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Screening for *Salmonella typhi* Serum Antibodies and Stool Antigen among Undergraduate Students of Babcock University, Ilishan-Remo, Ogun State, Nigeria

Seyi Samson Enitan[1*], John Cletus Ihongbe[1], John Okeleke Ochei[1], Emmanuel Ileoma[1], Esther Ngozi Adejumo[1] and Oluwadamilola Rosemary Ogunsola[1]

[1]Department of Medical Laboratory Science, Babcock University, Ilishan-Remo, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author SSE designed the study, wrote the protocol and the first draft of the manuscript. Authors JCI and JOO managed the analyses of the study. Authors EI and ENA managed the literature searches. Author ORO performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Salmonella typhi* infection is endemic in Nigeria with varied morbidity and mortality rates.
Aim: The aim of this study is to determine the prevalence rate of *Salmonella typhi* infection among the undergraduate Students of Babcock University, Ilishan-Remo, Ogun State using rapid diagnostic method.
Study Design: This is a descriptive-epidemiological survey.
Place and Duration of Study: Department of Medical Laboratory Science, Babcock University, Ilishan-Remo, Ogun State, Nigeria, between April and June, 2018.
Materials and Methods: Blood and stool specimens were randomly collected from 200 consenting

**Corresponding author: Email: enitans@babcock.edu.ng;*

undergraduate Students and screened using *Solid Rapid Diagnostic* Typhoid (United Kingdom) and *Accu-Chek S. typhi* antigen (India) Test Kits, respectively according to the manufacturer instruction.

Results: Out of the 200 participants screened, 7(3.5%) were positive for *S. typhi* serum immunoglobulin M antibody (IgM Ab), 68 (34.0%) were positive for *S. typhi* serum immunoglobulin G antibody (IgG Ab), while 18 (9.0%) were positive for *S. typhi* stool antigen (SAg). Percentage sero-positivity for *S. typhi* serum IgG antibody was significantly ($P < 0.05$) higher among participants who were male (29.0%), 16-20 years (17.0%) and Occupants of Hall 15 (8.0%). Risk factors associated with the occurrence of *Salmonella typhi* infection in this study include: lack of Typhoid fever vaccination, past history of typhoid fever, drinking of unsafe water and raw cow milk, consumption of beef, poultry and street vended food, as well as poor toilet hygiene.

Conclusion: The outcome of this study show that *Salmonella typi* infection exist among undergraduate Students of Babcock University, Ilishan-Remo, Ogun State; therefore, prompt treatment of all identified cases, in addition to a sustainable implementation of preventive measures is needed to halt the cycle of transmission.

Keywords: Salmonella typhi; serum antibodies; stool antigen; risk factors.

1. INTRODUCTION

The bacterium, *Salmonella typhi,* is the aetiologic agent of a worldwide life threatening enteric illness called typhoid fever [1]. It is transmitted via the fecal-oral route, by the ingestion of water and food contaminated with the faeces of an infected person. *S. typhi* is mainly water-borne, while *S. paratyphi* is mainly food-borne. The enteric fever caused by *S. typhi* leads to a series of severe pathologic conditions while that caused by *S. paratyphi*, A, B or C is usually milder [2,3].

There is no animal reservoir for *Salmonella typhi*. Humans are the only natural reservoir for the organism, and typhoid fever therefore must be acquired from convalescent or chronic carriers who excrete *S. typhi* for more than a year; especially older women with gallstones or biliary scarring, in whom *Salmonella typhi* may colonize the gallbladder or biliary tree. Factors outside the household like contaminated food from vendor and flooding help distribute the disease from person to person. Because of poverty and poor hygiene/sanitary condition, the disease is more common in less industrialized countries, principally, owing to the problem of unsafe drinking water, inadequate sewage disposal and flooding occasionally causing epidemics [3].

Typhoid fever is a disease of public health importance which affects people of all walks of lives in urban, peri-urban and rural areas. In 2013, the World Health Organization reported that typhoid fever kills about five million babies annually and make one sixth of the world population ill. Typhoid fever is found in large parts of Sub-Saharan Africa. There are about 16 million cases a year which result in about 25,000 deaths worldwide [3].

Globally, typhoid fever is an important cause of morbidity and mortality in many regions of the world with an estimated 12-33 million cases leading to 216,000 – 600,000 deaths annually [4]. Nigeria is not immune to the burden of typhoid fever and its associated complications. According to FMOH [5], the mortality rate of typhoid in Nigeria is as high as 30%. Reported cases of typhoid fever from 2000-2014 were about 103, 353 and 793 deaths from the same period of time with Lagos, Ogun and Abuja been one of the regions to record Figures higher than the national average. This is due to the influx of tertiary institutions, as well as problem of urbanisation within the region. Tuise [6], found a great impact of the typhoid fever on the wellbeing of University Students.

Regrettably, poor knowledge, attitude and practices of good hygiene, which have been overlooked; have contributed directly or indirectly to the burden of typhoid fever in Nigeria. This makes targeted public health interventions almost unattainable. Lack of clean water system, sanitation facilities and hygienic practices have made *Salmonella typhi* infection more difficult to control and prevent effectively. The outbreaks of typhoid fever do occur if control and preventive measures are not taken in a timely manner. Poor waste disposal and hygiene of University Students in food handling and preparation activities would provide an obvious infection route within the campus. The situation is complicated in that some Students may be carriers of the typhoid bacillus, so that although

they exhibit no outward signs of the disease, their feces contain the pathogens [6].

Normally, infection with the typhoid bacillus is confirmed through culture and serological methods, howbeit; wrong diagnosis is common with the later, and do lead to wrong reporting. Besides, self-diagnosis through experience or otherwise could lead to wrong diagnosis and medication. Purchase of drugs from the counter without appropriate laboratory test results could lead to development of antibiotic resistance to treatment of typhoid. Also, if diagnosis is correct and treatment is not accurate, the disease may thrive resulting in higher prevalence rate in the community. It is therefore important to diagnose and treat typhoid infection early since serious complications that may include severe intestinal bleeding or perforations can arise within a week. Currently, there is emergence and recurrence of typhoid fever due to floods, poor sanitation and emergence of strains that are resistant to antibiotics in the country. Lack of periodic epidemiological survey and incorrect reporting of data could give a wrong impression on the current prevalence rate of typhoid fever within a given community [7].

Furthermore, infected individuals must be correctly diagnosed and properly treated. Although febrile patients infected with typhoid bacillus often present with signs and symptoms compatible with typhoid fever, the situation is more difficult to identify among apparently healthy individuals that are carriers of typhoid pathogens. Although they exhibit no outward signs and symptoms of the disease, their feces contain the pathogens and therefore such people serve as crucial reservoir of infection within the community. Convalescent and chronic carriers of typhoid pathogens must be identified in order to halt the cycle of infection. Paucity of studies and information with regard to the prevalence rate of *Salmonella typhi* infection among undergraduate students of Babcock University necessitates this study. Hence, the study investigates the prevalence of typhoid infection and associated risk factors among undergraduate students of a private university.

Still, in most parts of Africa, Nigeria inclusive, the Widal agglutination test (a serological test), despite its limitations, is the most common diagnostic tool employed in the diagnosis of typhoid fever because it is relatively cheap, easy to perform and requires minimal training and equipment. However, a number of medical Practitioners have often raised alarm on the apparently "high rate" of typhoid fever diagnosed in healthcare facilities in Nigeria due to high false positivity associated with the test. With the continuous emergence of typhoid Rapid Diagnostic Test (RDT) Kits in the market, there is need to verify their effectiveness. This study is therefore designed to investigate the effectiveness of Rapid Diagnostic method in the detection of *Salmonella typhi* infection among apparently healthy and as well as febrile undergraduate students of Babcock University.

2. MATERIALS AND METHODS

2.1 Study Design

This was a descriptive-epidemiological survey.

2.2 Study Area

This cross-sectional institutional based study was carried out among undergraduate students of Babcock University, Ilishan-Remo, Ikenne Local Government Area, Ogun State, a first class Seventh-day Adventist Institution of higher learning located in the South-Western region of Nigeria, coordinates: 6.8862^{o} N, 3.7055^{o} E. The University has nine (9) schools with a total student population of about ten thousand (10, 000) offering different academic and professional courses; both at the undergraduate and postgraduate levels.

2.3 Study Population

Undergraduate students of Babcock University were the target population. They consist of young male and female adults within the age range of 16-35 years from different ethnic, religious and cultural background; studying different courses in various Departments. The University hosts eight female halls and seven male halls. Study participants were randomly selected from the various halls of residence.

2.4 Duration of Study

The study was carried out between the months of April and June, 2018.

2.5 Sample Size Calculation

The sample size (n) was estimated using the single population proportion formula described by Charan and Biswas [8]:

$$N = Z^2PQ/d^2$$

where;

N = Required sample size,
Z = Standard normal variate at 5% (*P <0.05*) error or 95% confidence interval is 1.96
P = Proportion of the population with typhoid fever infection from previous study,
Q = Proportion of the population without typhoid fever infection (1 – P) and
d = Absolute error margin is 0.05

For the calculation, a 95% confidence interval, a *P* value of 0.1407, *i.e,* a prevalence rate of 14.07% from previous study by Udujih et al. [9] and margin of error (d) set at 0.05 was used to determine the minimum sample size required.

The minimum sample size (N) calculated was 186.

However, in order to make our work robust, we decided to screen a total of 200 Students.

Sample size: A total of 200 blood and stool specimens were collected randomly from interested 200 undergraduate students (100 males and 100 females) of Babcock University, Ilishan-Remo, Ogun state.

2.6 Eligibility of Subjects

Inclusion criteria: Undergraduate students without history of antibiotics, antacids, histamine-2 receptor antagonists (H_2 blockers) or Proton Pump inhibitors therapy in the preceding two (2) weeks were recruited randomly for the study.

Exclusion criteria: Undergraduate students with the history of antibiotics, antacids, histamine-2 receptor antagonists (H_2 blockers) or Proton Pump inhibitors therapy in the preceding two weeks, as well as Postgraduate students were excluded from the study.

2.7 Data Collection

Prior to specimen collection, demographic and clinical information of the participants was obtained using prepared questionnaires which was administered to the participants. The first part of the questionnaires contained the biodata of the participants such as age, marital status, study level, tribe and hall of residence. The second part includes clinical data relating to history of typhoid fever (High fever, abdominal pain, constipation, nausea, vomiting, loss of appetite, diarrhea etc), risk factors (if any), personal hygiene and health care-seeking behavior. The study population was stratified by hall of residence. For each participant, only the personal identification number (PIDN) was recorded on the laboratory forms (no names) for the purpose of confidentiality. All the filled questionnaires were destroyed after data entry was completed.

Specimen collection: Both blood and stool specimens were collected from each participant.

Specimen storage: Upon arrival in the laboratory, the serum and stool specimens were processed within 2 hours of collection. Where immediate serological testing was not possible, the sera was stored at 2-8^{o}C for up to 3 days or frozen at -20°C if longer-term storage was required. Frozen specimens was completely thawed and mixed well prior to testing. Repeated cycle of freezing and thawing of sera was avoided. The stool specimen on the other hand was stored at 4^{o}C in the refrigerator if delay is also expected.

2.8 Laboratory Analyses

Detection of serum anti-*Salmonella typhi* antibody using Rapid Diagnostic Method: Serum anti-*Salmonella typhi* antibody was detected using a one-step *Salmonella typhi* antibody test cassette, Solid Rapid Diagnostic Typhoid *IgG/IgM* (United Kingdom) according to the manufacturer instruction. Positive and negative control samples were run simultaneously with the test samples.

2.9 Interpretation of Results

Positive Result: Coloured bands appeared at the Control line (C) and Test lines (T). The presence of test and control bands in the IgM column indicates early primary infection with *S. typhi*. The presence of test and control bands in the IgG column indicates late stage or latent infection with *S. typhi*; while the presence of test and control bands in both the IgM and IgG columns indicated active primary and repeat infection with *S. typhi*.

Negative result: The presence of only one pink color band (the control) within the result window indicated a negative result.

Invalid result: A total absence of color in either control and test regions or only one color band appearing on the test region indicates procedure error and/or the test reagent has deteriorated. If

this occurs, the assay was repeated using a new test cassette.

Detection of stool *Salmonella typhi* antigen using Rapid Diagnostic Test Kits: Stool *Salmonella typhi* antigen was detected using a one-step Accu-chek *Salmonella typhi* antigen test cassette supplied by Millennium Biotechnology Inc (India), according to the manufacturer instruction. Positive and negative control samples were run simultaneously with the test samples.

2.10 Interpretation of the Test

Positive result: The presence of two color bands ("T" band and "C" band) within the result window regardless of which band appeared first indicates a positive result.

Negative result: The presence of one pink color band only within the control result window indicates a negative result. No line appears in the test line region.

Invalid result: The test was invalid if control line fails to appear. If there was no distinct color visible both in the test and control region, or there was a visible line only in the test region but not control region, then the test is invalid. In this case, the specimen was re-tested.

2.11 Data Analyses

Statistical analysis was carried out using SPSS Statistics software package (version 18.0). One-way analysis of variance (ANOVA) and Tukey-Kramer Multiple Comparisons Test was used to test for significant differences in the prevalence of *Salmonella typhi* infection among the students using serum antibody and stool antigen detection method. *P* value *<0.05* was considered significant. Statistical analysis outputs were presented using tables and charts.

3. RESULTS AND DISCUSSION

The sero-prevalence of anti-*Salmonella typhi* IgM antibody in relation to the demographic characteristics of the study Participants is presented in Table 1. Out of the 200 participants screened. 7 (3.5%) were sero-positive for anti-S*almonella typhi* IgM antibody. Percentage sero-positivity among the male and female participants were 2.5% and 1.0%, respectively. The difference was not statistically significant *(P >0.05)*. Although, the highest sero-positivity for anti-*salmonella typhi* IgM antibody was recorded among participants within 16-20years age range (2.0%), the difference was not statistically significant (*P >0.05*) when compared with other age categories. Still, there were no significant differences *(P >0.05)* in the sero-prevalence rate of anti-*Salmonella typhi* IgM antibody among the study participants on the basis of marital status, study level, religion, tribe and Hall of Residence.

Table 2 shows the sero-prevalence of anti-*Salmonella typhi* IgG antibody in relation to the demographic characteristics of the study participants. The overall percentage sero-positivity of anti-*Salmonella typhi* IgG antibody among the study Participants was found to be 34.0%. There were no significant differences *(P >0.05)* in the sero-prevalence of anti-*Salmonella typhi* IgG antibody among the study participants on the basis of marital status, study level, religion and tribe, except for gender, age range and Hall of Residence. The proportion of male students (29.0%) who were sero-positive for anti-*Salmonella typhi* IgG antibody were significantly higher *(P <0.05)* than their female counterparts (5.0%). With regard to age range, participants between 16-20 years (17.0%) were more significantly *(P <0.05)* sero-positive for anti-*Salmonella typhi* IgG antibody than other age category.

Still, on the basis of Hall of Residence, the highest percentage sero-positivity for anti-*Salmonella typhi* IgG antibody was recorded among occupants of Winslow Hall (8.0%) which was found to be significantly higher.

Furthermore, the prevalence of stool *Salmonella typhi* antigen in relation to the demographic characteristics of the study Participants is presented in Table 3. The overall prevalence rate of stool S*almonella typhi* antigen among the study participants was 9.0%. Out of the 100 male and 100 female participants screened, 13 (6.5%) and 5 (2.5%), respectively, were positive for stool *Salmonella typhi* antigen. The difference was however, not statistically significant *(P >0.05)*. Although, the highest percentage positivity for stool *Salmonella typhi* antigen was recorded among participants within 16-20years age range (4.0%), the difference was not statistically significant (P>0.05) when compared with other age categories. Still, there were no significant differences *(P >0.05)* in the percentage positivity of stool *Salmonella typhi* antigen among the study participants on the basis of marital status, study level, religion, tribe and Hall of Residence.

Table 1. Frequency of occurrence of anti-*Salmonella typhi* IgM antibody in relation to the Gender, Age and Hall of residence of the study participants

Demographic characteristics	Category	Total Number of serum samples examined N=200 (100%)	Number of serum samples positive 7 (3.5%)	Number of serum samples negative 193 (96.5)	P -value
Gender	Male	100 (50.0)	5(2.5)	95(47.5)	0.251
	Female	100 (50.0)	2(1.0)	98(49.0)	0.249
Age range	16-20 yrs	96 (48.0)	4(2.0)	92(46.0)	0.933
	21-25 yrs	74 (37.0)	1(0.5)	73(36.5)	0.923
	26-30 yrs	26 (13.0)	2(1.0)	24(12.0)	0.898
	≥30 yrs	4 (2.0)	0(0)	4(2.0)	0.888
Hall of Residence	Hall 1	3 (1.5)	1(0.5)	2(1.0)	0.961
	Hall 2	19 (9.5)	0(0)	19(9.5)	0.970
	Hall 3	18 (9.0)	1(0.5)	17(8.5)	0.969
	Hall 4	17 (8.5)	0(0)	17(8.5)	0.969
	Hall 5	15 (7.5)	1(0.5)	14(7.0)	0.967
	Hall 6	15 (7.5)	0(0)	15 (7.5)	0.968
	Hall 7	14 (7.0)	0(0)	14(7.0)	0.967
	Hall 8	17 (8.5)	0(0)	17 (8.5)	0.969
	Hall 9	14 (7.0)	0(0)	14(7.0)	0.967
	Hall 10	14 (7.0)	0(0)	14(7.0)	0.967
	Hall 11	6 (3.0)	0(0)	6(3.0)	0.984
	Hall 12	3 (1.5)	1(0.5)	2(1.0)	0.961
	Hall 13	15 (7.5)	0(0)	15(7.5)	0.968
	Hall 14	13 (6.5)	0(0)	13(6.5)	0.967
	Hall 15	17 (8.5)	3(1.5)	14(7.0)	0.969

P value >0.05 is considered statistically not significant

Table 4 shows the distribution of symptomatic and asymptomatic *Salmonella typhi* infection in relation to the social demographic characteristics of the study participants. Out of the 200 participants screened, 32 (16%) were symptomatic, while 36 (18%) were asymptomatic. The percentage of male participants with symptomatic *Salmonella typhi* infection (14.5%) was significantly *(P <0.05)* higher than their female counterparts (1.5%). Meanwhile, there were no significant differences in the percentages of participants with symptoms and those without symptoms on the basis of age, marital status, study level, religion, tribe and Hall of Residence. Furthermore; the indications for typhoid fever in relation to the occurrence of serum anti-*Salmonella typhi* antibody and stool antigen positivity among the study Participants is represented with a histogram (Fig. 1).

Signs and symptoms indicated by the participants include: loss of appetite (16.0%), high fever (13.0%), abdominal pain (7.0%), vomiting (5.0%), diarrhoea (3.0%) and headache (2.5%). Meanwhile, 53.5% of the participants did not complain of any obvious signs and symptoms. Out of the 26 (13.0%) participants who indicated high fever, 3 (1.5%), 5 (2.5%) and 3 (1.5%) of them were positive for *S. typhi* serum IgM Ab, serum IgG Ab and stool Ag, respectively. Out of the 32 (16.0%) who indicated loss of appetite, none was positive for *S. typhi* serum IgM Ab. While 11 (5.5%) of the participants were positive for *S. typhi* serum IgG Ab, only 1 person (0.5%) was positive for the stool Ag. None (0%) of the participants who complained of headache was positive for *S. typhi* serum IgM Ab and stool Ag, howbeit, 2 of them tested positive for *S. typh i*serum IgG Ab.

In addition, among those who indicated vomiting, only one person (0.5%) was positive for *S. typhi* serum IgM Ab and stool Ag; while 4 (2.0%) of them were positive for *S. typhi* serum IgG Ab. Out of the 14 (7.0%) participants who complained of abdominal pain, 2 (1.0%), 6 (3.0%) and 3 (1.5%) of them were positive for *S. typhi* serum IgM Ab, serum IgG Ab and stool Ag, respectively. Lastly, among the 6 persons (3.0%) who indicated diarrhea, 1 person (0.5%) was positive for *S. typhi* serum IgM Ab and stool Ag, while 2 of them (1.0%) was positive for *S. typhi* serum IgG Ab.

Table 2. Frequency of occurrence of anti-*Salmonella typhi* IgG antibody in relation to the gender, age and Hall of residence of the study participants

Demographic characteristics	Category	Total Number of serum samples examined N=200 (100%)	Number of serum samples positive N=68 (34%)	Number of serum samples negative N=132 (66%)	P -value
Gender	Male	100 (50.0)	58(29.0)	42(21.0)	0.042*
	Female	100 (50.0)	10(5.0)	90(45.0)	0.063
Age range	16-20 yrs	96 (48.0)	34(17.0)	62(31.0)	0.047*
	21-25 yrs	74 (37.0)	22(11.0)	52(26.0)	0.130
	26-30 yrs	26 (13.0)	10(5.0)	16(8.0)	0.212
	≥30 yrs	4 (2.0)	2(1.0)	2(1.0)	0.305
Hall of Residence	Hall 1	3 (1.5)	1 (0.5)	2 (1.0)	0.383
	Hall 2	19 (9.5)	2 (1.0)	17 (8.5)	0.307
	Hall 3	18 (9.0)	2 (1.0)	16 (8.0)	0.307
	Hall 4	17 (8.5)	11 (5.5)	6 (3.0)	0.090
	Hall 5	15 (7.5)	5 (2.5)	10 (5.0)	0.212
	Hall 6	15 (7.5)	1 (0.5)	14 (7.0)	0.383
	Hall 7	14 (7.0)	11 (5.5)	3 (1.5)	0.070
	Hall 8	17 (8.5)	2 (1.0)	15 (7.5)	0.305
	Hall 9	14 (7.0)	2 (1.0)	12 (6.0)	0.307
	Hall 10	14 (7.0)	2 (1.0)	12 (6.0)	0.307
	Hall 11	6 (3.0)	3 (1.5)	3 (1.5)	0.314
	Hall 12	3 (1.5)	1 (0.5)	2 (1.0)	0.383
	Hall 13	15 (7.5)	2 (1.0)	13 (6.5)	0.300
	Hall 14	13 (6.5)	7 (3.5)	6 (3.0)	0.192
	Hall 15	17 (8.5)	16 (8.0)	1 (0.5)	0.039*

**P value <0.05 is considered statistically significant*

The risk factors associated with the occurrence of S*almonella typhi* infection among the study participants is presented in Table 5. Out of the 200 participants that were screened, 52 (26.0%) of them indicated that they have no knowledge of *S. typhi*. 5 (2.5%), 40 (20.0) and 5 (2.5%) were positive for *S. typhi* serum IgM Ab, serum IgG Ab and stool Ag, respectively. 96 (48.0%) indicated they have not received typhoid fever vaccine. 2 (1.0%), 46 (23.0%) and 10 (5.0%) of them were positive for *S. typhi* serum IgM Ab, serum IgG Ab and stool Ag, respectively. History of typhoid fever was indicated by 136 (68.0%) of the participants, among which 1 (0.5%), 49 (24.5%) and 5 (2.5%) participants tested positive for *S. typhi* serum IgM Ab, serum IgG Ab and stool Ag, respectively.

Furthermore; drinking of raw cow milk was indicated by 102 (51.0%) of the participants, among which 4 (2.0%), 41 (20.5%) and 9 (4.5%) participants were positive for *S. typhi* serum IgM Ab, serum IgG Ab and stool Ag, respectively. 162 (81.0%) responded that they consume beef. 7 (3.5%), 12 (6.0%) and 2 (1.0%) of them were positive for *S. typhi* serum IgM Ab, serum IgG Ab and stool Ag, respectively. Consumption of vegetables was indicated by 112 (56.0%), out of which 7 (3.5%), 26 (13.0%) and 2 (1.0%) of them were positive for *S. typhi* serum IgM Ab, serum IgG Ab and stool Ag, respectively. Still, 120 (60.0%) of the participants responded that they consume poultry/poultry products. 7 (3.5%), 37 (18.5%) and 5 (2.5%) were positive for *S. typhi* serum IgM Ab, serum IgG Ab and stool Ag, respectively.

Also, 112 (56.0%) of the participants indicated that they consume street vended food, out of which, 7 (3.5%), 41 (20.5%) and 6 (3.0%) of them were positive for *S. typhi* serum IgM Ab, serum IgG Ab and stool Ag, respectively. With regard to the type of water drank as a child, 0.5% *S. typhi* serum IgM Ab and stool Ag positivity was recorded among 14 (7.0%) participants who indicated they drank unsafe water (neither boiled nor filtered water) as a child. Still, 0.5% *S. typhi* serum IgM Ab and 3.0% stool Ag positivity was recorded among 43 (21.5%) participants who also indicated they drank unsafe water (neither boiled nor filtered water) as an adult.

Table 3. Frequency of occurrence of stool *Salmonella typhi* antigen in relation to the social demographic characteristics of the study participants

Demographic characteristics	Category	Total Number of stool samples examined N=200 (100%)	Number of stool samples positive N=18 (9%)	Number of stool samples negative N=182 (91%)	P -value
Gender	Male	100 (50.0)	13(6.5)	87(43.5)	0.064
	Female	100 (50.0)	5(2.5)	95(47.5)	0.067
Age range	16-20 yrs	96 (48.0)	8(4)	88(44)	0.669
	21-25 yrs	74 (37.0)	6(3)	68(34)	0.659
	26-30 yrs	26 (13.0)	3(1.5)	23(11.5)	0.636
	≥30 yrs	4 (2.0)	1(0.5)	3(1.5)	0.626
Hall of Residence	Hall 1	3 (1.5)	1(0.5)	2 (1.0)	0.954
	Hall 2	19 (9.5)	1(1)	18 (8.5)	0.961
	Hall 3	18 (9.0)	0(0)	18 (9.0)	0.886
	Hall 4	17 (8.5)	1(1)	16 (7.5)	0.973
	Hall 5	15 (7.5)	0(0)	15 (7.5)	0.881
	Hall 6	15 (7.5)	0(0)	15 (7.5)	0.890
	Hall 7	14 (7.0)	1(0.5)	13 (6.5)	0.984
	Hall 8	17 (8.5)	0(0)	17 (8.5)	0.900
	Hall 9	14 (7.0)	1 (1.5)	13 (5.5)	0.984
	Hall 10	14 (7.0)	1(0.5)	13 (6.5)	0.980
	Hall 11	6 (3.0)	1 (1)	5 (2.0)	0.974
	Hall 12	3 (1.5)	0(0)	3 (1.5)	0.984
	Hall 13	15 (7.5)	1(0.5)	14 (7.0)	0.903
	Hall 14	13 (6.5)	2(1)	11 (5.5)	0.623
	Hall 15	17 (8.5)	8 (4.0)	9 (4.5)	0.507

P value >0.05 is considered statistically not significant

Furthermore; out of the 12 (6.0%) participants who indicated that they wash hands less often after toileting, 4 (2.0%) and 1 (0.5%) of them tested positive for *S. typhi* serum IgG Ab and stool Ag, respectively. And finally, with regard to hand hygiene before eating, 18 (9.0%) of them indicated that they wash less often, among which 1 (0.5%), 10 (5.0%) and 2 (1.01%) were positive for *S. typhi* serum IgM Ab, serum IgG Ab and stool Ag, respectively.

The bacterium, *Salmonella typhi*, causes a life threatening illness called typhoid fever with approximately 26.9 million people affected globally. Typhoid fever is endemic in Nigeria and the mortality rate is as high as 30% [2,5,10]. For effective management of typhoid fever, diagnosis of the disease must be done with speed and accuracy. Laboratory diagnosis of typhoid fever requires isolation and identification of *Salmonella typhi*. However, in many areas where the disease is endemic, laboratory capability is limited. Recent advances in molecular immunology have led to the identification of sensitive and specific markers for typhoid fever and technology to manufacture practical and inexpensive kits for their rapid detection. Rapid diagnostic tests (RDTs) have the potential to be useful for clinicians working in resource-limited settings in the tropics [11,12].

The prevalence rate of *S. typhi* infection as observed in this present study was found to be either lower or higher than those of previous studies depending on the year of study, geographical location, study population and method of diagnosis employed. In a study conducted in Lagos, South-Western Nigeria, a prevalence of 45.2% was reported by Akinyemi et al. [13]. This was higher than the 3.5% (serum anti-*S. typhi* IgM Ab), 34% (serum anti-*S. typhi* IgG Ab) and 9.0% (stool *S. typhi* Ag) observed in this study. In 2008, Smith et al. [14] investigated the prevalence of *Salmonella typhi* infection among food handlers from local restaurants commonly refer to as "*Bukkas*" in Nigeria using culture technique. Out of the 53 stool samples examined, 5.7% of them were found to be positive for *S. typhi*, which was found to be lower when compared to the 9.0% obtained for stool *S. typhi* antigen in this study.

Table 4. The distribution of symptomatic and asymptomatic *Salmonella typhi* infection in relation to the gender, age and Hall of residence of the study participants

Demographic characteristics	Category	Number of participants examined N=200 (100%)	Number with Symptomatic *S. typhi* infection N=32 (16.0%)	Number with Asymptomatic *S. typhi* infection N=36 (18.0%)	P -value
Gender	Male	100 (50.0)	29 (14.5)	29 (14.5)	0.037*
	Female	100 (50.0)	3 (1.5)	7 (3.5)	0.481
Age range	16-20 yrs	96 (48.0)	18 (9.0)	16 (8.0)	0.057
	21-25 yrs	74 (37.0)	10 (5.0)	12 (6.0)	0.061
	26-30 yrs	26 (13.0)	4 (4.0)	6 (3.0)	0.064
	≥30 yrs	4 (2.0)	2 (0)	2 (1.0)	0.078
Hall of Residence	Hall 1	3 (1.5)	1 (0.5)	0 (0)	0.457
	Hall 2	19 (9.5)	0 (0)	2 (1.0)	0.500
	Hall 3	18 (9.0)	0 (0)	2 (1.0)	0.500
	Hall 4	17 (8.5)	7 (3.5)	4 (2.0)	0.337
	Hall 5	15 (7.5)	2 (1.0)	3 (1.5)	0.961
	Hall 6	15 (7.5)	0 (0)	1 (0.5)	0.500
	Hall 7	14 (7.0)	8 (4.0)	3 (1.5)	0.364
	Hall 8	17 (8.5)	0 (0)	2 (1.0)	0.500
	Hall 9	14 (7.0)	0 (0)	2 (1.0)	0.500
	Hall 10	14 (7.0)	0 (0)	2 (1.0)	0.500
	Hall 11	6 (3.0)	1 (0.5)	2 (1.0)	0.961
	Hall 12	3 (1.5)	0 (0)	1 (0.5)	0.500
	Hall 13	15 (7.5)	0 (0)	2 (1.0)	0.500
	Hall 14	13 (6.5)	2 (1.0)	5 (2.5)	0.482
	Hall 15	17 (8.5)	11 (5.5)	5 (2.5)	0.301

P-value <0.05 is considered statistically significant

Furthermore, Okonko et al. [15], reported a prevalence rate of 80.1% among Patients in Abeokuta, South-Western Nigeria using Widal agglutination technique. While, Ishaleku et al. [16], observed a prevalence of 73.0% in a study conducted over 3 years (2005-2007) among Students of a tertiary institution in Nasarawa State, Nigeria) using the same Widal agglutination technique. The prevalence reported by both studies above were found to be much higher than observed in this current study. However; a study conducted the same year by Aberal et al. [17], in Bahir Dar, North-Western Ethiopia, reported a prevalence rate as low as 1.6% among 384 food handlers using stool culture technique.

In another African country, Benedikt et al. [18], observed a prevalence of 23.7% among patients in Teule Hospital, Muheza District, Tanzania. They found 33 blood samples to be positive for *S. Typhi* in blood culture after testing 139 samples with Tubex. On one hand, their result was higher than the 3.5% and 9.0% reported in this study for serum anti-*S. typhi* IgM antibody and stool *S. typhi* antigen, respectively. On the other hand, it was lower than the 34% reported for serum anti-*S. typhi* IgG antibody in this study.

In 2013, Reuben et al. [19], undertook a retrospective and cross-sectional studies of *S. typhi* infection among pregnant women in a Community in Central Nigeria; their results show that 44 (88%) of the subjects were seropositive for *S. typhi* by widal test, whereas 33 (66%) were positive using stool culture technique. Their data were much higher than observed in this present study. The same year, Balakrishna et al. [20], compared the performance of three diagnostic methods for *S. typhi* infection. The outcome of their study shows that out of 200 clinically diagnosed typhoid cases,28 (14%) were blood culture positive for *S. typhi*, 43 (21.5%) were widal positive, while 55 (27.5%) were typhi dot positive. These results were not consistent with those of current study.

Table 5. Risk factors associated with the occurrence of S*almonella typhi* infection among the study participants

Characteristics	Responses	No. of participants examined N=200 (100%)	No. positive for *S. typhi* IgM Ab N=7 (3.5%)	No. positive For *S. typhi* IgG Ab N=68 (34%)	No. positive for *S. typhi* Stool Ag N=18 (9.0%)
Have knowledge about *S. typhi*	Yes	148 (74.0)	2 (1.0)	28 (14.0)	13 (6.5)
	No	52 (26.0)	5 (2.5)	40 (20.0)	5 (2.5)
Have received typhoid vaccine	Yes	104 (52.0)	5 (2.5)	22 (11)	8 (4.0)
	No	96 (48.0)	2 (1.0)	46 (23)	10 (5.0)
History of typhoid fever	Yes	136 (68.0)	1 (0.5)	49 (24.5)	5 (2.5)
	No	64 (32.0)	6 (3.0)	19 (9.5)	13 (6.5)
Drinking raw cow milk	Yes	102 (51.0)	4 (2.0)	41 (20.5)	9 (4.5)
	No	98 (49.0)	3 (1.5)	27 (13.5)	9 (4.5)
Consuming beef	Yes	162 (81.0)	7 (3.5)	12 (6.0)	2 (1.0)
	No	38 (19.0)	0 (0)	56 (28.0)	16 (8.0)
Consuming fish	Yes	170 (85.0)	1 (0.5)	5 (2.5)	3 (1.5)
	No	30 (15.0)	6 (3.0)	63 (31.5)	15 (7.5)
Consuming vegetables	Yes	112 (56.0)	7 (3.5)	26 (13.0)	2 (1.0)
	No	88(44.0)	0 (0)	42 (21.0)	16 (8.0)
Consuming poultry	Yes	120 (60.0)	7 (3.5)	37 (18.5)	5 (2.5)
	No	80 (40.0)	0 (0)	31 (15.5)	13 (6.5)
Consuming street vended food	Yes	112 (56.0)	7 (3.5)	41 (20.5)	6 (3.0)
	No	88 (44.0)	0 (0)	27 (13.5)	12 (6.0)
Type of water drank as a child	Portable	93 (46.5)	3 (1.5)	37 (18.5)	10 (5.0)
	Boiled or filtered	93 (46.5)	3 (1.5)	31 (15.5	7 (3.5)
	Not boiled nor filtered	14 (7.0)	1 (0.5)	0 (0)	1 (0.5)
Type of water being drank as an adult	Portable	104 (52.0)	4 (2.0)	46 (23.0)	4 (2.0)
	Boiled or filtered	53 (26.5)	2 (1.0)	22 (11.0)	8 (4.0)
	Not boiled nor filtered	43 (21.5)	1 (0.5)	0 (0)	6 (3.0)
Anal cleaning after toileting	Use tissue paper	92 (46.0)	3 (1.5)	25 (12.5)	5 (2.5)
	Wash with water	108 (54.0)	4 (2.0)	43 (21.5)	13 (6.5)
	Do nothing	0 (0)	0 (0)	0 (0)	0 (0)
Hand hygiene after toileting	Wash always	82 (41.0)	2 (1.0)	34 (17.0)	11 (5.5)
	Wash often	106 (53.0)	5 (2.5)	30 (15.0)	6 (3.0)
	Wash less often	12 (6.0)	0 (0)	4 (2.0)	1 (0.5)
	Never	0 (0)	0 (0)	0 (0)	0 (0)
Hand hygiene before eating	Wash always	111 (55.5)	5 (2.5)	37 (18.5)	8 (4.0)
	Wash often	71 (35.5)	1 (0.5)	21 (10.5)	8 (4.0)
	Wash less often	18 (9.0)	1 (0.5)	10 (5.0)	2 (1.0)
	Never	0 (0)	0 (0)	0 (0)	(0)

Key: Ab= Antibody, Ag= Antigen, IgM= Immunoglobulin M, IgG= Immunoglobulin G

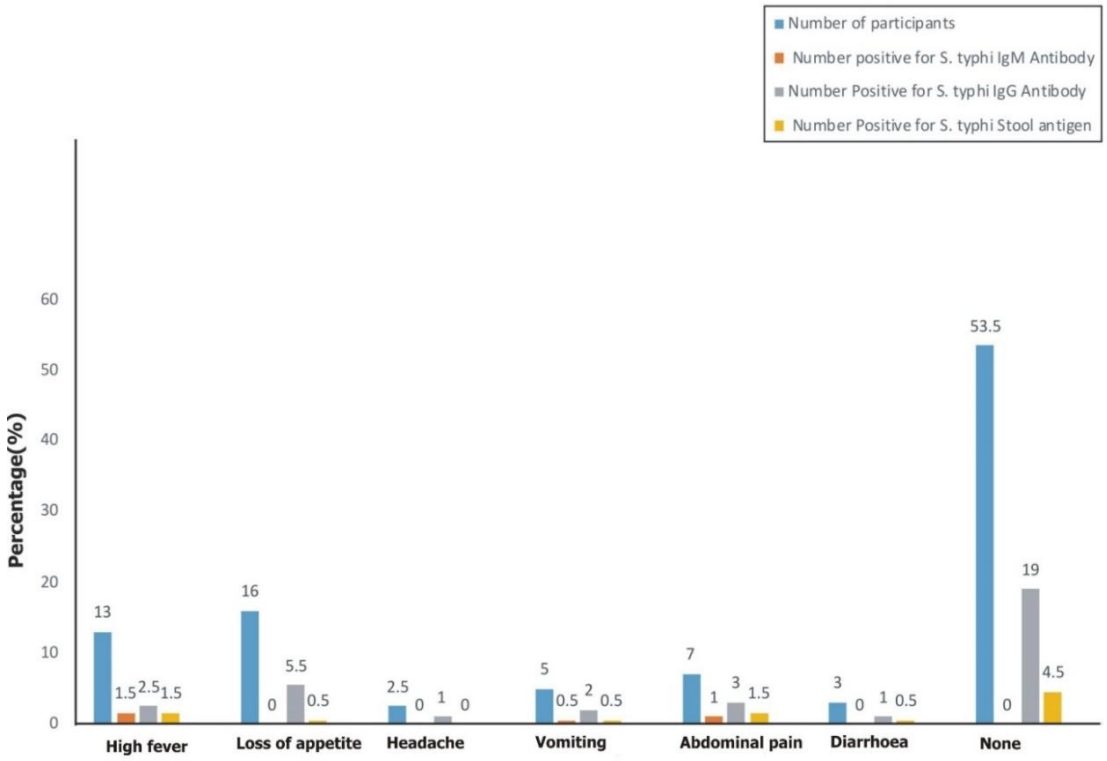

Fig. 1. Indications for typhoid fever in relation to the occurrence of *Salmonella typhi* serum antibody and stool antigen positivity among the study participants

Andualem et al. [21], in a work carried out among febrile patients at St. Paul's Hospital, Addis Abba, Ethopia, reported a prevalence of 2.6% using blood culture technique. 49.3% of the samples had reactive slide agglutination test for either or both O and H antigens. 71.4% of the samples were positive for *S. typhi* O, while 28.6% of the samples were positive for *S. typhi* H antigen.

A study conducted by Mehmood et al. [22], in Pakistan, reported a prevalence of 10.3% using blood culture. Out of the 145 patients they examined, forty-seven (32.4%) of them had only IgM positive on Typhidot, 7(4.8%) had both IgM and IgG positive, while 91(62.8%) had both IgM and IgG negative. These results also differ from the findings in this current study. A recent study by Udujih et al. [9], reported a prevalence rate of 31.9% among undergraduate Students of Imo State University Owerri, Nigeria using stool culture technique. This was higher than the 9.0% reported in this study using stool antigen detection technique. Another recent study by Ansari and Baravkar [23], reported a prevalence of 11.3% and 4.6% using Widal agglutination and Blood culture techniques, respectively. These data were lower than the 34% reported for serum anti-*S. typhi* IgG Ab, but higher than 3.5% serum anti-*S. typhi* IgM Ab observed in this study. Meanwhile, the 4.6% reported by Ansari and Baravkar [23] using blood culture technique was lower than 9.0% obtained using stool antigen detection technique in this study.

With regard to gender distribution of *S. typhi* infection among the under the undergraduate students of Babcock University, there were no significant differences (P>0.05) in the percentage positivity of anti-S*almonella typhi* serum IgM and stool antigen among the study participants on On the other hand, the percentage of male participants (29.0%) who were sero-positive for *Salmonella typhi* serum IgG antibody was significantly higher (P<0.05) than their female counterparts (5.0%).

The findings in this study fail to agree with the report of Ishaleku et al. [16], as well as of Ansari and Baravkar [23], who reported a non-significant difference (P>0.05) in *S. typhi* infection between the sexes, implying that either sex had an equal chance of infection. On one hand, the findings in this study contradicts the

report of Rasull et al. [24], who reported a higher prevalence of *S. typhi* infection in females (52.62%) as compared to males (42.32%). On the other hand, the study is consistent with those of Okonko et al. [15], who reported a higher prevalence of *S. typhi* infection among males (92.4%) than in their female counterparts (70.0%). It is also in accordance with the work of Ajayi et al. [7], who reported a higher prevalence in males, compared to the females. It also agrees with the findings of Udujih et al. [9], who reported a prevalence of 8.2% and 5.9% for males and females, respectively. Males are often known to pose a care-free attitude to the hygienic condition of the foods they eat or the environment where such is prepared. While females on the other hand, are often times more hygiene conscious than their male counterpart hence the low infection rate observed in them.

With regard to age distribution of *S. typhi* infection among the under the undergraduate students of Babcock University, there were no significant differences (P>0.05) in the percentage positivity of anti-S*almonella typhi* serum IgM and stool antigen among the study participants. On the other hand, the percentage of sero-positivity for *S. typhi* serum IgG antibody was significantly (P<0.05) higher among participants aged 16-20 years (17.0%) compared to other age categories. This is in discordance with the reports of previous studies. For instance, Ishaleku et al. [16], Reuben et al. [19] and Rasull et al. [24], reported a higher prevalence among older subjects: 21-30 years, 20-30 years and 21-30 years, respectively. On the other hand, the observation is consistent with those of Ajayi et al. [7], Ansari and Baravkar [23]; and Udujih et al. [9], who all reported a higher prevalence among younger subjects: 10- 25 years, 11-20 years and 18-21 years, respectively. This observation is not surprising as students within the younger age group are most vulnerable and tend to exhibit low hygienic practices, while the students within the higher age group might be more aware and enlightened on the need for good hygienic practice.

With regard to the symptomatology of *S. typhi* infection among the study participants, percentage of students without symptoms (18.0%) were non-significantly (P>0.05) higher than those with symptoms (16%). Report by Mallett et al. [25], revealed that most typhoid fever cases diagnosed with RDT kits are mostly asymptomatic and may go undiagnosed. In this study, we defined symptomatic *S. typhi* infection as the presence of one or more of the immunological marker (anti-*S. typhi* IgM Ab, IgG Ab or stool Ag) in the presence of one or more signs and symptoms of typhoid fever in the participants. On the other hand, we defined asymptomatic *S. typhi* infection as the presence of one or more of the immunological marker (anti-*S. typhi* IgM Ab, IgG Ab or stool Ag) in the absence of any signs and symptoms of typhoid fever in the participants.

The main signs and symptoms complained by the study Participants were loss of appetite (16.0%), high fever (13.0%) and abdominal pain (7.0%). This differ from the work of Abera et al. [17], in which participants complained mainly of diarrhoea (6.5%) and that of Rasull et al. [24], with fever (99.7%), diarrhoea (98.95%) and abdominal pain (98.42%) as the major complain.
Risk factors associated with the occurrence of *Salmonella typhi* infection among the study participants include: lack of knowledge of *S. typhi*, lack of Typhoid fever vaccination, past history of typhoid fever, drinking of raw cow milk, consumption of beef, poultry/poultry products, vegetables, and street vended food, ingestion of not boiled nor filtered water, washing hands less often after toileting and before eating among several others. This is consistent with the report of Ajayi et al. [7]; Udujih et al. [9], Smith et al. [14] and Rasull et al. [24].

Infection with *Samonella typhi* is mainly by the oral route through ingestion of faecal contaminated water and food, unclean hands, flies and meat from infected animals. The result is in tandem with the findings of Ibrahim [26] that students from middle income countries are susceptible to *Samonella typhi* due to contaminated food or water. Additionally, in West African sub-region Rukunga [27], reported that contaminated water can be extremely dangerous when it becomes the vehicle of the transmission of disease as it is the prime cause of premature deaths worldwide, especially for young adults. In regions with poor sanitation, faecal pathogens are frequently transferred to the water borne sewage system, through flush toilets and pit latrines subsequently contaminating surface and ground water [28,29].

Following appropriate antibiotic therapy, clinical cure from typhoid fever is defined as absence of signs and symptoms consistent with *S. typhi* infection. On the other hand, immunlogic cure is confirmed by non-detection of blood and stool

antigen, while microbiologic cure is confirmed by a negative blood and stool culture. Immunologically speaking, serum anti-*S. typhi* immunoglobulins M (IgM) and G (IgG) and stool antigen are important markers in the diagnosis of typhoid fever. Anti-*S. typhi* immunoglobulins M (IgM and G (IgG) are demonstrable in patient's serum 1-7 days and 7-21 days, respectively post-exposure to the typhoid pathogen. While IgM level decline very fast, IgG tend to persist for a much longer period, but does not confer a life immunity. The detection of serum anti-*S. typhi* IgM antibody, with or without stool antigen among the study participants indicates early, primary or current infection with *S. typhi*; while the detection of serum anti-*S. typhi* IgG with or without stool antigen indicates late stage, latent or past infection with *S. typhi*. The detection of both the anti-*S. typhi* IgM and IgG antibodies with or without stool antigen, indicates active primary and repeated infection with *S. typhi*.

Furthermore; the detection of serum anti-*S. typhi* IgG and stool *S. typhi* antigen in the absence of serum anti-*S. typhi* IgM is common among convalescent and chronic carriers of *S. typhi*, who although they exhibit no outward signs and symptoms of the disease, their feces contain the pathogens and therefore such people serve as crucial reservoir of infection within the community [7,30]. In the light of the above, convalescent and chronic carriers of typhoid pathogens must be identified and treated in order to halt the cycle of infection.

It is also very important to mention that anti-*S. typhi* IgM and IgG antibodies are also demonstrable in patient's serum in the absence of stool antigen following vaccination with Typhoid (Vi Polysaccharide) vaccine, given at 2 years old with re-vaccination at every 4 years interval. The other type of typhoid vaccine, Ty21a, has liquid and capsule forms. The liquid form is no longer available. The capsule form for individuals >5 years requires 3-4 orally administered doses, taken every other day. If the schedule is interrupted by an interval > 21 days, the series is restarted from beginning. If the delay is less than 21 days, series is resumed without repeating the previous dose. Booster doses are given after 3-7 years. The percentage of the participants without history of typhoid fever vaccine (48.0%) were found to be lower than the 64.8% reported by Rasull et al. [24]. This partly explains the variation in the prevalence rate of *S. typhi* infection observed in this study.

Meanwhile; absent of serum anti-*S. typhi* antibodies (IgM and IgG) and stool antigen among the study participants denotes absence of typhoid infection and that the individual is susceptible to infection upon exposure in the future. Diagnosis of typhoid fever using the serum antibody test only, without recourse to the blood and stool antigen test may result in over-diagnosis of the infection. Since anti-*S. typhi* serum antibodies are detectable following natural infection, as well as after vaccination with typhoid vaccines; it is therefore very important to obtain patient's history with regard to previous vaccination with typhoid vaccines.

4. CONCLUSION

Salmonella typi infection exist among undergraduate Students of Babcock University, Ilishan-Remo, Ogun State, Nigeria. Therefore, preventive measures including personal and environmental hygiene such as regular hand-washing after toileting and before eating, availability of hand washing facilities, purified water supplies, sewage control, and supervising of food handlers should be ensured and sustained. Health campaigns should be carried out by all the stakeholders with a view of creating awareness among the students on the importance of control and prevention of typhoid and seeking early treatment from health facilities. Students of the University should also be encouraged to receive typhoid vaccine. In addition to the above, all cases of typhoid fever among Students of the University should be identified and promptly treated to halt the cycle of transmission. It is important that every treatment of fever be preceded by adequate laboratory diagnosis that can establish the actual aetiology. Typhoid fever has been over-diagnosed and many patients have been placed on antibiotics against typhoid fever when it is not called for. Also, follow-up of cases of infection should be carried out, as well as proper documentation. This will ensure that infections are completely eliminated and the carrier status is reduced or eliminated if possible. Although, culture is and remains the gold standard for the diagnosis of typhoid fever, however, where and when facilities for culture are not available, RDT kits with high sensitivity and specificity appears to be a practical alternative to culture method in the diagnosis of typhoid fever, and can therefore be used in the resource limited settings, as it neither requires expensive laboratory equipment, nor expertise to conduct the test. But whenever feasible, confirmation with stool and blood

culture is strongly encouraged with the appearance of drug resistant strains.

CONSENT

All authors declare that 'written' informed consent was obtained from the participants with assurance of anonymity and confidentiality before the commencement of the study.

ETHICAL APPROVAL

Ethical approval for the study was obtained from the Babcock University Health Research Ethics Committee (BUHREC) with ethical approval registration number: BUHREC215/18.

DISCLAIMER

This manuscript was presented in a Conference.
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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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