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SCREENING FOR *Salmonella typhi* IgM, IgG ANTIBODIES AND *Salmonella typhi* STOOL ANTIGEN AMONG STUDENTS OF WESTERN DELTA UNIVERSITY, OGHARA.

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Abstract

There is so much controversy surrounding the use of Widal agglutination test in the diagnosis of enteric fevers (especially typhoid fever) currently in medical practice in Nigeria. One of the reasons for this, is the occurrence of cross-reactivity of *Salmonella typhi / paratyphi somatic* (O) or flagellar (H) antigens with antibodies raised against other disease agents like HBV (HBsAg), HIV (AIDS), *Treponema palladium* (syphilis) e.t.c. Alternative and more specific rapid diagnostic tools are being sourced for and used currently and include serum or plasma *Salmonella typhi* IgM/IgG antibodies and stool antigen markers. A total of 210 students of Western Delta University made up of 50(23.8%) and 160(76.2%) males and females respectively were enrolled for the study. Fourteen (6.7%) made up of 4(1.9%) and 10(4.8%) males and females were seropositive for immunoglobulin M while out of 25(11.9%) students who tested positive for immunoglobulin G, 7 (3.3) and 18(8.6%) were males and females respectively. *Salmonella typhi* stool antigen was found in 61(29.1) participants consisting of 20(9.5%) and 41(19.5%) males and females respectively. The sex distribution of IgM/IgG antibodies and stool antigen did not show any statistically significant difference (P>0.05) suggesting both sexes have equal chance of infectivity. Findings in this work imply that confirmed typhoid fever and enteric fevers are prevalent among the student population. The prevalence rates and their health implications are discussed.

Keywords: *Screening, Salm, Typhi, IgM/IgG Antibodies, Stool Antigen, Students*

INTRODUCTION

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

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. Nigeria is not immune to the burden of typhoid fever and its associated complications. According to FMOH, 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 being one of the regions to record figures higher than the national average. This is due to the influx of tertiary institutions, as well as the problem of urbanization within the region.

Tuise (2015), found a great impact of 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 has made *Salmonella typhi* infection more difficult to control and prevent effectively (Hook, 2005). 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.

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. 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 (Chart *et al*., 2000). 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 (Chart *et al*., 2000).

The situation is complicated in that some persons may be carriers of the typhoid bacillus, so that although they exhibit no outward signs of the disease, their faeces may contain the pathogens (Tuise, 2015). 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.

For a long time, the gold standard methods for detection of typhoid fever include blood, urine, bone marrow and stool cultures of which bone marrow was acclaimed to be the most reliable (Duthie and French, 1990). The sensitivity of blood/stool cultures however, ranged from 40-97% if the patient has not used antibiotics (Willke *et al*., 2002). Notwithstanding, blood and stool cultures are less frequently used in developing countries due to cost and requirement of highly trained professionals (Mengist and Tilahun, 2017). The most preferred or commonly used method therefore, in health facilities in tropical regions such as Nigeria (where laboratory services are still evolving), is the Weil – Felix agglutination test (otherwise known as WIDAL TEST) which is supposedly easy, cheaper and does not require HIGHLY trained laboratory personel (Mawazo *et al*., 2019).

The Widal Test has however, been associated with some controversies which include: inherent variabilities of the test, difficulty in establishing a steady – state baseline titre for the population, repeated exposures to *Salm. typhi* organisms in endemic regions, cross – reactivity with other non – Salmonella organisms and lack of reproducibility of the test results (Olopoenia, 2000). The test also relies on the demonstration of a rising titre of antibodies in paired samples (acute stage and convalescent stage of illness) 10 to 14 days apart (Andualem *et al.,* 2014). The Widal test is also affected by cross – reactivity with other Salmonella subspecies which may not be the direct cause of fever and may even turn positive in malaria infection (Willke *et al.,* 2002).

With regard to collecting paired sera (in other to demonstrate rising titre), it is often difficult to clinically prove a rise in blood cultures (Mengist and Tilahun, 2017). Moreover, it is not practicable as patients cannot be kept waiting without initiating treatment (Keddy *et al.,* 2011). Despite the above enumerated limitations, in most developing countries including Nigeria, the Widal Agglutination Test is the most common diagnostic tool (second requested test after malaria test) employed in the diagnosis of typhoid fever (Birhanie *et al*., 2014; Malisa and Nyaki, 2010).

A number of Medical Practitioners have however, often raised alarm at the apparently "high rate" of typhoid fever or enteric fevers diagnosed in healthcare facilities in Nigeria due to high false positivity associated with the test. An alternative diagnostic method which provides a check or provides a more direct and accurate method of diagnosing typhoid fever is by screening for specific immunoglobulin M and immunoglobulin G antibodies raised against *Salmonella typhi* organisms as well as *Salmonella typhi* stool antigen. This will be of great assistance to healthcare providers and practitioners.

This study is therefore aimed at screening for *Salmonella typhi* IgM, IgG antibodies and *Salmonella typhi* stool antigen among students of Western Delta University, Oghara with the following objectives:

1. To screen students of Western Delta University, Oghara, Delta State for presence of *Salmonella typhi* serum IgM/IgG antibodies using a rapid diagnostic method.

2. To screen students of Western Delta University, Oghara, Delta State for presence of *Salmonella typhi* stool antigen using a rapid diagnostic method.

MATERIALS AND METHODS

Ethical Clearance

Participating students were all adults aged between 18-37yrs (average of 22yrs) who gave their oral and written informed consent to take part in the study.

Study Design/Study Area

This study was a cross-sectional type of which samples were collected (obtained) from randomly selected consenting students of Western Delta University, Oghara, Delta State, Nigeria. The study was carried out from March to June, 2020 in the Department of Microbiology of the institution. The University which was established in 2008, is situated in the Western end of Delta State close to Koko junction along Benin-Sapele Road, Nigeria. The University has three Colleges and a student population of about one thousand.

Sample Size Determination/Sampling

Sample size for this study was computed in line with a scheme provided by Charan and Biswas (2013) of which the calculated minimum sample size N was 186. This was arrived at with a 95% confidence interval, a P value of 0.1407, a typhoid prevalence rate (based on report of Udijih *et al.* 2017) of 14.07% and a margin error set at 0.05. For convenience however, a total of 210 subjects were enrolled for this study. Two hundred and ten (210) whole blood samples were collected in sequestrinized anticoagulated containers and used to screen for *Salmonella typhi* IgM/IgG antibodies and stool antigen detection

Using 5ml sterile needles and syringes, about three millilitres (3ml) of venous blood was collected from 210 randomly selected Western Delta University, Oghara students made up of 50 (23.8%) male and 160 (76.2%) female students. Whole blood samples were collected by venipuncture (by tying a tourniquet around the upper arm and surface sterilizing the arm with 70% ethanol to sterilize and stimulate increased blood pressure in the veins) and were dispensed into ethylene diamine tetra acetic acid (EDTA) anticoagulated blood containers, properly mixed by standard method and labeled appropriately. Both symptomatic and asymptomatic students were recruited for the study because almost 90% of participants did not show or feel any visible signs/symptoms of enteric fevers especially typhoid fever.

All collected specimens were processed within 2-24hrs of collection. Where there was inevitable delay in screening, affected samples were refrigerated (at $4^{o}C$) in a functional fridge with steady power supply.

Salmonella typhi IgM/IgG Antibody Detection

Whole blood *Salmonella typhi* IgM/IgG antibodies was/were detected using a one-step *Salmonella typhi* antibody test cassette, Solid Rapid Diagnostic Typhoid IgG/IgM kit (Acro Biotech, USA) according to manufacturer's instruction. One drop of whole blood (about 0.04ml) was taken by a dropper which was held vertically and the blood was transferred to the specimen area. Two drops of buffer (about 0.08ml) were added and result was read within 15-20mins. All cassettes (before use) were

allowed to equilibrate to room temperature (15-30mins) on laboratory bench before testing by bringing the pouch to room temperature before opening it.

Interpretation of Results

Positive Results

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

Negative Result

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

Invalid Result

Absence of colour in either control or test regions or only one colour 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.

***Salmonella typhi* Stool Antigen Detection**

Salmonella typhi/paratyphi duo stool antigen was detected using one-step Quick Profile *Salmonella typhi/paratyphi* Antigen Duo test cassettes (Lumi Quick Diagnostics, USA). Screening of samples was done in line with manufacturer's instruction. Positive and negative control samples were run alongside test samples. One drop of whole blood (about 0.04ml) was taken by a dropper which was held vertically and the blood was transferred to the specimen area. Two drops of buffer (about 0.08ml) were added and result was read within 15-20mins. All cassettes (before use) were allowed to equilibrate to room temperature (15-30mins) on laboratory bench before testing by bringing the pouch to room temperature before opening it.

Interpretation of Results

Applies as in the case of *Salm. typhi* IgG/IgM antibody detection. In terms of Performance characteristics of test kits used, the kit for IgM detection has percentage sensitivity, specificity and accuracy of 93.9%, 99.0% and 98.5% respectively while that for IgG detection has 86.7%, 99.6% and 99.0% respectively.

Cross-Reactivity

All kits were tested for HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, HCV, HIV, Syphilis, *Helicobacter pylori*, CMV, Rubella and Toxoplasmosis positive specimens. The results were all negative thus suggesting there is no cross-reactivity.

Socio-demographic Information

This was obtained using a structured questionnaire which contained items such as: awareness about *Salm. typhi*, typhoid vaccine, personal hygiene, mode of consumption of raw cow milk, beef, vegetables, poultry foods etc.

Data Analyses

Tests were done to determine existence of significant differences in the prevalence of *Salm. typhi* infection among participating students at 95% confidence interval (0.05). Simple percentages were also used to analyze results obtained. The Fisher's

Exact Test four-fold Chi-square contingency table was used to analyze data at both 95% (0.05) and 99% (0.01) confidence intervals.

Results

Table 1 shows the distribution and prevalence of *Salmonella typhi* Immunoglobin M (IgM) antibody with respect to sex of the study population. Fourteen (6.67%) out of 210(100%) screened students were seropositive for *Salmonella typhi* IgM antibody.

Table 1: Sex Distribution of anti-Salmonella *Typhi* IgM antibody among Western Delta University students recruited for the study

Sex	Total no. Plasma samples processed N = 210	No. of positive plasma samples	No. of Negative samples	P – value
Male	50(23.8%)	4(28.6%)	46(23.5)	$X^2_{0.05,1}$ =3.841 $X^2_{0.01,1}$ = 6.635
Female	160(76.2%)	10(71.4%)	150(76.5)	
Total	210(100.0%)	14(6.67%)	196(93.3%)	

At 95% CI, $X^2_{0.05,1}$ or Book (P) value = 3.841
At 99% CI, $X^2_{0.01,1}$ or Book (P) value = 6.635
Critical (calculated) X^2 = 0.1875
P > 0.05
P > 0.01

Out of this number, 4(28.6%) and 10(71.4%) male and female students respectively were seropositive for the antibody. On the whole, 50(23.8%) and 160(76.2%) males and females were screened respectively of which 46(23.5%) and 150(76.5%) male and female students were seronegative respectively for the antibody. Statistically, a chi-square(x^2) analysis on obtained data showed that critical or calculated x^2 is 0.1875 as against $X^2_{0.05,1}$ =3.841 and $X^2_{0.01,1}$ = 6.635 and hence, P > 0.05 at 95% confidence interval and P > 0.01 at 99% CI suggesting that there is no significant association of *Salm. typhi* IgM antibody with sex of students enrolled for the study.

Table 2 shows the sex distribution (sero-prevalence) of *Salmonella typhi* immunoglobulin G (IgG) antibody among the students recruited for the study. A total of 81 (38.6%) students made of 22 (27.2%) and 59 (72.8%) males and females respectively were seropositive for IgG *Salmonella typhi* antibody. Out of the 50 (23.8%) and 160 (76.2%) male and female students population screened, 28 (21.7%) and 101 (78.3%) male and female students respectively were sero-negative for the antibody. Statistically, a chi-square(x^2) analysis on obtained data showed that critical or calculated x^2 is 8.162 as against $X^2_{0.05,1}$ =3.841 and $X^2_{0.01,1}$ = 6.635 and hence, P < 0.05 at 95% confidence interval

and P < 0.01 at 99% CI suggesting a significant association of *Salm. typhi* IgG antibody with sex of students enrolled for the study.

In **Table 3**, the occurrence rate of *Salmonella typhi* stool antigen with respect to sex of the study population is shown. A total of 91 (43.3%) participants made up of 34 (37.4%) and 57 (62.6%) males and females were seropositive respectively for *Salmonella typhi* stool antigen.

One hundred and nineteen (56.7%) participants made up of 16 (13.5%) and 103 (86.5%) males and females were seronegative respectively for the antigen.

Statistically, a chi-square(x^2) analysis on obtained data showed that critical or calculated x^2 is 16.260 as against $X^2_{0.05,1}$ =3.841 and $X^2_{0.01,1}$ = 6.635 and hence, P < 0.05 at 95% confidence interval and P < 0.01 at 99% CI suggesting a significant association of *Salm. typhi* stool antigen with sex of students enrolled for the study.

Table 2: Sex distribution of anti-salmonella IgM antibody among Western Delta University students recruited for the study.

Sex	Total no. Plasma samples processed N = 210	No. of positive plasma samples	No. of Negative samples	P – value
Male	50(23.8%)	22(27.2%)	28(21.7)	$X^2_{0.05,1}$ =3.841 $X^2_{0.01,1}$ = 6.635
Female	160(76.2%)	59(72.8%)	101(78.3)	
Total	210(100.0%)	81(38.6%)	129(61.4%)	

At 95% CI, $X^2_{0.05,1}$ or Book (P) value = 3.841
At 99% CI, $X^2_{0.01,1}$ or Book (P) value = 6.635
Critical (calculated) X^2 = 8.162
P < 0.05
P < 0.01

Table 3: Occurrence rate of *Salmonella typhi* stool antigen with respect to sex among study Population

Sex	Total no. Plasma samples processed N = 210	No. of positive plasma samples	No. of Negative samples	P – value
Male	50(23.8%)	34(37.4%)	16(13.5)	$X^2_{0.05,1}$ =3.841 $X^2_{0.01,1}$ = 6.635
Female	160(76.2%)	57(62.6%)	103(86.5)	
Total	**210(100.0%)**	**91(43.3%)**	**119(56.7%)**	

At 95% CI, $X^2_{0.05,1}$ or Book (P) value = 3.841
At 99% CI, $X^2_{0.01,1}$ or Book (P) value = 6.635
Critical (calculated) X^2 = 16.260
P < 0.05
P < 0.01

Lastly, **Table 4** shows a comprehensive or overall distribution of *Salmonella typhi* immunoglobin M (IgM) and immunoglobin G (IgG) antibodies as well as *Salmonella typhi* stool antigen among the student population in relation to some selected risk factors. The selected risk factors included awareness about *Salmonella typhi*, typhoid vaccine, personal hygiene, mode of consumption of raw cow milk, beef, vegetables, poultry foods, vended foods, types of drinking water (as a child and as an adult.), method of anal cleaning after toileting etc.

Table 4 indicates typhoid fever in relation to the occurrence of plasma *Salmonella typhi* IgM/IgG antibodies and stool antigen positivity among the students recruited for the study. Based on personal interview at point of sample collection, 40(19.1%) of participants were symptomatic of typhoid fever, while 170(80.9%) did not show any signs/symptoms of the enteric disease. Common symptoms manifested and communicated included high fever, headache, abdominal (stomach pain), fatigue, weakness, nausea, constipation/diarrhea and loss of appetite.

One hundred and forty-nine (70.9%) out of 210(100%) screened participants indicated that they had no knowledge of *Salmonella typhi* as against 61(29.1%) participants who asserted that they were aware of the pathogen. Four (1.9%), 7(3.3%) and 18(8.6%) participants who said they had no knowledge, were positive for *Salmonella typhi* IgM, IgG antibodies and stool antigen respectively while 6(2.9%), 3(1.4%) and 12(5.7%) participants who said they had no knowledge about the disease tested positive to *S. typhi* IgM, IgG antibodies and stool antigen respectively. Out of 180(85.7%) students who responded that they have not received typhoid vaccine, 13(6.2%), 12(5.7%) and 50(23.8%) tested positive for *Salmonella typhi* IgM, IgG antibodies and stool antigen respectively. Thirty (14.3%) participants responded in the affirmative that they had received typhoid vaccine at one time or the other and out of this number, 0(0.0%), 1(0.5%) and 4(1.9%) were seropositive to *Salmonella typhi* IgM, IgG antibodies and stool antigen respectively. This suggested that no single student in this group tested positive to IgM antibody.

Respondents who indicated that they knew the history of typhoid fever were 80(38.1%) out of which 2(1.0%), 1(0.5%) and 8(3.8%) recorded positive results for *Salmonella typhi* IgM, IgG Plasma antibodies and stool antigen respectively. Fifty-seven (27.1%) subjects indicated that they drank raw milk of which 0(0.0%), 2(1.0%) and 5(2.4%) tested positive to *Salmonella typhi* IgM, IgG antibodies and stool antigen respectively. Out of 205(91.6%) respondents who said they consumed beef, 31(14.8%), 25(11.9%) and 52(24.8%) were seropositive to *Salmonella typhi* IgM, IgG antibodies and stool antigens respectively.

Moreover, 195(92.9%) students penned that they consumed vegetables and out of this, 22(10.5%), 46(21.9%) and 71(33.8%) were positive to *Salmonella typhi* IgM, IgG antibodies and stool antigen respectively. Similarly, 145(69.1%) participants maintained that they ate poultry foods out of which 29(13.8%), 61(29.1%) and 70(33.3%) were seropositive to *Salmonella typhi* IgM, IgG antibodies and stool antigen respectively.

Table 4 also shows 182(86.7%) participants who indicated that they patronized and ate street vended foods of which 16(7.6%), 38(18.1%) and 48(22.9%) tested positive to *Salmonella typhi* IgM, IgG antibodies and stool antigen respectively. With respect to the type of water respondents drank as children, none of them drank (as indicated) unboiled/unfiltered water and out of the 140(66.7%) who indicated that they drank portable water as children, 18(8.6%), 33(15.7%) and 51(24.3%) were positive to *Salmonella typhi* IgM, IgG antibodies and stool antigen respectively. As adults, 150(71.4%) participants maintained that they drank portable water and out of this, 6(2.9%), 19(9.1%) and 25(11.9%) were seropositive to *Salmonella typhi* IgM, IgG antibodies and stool antigen respectively. None of these adults indicated that they drank unboiled, unfiltered water.

Out of 130(61.9%) respondents who penned that they adopt anal washing with water after toileting, 12(5.7%), 11(5.2%) and 31(14.8%) tested positive to *Salmonella typhi* IgM, IgG antibodies and stool antigen respectively.

Those who indicated they preferred and used tissue paper after toileting were 80(38.1%) out of which 4(1.9%), 6(2.8%) and 15(7.1%) were seropositive to *Salmonella typhi* IgM, IgG antibodies and stool antigen respectively.

Respondents who indicated that they often hand-washed after toileting were 180 (85.7%) of which 19(9.1%), 23(11.0%) and 30(14.3%) tested positive to *Salmonella typhi* IgM, IgG and stool antigen respectively. None of these participants indicated never doing hand washing after toileting. Lastly, 120(57.1%) students maintained that they often wash their hands before eating and 5(2.4%), 18(8.6%) and 27(12.9%) out of this group were seropositive to *Salmonella typhi* IgM, IgG antibodies and stool antigen respectively.

Also, none of the respondents in this category indicated never washing hands before settling down to eat any type of food.

Table 4: Influence of Risk factors on *Salmonella typhi* infection occurrence among study Population

Risk Factors	Responses	No. of Subjects recruited n=210	No. of positive for *Salmonella* IgM antibody	No. of positive for *Salmonella typhi* IgG antibody	positive for *Salm typhi* stool antigen
knowledge about *S. typhi* ?	Yes	61(29.1%)	6(2.90%)	3(1.4%)	12(5.7%)
	No	149(70.9%)	4(1.9%)	7(3.3%)	18(8.6%)
Received typhoid vaccine?	Yes	30(14.3%)	0(0.0%)	1(0.5%)	4(1.9%)
	No	180(85.7%)	13(6.2%)	12(5.7%)	50(23.8%
History of Typhoid fever	Yes	80(38.1%)	2(1.0%)	1(0.5%)	8(3.8%)
	No	130(61.9%)	12(5.7%)	10(4.8%)	8(3.8%)
Drink raw cow milk	Yes	57(27.1%)	0.00	2(1.0%)	5(2.4%)
	No	153(72.9%)	6(2.9%)	11(5.2%)	19(9.1%)
Consume Beef ?	Yes	205(97.6%)	31(14.8%)	25(11.9%)	52(24.8%
	No	5(2.4%)	0(0.00)	3(1.4%)	5(2.4%)
Consume vegetables?	Yes	195(92.9%)	22(10.5)	46(21.9%)	71(33.8%
	No	15(17.1%)	1(0.5%)	3(1.4%)	6(2.9%)
Consume poultry foods?	Yes	145(69.1%)	29(13.8%)	61(29.1%)	70(33.3%
	No	65(30.9%)	8(3.8%)	20(9.5%)	11(5.2%)
Consume street vended food	Yes	182(86.7%)	16(7.6%)	38(18.1%)	48(22.9%
	No	28(13.3%)	2(1.0%)	5(2.4%)	9(4.3%)
Type of drinking water as a child	Portable	140(66.7%)	18(8.6%)	33(15.7%)	51(24.3%
	Boiled/Filtered	70(33.3%)	8(3.8%)	14(6.7%)	0(0.00%)
	Unboiled/Unfiltered	0(0.00%)	0(0.00%)	0(0.00%)	0(0.0%)
Type of drinking water as an adult	Portable	150(71.4%)	6(2.9%)	19(9.1%)	25(11.9%
	Boiled/Filtered	60(28.6%)	1(0.5%)	3(1.4%)	9(4.3%)
	Unboiled/Unfiltered	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Anal cleaning after	Use of Tissue paper	80(38.1%)	4(1.9%)	6(2.8%)	15(7.1%)
	Wash with water	130(61.9%)	12(5.7%)	11(5.2%)	31(14.3)
toileting	Do nothing	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Hand hygiene after eating	Wash often	180(85.7%)	19(9.1%)	23(11.0%)	30(14.3%
	Wash less often	30(14.3%)	0(0.0%)	2(1.0%)	7(3.3%)0
	Never	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Hand Hygiene Before Eating	Wash often	120(57.1%)	5(2.4%)	18(8.6%)	27(12.9%
	Wash less often	90(42.9%)	4(1.9%)	9(4.3%)	13(6.2%)
	Never	0(0.0%)	0(0.0%)	0(0.0%)	

Symptomatic typhoidal participants: 40
Asymptomatic typhoidal participants: 80

DISCUSSION

Typhoid fever is a communicable disease found only in man and caused by *Salmonella typhi* (B.S Symposium, 2001). It is an acute generalized infection of the reticulo-endothelial system, intestinal lymphoid tissue and the gall bladder. For many decades, typhoid fever was not recognized as a separate clinical disease and this was because it was often confused with other prolonged febrile fevers such as typhus fever caused by Rickettsia organisms (BSS, 2001).

The "Widal Test" which was formerly known as Weil-Felix Test, is a serological test used mostly for the diagnostic investigation of enteric fevers (especially typhoid fever) in Nigeria. This is because it is relatively cheap, easy to perform and requires minimal training and equipment. As a result, the Widal Test has suffered a lot of abuse in terms of the high seropositive reports being churned out. In Nigeria today, if a patient is not positive for malaria, he is likely to have typhoid or both. Hence, Medical Practitioners often frown at the apparently high rate of typhoid fever diagnosed in healthcare facilities in Nigeria due to high false positivity associated with the test.

The consequences of false positive results include misuse of antibiotics, danger of increased antibiotic resistance, increased cost of treatment due to increased hospital stay for inpatients and non-detection of fatal febrile diseases (Mawazo *et al.,* 2019). Laboratory diagnosis of typhoid fever requires isolation and identification of *Salmonella typhi*. However, in many areas where the disease is endemic, laboratory input 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 and Rapid Diagnostic tests are useful for clinicians working in resource limited settings in the tropics (Wijedoru *et al.,* 2010; Mitra *et al.,* 2010).

This study was therefore geared towards verifying the accuracy of alternative rapid diagnostic *Salmonella typhi* antibody test kits in diagnosing enteric fevers especially typhoid fever. In this study, out of 210 (100.0%) students screened for *Salmonella typhi* IgM antibody, 14 (6.7%) were positive of which 4 (1.9%) and 10 (4.8%) were male and female participants respectively. The difference in the occurrence rate between males and females was not statistically significant ($P > 0.05$). This implied that the host immunological production of immunoglobulin M antibody to fight *Salmonalla typhi* antigen does not have sex discrimination (Table 1).

Similarly, 25 (11.9%) participants were seropositive to immunoglobulin G antibody which was raised against the *Salmonella typhi* antigen. Out of this, 7 (3.3%) and 18 (8.6%) were seropositive male and female students respectively. The occurrence rate of this antibody in females is not significantly different from that in males ($P > 0.05$).

Serum *Salm. typhi* IgM and IgG and stool antigen are important markers in the diagnosis of typhoid fever. *Salm. typhi* IgM and IgG are demonstrable in patients serum 1-7days and 7-21days respectively after exposure to *Salm. typhi* infection. Whereas IgM levels decline fast, IgG tends to persist for much longer period but does not confer immunity.

The detection of serum *Salmonella typhi* IgM antibody with or without stool antigen indicates early primary or current infection with *Salm. typhi* while detection of serum *Salm. typhi* IgG with or without stool antigen indicates latent or past infection with *Salm. typhi*. The detection of both *Salm. typhi* IgM and IgG antibodies with or without stool antigen indicates active primary and repeated infection with *Salm. typhi*. Moreover, the prevalence of *Salm. typhi/paratyphi* stool antigen among participants was 29.1% of which 20 (9.5%) males and 41 (19.5%) females were seropositive and there was however, no significant difference ($P > 0.05$).

With regard to gender distribution of *S. typhi* infection among the undergraduate students of Western Delta University, there was a significant difference ($P>0.05$) in the percentage positivity of *Salmonella typhi* serum IgM and stool antigen among the study participants. On the other hand, the percentage of male participants 50 (23.8%) who were sero-positive for *Salmonella typhi* serum IgG antibody was significantly lower ($P<0.05$) than their female counterparts 160 (76.2%).

Findings in this study are in agreement with the reports of Ishaleku *et al.* (2010). Ansari and Baravkar (2017) which stated a non-significant difference ($P>0.05$) in *Salmonella typhi* infection between the sexes, implying that either sex had an equal chance of infection. In this study, *Salm. typhi* IgM, IgG antibodies and *Salm. typhi* stool antigen seropositivity rates in females (i.e 4.8%, 8.6% and 19.5% respectively) were higher than those recorded for their male counterparts (i.e 1.9%, 3.3% and 9.5% respectively). This finding is supported by some previous authors whose reports stated a higher prevalence of *Salm. typhi* infection in females (52.6%) than in males (42.3%). Findings however, are not in agreement with the report of Okonkwo *et al.* (2010) which stated a higher *Salm. typhi* male infection rate of 92.4% than in the female participants (70.0%). The report in this study is also inconsistent with the work of Ajayi *et al.* (2015) and Udijih *et al.* (2017) who all reported a higher *Salm. typhi* infection rate in males than in females. They reasoned that males are often care-free to ensuring hygienic condition of the food they eat or the environment where such food is prepared as compared to the females who are more hygiene conscious. This however, may not be completely true as neatness or maintaining a personal hygienic lifestyle is not sex related.

In our present study, *Salm. typhi* IgM and IgG as well as stool antigen prevalence rates were 6.7%, 11.9% and 29.1% respectively. With respect to IgM, the finding in this work is low and inconsistent with the report of 4.1% IgM prevalence rate recorded by some previous authors (ONG et al., 1989). Prevalence rate of 6.7% in this work is higher and not in agreement with 3.5% IgM prevalence rate reported by Enitan *et al.* (2019). However, 11.9% and 29.1% IgG and stool antigen prevalence rates respectively reported in this study are low and high when compared with 34% and 9.0% reported respectively by some previous authors (Enitan *et al.*, 2019).

In similar studies, the reports of 80.1% typhoid fever prevalence rate using Widal Test by Okonkwo *et al.* (2010) in Abeokuta; 73% typhoid fever prevalence rate among selected tertiary students in Nasarawa State by Ishaleku *et al.* (2010); 88% typhoid fever prevalence rate by Widal agglutination test among pregnant women in Central Nigeria by Reuben *et al.*

(2013); 57.1% typhoid fever prevalence rate by Widal test by Wam *et al*. (2019); 68.4% and 20% typhoid fever Widal test and stool culture prevalence rates respectively by Gemechu *et al*. (2017); 45.2% typhoid fever prevalence rate in Lagos, Nigeria by Akinyemi *et al*. (2008); 39.3% typhoid fever prevalence rate by stool culture by Wam *et al.* (2019) and 23.7% typhoid fever prevalence rate among selected patients in an Hospital based in Tanzania by Benedikt *et al.* (2011); 31.9% typhoid fever prevalence rate using stool culture among Imo State University students by Udijih *et al.* (2017) are all very high and not in consonance with findings in this study.

Conversely, findings of 6.7% IgM, 11.9% IgG and 29.1% stool antigen prevalence rates in this current study are high when juxtaposed with 5.7% typhoid fever prevalence rate among food handlers (Bukas) using culture method by Smith *et al.* (2008); 1.6% typhoid fever prevalence rate among food handlers using culture method by Aberal *et al*. (2010) as well as 11.3% and 4.6% typhoid fever prevalence rates using Widal agglutination and blood culture respectively by Ansari and Baravkar (2017). The report of 49.3% typhoid fever prevalence rate among febrile patients in an Ethiopian Hospital using Widal test is also not consistent with present study finding (Andualem *et al.*, 2014).

In related studies, Balakrishna *et al*. (2013) used three diagnostic methods of blood culture, Widal test and Typhidot to establish typhoid fever prevalence of which 14% *Salm. typhi* infection was recorded by blood culture, 21.5% by Widal test and 27.5% by Typhidot. Typhoid fever prevalence rate of 10.3% has been reported by Mahmood *et al.* (2015) and these are also not in agreement with report of this present study.

CONCLUSION

Serum or plasma or whole blood *Salmonella typhi* IgM and IgG antibodies as well as stool antigen are important markers in the diagnosis of typhoid fever. Besides, the detection of 6.7%, 11.9% and 29.1% prevalence rates respectively of the above markers in the screened samples suggests that typhoid fever is prevalent among Western Delta University undergraduate students. Typhoid fever has however, been over-diagnosed and patients are often placed on antibiotics when it is not necessary. Notwithstanding, properly diagnosed cases of infection should be followed up and properly documented and treated appropriately. This will ensure that enteric fevers (especially paratyphoid ever) are well managed and carrier status is reduced or eliminated if possible. Besides, while students of the University are encouraged to take anti-typhoidal vaccine, strict 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 encouraged and adhered to as preventive measures.

REFERENCES

Abera B, Biadegelgen F, Bezabih B., (2010). Prevalence of *Salmonella typhi* and intestinal parasites among food handlers in Bahir Dar Town, Northwest Ethiopia. Ethiop. J. Health Dev. 24(1):46-50.

Acharya VI, Lowe CU, Thapa R, Gurubacharya V.L, Shrestha MB, Cadoz M, Schulz D, Armand J, Bryla DA, Trollfors B, Cramton T, Schneerson R, and Robbins JB., (1987). Prevention of typhoid fever in Nepal with the Vi capsular polysaccharide of *Salmonella typhi*. A preliminary report. *New England Journal of Medicine.* 317: 1101-4.

Ajayi O. E., Olukunle O. F., and Boboye B. E. Prevalence of typhoid fever among different socio-demographic groups in Ondo State, Nigeria. Journal of Applied Life Sciences International. 3(2):8995.

Akinyemi K. O., Smith A. O., Bola O, and Coker A. O. (2012). Multidrug resistance in *Salmonella enterica* serovar typhi isolated from patients with typhoid fever complications in Lagos, Nigeria. Public Health. 119: 321-327

Andualem G, Abebe T, Kebede N, GebreSelassie S, Mihret A, and Alemayehu H., (2008) A comparative study of widal test with blood culture in the diagnosis of typhoid fever in febrile patients. BMC Research Notes.; 7:653.

Ansari J.N and Baravkar A.S., (2017). Typhoid fever: Accuracy in laboratory diagnosis by Widal test and blood culture techniques. International Journal of Scientific Research. 6:72-73.

Arnold K, Hong C.S, Nelwan R, et al. (1993). Randomized comparative study of fleroxacinand chloramphenicol in typhoid fever. *The American Journal of Medicine*. 94: 195S-200S.

Balakrishna T.P, Sumathi S, Anuradha K, Venkatesh D, and Krishna S., (2013). A comparative study of typhidot and widal test in: The diagnosis of typhoid fever. Journal of Evolution of Medical and Dental Sciences; 2(21):3720-3725.

Benavente L, Gotuzzo J, Guerra O, Grados H, and Bravo N., (1984) Diagnosis of typhoid fever using a string capsule device. *Transactions of the Royal Society of Tropical Medicine and Hygiene.*78(3): 404-6.

Benedikt L, Kamala T, Shaali M, Ame G, Mtove M, Seidlein L, Ben Al, Hendriksen CE, Mwambuli A, Shoo A, Deok R, Kim L, Ochiai R, Favorov M, John D, Harald C, Jacqueline W, Deen L, and Ali S M., (2011). Assessment and comparative analysis of a rapid diagnostic test (Tubex®) for the diagnosis of typhoid fever among hospitalized children in rural Tanzania. BMC Infectious Diseases. 11:147150.

Bhutta Z. A, Khan I, and Molla A.M. (1994). Therapy of multidrug resistant typhoidal salmonellosis in childhood: a randomized controlled comparison of therapy with oral cefixime versus IV ceftriaxone. *The*

*Pediatric Infectious Disease Journal.*13: 990-4.

Bhutta Z.A, Khan I.A, and Shadmani M., (2000). Failure of short-course ceftriaxone chemotherapy for multidrug-resistant typhoid fever in children: a randomized controlled trial in Pakistan. *Antimicrobial Agents and Chemotherapy*. 44: 450-2.

Bhutta Z.A. (1996). Impact of age and drug resistance on mortality in typhoid fever. *Archives of Disease in Childhood.* 75: 214-7.

Bhutta ZA. (2002) Typhoid and paratyphoid. In: Southall D, Coulter B, Ronald C,Nicholson S, Parke S, editors. *International child health care: a practical manual for hospitals worldwide.* London: BMJ Books. p. 426-9.

Bhutta Z.A, Naqvi SH, and Suria A. (1991) Chloramphenicol therapy of typhoid fever and its relationship to hepatic dysfunction. *Journal of Tropical Pediatrics.* 37: 320-2.

Bhutta Z.A, and Mansurali N, (1999). Rapid serologic diagnosis of pediatric typhoid fever in an endemic area: a prospective comparative evaluation of two dot-enzyme immunoassays and the Widal test. *The American Journal of Tropical Medicine and Hygiene.* 61(4): 654-7.

Black R.E, Levine M.M, Ferreccio C, Clements M.L, Lanata C, Rooney J, and Germanier R, (1990). Efficacy of one or two doses of Ty21a *Salmonella typhi* vaccine in enteric-coated capsules in a controlled field trial. *Vaccine*. 8: 81-4.

Bodhidatta L, Taylor DN, Thisyakorn U, and Echeverria P., (1987). Control of typhoid fever in Bangkok, Thailand, by annual immunization of school children with parenteral typhoid fever. *Reviews of Infectious Diseases.* 9: 841-5.

Bopp C. A, Brenner F. W, Wells J.G, and Strockbine N.A., (1999) *Escherichia*, *Shigella*, and *Salmonella*. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. *Manual of clinical microbiology*. Seventh edition. Washington DC: American Society for Microbiology, ASM press. p. 459-74.

Brown J.C, Shanahan P.M, Jesudason M.V, Thomson C.J, and Aymes S.G, (1996). Mutations responsible for reduced susceptibility to 4-quinolones in clinical isolates of multi-resistant *Salmonella typhi* in India *The Journal of Antimicrobial Chemotherapy*. 37: 891-900.

Buckle G. C, Walker C. L, Black R. E. Typhoid fever and paratyphoid fever: Systematic review to estimate global morbidity and mortality for 2010. J. Glob Health.; 2(1):010401.

Charan J, and Biswas T, (2013). How to calculate sample size for different study designs in medical research? Indian Journal of Psychological Medicine. 121– 126.

Cheesbrough M. Salmonellae In: Cheesbrough, M. (ed.), District Laboratory Practice in Tropical Countries (Part 2), 2nd Edition Updated. Cambridge University Press, Cape Town, South Africa.; 186-186.

Choo K.E, Davies T.M.E, Ismail A, and Ong K.H., (1997). Longevity of antibody responses to a*Salmonella typhi* specific outer membrane protein: Interpretation of a dot enzyme immunosorbent assay in an area of high typhoid fever endemicity. *The American Journal of Tropical Medicine and Hygiene*. 57(4): 96-9.

Choo K.E, Davies T.M.E, Ismail A, Tuan Ibrahim T.A, and Ghazali W.N.W., (1999). Rapid andreliable serological diagnosis of enteric fever: comparative sensitivity and specificity of Typhidot and Typhidot-M tests in febrile Malaysian children. *Acta Tropica*. 72: 175-83.

Chinh N.T, Parry C.M, Ly .NT, et al., (2000). A randomised controlled comparison of azithromycin and ofloxacin for multidrug-resistant and nalidixic acid resistant enteric fever. *Antimicrobial Agents and Chemotherapy.* 44: 1855-9.

Cristiano P, Imparato L, Carpinelli C, et al., (1995). Pefloxacin versus chloramphenicol in the therapy of typhoid fever. *Infection.* 23: 103-5.

Clegg A, Passey M, Omena MK, Karigifa K., and Sueve N., (1994) Re-evaluation of the Widal agglutination test in response to the changing pattern of typhoid fever in the highlands of Papua New Guinea. *Acta Tropica.* 57:255-63

Coleman W, and Buxton B. H. (1907) The bacteriology of the blood in typhoid fever. *The American Journal of the Medical Sciences.* 133: 896-903.

Das U, and Bhattacharya S.S., (2000). Multidrug resistant *Salmonella typhi* in Rourkela, Orissa. *Indian Journal of Pathology & Microbiology.* 43: 135-8.

Dhanjee A, Sheikh MA, Yaqub M, and Alam S.E. Orelox (cefpodoxime) in typhoid fever. *Journal of the Pakistan Medical Association.* 49: 8-9.

Donald E., (2014) Deadly diseases and epidemic typhoid fever. Chelsea House Publishers, Philadelphia.

Doherty C.P, Saha S.K, and Cutting W.A., (2000). Typhoid fever, ciprofloxacin and growth in young children. *Annals of Tropical Paediatrics.* 20: 297-303.

Dutta P, Mitra U, Dutta S, De A, Chatterjee M.K, and Bhattacharya S.K., (2001). Ceftriaxone therapy in ciprofloxacin treatment failure typhoid fever in children. *The*

Indian Journal of Medical Research. 113: 210-3.

Dutta P, Rasaily R, Saha MR, et al., (1993). Ciprofloxacin for treatment of severe typhoid fever in children. *Antimicrobial Agents and Chemotherapy*. 37: 1197-9.

Edelman R, and Levine, M. M., (1986). Summary of an international workshop on typhoid fever. *Reviews of Infectious Diseases.*329-47.

Ferrecio C, Levine M.M, Manterola A, Rodriguez G, Rivara I, Prenzel I, Black R, Mancuso T, and Bulas D., (1984) Benign bacteremia caused by *Salmonella typhi* and *Para typhi* in children younger than 2 years. *The Journal of Pediatrics*. 104(6): 899-901.

Ferreccio C, et al., (1988) Efficacy of ciprofloxacin in the treatment of chronic typhoid carriers. *The Journal of Infectious Diseases*. 157: 1235-9.

Frenck R.W, Nakhla I, and Sultan Y., (2000). Azithromycin versus ceftriaxone for the treatment of uncomplicated typhoid fever in children. *The Journal of Infectious Diseases.* 31: 1134-8.

FMOH. Annual Health Statistic. Federal Ministry of Health, Abuja; 2015.

Gasem M.H, Smits H.L, Nugroho N, Goris M.A, and Dolmans W.M.V., (2002). Evaluation of a simple and rapid dipstick assay for the diagnosis of typhoid fever in Indonesia. *Journal of Medical Microbiology*. 51: 173-7.

Gasem, M.H, Dolmans, W.M, Isbandrio, B.B., Wahyono, H, Keuter M, and Djokomoeljanto R. (1995). Culture of *Salmonella typhi* and *Salmonella paratyphi* from blood and bone marrow in suspected typhoid fever. *Tropical and Geographical Medicine.* 47: 164-7.

Girgis N.I, Sutan Y, Hammad O, and Farid Z., (1995). Comparison of the efficacy, safety and cost of cefixime, ceftriaxone and aztreonam in the treatment of multidrug resistant *Salmonella typhi* septicemia in children. *The Pediatric Infectious Disease Journal.* 14: 603-5.

Girgis N.I, Tribble D.R, Sultan Y, Farid Z., (1995). Short course chemotherapy with cefixime in children with multidrug resistant *Salmonella typhi* septicemia. *Journal of Tropical Pediatrics.* 41: 364-5.

Gotuzzo E, et al., (1988). Use of norfloxacin to treat chronic typhoid carriers. *Journal of Infectious Diseases.* 157: 1221-5.

Gotuzzo E, Frisancho O, Sanchez J, Liendo G, Carillo C, Black R.E, and Morris J.G., (1991) Association between the acquired immunodeficiency syndrome and infection with *Salmonella typhi* or *Salmonella paratyphi* in an endemic

typhoid area. *Archives of Internal Medicine*. 381-2.

Gupta A.,(1994). Multidrug-resistant typhoid fever in children: epidemiology and therapeutic approach. *The Pediatric Infectious Disease Journal*. 13: 124-40.

Guerra-Caceres JG, Gotuzzo-Herencia E, Crosby-Dagnino E, Miro-Quesada M, and Carillo-Parodi C., (1979). Diagnostic value of bone marrow culture in typhoid fever. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 73: 680-3.

Gupta A, Swarnkar N.K, and Choudhary S.P., (2001). Changing antibiotic sensitivity in enteric fever. *Journal of Tropical Pediatrics*. 47: 369-71.

Hatta, M, Goris M.G.A, Heerkens G.C, Gooskens J, Smits H.L., (2002). Simple dip stick assay for the detection of *Salmonella typhi*-specific immunoglobulin M antibodies and the evolution of the immune response in typhoid fever. *The American Journal of Tropical Medicine and Hygiene*. 66: 416-21.

House D, Wain J, Ho V.O, Diep T.O, Chinh N.T, Bay P.V, Vinh H, Duc M, Parry C.M, Dougan G, White N.J, Hien T.T, and Farrar J.J., (2001). Serology of typhoid fever in an endemic area and its relevance to diagnosis. *Journal of Clinical Microbiology.* 39: 1002-7.

Hoffman S.L, Edelman D.C, Punjabi N.H, Lesmana M, Cholid A, and Sundah **S,Harahap J., (1986**). Bone marrow aspirate culture superior to streptokinase clot culture and 8 ml 1 :10 blood-to-broth ratio blood culture for diagnosis of typhoid fever. *The American Journal of Tropical Medicine and Hygiene.* 35: 836-9.

Hohmann E.L, Oletta C.A, Killeen K.P, and Miller S.I. (1996). *phoP/phoQ*-deleted *Salmonella typhi* (Ty800) is a safe and immunogenic single-dose typhoid fever vaccine in volunteers. *The Journal of Infectious Diseases.* 173: 1408-14.

Ibrahim I. O., (2014). Baseline Salmonella agglutinin titres in apparently healthy freshmen in Awka, South Eastern, Nigeria. Sci. Res. Essay. p225-230.

Institute of Medicine. (1986). *New vaccine development: establishing priorities. Vol.II. Diseases of importance in developing countries.* Washington DC: National Academy Press; (Appendix D 14, p. 1-10).

Ismail A, Kader S.A, and Ong K. H., (1991). Dot enzyme immunosorbent assay for the serodiagnosis of typhoid fever. *The Southeast Asian Journal of Tropical Medicine and Public Health.* 22(4): 563-6.

Ismail T.F, Smits H.L, Wasfy M.O, Malone J.L, Fadeel M.A, and Mahoney F., (2002). Evaluation of

dipstick serological tests for diagnosis of brucellosis and typhoid fever in Egypt. *Journal of Clinical Microbiology*. 40: 3509-11.

Islam A, Butler T, Kabir I, and Alam N.H., (1993). Treatment of typhoid fever with ceftriaxone for 5 days or chloramphenicol for 14 days: a randomized controlled trial. *Antimicrobial Agents and Chemotherapy*. 37: 1572-5.

Ivanoff B.N, Levine M.M, and Lambert P.H., (1994) Vaccination against typhoid fever: present status. *Bulletin of the World Health Organization*. 957-71.

Ivanoff B, Cordel J, Robert D, and Fontanges R., (1980). Importance de la voie respiratoiredans la salmonellose expérimentale de la souris Balb/c. *Comptes Rendus de l'Académie des Sciences* (Paris). 1271-4.

Ivanoff B, Levine M., (1997). Typhoid fever: Continuing challenges from a resilient bacterial foe. *Bulletin de l'Institut Pasteur/Research on. Infectious. Diseases.* 95(3): 129-42.

Jackson A. A, Ismail A, Afifah T, Tuan Ibrahim T.A, Abdul Kader Z, Mohd N., (1995) Retrospective review of dot enzyme immunosorbent assay test for typhoid fever in an endemic area. *The Southeast Asian Journal of Tropical Medicine and Public Health.* 26: 625-30.

Jegathesan M., (1983). Phages types of *Salmonella typhi* isolated in Malaysia over the 10-year period 1970-1979. *The Journal of Hygiene.* 90(1): 91-7.

Keddy K.H, Klugman K.P, Hansford C.F, Blondeau C, and Bouveret Le Cam N.N. Persistence of antibodies to the *Salmonella typhi* Vi capsular polysaccharide vaccine in South African school children ten years after immunization. *Vaccine.* 17: 110-3.

Kossaczka Z, Lin F, Ho V, Thuy N, Bay P, Thanh T, Khiem H, Trach D, Karpas A,Hunt S, Bryla D, Schneerson R., Robbins J, and Szu S., (1999). Safety and immunogenicity of Vi conjugate vaccines for typhoid fever in adults, teenagers, and 2- to 4-year-old children in Viet Nam. *Infection and Immunity*. 67: 5806-10.

Konadu E, Lin FY, Ho V, Thuy N, Bay P, Thanh T, Khiem H, Trach D, Karapas A, Li J, Robbins J, and Szu S., (2000). Phase 1 and phase 2 studies of *Salmonella enterica* serovar *paratyphi* A O-specific polysaccharide-tetanus toxoid conjugates in adults, teenagers, and 2- to 4-year-old children in Viet Nam. *Infection and Immunity.* 68: 1529-34.

Koul P.A, Wani J.I, Wahid A., (1995). Ciprofloxacin for multi resistant enteric fever in pregnancy. *Lancet.* 346: 307-8.

Klugman K, Hendrick J, Koornhof J, Robbins J.B, and Le Cam N.,

(1996). Immunogenicity, efficacy and serological correlate of protection of *Salmonella typhi* Vi capsular polysaccharide vaccine three years after immunization. *Vaccine.* 14(5): 435-8.

Kubin R., (1993). Safety and efficacy of ciprofloxacin in paediatric patients: a review. *Infection.* 21: 413-21.

Lanata C.F, Levine M.M, Ristori C, Black R.E, Jiménez L, Salcedo M, García J, and Sotomayor V., (1983). Vi Serology in the detection of chronic *Salmonella typhi* carriers in an endemic area. *Lancet*. 441-3.

Leung D, Venkatesan P, Boswell T, Innes J.A, and Wood M.J., (1995). Treatment of typhoid in pregnancy. *Lancet.* 346: 648.

Levine M.M, Black R, Lanata C, Chilean Typhoid Committee., (1982). Precise estimation of the number of chronic carriers of *Salmonella typhi* in Santiago, Chile, an endemic area. *The Journal of Infectious Diseases*. 724-6.

Lim P.L, Tam F.C.H, Cheong Y.M, and Yegathesan M., (1998) One-step 2-minute test to detect typhoid-specific antibodies based on particle separation in tubes. *Journal of Clinical Microbiology.* 36(8): 2271-8.

Lin FY, Ho V.A, Khiem H.B, Trach DD, Bay P.V, Thanh TC, Kossaczka Z, Bryla D.A, Shiloach J, Robbins J, Shneerson R, and Szu SC., (2001). The efficacy of a *Salmonella typhi* Vi conjugate vaccine in two to five years old children. *New England Journal of Medicine.* 344: 1263-9.

Lu-Fong M, Ludan A.C, Martinez M.M, and Raymundo J.G., (1999). Dot EIA (Typhidot): an aid to the diagnosis of typhoid fever among Filipino children. *Malaysian Journal of. Child Health.* 8: 163.

Mallett S, Parry C.M, Wijedoru L., (2017). Rapid diagnostic tests for typhoid and paratyphoid (enteric) fever. Cochrane Database Syst Rev.;5:CD008892. DOI: 10.1002/14651858.CD008892.pub 2

Mehmood K, Sundus A, Naqvi I.H, Ibrahim MF, Siddique O, and Ibrahim N.F., (2015). Typhidot - A blessing or a menace. Pak J Med Sci.; 31(2):439-443. DOI:http://dx.doi.org/10.12669/pj ms.312.5934

Mitra R, Kumar N, Trigunayat A, and Bhan S., (2010). New advances in the rapid diagnosis of typhoid fever. African Journal of Microbiology Research. ;4(16):16761677.

Murdoch D.A, Banatvala N, Shoismatulloev B.I, Ward LR, Threlfall E.J, and Banatvala N.A., (1998). Epidemic ciprofloxacin-resistant *Salmonella typhi* in Tajikistan. *Lancet.* 351: 339.

Pang T., (1989). False positive Widal test in nontyphoid Salmonella infection. *Southeast Asian Journal of Tropical Medicine and Public Health.* 20: 163-4.

Pruss U. (2006) Preventing diseases through healthy environment towards an estimate of the environmental burden of diseases. World Health Organization, France.

Punjabi N.H., (1998) Cost evaluation of typhoid fever in Indonesia. *Medical Journal of Indonesia.* 90-3TR.

Phoung C.X.T, Kneen R, Anh N.T, Luat T.D, White N.J, Parry C.M, Dong Nai Paediatric Center Typhoid Study Group., (1999). A comparative study of ofloxacin and cefixime for treatment of typhoid fever in children. *The Pediatric Infectious Disease Journal.* 18: 245-8.

Popoff M. Y, Le Minor L., (1997) *Antigenic formulas of the salmonella serovars.* Seventh edition. Paris: Pasteur Institute.

Punjabi N.H., (2000). Typhoid fever. In: Rakel RE, editor. *Conn's Current therapy*. Fifty-second edition. Philadelphia: WB Saunders. p.161-5.

Punjabi N.H, Hoffman S. L, Edman D.C, et al., (1988). Treatment of severe typhoid fever in children with high dose dexamethasone. *The Pediatric Infectious Disease Journal.* 7: 598-600.

Rasul F, Sughra K, Mushtaq A, Zeeshan N, Mehmood S, and Rashid U., (2008). Surveillance report on typhoid fever epidemiology and risk factor assessment in district Gujrat, Punjab, Pakistan. Biomedical Research.;28(8):1-6.

Reuben C, Rine H, Suleiman C, and Jatau A.G., (2013). Retrospective and cross-sectional studies of typhoid fever in pregnant women in a community in Central Nigeria. International Journal of Advanced Research 3: 66-72.

Rogerson S.J, Spooner V.J, Smith T.A, Richens J., (1991). Hydrocortisone in chloramphenicol-treated severe typhoid fever in Papua New Guinea. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 85: 113-6.

Rowe B, Ward L.R, and Threlfall E.J., (1997). Multidrug-resistant *Salmonella typhi*: a worldwide epidemic. *Clinical Infectious Diseases*. 24(Suppl 1): S106-9.

Rukunga G.K. (2011), Environmental Health for East Africa. African Medical and Research Foundation (AMREF) Nairobi, Kenya.

Saha S.K, Baqui A.H, Hanif M, Darmstadt G.L, Ruhulamin M, Nagatake T, Santosham M, and Black R., (2001). Typhoid fever in Bangladesh: implications for vaccination policy. *The Pediatric Infectious Disease Journal*.521-4.

Saha S.K, Talukder S.Y, Islam M, and Saha S., (1999). A highly ceftriaxone resistant *Salmonella. typhi* in Bangladesh. *The Pediatric Infectious Disease Journal.* 18(3): 297-303.

Saha S.K, Saha S, Ruhulamin M, Hanif M, and Islam M., (1997). Decreasing trend of multi resistant *Salmonella typhi* in Bangladesh.

The Journal of Antimicrobial Chemotherapy. 39: 554-6.

Seoud M, Saade G, Uwaydah M, Azoury R. Typhoid fever in pregnancy. *Obstetrics and Gynecology* 1988; 71: 711-4.

Sinha A, Sazawal S, Kumar R, Sood S, Reddaiah V.P, Singh B, Rao M, Naficy A, Clemens J, and Bhan M.K., (1999). Typhoid fever in children aged less than 5 years. *Lancet.* 354: 734-737.

Smith S.I., Alao F., Goodluck H.T., Fowora M., Bamidele M., Omonigbehin E., and Coke A.O., (2010) Abeokuta, South-Western Nigeria. British Journal of Pharmacology and Toxicology. 1(1):6-14.

Soewandojo E, Suharto U, Hadi U, Frans P, and Prihartini E., (1998). Comparative results between bone marrow culture and blood culture in the diagnosis of typhoid fever. *Medical Journal of Indonesia.* 7(S1): 209.

Tacket CO, Hone DM, Losonsky G, Guers L, Edelman R, and Levine M.M. Clinical acceptability and immunogenicity of CVD 908 *Salmonella typhi* vaccine strain. *Vaccine* 1992; 10(7): 443-6.

Tacket C.O, Sztein M.B, Losonsky G.A, Wasserman S.S, Nataro J.P, Edelman R,Pickard D, Dougan G, Chatfield S.N, and Levine M.M., (1997). Safety and immune response in humans of live oral *Salmonella typhi* vaccine strains deleted in *htrA* and *aroC, aroD*. *Infection and Immunity.* 65: 452-6.

Tacket CO, Kelly SM, Schodel F, Losonsky G, Nataro J.P, Edelman R, Levine M, and Curtiss R., (1997). Safety and immunogenicity in humans of an attenuated *Salmonella typhi* vaccine vector strain expressing plasmid-encoded hepatitis B antigens stabilized by the ASD balanced lethal system. *Infection and Immunity*. 65(8): 3381-5.

Tarr P.E, Kuppens L, Jones T.C, Ivanoff B, and Heymann D.L., (1999). Considerations regardingmass vaccination against typhoid fever as adjunct to sanitation and public health measures. *The American Journal of Tropical Medicine and Hygiene.* 61:163-70.

Threlfall E.J, Ward L.R, Skinner J.A, Smith H.R, and Lacey S., (1999). Ciprofloxacin-resistant *Salmonella typhi* and treatment failure. *Lancet.* 353: 1590-1.

Thisyakorn U, and Mansuwan P., (1992). Comparative efficacy of mecillinam, mecillinam/amoxycillin and trimethoprim-suphamethoxazole for treatment of typhoid fever in children. *The Pediatric Infectious Disease Journal.* 11: 979-80.

Tuise F. (2015) Safety and immunogenicity of vi conjugate vaccines for typhoid fever in adults, teenagers, and 2- to 4-year-old children in Viet Nam. Infection and Immunity; 67:5806-5810.

Twort A., (2009) Water supply (Third edition). Holder and Stonington Limited, London.

Udujih H., Ibe S.N., Udujih O.G., Iwuala C.C., Edward U.C., and Kenechukwudozie Q.O., (2017). Salmonella infection among undergraduate students of Imo State University Owerri, Nigeria. ASJ: International Journal of Health, Safety and Environments; 3(6):128-133.

Van Basten JP, and Stockenbrugger R., (1994). Typhoid perforation. A review of the literature since 1960. *Tropical and Geographical Medicine.* 46: 336-9.

Vallenas C, Hernandez H, Kay B, Black R, and Gotuzzo E., (1985) Efficacy of bone marrow, blood, stool and duodenal contents cultures for bacteriologic confirmation of typhoid fever in children. *Pediatric Infectious Disease. (*5): 496-8.

Wain J, Diep T.S, Ho V.A, Walsh A.M, Hoa T.T.N, Parry C.M, and White N.J, (1998). Quantitation of bacteria in blood of typhoid fever patients and relationship between counts and clinical features, transmissibility, and antibiotic resistance. *Journal of Clinical Microbiology.* 36: 1683-7.

Wain J, Bay P.V, Vinh H, Duong N.M, Diep T.S, Walsh A.L, Parry C.M, Hasserjian R.P, Ho V.A, Hien TT, Farrar J, White N.J, and Day N.P., (2001). Quantitation of bacteria in bone marrow from patients with typhoid fever; relationship between counts and clinical features. *Vaccine*; 39: 1571-6.

WHO. Common-source water-borne outbreak of multidrug-resistant typhoid Organization (WHO) Geneva, Switzerland. 2015.

WHO. Endemic typhoid fever- a diagnostic pitfall. World Health Organization. Geneva, Switzerland; 2012.

Yang H.H, Wu C.G and Xie G.Z. (2001). Efficacy trial of Vi polysaccharide vaccine against typhoid fever in southwestern China. *Bulletin of the World Health Organization*; 79 (7):625-631 .

Yang H.H, Kilgore P.E, Yang L.H, Park J.K, Pan Y.F, Kim Y, Lee Y.J, Xu Z.Y, and Clemens J. (2001). An outbreak of typhoid fever, Xing-An County, People's Republic of China, 1999: Estimation of the field effectiveness of Vi polysaccharide typhoid vaccine. *The Journal of Infectious Diseases.* 183: 1775-80.