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Prevalence of *Salmonella typhi* among Patients in Abeokuta, South-Western Nigeria

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Abstract: This study reports on the prevalence of typhoid fever between genders among patients in Abeokuta, Nigeria. Typhoid fever caused by *Salmonella typhi* is an endemic disease in the tropic and sub-tropic and has become a major public health problem in developing countries of the world with an estimated annual incidence of 540 per 100,000. The annual incidence of typhoid is estimated to be about 17 million cases worldwide. It is often encountered in tropical countries including Nigeria where they constitute serious sources of morbidities and mortalities. Blood samples were collected from 840 apparently healthy people; 460 (54.8%) females and 380 (45.2%) males. The samples were examined for the presence and levels of *Salmonella typhi* antibodies by Widal agglutination technique. The standard Samonella 'O' and 'H' suspension (ANTEC diagnostic products) were used as antigens. Of the 840 sera tested, agglutinins to *Salmonella typhi* were most prevalent in female subjects accounting for [426(92.6%)] of the 'H' antigens and [322(70.0%)] of 'O' antigens at the various dilutions while in the male subjects, [351(92.4%)] accounts for the 'O' and [327(86.1%)] for the 'H' antigens. There was a female preponderance (F/M 2:1). The levels of agglutinin of *Salmonella paratyphi* C-H [93(24.5%)] and *Salmonella typhi* C-O [112(29.5%)] in the males were however, low. In the females, the low significant agglutinin titres for *Salmonella typhi* O and *Salmonella paratyphi* A-O were observed in 27.6% and 36.1% of the sera respectively. The results of this study showed that more males had *Salmonella agglutinin* titres for *S. typhi* O [351(92.4%)] and *S. typhi* H [327(86.1%)]. More so, 132 (34.7%) males had *Salmonella agglutinin* titres for *S. paratyphi* A-O, 119 (31.3%) for *S. paratyphi* B-O, 112 (29.5%) for *S. paratyphi* C-O, 117 (31.0%) for *S. paratyphi* A-H, 125 (33.0%) for *S. paratyphi* B-H, and 93 (24.5%) for *S. paratyphi* C-H. It also showed that more females had *Salmonella agglutinin* titres for *S. typhi* H [426 (92.6%)] followed by *S. typhi* O [322(70.0%)], *S. paratyphi* B-H [168 (36.5%)], *S. paratyphi* B-O [163(35.4%)], *S. paratyphi* B-O [147(32.0%)], *S. paratyphi* C-O [145(31.5%)], *S. paratyphi* A-H [142 (31.0%)], and *S. paratyphi* C-H [130 (28.3%)]. Since the positive sera with titres of less than 1:80 occurred in more than 5% of the samples tested, this study therefore suggests that such titres be regarded as normal among the communities studied while there should a high index of suspicion of clinical infections in titres above 1:80 when a second serum is impractical. The findings of this study further establishes the *Salmonella typhi* titres that are not diagnostically significant but normal in the study population and the titre that could be used as presumptively diagnostic of typhoid fever. This will improve accurate diagnosis. Improving accurate diagnosis is the surest way to reverse the deteriorating

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health status of Nigerians. Poor diagnosis leads to emergence of resistant strains of diseases. It also further establishes the *Salmonella typhi* titres that are not diagnostically significant but normal in the study population and the titre that could be used as presumptively diagnostic of typhoid fever.

Key words: Agglutinin, *Salmonella tyhi*, *Salmonella paratyhi*, typhoid fever, widal test, Nigeria

INTRODUCTION

Typhoid fever (enteric fever) caused by *Salmonella typhi* is an endemic disease in the tropic and sub-tropic and has become a major public health problem in developing countries of the world with an estimated annual incidence of 540 per 100,000. The annual incidence of typhoid is estimated to be about 17 million cases worldwide (WHO, 2008). It is often encountered in tropical countries including Nigeria where they constitute serious sources of morbidities and mortalities (Ibekwe *et al*., 2008). Typhoid and paratyphoid fevers are infections caused by bacteria, which are transmitted from faeces to ingestion. Clean water, hygiene and good sanitation prevent the spread of typhoid and paratyphoid. Contaminated water is one of the pathways of transmission of the disease (WHO, 2008). Typhoid and paratyphoid fevers are caused by the bacteria *Salmonella typhi* and *Salmonella paratyphi*, respectively. *Salmonella typhi* is Gram-negative bacteria, which are motile, though non-flagellate variants, occur. Capsules are not formed. They are intestinal pathogens, which comprises of a species *Salmonella typhi*, which causes an enteric fever known as typhoid fever (Philip, 2000). It is pathogenic to both man and mammals with associable inflammatory reaction in the intestinal tract.

Typhoid fever is among the water-borne infections (Singh and Mcfeters, 1992) characteristic of environments with poor sanitation and hygiene. It is a health problem that has been associated with development (Jegathesan, 1984). Human infection with *Samonella* is mainly by the oral route through ingestion of faecal contaminated food and water, unclean hands, flies and meat from infected animals (Carol *et al*., 1989). Typhoid and paratyphoid germs are passed in the faeces and urine of infected people. People become infected after eating food or drinking beverages that have been handled by a person who is infected or by drinking water that has been contaminated by sewage containing the bacteria. Once the bacteria enter the person's body they multiply and spread from the intestines, into the bloodstream (WHO, 2008). Even after recovery from typhoid or paratyphoid, a small number of individuals (called carriers) continue to carry the bacteria. These people can be a source of infection for others. The transmission of typhoid and paratyphoid in less-industrialized countries may be due to contaminated food or water. In some countries, shellfish taken from sewage-contaminated beds is an important route of infection. Where water quality is high, and chlorinated water piped into the house is widely available, transmission is more likely to occur via food contaminated by carriers handling food (WHO, 2008). Infection through contaminated surgical equipment and person-to-person contact in hospital has also been reported (Carol *et al*., 1989).

Salmonella typhi have somatic antigens and glycolipid microcapsule the vi or virulence antigen. Phage typing can distinguish different strains of the organism. Enteric fever caused by *Salmonella typhi* is often encountered in tropical countries including Nigeria where they constitute serious sources of morbidities and mortalities (Baver, 1995). It is a major public health problem in the developing countries of the world with an estimated annual incidence of 540 per 100,000 (Ibekwe *et al*., 2008). Salmonella are divided into distinct serologic groups (A through E) on the basis of their somatic O antigens. While all group D organisms, such as *S. typhi* possess O antigen 9, about 60 of the 78 groups D serotypes including *S. typhi* also have O antigen 12 (Hook, 1985). Thus, infection by any of the group D serotypes can produce antibodies that can react with the O antigen used in the Widal reaction (Olopoenia *et al*., 2000). Also, since all groups A and B organisms possess O antigen 12, cross-reactions with O antibody of group D serotype can occur with any of the group A and B serotype O antigens. Depending on the relative quality and quantity of antigenicity of the O antigens 9 and 12 contained in other common non-typhoidal *Salmonella serotypes*, cross-reaction may occur frequently enough to lessen considerably the diagnostic specificity of the Widal agglutination reaction (Olopoenia *et al*., 2000). In endemic areas, most individuals are careers. Thus, 35.9% of such apparently healthy persons have been detected with normal antibody titres of up to 1:40 and 1:80 for O and H *Salmonella antigens* (Tanyigna *et al*., 1999) and the levels reflected severity of infection with *Salmonella*. Even though the associable mortality rate is very low, the high prevalence of salmonellosis has caused major economic and health impacts. As such, vaccines have been developed against strains of *Salomonella* (Myron *et al*., 1976).

Based on the immunology of *Salmonella* infection, serological diagnostic tests relying on *Salmonella antigens* as a tentative evidence of salmonellosis have been developed, notably, the Widal agglutination test (Outi *et al*., 1989). Agglutination is a classic serologic reaction that results in clumping of a cell suspension by a specific antibody, directed against a specific antigen. Such tests have been widely used for detection of antibodies against various disease-producing microorganisms in

serum for a long time (Olopoenia *et al*., 2000). The Widal agglutination test, developed by Widal in 1896 to aid in the diagnosis of typhoid fever, utilizes a suspension of killed *Salmonella typhi* as antigen, to detect typhoid fever in serum from suspected S typhi-infected patients who present with febrile illness. The value and clinical application of the Widal test in developed countries has diminished considerably in recent years (Washington and Henry, 1991) and a large number of antigenically related determinants of both typhoid and non-typhoid *Salmonella* organisms are now recognized (Olopoenia *et al*., 2000).

The Widal test is a presumptive serological test for Enteric fever or Undulant fever. In case of *Salmonella* infections, it is a demonstration of agglutinating antibodies against antigens O-somatic and H-flagellar in the blood. Two types of agglutination techniques are available: the slide test and the tube test. The slide test is rapid and is used as a screening procedure. Using commercially available antigens of *S. typhi*, a drop of the suspended antigen is added to an equal amount of previously prepared serum. An initial positive screening test requires the determination of the strength of the antibody. This is done by adding together equal amounts of antigen suspension and serially diluted serum from the suspected patient. Agglutinations are visualised as clumps. Weakly reactive agglutinations may require an adequate light source for proper visualisation, while strongly reactive agglutinations are easily seen. The result of the tests is scored from 0 to 4+, i.e., 0 (no agglutination), 1+ (25% agglutination), 2+ (50% agglutination), 3+ (75% agglutination) or 4+ (100% agglutination). The smallest quantity of serum that exhibits a 2+ or 50% agglutination is considered the end-point of serum activity or titre (Olopoenia *et al*., 2000).

The tube agglutination test requires much more technical work than the rapid slide test, and is a macroscopic test (Gaultney *et al*., 1971). It also serves as a means of confirming the results of the slide test. A mixture of suspended antigen and antibody is incubated for up to 20 h at 37ºC in a water bath. Agglutinations are visualised in the form of pellets, clumped together at the bottom of the test tube. Results are scored from 0 to 4+ positive agglutination as described above for the slide test. The tube test is useful to clarify erratic or equivocal agglutination reactions obtained by the more rapid slide test (Olopoenia *et al*., 2000). Widal agglutination was introduced as a serologic technique to aid in diagnosis of typhoid fever. The test was based on demonstrating the presence of agglutinin (antibody) in the serum of an infected patient, against the H (flagellar) and O (somatic) antigens of *Salmonella typhi* (Olopoenia *et al*., 2000). It is not a very accurate method, since patients are often exposed to other bacteria (e.g. *Salmonella enteritidis*, *Salmonella typhimurium*) in this species that induce cross-reactivity; many people have antibodies against these enteric pathogens, which also react with the antigens in the Widal test, causing a false-positive result. Test results need to be interpreted carefully in the light of past history of enteric fever, typhoid vaccination, and general level of antibodies in the populations in endemic areas of the world.

Other means of diagnosing *Salmonella typhi* (and paratyphi) include cultures of blood, urine and feces. The organism also produces H_2S from thiosulfate. Often 2-marceptoethanol is added. This agent binds to the IgM class of antibodies, so if a decrease in the titer is seen after using this agent, it means that it's IgM that's high but not IgG. This differentation of antibody classes is important; as it allows for the distinction of a recent (IgM) from an old infection (IgE). Typhidot is the other test used to ascertain the diagnosis of typhoid fever. As with all serological tests, the rise in antibody levels needed to make the diagnosis takes 7-14 days, which limits their use (Olopoenia *et al*., 2000). While the definitive diagnosis of typhoid fever depends on the isolation of *S. typhi* from blood, stools, urine or other body fluids (Gilman *et al*., 1975; Manson-Bahr and Bell, 1987), the role of the Widal test had been to increase the index of suspicion for the presence of typhoid fever by demonstrating a positive agglutination during the acute and convalescent period of infection with evidence of a four-fold rise of antibody titre (Somerville *et al*., 1981).

In developed countries, the use of Widal agglutination as a laboratory tool to aid in the diagnosis of typhoid fever during the acute phase of the illness has largely been abandoned (Washington and Henry, 1991), as the need for such a test is minimal, especially in view of the low prevalence of typhoid fever. In addition, adequate and improved sanitation, sewage systems, proper hygiene and better means of isolating the organism from culture are available (Olopoenia *et al*., 2000). Unfortunately, in some developing countries, the situation is quite different, and the Widal test appears to be the only laboratory means employed in the diagnosis of typhoid fever among suspected patients (Olopoenia *et al*., 2000). As the test suffers from serious cross-reactivity with other infectious agents, it may produce false-positive results, leading to an over-diagnosis of typhoid fever. Reynolds *et al*. (1970) concluded that diagnosis of typhoid fever based on serology (Widal agglutination) alone is frequently inaccurate. Concomitant with this increase in diagnosis is the abuse of the first-line drug of choice (chloramphenicol), which has led to the selection of resistant strains of *S. typhi* (Olopoenia *et al*., 2000).

Over 100 years since its introduction as a serologic means of detecting the presence of typhoid fever, the Widal test continues to be plagued with controversies involving the quality of the antigens used and interpretation of the result, particularly in endemic areas. The significance of the Widal agglutination test in the

diagnosis of typhoid fever has been reviewed by Olopoenia *et al.* (2000). Georges-Fernand Widal test if TO antigen more than 1.160 in active infection widal test if TH antigen more than 1.160 in past infection or in immunized (Olopoenia *et al.*, 2000). Areas of concern with clinical and laboratory significance of Widal test has been reviewed in the past and this include: the techniques of test performance, interpretation of results, limitation of the value of the test results in endemic typhoid areas, the quality of the antigens used, and alternative diagnostic tests. Similarly, the reliability of serologic test in solely diagnosing typhoid fever has suffered doubt. It has been reported to remain positive, months after an effective therapy of the infection (Outi *et al.*, 1989) such that a positive test may not necessarily indicate active infection, making the test be of relevance in diagnosing post-infection complications. Also, the quality of *Salmonella antigens* and interpretation of results, specifically in the Widal agglutination test have been identified as areas of controversy (Olopoenia *et al.*, 2000), hence, the suitability of stool culture alongside serologic test in diagnosing active infection (Adeleke *et al.*, 2006).

In Nigeria, the Widal agglutination test is about the sole laboratory diagnostic tool employed to buttress clinical diagnosis of enteric fever for the purpose of directing therapeutic measures specifically against this malady (Ibekwe *et al.*, 2008). As is generally known, the results of this serological test only become reliable if at least two properly staggered tests show about four-fold rise in antibody levels (Gilles, 1975). While performance of the test may require some detailed technical work, interpreting the test result is the more arduous task (Olopoenia *et al.*, 2000). Since the ultimate goal of the test is antigen-antibody complex reaction, cross-reactions are encountered when antibody produced by non-typhoidal antigens reacts with typhoid-specific antigens. Several other diseases caused by non-Salmonella organisms (malaria, dengue, miliary tuberculosis, endocarditis, chronic liver disease, brucellosis, etc) have been shown to exhibit this cross-reactivity in typhoid endemic regions, and these cross-reactions increase the error rate of the result of the Widal test (Olopoenia *et al.*, 2000). However, the scientific turism remains that only the bacteriological isolation of enteric fever bacteria from the patients' blood, faeces or urine constitute unequivocal evidence of the infection (Opera and Nweke, 1991; Ibekwe *et al.*, 2008).

The use of the Widal test to diagnose typhoid fever should therefore be limited to situations in which there is no other confirmatory supportive test, such as positive culture, available (Olopoenia *et al.*, 2000). Similarities between typhoidal and non-typhoidal *Salmonella antigens* mean that a serological method of diagnosis is the least accurate for typhoid fever. Due to the inexperience of some clinicians in typhoid endemic countries, many cases of pyrexia of unknown origin receive the diagnosis of typhoid fever, based upon a false-positive Widal test result rather than a positive culture of *S. typhi* (Olopoenia *et al.*, 2000). In Nigeria, the harsh economic climate has encouraged a cancerous rate of household production of various food products with the attendant risk to public health (Adeleke *et al.*, 2006). As a matter of fact, the unreported cases of water-borne infections, particularly typhoid fever, have been more than those reported to hospitals for treating 7 female patients (77 samples) of different age groups (Adeleke *et al.*, 2006). Thus, this study reports on the prevalence of typhoid fever caused by *Salmonella typhi* between genders in Abeokuta, Nigeria. It also further establishes the *Salmonella typhi* titres that are not diagnostically significant but normal in the study population and the titre that could be used as presumptively diagnostic of typhoid fever.

MATERIALS AND METHODS

Study area: This study was conducted among patients attending the Department of Health Services, University of Agriculture, Abeokuta, South-western, Nigeria. The University of Agriculture, Abeokuta with the acronym UNAAB is one of the three Universities of Agriculture in Nigeria. It was established in January 1988. The University started at its mini-campus in Isale-Igbein right in the heart of Abeokuta, the Capital of Ogun State located in the forest zone of Southwestern Nigeria, which borders Lagos State to the South, Oyo and Osun states to the North, Ondo State to the east and the republic of Benin to the west. The University moved in December 1997 to its permanent site, a 10,000-hectare Campus, which is located next to the Ogun-Oshun River Basin Development Authority on the Abeokuta-Ibadan road in the North Eastern end of the city, 15 km from Abeokuta City Centre.

Sample collection: This study was conducted from January 2002 to December 2004. A total of 840 blood samples were collected from 840 patients (460 females and 380 males) attending the Department of Health Services, University of Agriculture, Abeokuta, South-western, Nigeria. The samples were collected in sterile containers and transported to the laboratory for processing and to be analyzed. The samples were obtained by informed consent of the patients used for this study and the permission to that effect was obtained from the ethical committee. Two milliliters of the blood samples were centrifuged at a high speed for 5 min in order to separate the serum from the blood cells.

Widal agglutination test: ANTEC febrile antigen kit (United Kingdom) was used for the Widal test. The rapid slide screening test was first carried out, followed by the

Table 1: Incidence of Widal positive sera (*Salmonella agglutinin* titres) in relation to sexes of the subjects in Abeokuta, South-Western Nigeria

Sex	No. of sera tested (%)	No. of widal positive (%)	No. of widal negative (%)
Female	460(54.8)	322(70.0)	138(30.0)
Male	380(45.2)	351(92.4)	29(07.6)
Total	840(100.0)	673(80.1)	53(13.9)

Table 2: Distribution of *Salmonella agglutinin* titres in 380 male subjects in Abeokuta, South-Western Nigeria

Salmonellae	No. of sera tested	No. of widal positive (%)	No. of widal negative (%)
S. paratyphi A-O	380	132(34.7)	248(65.3)
S. paratyphi B-O	380	119(31.3)	261(68.7)
S. paratyphi C-O	380	112(29.5)	268(70.5)
S. typhi O	380	351(92.4)	29(07.6)
S. paratyphi A-H	380	117(31.0)	263(69.0)
S. paratyphi B-H	380	125(33.0)	255(67.0)
S. paratyphi C-H	380	93(24.5)	287(75.5)
S. typhi H	380	327(86.1)	53(13.9)

tube agglutination test according to the manufacturer's specifications. The ANTEC febrile antigens are suitable for both the rapid slide and tube agglutination tests against human sera for the detection of these agglutinins. The stained antigen suspensions are killed bacteria, stained to enhance the reading of agglutination tests. The blue stained antigens are specific to the somatic 'O' antigens whilst the red stained antigens are specific to the flagellar 'H' antigens. Using a pipette, 0.08, 0.04, 0.02, 0.01 and 0.005 ml of undiluted serum was dispensed onto a row of 3cm diameter circles. The reagent bottle was rigorously shaken and a drop of the undiluted antigen suspension was added to each serum aliquot. This was thoroughly mixed with the aid of a stirring stick and the slide was gently rotated. The reactions were observed after a minute. The agglutination observed in any circle was indicative of the following results in a test tube. 0.08 ml = 1:20, 0.04 ml = 1:40, 0.02 ml = 1:80, 0.01 ml = 1:160 and 0.005 ml = 1:320.

RESULTS

The distribution of *Salmonella agglutinin* titres obtained from 840 (100%) subjects' sera from the University Health Centre; 460 (54.8%) females and 380 (45.2%) males is shown in Table 1-5. Table 1 shows the incidence of Widal positive sera (*Salmonella agglutinin* titres) in relation to sexes of the subjects in Abeokuta, South-Western Nigeria. It showed that 673 (80.1%) of the samples analyzed for *Salmonella agglutinin* titres were Widal positive while 53 (13.9%) were Widal negative as shown in Table 1. It also showed that 92.4% (n = 351) of the sera from males were Widal positive and 70.0% (n = 322) of the sera from females were also Widal positive (Table 1).

Table 2 shows the distribution of *Salmonella agglutinin* titres in 380 male subjects in Abeokuta, South-Western Nigeria. The result showed that more males had *Salmonella agglutinin* titres for *S. typhi* O [351(92.4%)] and *S. typhi* H [327(86.1%)]. More so, 132 (34.7%) males had *Salmonella agglutinin* titres for *S. paratyphi* A-O, 119 (31.3%) for *S. paratyphi* B-O, 112 (29.5%) for *S. paratyphi* C-O, 117 (31.0%) for *S. paratyphi* A-H, 125 (33.0%) for *S. paratyphi* B-H, and 93 (24.5%) for *S. paratyphi* C-H as shown in Table 2.

Table 3 shows the frequency and percentage of sera with end-titres in 380 male subjects in Abeokuta, South-Western Nigeria. It was observed that with exception of *S. paratyphi* B-O and *S. paratyphi* A-H, *S. paratyphi* B-H, and *S. paratyphi* C-H, all other agglutinins tested were present in the sera of males up to the titre of 320 and at frequencies/percentages ranging from 1 (0.3%)- 351 (92.4%). However, the frequency of *Salmonella agglutinins* titre of <1:80 ranged from 4(1.1%) to 34(27.2%); 1:80 from 46(13.1%) to 178(54.4%); 1:160 from 7(6.0%) to 189(53.8%) and 1(0.3%) to 29(8.3%) for 1:320. The results showed that titres of 1:80 and 1:160 occurred in a significant proportion of the samples as shown in Table 3.

Table 4 shows the distribution of *Salmonella agglutinin* titres in 460 female subjects in Abeokuta, South-Western Nigeria. The result also showed that more females had *Salmonella agglutinin* titres for *S. typhi* H [426 (92.6%)] followed by *S. typhi* O [322(70.0%)], *S. paratyphi* B-H [168 (36.5%)], *S. paratyphi* B-O [163(35.4%)], *S. paratyphi* B-O [147(32.0%)], *S. paratyphi* C-O [145(31.5%)], *S. paratyphi* A-H [142 (31.0%)], and *S. paratyphi* C-H [130 (28.3%)] as shown in Table 4.

Table 5 shows the frequency and percentage of sera with end-titres in 460 female subjects in Abeokuta, South-Western Nigeria. It was observed that with exception of *S. paratyphi* A-H, *S. paratyphi* B-H and *S. paratyphi* C-H, all other agglutinins tested were present in the sera of females up to the titre of 320 and at frequencies/percentages ranging from 4(0.9%)-322(100.0%). However, the frequency of *Salmonella agglutinins* of <1:80 titre ranged from 5(1.6%) to 26(17.9%); 1:80 from 53(36.1%) to 307(72.1%); 1:160 from 19(11.3%) to 322(100.0%) and 4(0.9%) to 42(13.0%) for 1:320. The results showed that titres of 1:80 and 1:160 occurred in a significant proportion of the samples as shown in Table 5.

Table 3: Number and percentage of sera with end titres in 380 male subjects in Abeokuta, South-Western Nigeria

		End titres (%)			
Salmonellae	No. of widal positive (%)	< 80	80	160	320
S. paratyphi A-O	132(34.7)	14(10.6)	59(45.0)	39(29.5)	03(02.3)
S. paratyphi B-O	119(31.3)	13(10.9)	42(35.3)	34(28.6)	00(00.0)
S. paratyphi C-O	112(29.5)	28(25.0)	36(32.1)	21(18.8)	04(03.6)
S. typhi O	351(92.4)	04(01.1)	46(13.1)	189(53.8)	29(08.3)
S. paratyphi A-H	117(31.0)	08(06.8)	60(51.3)	07(06.0)	00(00.0)
S. paratyphi B-H	125(33.0)	34(27.2)	61(48.8)	10(08.0)	00(00.0)
S. paratyphi C-H	93(24.5)	18(19.3)	50(53.8)	07(07.5)	00(00.0)
S. typhi H	327(86.1)	20 (6.1)	178(54.4)	42(12.8)	01(00.3)

Table 4: Distribution of *Salmonella agglutinin* titres in 460 female subjects in Abeokuta, South-Western Nigeria

Salmonellae	No. of sera tested	No. of widal positive (%)	No. of widal negative (%)
S. paratyphi A-O	460	147(32.0)	313(68.0)
S. paratyphi B-O	460	163(35.4)	297(64.6)
S. paratyphi C-O	460	145(31.5)	315(68.5)
S. typhi O	460	322(70.0)	138(30.0)
S. paratyphi A-H	460	142(31.0)	318(69.0)
S. paratyphi B-H	460	168(36.5)	292(63.5)
S. paratyphi C-H	460	130(28.3)	330(71.7)
S. typhi H	460	426(92.6)	34(07.4)

Table 5: Number and percentage of sera with end titres in 460 female subjects in Abeokuta, South-Western Nigeria

		End titres			
Salmonellae	No. of positive (%)	< 80	80	160	320
S. paratyphi A-O	147 (32.0)	17(11.6)	53(36.1)	68(46.3)	15(10.2)
S. paratyphi B-O	163 (35.4)	14(08.6)	71(43.6)	75(46.0)	04(02.5)
S. paratyphi C-O	145 (31.5)	26(17.9)	68(47.0)	47(32.4)	04(02.8)
S. typhi O	322 (70.0)	05(01.6)	89(27.6)	322(100.0)	42(13.0)
S. paratyphi A-H	142 (31.0)	12(08.5)	110(77.5)	13(09.2)	00(00.0)
S. paratyphi B-H	168 (36.5)	25(15.0)	124(74.0)	19(11.3)	00(00.0)
S. paratyphi C-H	130 (28.3)	16(12.3)	95(73.1)	19(14.6)	00(00.0)
S. typhi H	426 (92.6)	09(02.1)	307(72.1)	107(25.1)	04(00.9)

DISCUSSION

In this study, 673(80.1%) of the 840(100.0%) blood samples gave positive Widal reaction. This indicates a high prevalence of typhoid fever in the sampled population. However, some of the subjects may not be having the active disease. This is in agreement with the observations of Outi *et al.* (1989) and Adeleke *et al.* (2006) in a similar study on Widal reaction as being more relevance in diagnosing post-infection complications when *S. typhi* may not be isolated. The Widal test reaction involves the use of bacterial suspensions of *S. typhi* and *S. paratyphi* `A' and `B', treated to retain only the `O' and `H' antigens. These antigens are employed to detect corresponding antibodies in the serum of a patient suspected of having typhoid fever. The IgM somatic O antibody appears first and represents the initial serologic response in acute typhoid fever, while the IgG flagella H antibody usually develops more slowly but persists for longer (Hoffman *et al.*, 1986; Washington and Henry, 1991; Olopoenia *et al.*, 2000). While bacteriological culture remains the gold standard for definitive diagnosis of typhoid fever, lack of its immediate availability during the acute febrile illness may limit its use. In an acute febrile illness in an endemic typhoid region where the clinical picture is ambiguous, a rapid, accurate, specific and sensitive test should be used to differentiate typhoidal from non-typhoidal febrile illnesses. Clinicians usually elect to treat, rather than wait for blood or stool culture results, which may take 3-5 days. While there might be some merit in this approach, particularly in areas where culture facilities are either poor or not available, and where Widal testing is the norm, the use of rapid antigen screening directly from the stool of the suspected patient would be more useful (Olopoenia *et al.*, 2000).

Also in this study, more sera from males were more Widal positive than sera from females. This is probably as a reflection of different eating habits and level of personal hygiene. This is also in agreement with the findings of Adeleke *et al.* (2006). In 380 males, the titre of Salmonella 'O' were higher than those of the 'H' whereas in 460 females, Salmonella 'H' titres were higher than those of 'O'. This differs from what was reported in a similar study by Ibekwe *et al.* (2008) where 82 apparently normal males had higher titre of Salmonella 'H' and 118 apparently normal females had higher Salmonella 'O' titres (Ibekwe *et al.*, 2008). Agglutinins to *S. typhi* were the most prevalent among the sera tested at various dilutions in both males and females. Three hundred and fifty-one [351(92.4%)] had higher titre for *S. typhi* 'O'

and 327 (86.1%) for *S. typhi* 'H' than in the females with 322 (70.0%) for the *S. typhi* 'O' and 426 (92.6%) for the *S. typhi* 'H'. Agglutinin level for the typhoid and paratyphoid group tested in this study were evidently very frequently found in the sera of the subjects. The levels of agglutinin of *Salmonella paratyphi* C-H [93(24.5%)] and *Salmonella typhi* C-O [112(29.5%)] in the males were however, low. Agglutinin titres of 80 were observed in only 13.1 and 32.1% for *Salmonella typhi* O and *Salmonella paratyphi* C-O respectively. In the females, the low significant agglutinin titres for *Salmonella typhi* O and *Salmonella paratyphi* A-O were observed in 27.6 and 36.1% of the sera respectively.

The value of Widal test depends upon the standardization and maintenance of the antigens to produce consistent results, and it has become evident from work done in recent years on standardization of the Widal test and interpretation of the results that both the O and H antigens are necessary for proper serologic analysis of the suspected serum. However, according to Welch in 1936 (reviewed in Olopoenia *et al*., 2000), no Widal test, regardless of the composition and standardization of the antigens used, is infallible, and thus it is unlikely that any will be developed that will lower the validity of the isolation of the aetiologic agent. Sansone *et al.* (1972) published a case report where the Widal reaction to typhoid O antigen on admission for an unexposed patient was 1:320, with an increase in titre to 1:20 480 by the fourth day. In an individual with no prior exposure to *S. typhi* infection (either lack of active infection or absence of passive immunization), a higher than 1:50 or 1:100 titre on an initial single test, usually correlates fairly well with exposure to typhoid fever (Olopoenia *et al*., 2000). However, even these single high-value titres in an endemic area where repeated exposures to *S. typhi* may have occurred, do not have any clinical relevance in the absence of a positive isolate of the causative organism or its antigen. A second sample collection will proof useful. But, in a situation where second sample collection is not feasible, knowledge of the agglutinin levels in the sera of normal subjects from the patients' community can form the baseline on which a diagnosis can be made (Opera and Nweke, 1991; Ibekwe *et al*., 2008).

For practical purposes, titres occurring in more than 5% of the subjects under study were not diagnostically significant and should be regarded as normal in that population (Collard, 1959 reviewed in Ibekwe *et al*., 2008). Based on this premise, it would seem that Salmonella titres of 80 occurred in significant proportion of the sample and in the males and females respectively. Titres above 1:80 occurred in more than 92.4 and 92.6% of the male and female samples respectively. Therefore, titres above 1:80 could be used in the presumptive diagnosis of enteric fevers in the study area, but should be confirmed if a second sample is possible. Although there are controversies surrounding the increase in titre beyond the first week of illness in some endemic areas, it is generally accepted by clinicians that, toward the end of the first week of illness, titres of either O or H antibody may rise to as high as 1:160. However, the lack of paired sera may lead to an erroneous interpretation of test results (Hoffman *et al*., 1986; Olopoenia *et al*., 2000).

Schroeder (1968) concluded in a review of clinical interpretation of serologic tests for typhoid fever that the tests are nonspecific, poorly standardized, confusing and difficult to interpret. Erroneous interpretation of the test result may lead to misdiagnosis and mismanagement of the patient, resulting in major morbidity and mortality (Olopoenia *et al*., 2000). A negative agglutination test may be for one of several reasons which include: 1) absence of infection by *S. typhi*, 2) the carrier state, 3) an inadequate inoculum of bacterial antigen in the host to induce antibody production, 4) technical difficulty or errors in the performance of the test, 5) previous antibiotic treatment and 6) variability in the preparation of commercial antigens. A negative Widal test result does not therefore necessarily rule out the absence of infection. Such results are best kept as a reference for subsequent comparative analysis (Olopoenia *et al*., 2000). A positive agglutination tests (on two successive occasions) on the other hand, may also be open to several different interpretations. 1) the patient being tested has typhoid fever, 2) previous immunization with *Salmonella antigen*, 3) cross-reaction with non-typhoidal Salmonella, 4) variability and poorly standardised commercial antigen preparation, 5) infection with malaria or other enterobacteriaceae, 6) other diseases such as dengue (Olopoenia *et al*., 2000). This could lead to confusion in the serological diagnosis of typhoid fever. Therefore, serological findings have to be interpreted with a lot of caution particularly in country like Nigeria where there are yet to be laid down standard baseline titres (Ibekwe *et al*., 2008).

In endemic typhoid regions, a single testing of a serum specimen for Widal agglutinin cannot provide a reliable diagnosis due to: repeated exposure to small inocula of *S. typhi* or to other *Salmonella* spp. that contain type 9 or 12 antigens, previous typhoid fever immunization and other infectious agents such as malaria (Olopoenia *et al*., 2000). Although a number of reports from some developing countries have suggested that a single Widal test is sufficient to make the diagnosis of typhoid fever (Mohammed *et al*., 1992; Rasaily *et al*., 1993; Choo *et al*., 1993), others have disputed the usefulness of such a single test result (Hoffman *et al*., 1986; Aquino *et al*., 1991). In some developing countries where the use of a single Widal test appears to be the norm, there has been an increase in the rate of false-positive results (Olopoenia *et al*., 2000).

Typhoid and paratyphoid fevers are common in less-industrialized countries, principally owing to the problem of unsafe drinking-water, inadequate sewage disposal and flooding. Public health interventions to prevent typhoid and paratyphoid include: 1) health education about personal hygiene, especially regarding hand-washing after toilet use and before food preparation; provision of a safe water supply; 2) proper sanitation systems; 3) excluding disease carriers from food handling. Control measures to combat typhoid include health education and antibiotic treatment. A vaccine is available, although it is not routinely recommended except for those who will have prolonged exposure to potentially contaminated food and water in high-risk areas. The vaccine does not provide full protection from infection (WHO, 2008).

The review of Olopoenia *et al.* (2000) and Adeleke *et al.* (2006) suggesting Widal agglutination test as being bedeviled with controversies in term of quality of *Salmonella antigens* and interpretation of results is also pertinent. It should be stressed that a single Widal agglutination test has no diagnostic significance. According to Hoffman *et al.* (1986), the results of a single Widal test, tube dilution, micro-agglutination or slide agglutination are virtually un-interpretable unless the sensitivity and specificity of the test for the specific laboratory and patient population are known, as well as predictive values. Even in the extreme case of a high titre in a single Widal agglutination test, the causative organism may often be due to other species of *Salmonella*, rather than *S. typhi* (Olopoenia *et al.*, 2000). Thus, for a more definite diagnosis of typhoid fever, serologic test and blood culture as well as stool culture from every patient are quite relevant. Therefore, efforts must be made however, to confirm the diagnosis by paired sera investigation more than in presently the case.

CONCLUSION

In conclusion, it is clear that *Salmonella agglutinins* are common among apparently healthy people. Obviously therefore, the prevalence of typhoid fever and the increasing menace of Multi-Drug Resistance (MDR) of its causative agent are seriously constituting a menace in poor developing tropical countries. The resultant effect on health status would affect productivity, intellectual development and other aspects of life (Adeleke *et al.*, 2006). There is therefore an urgent need, for measures to curtail the spread of the disease. However, Widal agglutination titres higher than 1:80 should be an index of presumptive diagnosis of typhoid fever (Ibekwe *et al.*, 2008). Serologic studies are helpful in typhoid fever cases in endemic regions only if patients have four-fold or greater increases in O or H agglutinin titres in serum specimens obtained 2-3 weeks apart (Olopoenia *et al.*, 2000). Therefore, in interpreting Widal test results, it is important that there should be close communication between the physician requesting the test and the laboratory, since modifications of technique in individual laboratories may affect the Widal titres and some patients with bacteriologically confirmed typhoid fever may fail to develop the usual rise of antibody titres. The results of the tests should be reported as either `no agglutination' or, if agglutination is present, in titres (1:20, 1:40 or 1:80) rather than in descriptive (negative or positive) terms, as the latter may be misleading and contribute to the false interpretation of the test result by the physician. The function of the laboratory is to perform and report the test result to the requesting physician, who in turn will use the data to help make the proper diagnosis. Unfortunately, in several areas of developing countries, the laboratory performs the test, makes the diagnosis and prescribes the antibiotics (Olopoenia *et al.*, 2000). Doctors should be more meticulous in their clinical assessment of patients before requesting Widal tests (Ibekwe *et al.*, 2008).

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