Baseline Agglutinin Titre of *Salmonella Enterica* among Healthy Subjects in Idemili South, Anambra, Nigeria: An Aid in Medical Diagnosis

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ABSTRACT

Sequel to the abundant use of agglutinin titre as diagnostic test for typhoid fever in Nigeria (and most African countries), the prevalence of typhoid and paratyphoid fever caused by *Salmonella enterica* (serovar: Typhi and Paratyphi) between genders was investigated and the baseline agglutinin titres for both healthy and presumptively diagnostic states were determined. Blood samples (sera / plasma) were collected from equal number of both genders and were examined for the presence and levels of *Salmonella* antibodies by Widal agglutination technique. Standard *S. typhi* and *S. paratyphi* "O" and "H" suspensions were used as antigens. Agglutinins to *S. typhi* "O" and "H" antigens occurred in 10% and 16% respectively in the total male subjects tested while in the female subjects tested, agglutinins to *S. typhi* "O" and "H" antigens occurred in 26% and 8% respectively. Also, agglutinins to *S. paratyphi C* ("O" and "H") antigens occurred in 40% and 10% respectively in the male subjects while in the female subjects tested, agglutinins to *S. paratyphi C* ("O" and "H") antigens occurred in 20% and 16% respectively. Generally, there was no gender predominance based on statistical analysis (Chi square) of the data generated from this study except for the *S. paratyphi* A-O antigen (higher in female), *S. paratyphi* C-O antigen (higher in male), and *S. typhi* O antigen which was also observed to be significantly higher in female. Since the positive sera / plasma with titres of 40 occurred in more than 5% of the samples, this study

therefore suggests that such titres be regarded as normal among the communities studied while there should be a high index of skepticism of clinical infections in titres above 40 when a second serum or plasma is impracticable as this will improve diagnosis.

Key Words: Baseline Agglutinin Titre, Widal Test, Enteric Fever

INTRODUCTION

Typhoid fever is an acute life-threatening febrile illness caused by the bacterium *Salmonellaenterica* serotype Typhi. As a basis for its pathogenicity, *S. typhi* has somatic antigens, glycolipid microcapsule, and the VI or virulence antigen. There are over 1600 serotypes and about 40 occur commonly (Uraih, 2004).An estimated 22 million cases of typhoid fever and 200,000 related deaths occur worldwide each year (Crump *et al.,* 2004). The disease is a cause for concern and a major public health problem in developing countries due to poor sanitary conditions and lack of or inadequate potable water. Risk is high for travelers to South Asia and other developing countries in Asia, Africa, the Caribbean, and Central and South America.The disease is endemic in all parts of India (Pal et al., 2013). In India, the disease is endemic with an incidence rate which ranges from 102 to 2219 per 100,000 populations (Chowta and Chowta, 2005). Enteric fever disturbs the local community and travelers to such endemic areas; the incidence rate increases during the rainy season due to water logging and the contamination of the water bodies with faecal material (Pokhrel et al., 2009). Travelers to South Asia are at highest risk for infections that are multidrug-resistant (ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole) (Ackers *et al.*, 2000). Travelers who are visiting relatives or friends and who may be less likely to eat only safe foods (cooked and served hot) and beverages (carbonated beverages or those made from boiled water) are at greater risk. Travelers have acquired typhoid fever even during brief visits of less than 1 week to countries where the disease is endemic (Steinberg *et al.,* 2004).

S. typhi is entirely parasitic and differs from other species by having man as its only natural host and being of low virulence to mice. Symptoms and signs (high fever, malaise, headache, constipation or diarrhea, rose-colored spots on the chest, and enlarged spleen and liver) usually develop 1–3 weeks after infection, and may be mild or severe. Healthy carrier state may follow acute illness. The IgM

somatic "O" antibody appears first and represents the initial serologic response in acute typhoid fever, while the IgG flagellar "H" antibody usually develops more slowly but persists for longer (Pokhrel et al., 2009). Although it can be treated with antibiotics, resistance to common antimicrobials is widespread (CDC, 2008). The gold standard for its diagnosis rests on the recovery and identification of the causal organisms, from blood during the first few days of the illness, or from feces during the second and third weeks of the illness or from urine during the third and fourth week (Easmon, 2002). It is important to state that in Widal test, the first serum sample should be drawn on the first day of admission, and a second sampling is required one week later. A rising titre over a 7-10 days period is necessary for a firmer diagnosis. However, a four-fold rise rarely occurs possibly due to the fact that titres are already significantly raised when a patient's serum is first tested (Cheesbrough, 2006).

Recovery from typhoid confers a permanent immunity (Willey *et al.,* 2008). Re-infection may occur but is often milder. Circulating antibodies to "O" or "Vi" are related to resistance to infection and disease. Though, relapses may still occur in 2-3 weeks after recovery in spite of antibodies. Secretary IgA antibodies may prevent attachment of *Salmonella* to intestinal epithelium (Brooks *et al.,* 2004). Typhoid vaccination is not required for international travel, but CDC recommends it for travelers to areas where there is a recognized risk of exposure to *S. typhi*, particularly for those who will be traveling in smaller cities, villages, and rural areas off the usual tourist itineraries, where food and beverage choices may be limited. While immunization is recommended, travelers should be cautioned that none of the available typhoid vaccines is 100% effective, nor do they provide cross protection against other common causes of gastrointestinal infections of *Salmonella.* Typhoid vaccination is not a substitute for careful selection of food and drink (CDC, 2008).

In endemic countries where there are yet to be laid down standard baseline, serological findings need to be interpreted with cautiousness for different geographical regions. An awareness of local normal O and H agglutinin titres is necessary during the interpretation of Widal test results. The antibody levels found in a healthy population however, may vary from time to time and in different areas, making it difficult to establish a cut off level of baseline antibody in a

defined area and community. Weak and delayed O and H antibody responses limit the usefulness of the Widal test in low typhoid endemic areas. Variations among laboratories in the performance and reading of Widal tests further affects the reliability of the test (Martins and Uraih, 2010). Antibiotics such as ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, amoxicillin and ciprofloxacin, have been commonly used to treat typhoid fever. The gold standard for the treatment of typhoid fever is choramphenicol (Easmon, 2002) although resistant strains are emerging with speed. Resistance to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole and streptomycin is now common. Ciprofloxacin resistance is an increasing problem, especially in the Indian subcontinent and Southeast Asia.

Many centres are therefore moving away from using ciprofloxacin as first line drug for treating suspected typhoid originating from South America, India, Pakistan, Bangladesh, Thailand or Vietnam. For these patients, the recommended first line treatment is ceftriaxone. It has also been suggested that Azithromycin, which significantly reduces relapse rates compared with ceftriaxone, is better at treating typhoid in resistant population than both fluoroquinolone drugs and ceftriaxone (Effa & Bukirwa, 2008; 2011).Prompt treatment of the disease with antibiotics reduce the case fatality rate to approximately 1%. Death occurs between 10% and 30% of untreated cases. In severe diarrhea, replacement of fluid and electrolyte is essential (Brooks, 2004). It is thought that cystic fibrosis may have risen to its present levels (1 in 1600 in UK) due to the heterozygous advantage that it confers against typhoid fever (Weinberg, 2008). The CFTR protein is present in both the lungs and the intestinal epithelium, and the mutant cystic fibrosis form of the CFTR protein prevents entry of the typhoid bacterium into the body through the intestinal epithelium. Even though cultural isolation of the organism and PCR technique are more accurate in diagnosis, time of result, required expertise, and cost are some of the delineating factors.

In healthy individuals with antigens from malaria, dengue fever, brucellosis, chronic liver disease, endocarditis or other enterobacteriaceae infections, antibody titre may be high (Colle et al., 1996) and may cross-react with *Salmonella* antigens. For that reason, a four-fold rise in antibody titres between acute and convalescent phases is considered as a significant change in a given person and since this type of comparison is not practically helpful in establishing diagnosis of an acute illness, a

single cutoff value is widely used (Pokhrel et al., 2009). In a given population, interpretation of a single Widal test result needs to be based on average baseline titre among the healthy individuals. Antibody titres beyond a baseline value should be regarded as significantly elevated titres which may be used for clinical diagnosis (Pokhrel et al., 2009).

MATERIALS AND METHODS

Study Area

Idemili South Local Government Area in Anambra, South-eastern Nigeria has geographical coordinates of 6° 4' 0'' North, 6° 50' 0'' East. It has a close proximity with Nnewi and Onitsha –some of the most Nigerian important business cities. The average annual rainfall of the region is approximately the same with that of Onitsha, which is about 1830mm (73.2 inches) (www.springerlink.com/index/k2605475). Major occupation of the region includes farming and trading. Blood specimen of 500 healthy subjects was collected at random from different towns in the study area through free will volunteering. Following aseptic techniques, Two (2) milliliters of blood specimen were collected from each subject, transferred to an Ethylenediamine tetra-acetic acid (EDTA) container and centrifuged at a high speed for five minutes in order to separate the plasma from the blood cells. Undiluted serums of subjects with close proximity to the laboratory were also used.

Widal Agglutination Test

ANTEC febrile antigen kit (United Kingdom) with positive and negative controls was used for the Widal test. The rapid slide screening test was first carried out, followed by the tube agglutination test according to the manufacturer's specifications. Rapid slide titration was carried out using a micropipette and 0.08, 0.04, 0.02, 0.01 and 0.005 ml of undiluted serum were dispensed onto a row of 3 cm diameter circles. The reagent bottle was rigorously shaken and a drop of the undiluted antigen suspension was added to each plasma or serum aliquot. This was thoroughly mixed with the aid of a stirring glass rod 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 (Cheesbrough, 2002).

Tube Agglutination Test

The positive results obtained through the rapid slide test were confirmed using the Standard Widal Confirmatory Quantitative Tube test. Eight (8) small plastic test tubes were labeled accordingly in a rack and 0.9 ml of 0.85% normal saline was dispensed into the first tube while 1 ml was dispensed into the remaining seven tubes. 0.1 ml of the subject's undiluted serum or plasma was dispensed into the first tube. This was thoroughly mixed and then 1 ml aqueous solution of the plasma or undiluted serum and 0.85% normal saline was pipetted from the first tube and dispensed into the second tube then thoroughly mixed. This doubling dilution technique was continued serially up to the seventh tube. 1 ml from the seventh tube was discarded.

The eight tube contained only saline as a control. The reagent bottle was shaken vigorously and a drop of the appropriate antigen suspension was added into each tube, thoroughly mixed and incubated as follows: *Salmonella* 'O' Antigens were left at 50 ^{0}C for 4 hours, *Salmonella* 'H' Antigens were left at 50 ^{0}C for 2 hours. They were then left overnight in the fridge and was allowed to reach room temperature before the tubes were read. Agglutination was then checked for in the last tube to show agglutination on each row of serial dilution (Cheesbrough, 2002). All subjects used for this test were not positive for malaria using the Rapid Diagnostic Test (for *Plasmodium falciparum*).

RESULTS

The distribution of *Salmonella* agglutinin titres obtained from 500 healthy individuals' normal sera / plasma is shown in Tables 1.Table 2 shows the number and percentage of sera / plasma with end titres in 250 healthy male subjects. It was observed that none of the agglutinin was present in the sera / plasma of the healthy males, up to the titre of 80 unlike as seen in Table 3. More so, with the exception of *S. paratyphi* A-O, *S. paratyphi* C-O, *S. typhi* O and *S. paratyphi* B-H, the frequency of agglutinin titre of 40 ranged from 6-10%. It would seem that titres from 20 – 40 occurred in a significant proportion of the sample as shown in Table 2. Table 3 shows the number and percentage of sera with end titres in 250 healthy females. Apart from *S. paratyphi* A-O, *S. paratyphi* B-O, *S. paratyphi* B-H and *S. typhi* H, all other agglutinins tested were present in the sera of healthy females up to the titre of 80 at frequencies ranging from 2 – 4%. For agglutinin at titre of 40, the frequency ranged from 2 – 8% (Table 3).

Table 1: Distribution of *Salmonella* Agglutinin Titres in 500 Healthy Subjects.

Salmonellae	Male		Female	
	Total Number Tested	Number Positive (%)	Total Number Tested	Number Positive (%)
Salmonella paratyphi A-O	250	0 (0%)	250	20 (8%)
Salmonella paratyphi B-O	250	50 (20%)	250	20 (8%)
Salmonella paratyphi C-O	250	100 (40%)	250	50 (20%)
Salmonella typhi O	250	25 (10%)	250	65 (26%)
Salmonella paratyphi A-H	250	25 (10%)	250	15 (6%)
Salmonella paratyphi B-H	250	25 (10%)	250	15 (6%)
Salmonella paratyphi C-H	250	25 (10%)	250	40 (16%)
Salmonella typhi H	250	40 (16%)	250	20 (8%)

Table 2: Number and Percentage of Sera with End Titres in 250 Healthy Male Subjects.

Salmonellae	Number Positive (%)	End Titre <20	20	40	80	160	320
Salmonella paratyphi A-O	0 (0)	0 (0)	-	-	-	-	-
Salmonella paratyphi B-O	50 (20)	0 (0)	25 (10)	25 (10)	-	-	-
Salmonella paratyphi C-O	100 (40)	35 (14)	65 (26)	-	-	-	-
Salmonella typhi O	25 (10)	25 (10)	-	-	-	-	-
Salmonella paratyphi A-H	25 (10)	10 (4)	-	15 (6)	-	-	-
Salmonella paratyphi B-H	25 (10)	0 (0)	25 (10)	-	-	-	-
Salmonella paratyphi C-H	25 (10)	0 (0)	-	25 (10)	-	-	-
Salmonella typhi H	40 (16)	0 (0)	25 (10)	15 (6)	-	-	-

Table 3: Number and Percentage of Sera with End Titres in 250 Healthy Female Subjects.

Salmonellae	Number Positive (%)	Titre <20	20	40	80	160	320
Salmonella paratyphi A-O	20 (8)	15 (6)	5 (2)	-	-	-	-
Salmonella paratyphi B-O	20 (8)	15 (6)	5 (2)	-	-	-	-
Salmonella paratyphi C-O	50 (20)	10 (4)	20 (8)	10 (4)	10 (4)	-	-
Salmonella typhi O	65 (26)	20 (8)	20 (8)	20 (8)	5 (2)	-	-
Salmonella paratyphi A-H	15 (6)	0 (0)	5 (2)	5 (2)	5 (2)	-	-
Salmonella paratyphi B-H	15 (6)	0 (0)	10 (4)	5 (2)	-	-	-
Salmonella paratyphi C-H	40 (16)	15 (6)	15 (6)	5 (2)	5 (2)	-	-
Salmonella typhi H	20 (8)	0 (0)	20 (8)	-	-	-	-

Table 4: Significant Difference of Mean Percentage of Agglutinin Titres between Salmonellae of Males and Female in Idemili South, Anambra, Nigeria.

Salmonellae	Gender	Mean percentage of agglutinin titres
Salmonella typhi O	Male	3.33[a]
	Female	8.00[b]
Salmonella typhi H	Male	5.30[c]
	Female	2.70[c]
Salmonella paratyphi C-O	Male	13.33[d]
	Female	5.33[e]

Chi-square ($P \leq 0.05$)

Table 5: Presumptive Significant Titres for the Diagnosis of Different *Salmonella* Serogroups in Idemili South, Anambra, Nigeria.

Salmonellae	Male	Female
Salmonella paratyphi A-O	20 and above	20 and above
Salmonella paratyphi B-O	80 and above	20 and above
Salmonella paratyphi C-O	40 and above	40 and above
Salmonella typhi O	20 and above	80 and above
Salmonella paratyphi A-H	80 and above	20 and above
Salmonella paratyphi B-H	40 and above	20 and above
Salmonella paratyphi C-H	80 and above	40 and above
Salmonella typhi H	80 and above	40 and above

DISCUSSION

In this study, the titres of *Salmonella* "O" were higher than those of the "H" in both genders (Table 1). Agglutinins to *S. paratyphi* C-O was the most prevalent among the sera / plasma tested at various dilutions in males, summing up to 40%, while agglutinins to *S. typhi* O was the most prevalent among the sera tested at various dilutions in females with 26% followed by *S. paratyphi* C-O with 20% (Table 1). In Table 1, it was observed that *S. paratyphi* agglutinins seem to be prevalent in healthy males than in healthy females. This might be due to genetic make-up and or the prevalent consumption habit of ill-prepared in-between meals among males in the study area. Also, it seems that *S. typhi*O agglutinin was prevalent in healthy females than in healthy males. It could be that males consume more of potable water than females. Although the *S, typhi* H agglutinin was higher in males, there was no gender preponderance based on statistical analysis of data generated from this study at $P \leq 0.05$(Table 4).

In a situation where second sample is not feasible, knowledge of the agglutinin levels in the sera of normal subjects from patients' community can form the baseline on which a diagnosis can be made (Opara and Nweke, 1991). 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 *et al.*, 1959). Based on this premise, it would seem that *Salmonella* titres of 40 occurred in significant proportion of the male (6 – 10%) and female (2 – 8%) samples. No titre above 40 occurred more than 4% in female subjects which seems to attain higher level than the males. Therefore, the general baseline titre to *S. typhi* and *paratyphi* for both O and H antibody is 1:40.

Consequently, the general significant titre for O and H antibodies presumptive for the diagnosis of enteric fever in the study area is 1:80 and above but tests should be confirmed if second sample is possible. In comparison, this baseline titre is lower than that of Awka (Anambra, Nigeria) in a study carried out by Ibekwe *et al* (2008), which showed that Awka had a baseline titre of 80.Of the 200 sera tested in the study, agglutinins to *S. typhi* were most prevalent in male subjects accounting for 39% of the "O" antigens and 41.5% of "H" antigens at the various dilutions while in the 118 female subjects, 10.7% accounts for the "O" and 29.5% for the "H" antigens.

There was a male preponderance (M/F 2:1) although statistical analysis of their result was not stated. This is not in agreement with our result which had a lower baseline value with no gender predominance ($P \leq 0.05$) except for the *S. paratyphi* A-O antigen (higher in female), *S. paratyphi* C-O antigen (higher in male), and *S. typhi* O antigen (higher in female). The disagreement is likely due to urbanization, population density and poor handling of food cum water especially by waitrons at restaurants and other rampant roadside eating places in Awka. Also, in Ile-Ife, Nigeria, the baseline titre to *S. typhi* and *paratyphi* for both O and H antibody is 1:80 whilst the significant titre for O and H antibodies is 1:160 and above (Zailani et al., 2003). The disease is endemic in all parts of India and the healthy people in such regions may contain antibodies which are capable of reacting up to a variable titre due to past exposure, TAB vaccination, and cross reacting antigens.

Therefore it varies widely from place to place (Pal et al., 2013). Pokhrel et al (2009), in their work had concluded that when a single Widal agglutination titre is used for the diagnosis of enteric fever, it will be more appropriate to change the currently used cutoff levels against *S. enterica* serotype T*yphi* to > 1:80 for anti-O and > 1:160 for anti-H titres for Nepal, Northeast India.

Nepal agglutinin tire seems not to vary much over time as the work of Acharya et al (2013), showed that *Salmonella* agglutinins common among apparently healthy blood donors in Nepal have an average of >1:160 for both O and H agglutinins as diagnostically significant for the presumptive diagnosis of enteric fever. In Delhi, Northern India, anti-O titres of 100 and anti-H titres of 200 for *S. typhi* and anti-H titres of 400 for *S. paratyphi* A are considered presumptive for enteric fever (Malhotra and Khandpur, 2004).Also, a study carried out in Amlapuram, Andhra Pradesh, India had a baseline titre for the O and H antibodies of *S. typhi* of 1: 40.

The baseline titre for the *Salmonella enterica serovar paratyphi A* 'H' antigen was found to be ≤ 1:40 while the significant titre of the 'H' agglutinins and the 'O' agglutinins of *Salmonella enterica serotype typhi* was ≥ 1: 80. While the significant titre of the 'H' agglutinins of *Salmonella enterica serotype paratyphi A* was ≥ 1: 40, the significant titre of the 'H' agglutinins of *Salmonella enterica serotype paratyphi B* was ≥ 1: 20 (Peshattiwar, 2012). In district of Kerala, South India, Aruni et al (2014), found out that the cut-off value of antibody titers against *Salmonella enterica* typhi, paratyphi *A* and paratyphi B is 1:80 in case of O antigen and 1:160 in case of H antigen. Their finding is also is comparable to the work of Pal et al (2013), which had a cut-off titre of 1:80 for the anti-O antibodies and of 1:160 for the anti-H antibodies for the diagnosis of enteric fever in the Garhwal region of Uttarakhand, India.

On the average, this is akin to our findings (in males only) since the titre of 'H' was more than the 'O' antigen. This shows and confirms that different regions of a country have dissimilar baseline and significant titres. Based upon the results of the study carried out by Kataria et al (2013), it has been recommended that a single Widal test can be significant in an endemic region when higher titre of 1:160 is obtained. A higher titre of ≥ 1:320 for TO and TH antigen has also been recommended as diagnostic in Karnataka (Bahadur and Peerapur, 2013). The study of Mankodi and Aring (2013),had Widal agglutination titre for the presumptive

diagnosis of enteric fever against *S. typhi* to be> 1:80 for anti "O" and "H", while *S. paratyphi* A was found to be > 1:30 for anti "AH"; *S. paratyphi* B was > 1:20 for anti "BH". Their finding was more similar to our result and may likely mean that the environment, hygiene level and some demographic attributes of their study area and ours are similar. Kogekar et al (2015), in their recent work with a higher "H" agglutinin titre than ours, recommended that the significant titre of 'H' agglutinins and 'O' agglutinins of *Salmonella enterica serotype typhi* to be 1 in 160 and 1 in 40 respectively while the significant titre of 'H' agglutinins of both *Salmonella enterica* serotype paratyphiA and B to be 1 in 40 in central India. This also supplements that India is a more endemic country than Nigeria although there have been relatively scarce published baseline in Nigeria unlike in India.

Generally, the plausible cause of differences in the baseline titres and the titres presumptive for the diagnosis of enteric fever in different regions of the country and the globe at large might be due to factors like pollution of water bodies as a result of urbanization, population density, poor sanitary vicinity, and lack of good personal hygiene inter alia. Hence, the baseline titre value is proportional to endemicity (endemic rate). These factors have elicited an increase in the production of antibodies after the entry and production of antigens by *S. enterica*. It is reasonable that there should be different baseline titres for all *Salmonella*sero groups since Tables 2 and 3 have wide range levels of *Salmonella* agglutinin with obviously different titres that could be used in the presumptive diagnosis of enteric fever. Therefore, Table 5 shows other baseline titres that could be used for presumptive diagnosis of enteric fever.

CONCLUSION AND RECOMMENDATION

The clinical history of the subjects was not documented. The general baseline titre to *S. typhi* and *paratyphi* for both O and H antibody is 1:40 whilst the significant titre for O and H antibodies presumptive for the diagnosis of enteric fever in Idemili South (Anambra, Nigeria) is 1:80 and above but tests should be confirmed if second sample is possible. Poor diagnosis and drug abuse should be avoided so as to prevent future multi-antibiotic resistant strains of *Salmonella enterica*. Efforts must be made, to confirm the diagnosis by paired sera investigation. Doctors should be more meticulous in their clinical assessment of patients before requesting Widal test in typhoid endemic areas. Medical practitioners should

always make reference to the baseline value of their area of practice while more epidemiological surveillance should be carried out in other typhoid endemic areas without baseline value. Therefore, serological findings have to be interpreted with a lot of caution particularly in typhoid and paratyphoid endemic countries where there are no or paucity of standard baseline titres. So far, it is no doubt that *S. enterica* has done more harm than good although future findings might prove that it may convey or initiate immunity against other infection(s). Recently, the organism is noxious and the major cause of the enteric fever cum food poisoning. It is germane that public education, aimed at the orientation of the people on the effect of poor or lack of sanitation, be organized. More so, since the route of entry of *S. enterica* is through the gastrointestinal tract, and prevention centers on good hygiene, especially in the kitchen, the "boil it, cook it, peel it or forget it" recommended by the CDC is of high preventive measure. Travelers to endemic areas should seek for the vaccine before embarking on their journey.

REFERENCES

Acharya, T., Tiwari, B.R., & Pokhrel, B.M. (2013). Baseline Agglutinin titre in Apparently Healthy Nepalese Blood Donors. *JHAS,* 3(1), 27-30.

Ackers, M.L., Mintz, E.D., Puhr, N.D., & Tauxe, R.V. (2000). Laboratory-Based Surveillance of *Salmonella* Serotype Typhi Infections in the United States: Antimicrobial Resistance on the Rise. *Journal of the American Medical Association*, 283(20), 2668-2673.

Aruni, I.S., Prabakaran, P., & Kumaran, J. (2014). Study of Baseline Widal titre against *Salmonella* Species amongst Healthy Individuals in Thiruvananthapuram District of Kerala, South India. *The Experiment,* 20(1), 1380-1383. ISSN-2319-2119

Bahadur, A.K., & Peerapur, B.V. (2013). Baseline titre f Widal amongst Healthy Blood Donors in Raichur, Karnataka. *Journal of Krishna Institute of Medical Sciences University,* 2(2), 30-36. ISSN 2231 4261

Brooks, G.F., Butel, J.S., & Morse, S.A. (2004). *Jawetz, Melnick, & Adelberg's Medical Microbiology* (23rd Ed.). New York: McGraw Hill.

Centre for Disease Control and Prevention (2008). Health Information for International Travel. Available at: http://www.cdc.gov/travel/index.htm.

(Accessed 20/06/2008). Center for Disease Control and Prevention (CDC, 2008). Prevention of Specific Infectious Diseases.

Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries Part 2*. Cambridge: Cambridge University Press.

Chowta, M.N., Chowta, N.K., (2005). A Study on the Clinical Profile and the Antibiotic Response in Typhoid Fever. *Indian Journal of Medical Microbiology,* 23(2), 125-127.

Cheesbrough, M. (2002). *Medical Laboratories Manual for Tropical Countries*. 2, 479.

Collard, P., Sen, R., & Montefiore, D. (1959). The Distribution of Salmonella Agglutinins in Sera of Healthy Adults at Ibadan. *Journal of Hygiene* 57, 427-433.

Colle, J.G., Frason, A.G., Marmion, B.P., Simmons, A. (1996). *Mackie and Mc Cartney Practical Medical Microbiology.* New York: Churchill Livingston

Crump, J.A., Luby, S.P., & Mintz, E.D. (2004). The Global Burden of Typhoid Fever. *Bull World Health Organization*. 82 (5), 346-353

Easmon, C. (2002). Specialist Advise in Travel Medicine. *Shiraz E Medical Journal* 27, 7-13.

Effa, E.E. & Bukirwa, H. (2008). Azithromycin for Treating Uncomplicated Typhoid and Paratyphoid Fever (Enteric Fever). *Cochrane Database of Systematic Reviews*, Issue 4. Art. No.: CD006083. DOI: 10.1002/14651858.CD006083.pub2.

Effa, E.E. & Bukirwa, H. (2011). Azithromycin for Treating Uncomplicated Typhoid and Paratyphoid Fever (Enteric Fever). *Cochrane Database of Systematic Reviews*, Issue 10. Art. No.: CD006083. DOI: 10.1002/14651858.CD006083.pub3.

Ibekwe, A.C., Okonko, I.O., Onunkwo, A.U., Donbraye, E., Babalola, E.T., & Onoja, B.A. (2008). Baseline *Salmonella* Agglutinin titres in Apparently Healthy

Freshmen in Awka, South Eastern, Nigeria. *Scientific Research and Essay*, 3 (9), 425-430.

Kataria, V.K., Bhai, N., Mahawal, B.S. & Roy, R.C. (2013).Determination of Baseline Widal Titre among Apparently Healthy Population in Dehradun City. *Journal of Pharmacy and Biological Sciences,* 7(2), 53-55. ISSN:2278-3008

Kogekar, S., Peshattiwar, P., Jain, K., Rajput, M., & Shahi, P.K. (2015). Study of Baseline Widal titre amongst Healthy Individuals in and Around Indore, India. *International Journal of Current Microbiology and Applied Sciences,* 4(7), 775-778. ISSN: 2319-7706.

Malhotra, V.L., & Khandpur, N. (2004). Significance of Knowing Baseline Widal Test Titres in Pediatric Population in Delhi. *The Journal of Communicable Diseases*, 36(1), 70-71.

Mankodi, P.J., & Aring, B.J. (2013). Baseline Antibody Titer against *Salmonella Enterica* in Healthy Population of Gujarat, India. *International Journal of Scientific Research.* 2(5), 493-494. ISSN-2277-8179.

Martins, P.E., and Uraih, N. (2010). *Baseline Agglutinin titre of Salmonella Enterica in Healthy Individuals in Oba and Environs*. Unpublished BSc Project Report, Tansian University, Umunya.

Pal, S., Prakash, R., Juyal, D., Sharma, N., Rana, A., & Negi, S. (2013).The Baseline Widal titre among the Healthy Individuals of the Hilly Areas in the Garhwal Region of Uttarakhand, India. *Journal of Clinical and Diagnostic Research,* 7(3), 437-440. Doi: 10.7860/JCDR/2013/4889.2793. ISSN-0973-709X

Peshattiwar, P. (2012). Study of the Baseline Widal titre amongst Healthy Individuals in Amlapuram, India. *Journal of Clinical and Diagnostic Research,* 6(3), 416-417.

Pokhrel, B.M, Karmacharya, R., Mishra, S.K., & Koirala, J. (2009). Distribution of Antibody Titre against *Salmonella entericia* among Healthy Individuals in Nepal. *Annals of Clinical Microbiology and Antimicrobials,* 8(1). Doi: 10.1186/1476-0711-8-1

Steinberg, E.B., Bishop, R., Haber, P., Dempsey, A.F., Hoekstra, R. M., Nelson, J.M., Ackers M., Calugar, A., & Mintz, E.D. (2004). Typhoid Fever in Travelers: who Should be Targeted for Prevention? *Clinical Infectious Diseases,* 39(2), 186-191.

Uraih, N. (2004). *Food Microbiology*. Benin, Edo: Bobpeco Publishers.

Weinberg, E.D. (2008). Survival Advantage of the Hemochromatosis C282Y Mutation. *PERSPECTIVES in Biology and Medicine*, 51 (1), 98-102.

Willey, J.M., Sherwood, L.M., & Woolverton, C.J. (2008). *Prescott, Harley, and Klein's Microbiology.* New York: McGraw Hill.

Zailani, S. B., Oyelese, A.O., & Aboderin, A.O. (2003). Determination of Baseline Antibody Titre to *Salmonella typhi / paratyphi* in Ile-Ife, Nigeria. *African Journal of Medical Sciences,* 32(3), 307-310.

Reference to this paper should be made as follows: Martins, P.E.; et al. (2016), Baseline Agglutinin Titre of *Salmonella Enterica* among Healthy Subjects in Idemili South, Anambra, Nigeria: An Aid in Medical Diagnosis. *J. of Medical and Applied Biosciences,* Vol. 8, No. 1, Pp. 16 – 30.