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Performance of TUBEX® TF IgM Antibody Test Against Culture to Detect Typhoid Fever Among Hospitalized Patients in Nairobi County

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Abstract
Typhoid fever has remained endemic and a major health problem in developing countries like Kenya due to poor sanitation, overcrowding and limited access to diagnostic services. The main aim of this study was to determine the diagnostic value of TUBEX® TF IGM antibody tests for early diagnosis of typhoid fever among hospitalized patients in Nairobi County. TUBEX® TF is a competitive immunoassay which detects presence of anti-09 IgM *Salmonella* Typhi antibodies in a patient's serum. We studied TUBEX® TF a rapid sera diagnostic test for its usefulness in early diagnosis of typhoid fever and we compared its sensitivity and specificity to that of Widal test. The study was conducted on 92 (Gp-I) febrile patients who had clinically suggestive signs and symptoms of typhoid fever. Two groups of controls were created; 45 (Gp-II) and 15 (Gp-III) age and sex matched febrile and healthy controls, respectively. Blood culture was performed in all cases while TUBEX® TF and Widal tests were performed in both cases and controls. The sample size was based on convenience at the two health facilities of our study. Gp-I had 9 (9.78%) blood culture positives for *S.*Typhi, 11(11.99%) were positive for TUBEX® TF while 17 (18.48%) were positive for Widal test. 3 (6.67%) Gp-II were positive on both TUBEX® TF and Widal test while none of the two tests tested positive on Gp-III. Among 9 culture positive cases, TUBEX® TF was positive in 8 cases same as Widal test with sensitivity, specificity, PPV and NPV values of 88.9% (95% CI: 51.18-99.7), 97.6% (95% CI: 91.6-99.7), 80.0 %(95% CI: 44.4-97.5), 98.8 %(95% CI: 93.4-100), respectively. Widal test had 88.89% (95% CI: 51.8-99.7), 90.4% (95% CI: 81.9-95.7), 50.00% (95% CI: 24.7-75.3), 98.7% (95% CI: 92.9-100) respectively. This study demonstrated better results with TUBEX® TF test compared to Widal test when blood culture was used as a gold standard. However, these results should be further confirmed by using multiple gold standard tests such as molecular and enzyme-linked immunosorbent assays and carried out on large scale cross-sectional studies with varied prevalence of typhoid fever in the population.
Keywords: Typhoid fever, TUBEX® TF, Widal, Cases and Controls.
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1. Introduction

Enteric fever, an infection caused by *Salmonella enterica* serovar Typhi and serovar *Paratyphi* A remains a major public health challenge. The occurrence of enteric fever in poor populations with limited access to diagnostic services means that the disease burden is poorly quantified (WHO, 2003). It is estimated that by the year 2000, typhoid fever had caused 21.6 million illnesses and 216, 500 deaths globally (Crump *et al*., 2004). It has continued to be a major cause of morbidity, particularly in children and young adults in South East Asia, although accurate assessments are still hindered by the lack of reliable surveillance data (Darton *et al*., 2014). In Africa, Crump *et al*., 2004 estimates incidence of typhoid infections of 10-100 cases/100, 000 person years in most African countries, with the incidence highest in childhood. In East Africa, the incidence was estimated at 39/100, 000 person years. The high rate of infection and dominance of paratyphoid fever in several geographical areas in Africa are of particular concern (Darton *et al*., 2014). The global concern in Kenya is reflected in perceptions that typhoid is a common and serious disease among children and adults, where highly publicized outbreaks have strengthened this view among the public and health professionals (Mweu *et al*., 2008). The use of Widal test has strengthened this concern for it is the rapid test mostly performed in most primary health settings and inability of these settings to perform blood culture (Willke *et al.,* 2002). This is because the frequent use of Widal test is not only unlikely to be helpful but can also be misleading resulting to misdiagnosis and worsening antibiotic resistance (Mweu *et al.,* 2008). Therefore, accurate diagnosis of enteric fever has remained a challenge due to the nonspecific clinical presentation of cases and the low sensitivity and specificity of the commonly available tests (Darton *et al*., 2014). The control strategies utilizing cheap accurate diagnostics and effective vaccines are urgently required, and their development should be accelerated by the use of a human challenge model (Bhutta, Z.A, 2006). There have been few attempts to establish simple clinical case definitions for typhoid fever to help in case identification at presentation to health facilities (Tam *et al*., 2003).

For effective management of disease, there is a need for better data on typhoid fever among hospitalized patients. This data would be best generated by effective, broadly hospital based systems that could capture data on a range of serious bacterial diseases in urban and rural settings. Few or no such systems exist in the poorer countries of Africa and Asian countries where disease burdens are the highest (Mweu *et al*., 2008). Missed diagnoses and worsening antibiotic resistance may result, although the higher prevalence of non-typhoidal *Salmonellae* (NTS) makes it likely that on some occasions a child falsely diagnosed as having typhoid will by chance receive appropriate treatment for NTS (Graham SM., 2002). While newer, improved diagnostics are therefore urgently required, it is clear that they will have to be highly sensitive and specific (>90%) serological tests (Mweu *et al*., 2008).

Serologic laboratory testing of typhoid fever is important and can be a very useful complement to blood culture in order to increase the clinical information. This prospective validation study sought to evaluate the performance of rapid point of care diagnostic test TUBEX® TF for rapid detection of anti-*Salmonella* Typhi antibody test for early diagnosis of typhoid fever among hospitalized well-defined cohorts at Mbagathi County and Kenyatta National hospitals and selected control subjects to give a better sensitivity and specificity results for the TUBEX® TF kit. The core objective of this study was exhaustively met.

2. Materials and Methods

2.1 Study Participants and Sample Collection

Patients were recruited from two hospitals: Mbagathi County and Kenyatta National hospitals in Nairobi County, Kenya. The study took place between the period of January, 2018 and July, 2018. Majority of the patients presenting at these hospitals came from the neighboring informal settlement, Kibera. We obtained blood from consecutive clinically suspected typhoid fever cases, febrile patients and healthy individuals. Those that fulfilled criteria for suspected typhoid fever case had ≥37.5°C axillary temperature, abdominal pain, headache, constipation, diarrhea, vomiting, rash and who reported not to have taken antibiotic in the last 1 to > 2 days. All the patients were briefed on the significance and the aim of the study before they gave both oral and written informed consent. They were also asked to answer a brief questionnaire on clinical symptoms, vaccination history and antimicrobial use on a patient history form. Blood samples were drawn aseptically for blood culture following serological sample collection. For children 5-12 years of age, up to 5mls of blood was collected, 1- 3mls of this was inoculated immediately into a BACTEC Ped Plus/F (enriched Soybean-casein digest broth with CO_2; Becton-Dickenson) blood culture bottle (for children) while 2mls was put in plain vacutainer tube. For participants ≥ 13 years, we attempted to collect 8-10mls of blood, which was placed into a BACTEC Plus/F (for adults) bottle. Blood and broth were made to mix by inverting the inoculated bottles 4-5 times.

The collected samples were coded for reference in the laboratory for testing. Blood samples were then transported from the respective hospitals to the laboratories at the Center for Microbiology Research (CMR) at Kenya Medical Research Institute (KEMRI) while placed in a cooler box. Upon receipt at the laboratory, inoculated BACTEC Plus/F bottles were placed into BACTEC™ 9050(Becton, Dickinson and Company) blood culture system while vacutainer tubes containing blood for serology were centrifuged at 3000 x *g* for 10 minutes. The serum aliquots were separated from whole blood and placed in cryotubes then stored at -20^{O} C for later serological testing.

2.2 Laboratory Methods Performed

2.2.1 Blood Culture

Blood placed in BACTEC™ 9050 were incubated at 37^{0}C for seven days or until growth was observed through the Bactec automated signal according to the current standard operating procedures of the Kenya Research Institute (KEMRI). The positive samples were then subcultured on MacConkey agar and blood agar. Suspected colonies for *S.* Typhi were identified by Gram stain, standard biochemical tests including Oxidase test, Triple test sugar-iron sugar agar (TSI), Urease, motility test, Citrate and Indole and agglutination test with *Salmonella*-specific antisera

2.2.2 TUBEX® TF Test

All analysis using TUBEX® TF and blood culture were performed according to instruction for use as supplied together with the kit (http://www.idl.se). TUBEX® TF is a semi quantitative colorimetric test that detects titers of *Salmonella* enterica serovar Typhi lipopolysaccharide (LPS) O9 antigens in patient serum (Tam *et al*., 2003). It was run in parallel and independently with blood culture and processed on a blinded basis according to manufacturer's instructions, with the operator unaware of the result from blood culture and status of the patients. The score range from 0 (clear pink) to 10 (intense blue). Interpretation of TUBEX® test was done by at least 3 independent observers (The PI and 2 qualified technical persons) to validate the results. 2 out of 3 concordant results were recorded as either negative or positive with corresponding semi quantitative scores using the magnetic color scale. The score of 0-2 was interpreted as negative while the score of ≥ 4 was interpreted as positive TUBEX® result as per the manufacturer's instructions.

2.2.3 Widal Test

Semi quantitative slide and standard tube method using EUROPATH™ antigen containing *S.* Typhi O & *S.* Typhi H were performed. Generally antibodies having titers of 1:80 or more were considered clinically and diagnostically significant.

2.3 Data Analyses

The indeterminate results of TUBEX® TF were handled by either doing the retest or by using TUBEX® TF wash buffer in accordance to manufacturer's instructions. The missing data were traced back to laboratory results form used initially during results recording. The sample size was based on convenience at two health facilities of our study. The data were captured in Microsoft Excel 2016 (Microsoft Corporation, Redmond, United States of America) and converted to STATA version 15 (Stata LP, Collage Station, USA). Stata's diagt was used to determine the sensitivity, specificity, positive predictive values (PPVs) and negative predictive values (NPVs). Two sided significance level of 5% analyses of the performance of the two rapid diagnostic tests were also done. Analyses of 5% and 50% pretest probabilities of background typhoid fever rates were also performed to ensure that the results were applicable even in conditions of a few typhoid fever outbreaks – since incidence would be higher during outbreaks – and of lower endemicity, given that study patients were selected on the probability of having typhoid fever (Keddy *et al*., 2011). Cohen's kappa coefficient (k) analysis was used to test the agreement of the two sera diagnostic tests against a gold standard.

2.4 Ethical Approval

This study was approved by the Scientific Ethics and Review Unit at KEMRI (KEMRI/SERU/CMR/P00059/3546). Informed consent was obtained from all the research participants where adults obtained their own informed consent while parents or guardians consented for the children.

3. Results

3.1 Participants

In total, this study was conducted on 152 participants, including 92 participants who had clinically suggestive signs and symptoms of typhoid fever. 45 and 15 participants were age and sex matched febrile and healthy controls respectively. The diagram showing the flow of participants in the study is as shown in figure 1 below.

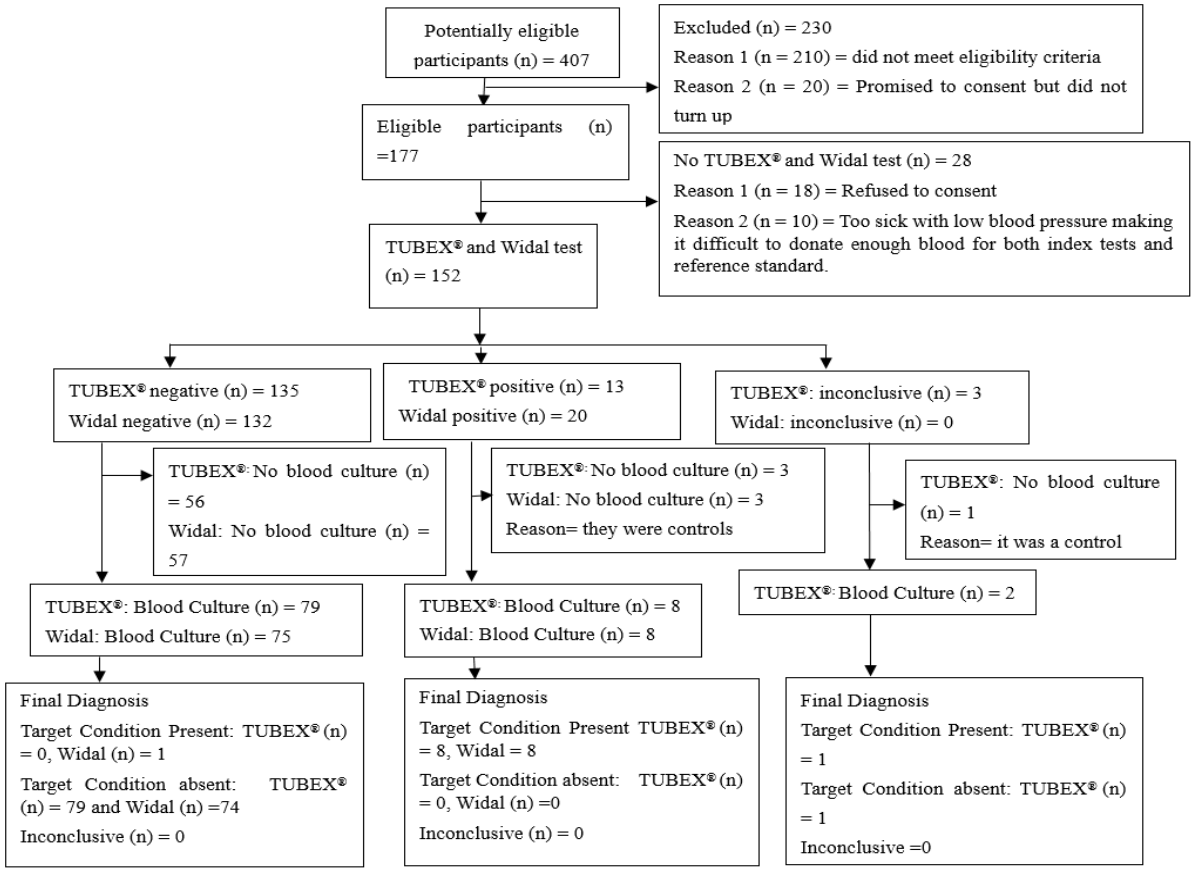

Figure 1: Diagram showing flow of participants during the study

Headache, abdominal pain and constipation were the main clinical characteristics recorded for our typhoid fever cases. Those with slow heart rate and muscle pain were few (Table 1)

Table1. Characteristics of patients with signs and symptoms for typhoid fever (n=92)

Characteristics of Patients	Values
Headache	78%
Abdominal Pain	62%
Constipation	40%
Diarrhea	16%
Vomiting	7%
Disorientation (Confusion)	4%
Cough	4%
Slow heart rate	3%
Myalgia (sore muscles) and Rash	2%

Generally, there were a total of 66 males (43.42%) and 86 females (56.58%). Total cases for male were 55 (59.78%) while female were 37 (40.22%) and corresponding values for febrile controls were 18 (40.00%) males and 27 (60%) females and healthy controls were 10 (66.67%) and 5 (33.3%) respectively. The frequencies of cases and controls are as shown in Figure 2-4 below. The participants were of ages between 5-60 years with a median age of 36.5 years.

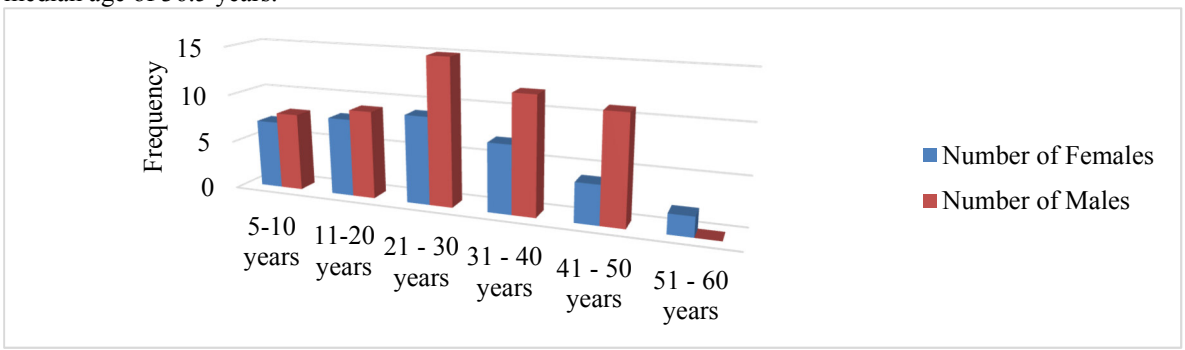

Figure 2: Cases Distribution of Males and Females

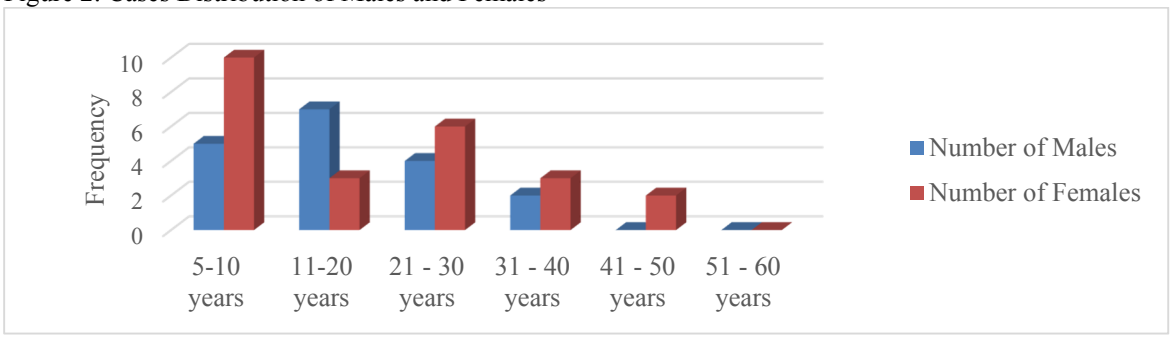

Figure 3: Febrile Controls Male and Female Distribution

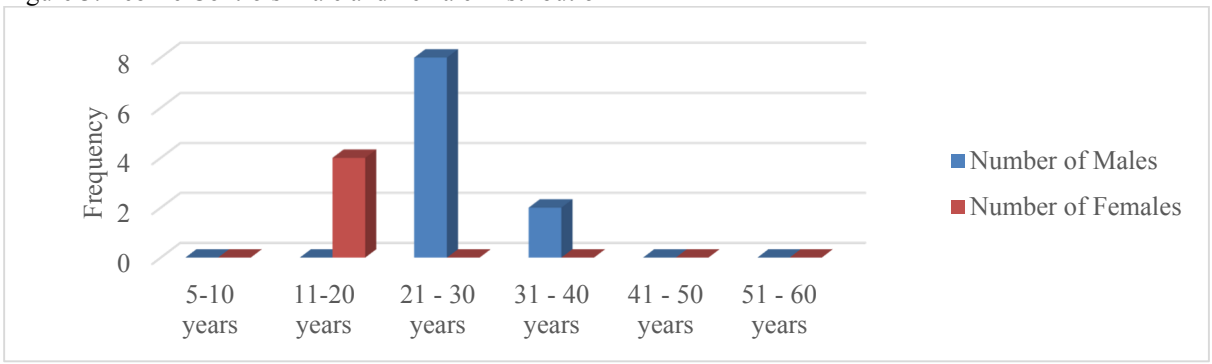

Figure 4: Healthy Controls Male and Female Distribution

3.2 Blood Culture

Among 92 clinically suspected typhoid fever cases, 9 (9.78%) were positive for *S.* Typhi with the highest rate of

blood culture positivity for *S.* Typhi 4 (4.34%) in children between ages 5-12 years, followed by adults 3 (3.26%) between ages18-60 years and children aged 13-17 years with 2 (2.17%) consequently. Of this culture positive patients, 5 were male (55.50%) while 4 (4.44%) were female. 13 (14.13%) sample cases grew other pathogens including *Micrococcus, Klebsiella* and *Bacillus.* 21(22.83%) were positive on BACTEC™ 9050 blood culture system but did not grow any microorganisms when sub-cultured on blood and MacConkey agar while 49 (53.26%) were negative on BACTEC™ 9050 blood culture system after 7 days. In compliance with our eligibility criteria no participant was taking any antibiotic upon visiting the health facility and before collection of blood samples.

3.3 Serology

Among 152 of our research participants, eleven (11.95%) tested positive using TUBEX® TF while 17 (18.48%) tested positive using Widal test. Both TUBEX® TF and Widal test were positive in 8/9 (88.89%) of blood culture positive cases. Of 83 culture negative cases, TUBEX® TF was positive in 3 (3.61%) and negative in 81 (97.45%) while Widal test was positive in 9 (10.84%) and negative in 75 (90.36%). Both TUBEX® TF and Widal test were positive in 3 (6.6%) out of the 45 febrile controls. None of the healthy controls were positive by either TUBEX® TF or Widal test (Table 2).

Table 2: Results of TUBEX® TF test and Widal test in different study groups

Study groups	No. of individual	TUBEX® TF positive	Widal test positive
Group I	09	08 (88.89)	08 (88.89)
Group II	83	03 (03.61)	09 (10.84)
Group III	45	03 (06.67)	03 (06.67)
Group IV	15	00 (00.00)	00 (00.00)

Figures in parenthesis indicate percentage.

Group I - Culture positive typhoid fever cases
Group II - Culture negative typhoid fever cases
Group III - Febrile controls
Group IV - Healthy control.

3.3.1 Diagnostic tests Evaluation

The two serological diagnostic techniques, TUBEX® TF and Widal test were evaluated using blood culture as a lone reference standard. The sensitivity, specificity, PPV and NPV of the two kits were as shown in table 3 below. Controls were from febrile patients and apparently healthy individuals and were considered culture sterile. If all the 92 cases with clinically suspected symptoms of typhoid fever were tested with both TUBEX® TF and Widal test, sensitivity, specificity, PPVs and NPVs were, 88.9% (95% CI 51.8-99.7), 97.6% (95% CI 91.6-99.7), 80.0% (95% CI 44.4-97.5), 98.8% (95% CI 93.4-100.0) and 88.9% (95% CI 51.8-99.7), 90.4% (95% CI 81.9-95.7), 50.0% (95% CI 24.7-75.3), 98.7% (95% CI 92.9-100.0), respectively. If all the cases and controls were included, sensitivity remained constant but PPVs declined. TUBEX® TF had 61.5% (95% CI 31.6-86.1) while Widal test had 42.1% (95% CI 20.3-66.5). If neither febrile and healthy controls were separately combined with cases and tested, the resulting diagnostic values had no statistical significance as the values were similar as to those recorded when cases were tested alone and when the cases were combined with both of these two groups of controls.

Table 3: Sensitivity, Specificity and Predictive values of TUBEX® TF test and Widal test using blood culture as a reference standard

		Sensitivity % (95% CI)	Specificity % (95% CI)	PPV% (95% CI)	NPV% (95% CI)	Prevalence (95% CI)
Cases	TUBEX® TF	88.9 (51.8-99.7)	97.6 (91.6-99.7)	80.0 (44.4-97.5)	98.8 (93.4-100.0)	9.8 (4.6-17.8)
	WIDAL	88.9 (51.8-99.7)	90.4 (81.9-95.7)	50.0 (24.7-75.3)	98.7 (92.9-100.0)	9.8 (4.6-17.8)
Cases+ all Controls	TUBEX® TF	88.9 (51.8-99.7)	96.5 (92.0-98.9)	61.5 (31.6-86.1)	99.3 (96.1-100.0))	5.9 (2.7-10.9)
	WIDAL	88.9 (51.8-99.7)	92.3 (86.7-96.1)	42.1 (20.3-66.5)	99.3 (95.9-100.0)	5.9 (2.7-10.9)
Cases + Sick Controls	TUBEX® TF	88.9 (51.8-99.7)	96.1(91.1-98.7)	61.5 (31.6-86.1)	99.2 (95.6-100.0)	6.6 (3.0-12.1)
	WIDAL	88.9 (51.8-99.7)	91.4 (85.1-95.6)	42.1 (20.3-66.5)	99.2 (95.4-100.0)	6.6 (3.0-12.1)
Cases + Healthy Controls	TUBEX® TF	88.9 (51.8-99.7)	98.0 (92.8-99.8)	80.0 (44.4-97.5)	99.0 (94.4-100.0)	8.4 (3.9-15.4)
	WIDAL	88.9 (51.8-99.7)	91.8 (84.5-96.4)	50.0 (24.7-75.3)	98.9 (94.0-100.0)	8.4 (3.9-15.4)

CI-Cumulative Intervals, NPV-Negative Predictive Value, PPV- Positive Predictive Value.

When these results were subjected to 5% pretest probability, PPVs and NPVs for TUBEX® TF and Widal tests were 66.0% (95% CI 32.6-88.6), 99.4% (95% CI 96.3-99.9) and 32.7% (95% CI 19.5-49.4), 99.4% (95% CI 96.0-99.9) respectively. When cases, febrile and apparently healthy controls were subjected to this pretest probability, PPVs showed varied decline while NPVs remained constant with little or no variation. When these results were further subjected to 50% pretest probability, PPVs rose while NPVs seemed to decline. When cases were subjected alone , TUBEX® TF had a PPVs of 97.4% (95% CI 90.2-99.3) and NPVs of 89.8% (95% CI 58.0-98.2) while Widal test had 90.2% (95% CI 82.1-94.9) and 89.1% (95% CI 56.1-98.1) respectively. On putting together cases, febrile and apparently healthy controls under this pretest probability, there seemed to be minimal to no variation on both PPVs and NPVs (Table 4).

Table 4: Predictive values of TUBEX® TF test and Widal test using blood culture as a reference standard under assumed pretest probabilities of 5% and 50%

		5% PPV% (95% CI)	5% NPV% (95% CI)	50% PPV% (95% CI)	50% NPV% (95% CI)
Cases	TUBEX® TF	66.0 (32.6-88.6)	99.4 (96.3-99.9)	97.4 (90.2-99.3)	89.8 (58.0-98.2)
	WIDAL	32.7 (19.5-49.4)	99.4 (96.0-99.9)	90.2 (82.1-94.9)	89.1 (56.1-98.1)
Cases + all Controls	TUBEX® TF	57.2 (35.4-76.5)	99.4 (96.3-99.9)	96.2 (91.2-98.4)	89.7 (57.8-98.2)
	WIDAL	37.8 (24.8-52.9)	99.4 (96.1-99.9)	92.0 (86.2-95.5)	89.3 (56.7-98.1)
Cases + Sick Controls	TUBEX® TF	54.5 (33.0-74.5)	99.4 (96.3-99.9)	95.8 (90.3-98.2)	89.6 (57.7-98.2)
	WIDAL	35.2 (22.8-50.1)	99.4 (96.1-99.9)	91.2 (84.9-95.0)	89.2 (56.4-98.1)
Cases + Healthy Controls	TUBEX® TF	69.6 (36.3-90.2)	99.4 (96.3-99.9)	97.8 (91.6-99.4)	89.8 (58.2-98.2)
	WIDAL	36.4 (22.1-53.7)	99.4 (96.1-99.9)	91.6 (84.4-95.7)	89.2 (56.5-98.1)

CI-Cumulative Intervals, NPV-Negative Predictive Value, PPV- Positive Predictive Value.

On testing the agreement of the two diagnostic tests with gold standard, TUBEX® TF test demonstrated almost perfect agreement while Widal test demonstrated substantial agreement (Table 5).

Table 5: Agreement of the TUBEX® TF and Widal test with blood culture using Cohen's kappa coefficient (k)

Test	Blood culture	
	Cohen's k	% of Agreement
TUBEX® TF	0.82	91.19%
Widal test	0.74	87.13%

4. Discussion

The diagnosis of typhoid fever is a challenge largely because its signs and symptoms are similar to those of other febrile illness (Bhutta ZA, 2006). Isolation of *S.* Typhi and *S.* Paratyphi from microbiologic culturing of bone marrow is considered a gold standard for the confirmation of typhoid fever (Islam *et al.,* 2106). Considering that typhoid fever is very prevalent in very young children in low income and endemic countries, bone marrow culture is rarely performed. Therefore, diagnosis of typhoid fever is made principally by blood, stool and urine cultures (Bhattacharya *et al.,* 2003), as an alternative to bone marrow culture. Unfortunately, blood culture has a diagnostic yield of 70-75% during the first week of illness and it is affected by antimicrobial use ((Krishna *et al*., 2011). Results require 2-7 days to be available and it entails technical procedures (Bhan *et al*., 2005). Therefore, rapid sera diagnostic tests have been the most preferred methods for diagnosis of typhoid fever in low resource settings of endemic countries (Keddy *et al*., 2011). Widal test has been one of these diagnostic tests and it has been in use for over a century in developing countries, but has remained a sera diagnostic test with limited diagnostic utility due to low sensitivity, specificity and positive predictive values which changes with geographical areas (Sherwal *et al*., 2004). Sharing of O and H antigens by other *Salmonella* serotypes and other members of *Enterobacteriaceae* has made the role of Widal test even more controversial in diagnosis of typhoid fever (Parry *et al*., 2002). TUBEX® TF is a new, inexpensive and reliable sera diagnostic test which is still under evaluation and is yet to be commercially available. It has been studied in many typhoid endemic countries with reports of significant higher sensitivities and specificities. In the present study, we studied TUBEX® TF test in our country for its usefulness in diagnosis of typhoid fever in patients presenting in our hospital. We also evaluated Widal test so as to compare its performance with that of TUBEX® TF test.

Among 92 clinically suspected typhoid fever cases in our study, 59.78% were males and 40.22% were females. Among them, the isolation of *S.* Typhi from blood of male patients was 55.5% and 44.44% in females. We had more female febrile participants compared to their male counterparts (Fig.3). Fig.4 shows apparently healthy endemic zone controls. These findings were in consistent with observation made by Begum (2008) who reported 60% male and 40% female. These findings also were similar to Roxas and Mendoza (1989) who reported 56% male and 44% female cases. Devaranavadagi and Srinivasa (2017) also reported 63.8% male and 36.2% female cases. The findings also correlates with the findings reported in Asia. In a study done in Bangladesh comparing differences in typhoid fever distribution in age and gender among pediatric patients, the isolation of *S.* Typhi in blood of male patients was 25.7% while for females was 14.9% (Sattar *et al.,* 2016). The high rate of typhoid fever infection in males than females, perhaps could due to published observation that places male less adherence to hygiene measures whether inside or outside home environment and washing of hands after using public restrooms or bathrooms at home, handling food and their greater propensity to eat raw or undercooked foods and at roadside locations (Hosoglu *et al*., 2006; Jeong *et al*., 2007). For this reason, the hypothesis that sex-linked differences in the degree of natural exposure of Peyer's patches to *S. Typhi contribute to high typhoid infection in males than in females maybe testable (Khan, 2012).*

The disease affected all ages; however most of the typhoid fever suspected cases came from the age group of 21-30 years (Fig.2). The high number of cases in this age group may have been partly due to other infections having similar clinical symptoms to typhoid fever. In our findings, we found children 5-12 years to be more susceptible to typhoid fever with an of isolation rate of *S.*Typhi of 4.34%. These findings place children of this age group in brackets of high risk group. This may due to a lack of immunity transferred by mother's milk or consumption of potable drinking water a common practice in rural/urban settlements (Saha *et al.,* 2003). Despite the fact that, typhoid fever is known to be sufficiently severe among children < 5 years old to require outpatient and inpatient care (Crump *et al.,* 2004), this study recruited children aged ≥ 5years because very young children still have their immune system under development. For this reason their immune antibodies are still weakly expressed. Therefore, placing them as part of our participants to evaluate new diagnostic technology could have affected the results of our test. This is supported by previous studies that have evidently shown that immunological responses and clinical characteristics in young children less than 5 years with *S.* Typhi bacteremia have to date remained poorly characterized (Sattar *et al.,* 2016).

Our findings were similar to other findings reported by Breiman *et al.,* (2012) done in Kibera slums, Kenya where the highest rates of typhoid fever were in children between age group of 5–9 years old (596 per 100,000 person-years of observation). Our findings were also consistent with the findings reported by studies done in five Asian countries, where high rate of isaltion *S.* Typhi (57%) was from children between the age group of 5-15 years (Ochiai *et al.,* 2008). Another study done in Bangladesh among pediatric patients, highest rate of blood culture

positivity of *S*. Typhi was found among children participants within 6-5 years of age (19.14%)(Sattar *et al.,* 2016). The similarity of these findings with that from Asian countries is partly due to dense population and severely limited options for sanitation and safe water for people who live in overcrowded areas in Africa and in Asia that places them at higher risk for typhoid fever (Breiman *et al.,* 2012). These findings proposes immunization programs against typhoid fever to be introduced and implemented with an aim of vaccinating younger children below 12 years in the first phase. Similarly, this will serve to arrest typhoid infection in other age groups. However, since the other young and elderly participants of age group between 13-17 years and 18-60 years respectively demonstrated significant percentage of typhoid fever infections, administration of typhoid vaccine booster dose should also be done in a potentially endemic population of the disease burden within these age groups (Saha *et al.,* 2003).

When we screened 92 febrile patients for *S.* Typhi by blood culture in our study, 9 (9.78%) constituted bacteriologically proven typhoid fever cases and were positive for *S.* Typhi (Table 2). The remaining 83 patients were blood culture negative but with clinically suggestive symptoms of typhoid fever. Our findings recorded a little higher prevalence of typhoid fever when compared to previous similar studies done in Kenya. A study on typhoid fever incidence in urban settlements and rural area in Kenya reports incidence of typhoid fever in Kibera to be 6.4% (Breiman *et al*., 2012). Another study done in Maina slum, Nyahururu municipality reports a prevalence of 6.3% of typhoid fever infections (Nguri, 2011). These two studies were done in Kenya and the differences in prevalence between them and our study is noticeable. However, these differences can be explained by two hypotheses: (i) The current study was run on a small scale crossectional area and samples were collected within a period of six months which may have been the pick season of typhoid infections (Dewan *et al*., 2013), (ii) Typhoid fever prevalence may have rose since when the stated studies were carried out around a decade ago. The association of typhoid fever with overcrowding (Kariuki *et al*., 2006), could prove the second hypothesis since Kenya's population is known to increase by 10 million people every decade, due to improve healthcare systems that increase life expectancy and reduces mortality rates.

In contrast with the data from different geographical areas, the current findings correlate with the findings from these areas. A study done in endemic site in Papua New Guinea reported a prevalence of 4% of typhoid fever (Arya & Agarwal, 2013). Bhattacharya *et al*., (2003) reported a prevalence of 2.6% in India. In Egypt, a study evaluating Enterocheck WB® test in diagnosis of typhoid fever among Egyptian adults reported a high prevalence of 13.6% of typhoid fever (Hamdy *et al*., 2014). The relatively high prevalence of typhoid fever in this study may have been contributed by the fact that, the largest number of the research participants came from the surrounding informal settlement with low sanitary hygiene levels. These findings suggest prevalence of typhoid fever to be done on a large scale cross sectional study so as to determine the definitive disease burden among population for prober planning of control programs

The performance of diagnostic tests needs to be continuously evaluated to establish if their performance is adequate for the diagnostic purposes that are intended. They should however be evaluated under conditions they are most likely to be used and in particular human population or clinical setting (Keddy *et al*., 2011). In our current study, we evaluated accuracy of TUBEX® TF rapid diagnostic test and compared its sensitivity with that of Widal test. The sensitivity and specificity of TUBEX® TF was comparable to that of Widal test. TUBEX® TF had a sensitivity of 88.9% (95% CI: 51.18-99.7) and specificity of 97.6% (95% CI: 91.6-99.7) while Widal test had 88.89% (95% CI: 51.8-99.7) and 90.4% (95% CI: 81.9-95.7) respectively. However, PPVs of TUBEX® TF and Widal test was 80.0 % (95% CI: 44.4-97.5) and 50.00% (95% CI: 24.7-75.3) respectively (Table 3). TUBEX® TF demonstrated superior Positive predictive value when compared to Widal test. The low PPV for Widal test was clear evidence that the kit was not doing well as compared to the reference standard and also as compared to TUBEX® TF. However, the fact that the PPV increases with increasing prevalence while NPV decreases with increase in prevalence (Parikh *et al*., 2008), Widal test could not demonstrate that it is even better than TUBEX® TF in our current setting as TUBEX® had higher PPV. Inclusion of controls lowered the PPVs for both TUBEX® TF and Widal test and on this regard, both kits performed poorly. Therefore, the decision to use them at primary health setting cannot be recommended in such a case. Nevertheless, the two sera diagnostic tests performed well in typhoid confirmed cases in this study. The 9.8% prevalence in our study may not be consistent with the prevalence of typhoid fever in other Sub-Saharan African countries. Therefore, when the pretest probability for typhoid fever was lowered to 5%, the performance of the two rapid tests declined further suggesting that the two rapid tests cannot be of any diagnostic significance in a clinical setting of low endemicity (Table 4). At a 50% pretest probability which is higher than the actual fraction of the blood culture positive cases in our study, the performance of the two sera diagnostic tests improved greatly suggesting that the tests can be used judicially during outbreaks (Keddy *et al*., 2011). These results were comparable to other results reported in a study carried out during the typhoid outbreak in Harare, Zimbabwe where TUBEX® TF was reported to have 100% sensitivity and 94.12% specificity (Tarupiwa *et al*., 2015). In another study carried out in two Sub-Saharan countries South Africa and United republic of Tanzania, TUBEX® TF had sensitivity of 73.0 % (95% CI: 63.3-83.4) and a specificity of 69.0% (95% CI: 49.2-84.7) (Keddy *et al*., 2011). However despite our findings on Semi-quantitative slide and

single-tube Widal test being quit promising, it contradicted the findings on these two Sub-Saharan countries as these countries reported poor performance. In India, TUBEX® TF test was recommended for the diagnosis of acute stage of the disease in a clinical setting with 76% sensitivity and 96-99% specificity (Khanna *et al*., 2015). These results were in agreement with our findings on TUBEX® TF. Blood culture demonstrated a good agreement with both TUBEX® TF and Widal test when it was used to form a single predictive value with either of the two index tests (Table 5). Therefore, the two tests can be utilized together with blood culture for typhoid diagnosis. We performed the TUBEX® TF and Widal tests on unpaired sera though previously it had been reported that sensitivity and specificity of a sera diagnostic test for typhoid fever improves with the use of paired sera (House *et al.,* 2005). In our setting, it was not possible to use paired sera as we chose to apply the tests under the conditions that are found in a clinical practice. In our experience, patients rarely return for outpatient follow-up once treated so that, obtaining paired sera in a routine clinical setting is unlikely (Keddy *et al*., 2011).

TUBEX® TF test has been evaluated in several other countries with varied level of performance being reported. A study in Papua New Guinea evaluated TUBEX® TF using blood culture as gold a standard and it demonstrated a high sensitivity of 76% and specificity of 96-99% which was higher than Typhidot and comparable to Widal test (Siba *et al*., 2012). In Egypt, TUBEX® TF had sensitivity of 74.6% and specificity of 75% but it was not superior to Widal test as it had a higher result (Bakr *et al*., 2010). In Asia, the performance of TUBEX® TF in India was evaluated and compared with Widal and Typhidot and study reported TUBEX® TF and Typhidot during the acute stage of fever is not better than Widal test (Dutta *et al*., 2006). Moreover, TUBEX® TF produced low sensitivity and specificity of 69% and 95% respectively in China and this study concluded that the newer serological tests did not offer substantial advantages over the established Widal test (Dong *et al.,* 2007). With continued studies to discover better rapid test to diagnose typhoid fever facing various shortcomings, TUBEX® TF test has been making a positive progress. One example of this progress is a recent study carried out in Uganda; TUBEX® TF was used for epidemiological investigation where a probable typhoid case was defined as a suspected case with a positive TUBEX® TF test while blood culture was being used to define a confirmed case (Kabwana *et al*., 2017).

A sera diagnostic test can be of diagnostic value if its limitations are well known. In endemic areas, the Widal test which is an easy inexpensive test with its known shortcomings has been reported to be of diagnostic value in unvaccinated individuals where blood culture cannot be obtained especially in developing countries (Khanna *et al*., 2015). Our study had several limitations. Review of data on recent literature suggests that typhoid fever is uncommon, perhaps 100 times or 250 times less common than invasive diseases, and this could been brought about by of the use of Widal test that may result in many hundreds of over-treatment episodes for every true case treated and may perpetuate the perception that typhoid is common (Mweu *et al.,* 2008: Reddy *et al*., 2010).). The 9.8% prevalence of bacteriologically proven *S*. Typhi cases may not be consistent with prevalence of typhoid fever in other Sub-Saharan countries. Therefore, 5% and 50% pretest probabilities were performed to give results of the tests if applied in different areas with low typhoid incidences and during typhoid fever outbreaks that normally records high incidences.

An important limitation for TUBEX® TF test was the absence of cases of other non-typhoidal *Salmonella* such as *S. typhimurium* and *S. enteritidis.* Previous studies have shown that TUBEX® TF test may give false positive results due to bacteremia caused by *S. enteridities* because they have 09 antigens (Tam et *al.,* 2008). Scoring system of the TUBEX® TF test may have led to operator bias. The scoring system should be standardized and operator bias should be removed by taking the readings with the help of machine only (Khanna *et al.,* 2015). The results were read by at least 3 independent observers (The PI and 2 qualified technical persons) to validate the results. 2 out of 3 concordant results were recorded as either negative or positive with corresponding semi quantitative scores using the magnetic color scale.

Another limitation of this study was that, during an early infection of typhoid fever, immune system may not produce enough IgM antibodies to be detected by TUBEX® TF test hence this could give false negative results. Additionally, TUBEX® TF test has been designed to detect IgM antibodies; it may detect IgG antibodies when it synergistically binds to the antigen coated particles (http://www.idl.se). This may lead to recording of false positive results. In our study, there were only three patients who tested positive though their blood cultures tested negative. While these patients may have affected our results alittle, it cannot be justified that three positive results on TUBEX® TF test were due IgG antibodies synergistically binding to the antigen coated particles alone.

Widal test had several limitations. The most important of all was lack of demonstration of four-fold rise in antibody concentration. Widal test requires acute-and convalescent-phase serum samples collected approximately 10 days apart where positive result is determined by a 4-fold increase in antibody titer (House *et al.,* 2005). We performed the test only at acute stage samples for it was challenging to obtain a convalescent samples consistenly in our setting (Andrew *et al.,* 2015). Another limitation for Widal test was difficulties in interpretations of test results. Interpretation of results using transparent slides was done on black or white background and bringing them close to lighten electric lump for easy visualization of agglutination reaction.

This study did not include aspiration from bone marrow in our study. Blood culture is known to be less than

100% sensitive even in absence of antimicrobial exposure (Andrews and Ryan, 2015). We instead use modern blood culture technique BACTEC™ 9050 blood culture system (Becton, Dickinson and Company) to improve sensitivity of our blood culture tests by optimizing it (Mirret *et al*., 2003). This may have limited diagnostic values recorded in the study as blood culture was the only sole reference standard. Moreover, antimicrobial susceptibility profiles were not perform in this study on the isolates of *S.* Typhi. This was a limitation because confirmed typhoid fever cases could not be ascertained if they were from circulating resistant *S*. Typhi in the population.

Our study only covered very selective and small groups of individuals making it relatively small and limiting to access exhaustively the impact of socioeconomic status of participants and other factors. This would have served to make a more definitive conclusion on the impact of typhoid fever in different economy class of population. However, the main objective of our study was to evaluate the effectiveness of the sera diagnostic tests for early diagnosis of typhoid fever on hospitalized patients in a clinical setting. In our study TUBEX® TF test showed to be significantly better than Widal test and demonstrated shorter turnaround time than both blood culture and Widal test. Nonetheless, despite these limitations, this study describes comparison of the newly developed TUBEX® TF diagnostic assay and the mostly used Widal test for typhoid fever diagnosis and our results strongly support development of the new diagnostic tests.

5. Conclusion

Typhoid fever in developing countries like Kenya is a major public health problem. The findings from these study can be used to deduce the burden of typhoid fever in Kenya and can help in designing, planning and implementing immunization programs on typhoid fever targeting specific population at risk especially children below 12 years. This study strongly support continued evaluation of performances of the new and old sera diagnostic tests to be done on regular basis. This will play a critical role in diagnosis, management of antimicrobial resistance and treatment of typhoid fever. TUBEX® TF test proved superior to Widal test when blood culture was used as a reference standard. However, these results should be further confirmed by using multiple reference standards like molecular and ELISA and carried out on large scale cross-sectional studies with varied prevalence of typhoid fever in the population. Our findings showed that either TUBEX® TF or Widal test can be used to make a single predictive value with blood culture to diagnose typhoid fever. Performance of TUBEX® TF was promising unlike that of Widal test. However, none of the two sera diagnostic tests can justifiably be used alone in a clinical set up to diagnose typhoid fever. With increasing need to introduce typhoid vaccine as a means of managing typhoid infections, surveillance will be critical in providing the guidance for the deployment of vaccines. Diagnostic speed may be sacrificed but accuracy will be critical (Andrew *et al*., 2015). Therefore, there is an urgent need for a highly sensitive and specific diagnostic technique to be developed for the purposes of surveillance, correct diagnosis and effective treatment of typhoid fever.

6. Conflict of Interests

The authors have no any conflict of interests.

7. Acknowledgements

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