above. S. typhi O antibodies among suspect indicated a reduction of proportion with increase in the titre. A similar trend was observed for S. typhi H antibodies (Table 2).

Table 2: Retrospective data (Jan 2013-Dec 2013) for *S. typhi* O and H antibodies among patients suspected of Malaria/typhoid fever co-infection. (N=2362)

Antibodies Type

Titre Value	O		H	
	Number	%	Number	%
< 80	1423	60.2	1336	56.6
≥ 80	939	39.8	1026	43.4
≥ 160	722	30.6	727	30.8
≥320	133	5.6	146	6.2

Retrospective data on the co-infection rate indicated that, 120 (5.1%) of the subject were positive for malaria (RDT), and showed a titre of 80 and above for *S. typhi* O antibodies. The number co-infected decrease 89(3.8%) at a Widal test titre of 160 and above. When the Widal test titre considered positive increased to 360 the rate further dropped to 19 (0.8%). Higher rates were established in the three titres with little increase in the co-infection rate when the H antibody is considered (Table 3).

Table 3: Retrospective data on the co-infection rate for Malaria and *S. typhi* O and H antibodies at various titres. (N=2362)

Antibodies Type

Titre Value	O		H	
	Number	%	Number	%
≥80	120	5.1	127	5.4
≥160	89	3.8	92	3.9
≥320	19	0.8	18	0.76

Out of 2362, data analyzed, the profile for the subjects indicated that up to 2044 (86.5%) had no Malaria, 1423 (60.2%) had no *S. typhi* O antibodies at titre of 80 and above, whereas 1225 (51.86%) had neither malaria nor typhoid. When the titre is increased to 160 and above the interactions indicated that 1640 (69.4%) had no typhoid, and 1411 (59.7%) had no malaria or typhoid (Table 4)

Table 4: Retrospective analysis for the interactions between Malaria parasite and O antibodies titre (≥160) for *S. typhi*

Malaria	Insignificant Titre		Insignificant Titre		Total	
	Number	%	Number	%	n	%
positive	89	3.8	229	9.7	318	13.5
negative	633	26.8	1411	59.7	2044	86.5

The retrospective study indicated that higher proportion of subjects whom were malaria negative and had no evidence of O antibodies to *S. typhi* were found to increase with increase in the titre for the antibody.

Result obtained from the prospective analysis showed that; out of 200 subjects suspected of Malaria/typhoid co-infection, 42(21%) and 32(17%) were positive for malaria using microscopy and RDT respectively, whereas the control group showed 8.0(10%) and 2.0(2.5%) positive for microscopy and RDT respectively. However there is significant difference among the tests and controls for the diagnosis using microscopy (p=0.0373) and RDT (p=0.0006) (Table 5).

On the other hand, results from the prospective analysis for *S. typhi* O and H antibodies among 200 subjects suspected of malaria and/or typhoid fever showed that, the number of subjects with O antibodies at a titre of 80 and above was 130(65%), while at 160 and above the proportion was 89 (45%), and only 17 (8.5%) at a titre of 320 and above. For the 80 apparently healthy subjects however, 39(49%) had titre of 80 and above for *S. typhi* O antibodies, only 17(21.3%) had 160 and above, none of them had a titre of 320 and above for *S. typhi* O antibodies. The trend of *S. typhi* O antibodies among the patients and the controls indicates a decrease of proportion with increase in the titre. A similar trend was observed for *S. typhi* H antibodies. There exist a significant difference (p=0.0002) in the proportion of the titres between the tests and the controls (Table 5).

The prospective analysis also revealed the co-infection rate among the subjects suspected of Malaria/or typhoid at a titre of 80 and above for O antibodies to *S. typhi* as 13% and 3.8% for the tests and controls respectively. The proportion decreases to 8.5% and 1.3% for the tests and the controls as the titre increases to 160 and above. At a titre of ≥320, the proportions were 1.5% and 0% for the tests and the controls respectively. The reduction in the proportion was observed when RDT is used for malaria parasite diagnosis. At a titre of 80 and above for *S. typhi* antibody, the co-infection rate obtained was 10% and 1.3% for tests and controls respectively. The rate decreases to 7% and 0% at 160 and above for tests and controls respectively. The rate was lowest for the test at a titre of 320 and above; 1%, whereas no co-infection recorded among the control group at same titre (Table 5).

Considering the titre of 160 and above for *S. typhi* O antibodies as diagnostic, 111(55.5%) of the subjects had no typhoid and as much as 86(43%) had neither malaria nor typhoid (Table 5).

Table 5: Prospective analysis for Malaria parasite, *S. typhi* O antibodies and their co-infections among Patient suspected of Malaria/typhoid.

	MALARIA				*S.typhi* O antibodies		CO-INFECTION			
	Microscopy		RDT		(Titre ≥160)		Microscopy		RDT	
Number	Tests	Cntrls	Tests	Cntrls	Tests	Cntrls	Tests	Cntrls	Tests	Cntrls
Positive (%)	42 (21)	8 (10)	34 (17%)	2 (2.50)	89 (45)	17 (21.3)	17(8.5)	1 (1.30)	14 (7.0)	0
Negative (%)	158 (79)	72 (9)	166 (83)	78 (97.5)	111(55.5)	63 (78.7)	183(91.5)	79 (98.7)	186 (93)	100

Key: RDT = rapid Diagnostic test; Contrls = Controls; Total Test Subjects = 200; Total Control Subjects = 80, Contrls= Controls

The diagnostic value for microscopy and RDT in the diagnosis of Malaria showed a sensitivity of 21% and 17% respectively, the specifity on the other hand was 90% and 94% accordingly. (Figure 1).

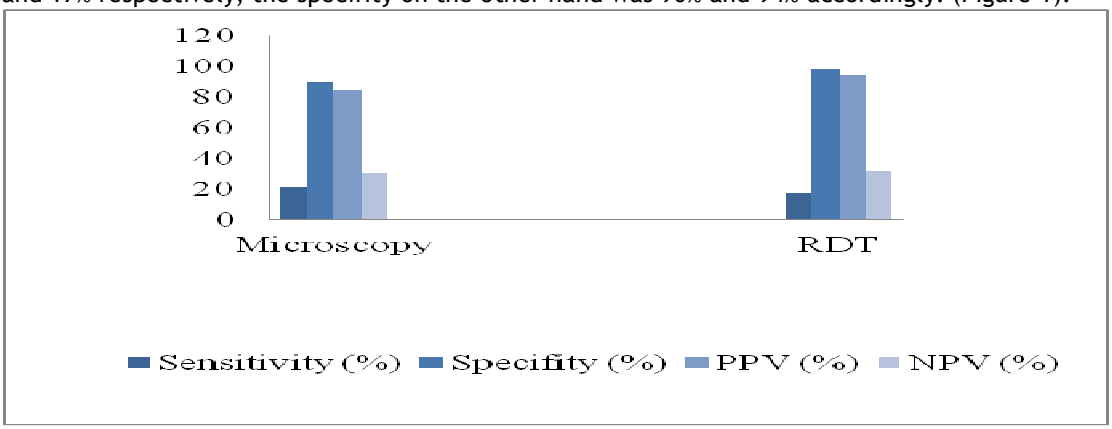

Figure 1: Diagnostic value for Microscopy and RDT

The diagnostic value of *S. typhi* O antibodies in detecting typhoid fever indicates that at a titre of ≥80 the sensitivity was 65% and specifity of 51%, whereas at ≥160, the sensitivity and specifity were 41% and 70% respectively. The sensitivity was 16% and the specifity remain 100% when the titre is ≥320. The trend for the diagnostic value indicates a decrease of the sensitivity and increase of the specifity as the antibody titre increases (Figure 2).

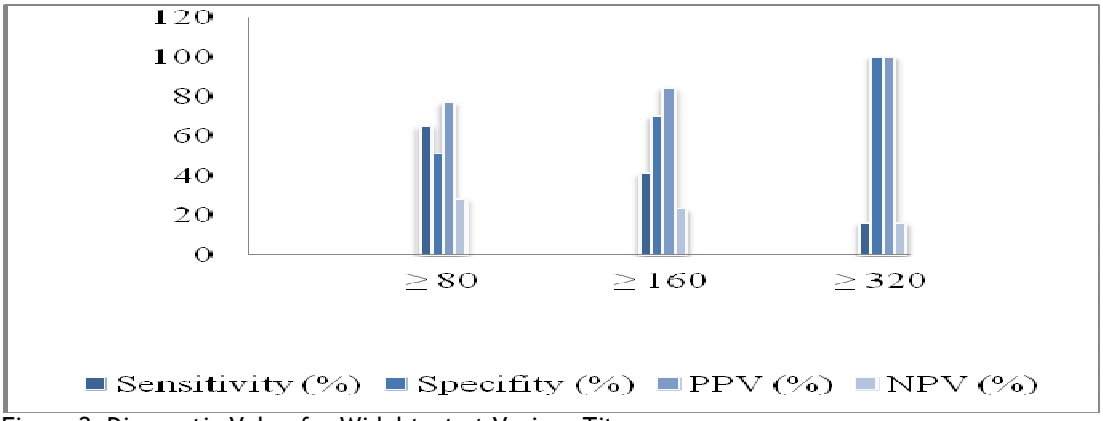

Figure 2: Diagnostic Value for Widal test at Various Titres.

Only three out of the 200 stool samples cultivated yielded growth for Salmonella specie. However, biochemical confirmation of the isolates obtained there from revealed these non-typhoid salmonella (NTS). No positive plate was obtained from the 80 control subjects evaluated for salmonella specie.

Blood cultures for the 200 experimental subjects were negative for salmonella specie. However, three other bacterial species were encountered namely; *Klebsiella specie*, *Staphylococcus specie*, and coagulase negative *Staphylococcus specie*. Cultures from blood samples of the control subjects yielded no growth.

DISCUSSION

The frequency of the request for Malaria parasite test and the evidence of typhoid by Widal test in our health facilities could not reflect the actual need for these investigations. Most cases of Malaria/typhoid co-infections are based on clinical suspicion alone or test results that could be non-specific, inaccurate as such unreliable. The prevalence of Malaria, typhoid fever and their co-infection may therefore not reflect the actual burden of the disease in the community. Establishing the laboratory profile of malaria, typhoid fever and their co-infection in suspected patients is therefore important to identify the true index of the disease in our community.

A retrospective analysis of Malaria and typhoid tests in BUHC showed that 2362 requests for Malaria and Widal tests were made. Up to 318(13.5%) were positive for Malaria parasite, while 722 (30.6%) had a titre of 160 and above for *S. typhi* O and H antibodies. The evidence of coinfection of Malaria and typhoid from data obtained was 89 (3.8%). The lower rates of malaria positive 13.5%, 30.6% for typhoid, and 3.8% for the co-infection suggest that the majority of patients requesting not needed. In this study evidence of co-infection of Malaria and typhoid fever was consider as a positive test for Malaria using microscopy or RDT, together with a titre of 160 and above for *S.*

The laboratory diagnosis of typhoid in the study area is primarily serological based therefore no data for blood and stool cultures were available.

The prospective study rates revealed that of 200 the suspected Malaria/typhoid co-infection, 42 (21%) and 34 (17%) were positive for Malaria using microscopy and RDT respectively. These proportions were higher than the retrospective, indicating an improvement in the positivity due the intervention of this study through implementation of quality assurance scheme. Other workers, Alhassan *et al.*, (2012) reported 17% in Sokoto, Mbuh *et al.,* (2003) reported 27% among febrile subject in Zaria. While a rate of 18.6% was reported by Richmond *et al.,* (2011) in Ghana, Jonathan *et al.*, (2011) in Oyo reported a rate of 5% using microscopy. From Imo state, Opara *et al.*, (2011) used microscopy and obtained 39% for malaria parasite whereas a very high rate (78.9%) was established in Anambra State, Nigeria (Ekesiobi *et al.*,2008). In Delta state however, Esohe *et al.* (2012) uses microscopy and recoded 37.6% positivity rate for malaria parasite.

Considering a titre of 160 and above for O antibodies as an evidence for typhoid fever 89 (45%) and 17 (21.3%) were positive among the febrile and controls respectively. The 89 (45%) rate for the evidence of typhoid fever among the febrile subjects is higher than the 722 (30.6%) obtained in the retrospective data analysis indicating an improvement. Other workers however reported their findings. Mbuh *et al.* (2003) reported a rate of 36.7% among the febrile and 30.8% for the controls group in Zaria, Nigeria. Igbeneghu *et al.* (2009) in Oyo State, Nigeria, reported 16.7% for the tests group and 11% for the controls. Higher rate for the antibodies 57.4% was reported from Ekesiobi *et al.* (2008) reported in Anambra, Nigeria.

typhi O antibodies (Sarkinfada *et al.*, 2003). Up to 17 (8.5%) had an evidence of malaria and typhoid co-infection, which is higher than the 89 (3.8%) recorded in the retrospective study in the same facility. The higher rate of the co-infection reported in the prospective study is a result of the intervention with a quality assurance on the diagnostic tests used in the study. Furthermore, the profile of the subject presented with febrile illnesses suspected of malaria and/or typhoid in this study shows 111 (55.5%) of the subjects had no typhoid, 158 (79%) had no malaria and 86 (43%) had neither malaria nor typhoid.

Overall, the normal trends indicate a higher rate of Malaria, typhoid and the co-infection in the prospective than in the retrospective analysis. It further indicated that up to 86.5%, 69.4% and 96.2% of the suspected febrile patients had no Malaria, typhoid and co-infection in the retrospective analysis respectively. While in the prospective data, 79%, 55.5% and 91.5% of the suspect had no Malaria, typhoid and co-infection respectively. Richmond *et al*. (2011), reported 79 (61.2%) had neither malaria nor typhoid, 105 (81.4%) had no malaria and 97 (75.2%) had no typhoid. Igbeneghu*et al*. (2009) reported that 85 (32.9%) of the febrile had neither malaria nor typhoid, 97 (37.6%) had no malaria and 215 (83.3%) had no typhoid.

The diagnostic value for Microscopy in the diagnosis of malaria showed a sensitivity of 21% specifity of 90%, positive predictive value 84% and a negative predictive value of 31% respectively. The RDT on the other hand shows 17%, 98%, 92% and 32% for the sensitivity, specifity, PPV and NPV respectively. This implies that the microscopy has more sensitivity but less specific compared to the RDT in our findings. The sensitivity and specifity of the widal test at a titre of 160 and above were 41% and 70% respectively. The PPV and NPV were 84% and 23% respectively. The parameters vary with the change in the antibodies titre. Sensitivity of the test increases as the titre is reduce while the specifity decrease with decrease in the titre for antibodies.

Conclusion

Despite the fact that malaria and typhoid fever are indistinguishable regarding their clinical signs and symptoms and there are some overlaps in their pathology, Plasmodium and Salmonella are not of the same phylum, do not share antigens nor have same method of transmission. Some clinicians treat malaria and typhoid concurrently once they have high antibody titre for Salmonella serotypes, even without adequate laboratory diagnoses for malaria and vice versa. Higher percentage of such patients may not need one or both the treatments. Retrospective study indicated rates of 13.5%, 30.6% and 3.8% for the Malaria (RDT) positive, S. *typhi* O antibodies titre 160 and above and co-infection respectively. No evidence for any of the two infections in about 59.7%.

Prospective analysis however indicated that 21%, 45%, and 8.5% were positive for Malaria, typhoid and the co-infection respectively. S. *typhi* O antibodies titre 160 and above and co-infection respectively. Fourty- three (43%) had no evidence of any of the two infections. The baseline titre for widal test determine in this work was 160 and above for O antibodies to S. typhi. This is because 39(49%) of the apparently healthy individuals had a titre of ≤80 for S. *typhi* O antibodies in their serum. Furthermore, laboratory evidence shows a higher proportion 86(43%) of the patients whom where suspected to have malaria/or typhoid fever, be negative for both malaria and a significant titre for S. *typhi* O antibodies. Laboratory evidence of malaria and typhoid coinfection rate at a cut-off titre of ≥160 was 8.5% using microscopy and 7% using RDT, suggesting that at a given titre, RDT is less sensitive than microscopy in detecting coinfection. A higher proportion of the study subjects 86 (43%) indicated neither malaria nor typhoid, who were earlier suspected to have malaria/or typhoid fever.

Recommendations

a. Co-infection should be confirmed with culture method, where not feasible serology could be used with strong clinical evidence.

b. Titre of ≥160 for S. *typhi* O antibodies obtained using quantitative or semi quantitative method for widal test could be considered to diagnose at the B.U.K community.

c. Use of RDT is an alternative way of quickly establishing the diagnosis of malaria, but it does not eliminate the need for malaria microscopy. Therefore, its use should be matched with a proper Quality Control due to its lower sensitivity that may leave some suspect with low parasitemia undiagnosed.

d. Quality assurance scheme should be introduced.

e. Newer methods of diagnosis (immunological methods) which are more sensitive and specific should be introduced to replace Widal test.

f. Causes of febrile illnesses other than malaria or typhoid should be revisited by our clinicians, to avoid delay of the treatment due to misdiagnosis.

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