# BASELINE ANTIBODY TITRE TO SALMONELLA TYPHI AND SALMONELLA PARATYPHI *A* AMONG APPARENTLY HEALTHY INDIVIDUALS IN ZARIA, KADUNA STATE, NIGERIA

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**OCTOBER, 2016.**

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**IMMUNOLOGY UNIT**
**DEPARTMENT OF MEDICINE**
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**OCTOBER, 2016.**

## DECLARATION

I declare that the work in this dissertation entitled **"Baseline Antibody Titre to *Salmonella typhi and Salmonella paratyphi A* Among Apparently Healthy Individuals In Zaria, Kaduna State, Nigeria"** has been carried out by me in the Immunology unit of the Department of Medicine, ABU Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references has been provided. No part of this dissertation was previously presented in this institution or any other institution for the award of a degree or diploma.

_____ _____

**ODUGU JUDE AMAKAEZE** **Date**

## CERTIFICATION

This dissertation titled **"Baseline Antibody Titre to *Salmonella typhi and Salmonella paratyphi A* Among Apparently Healthy Individuals in Zaria, Kaduna State, Nigeria"** by Jude Amakaeze ODUGU meets the regulations governing the award of the degree of Master of Science (M.Sc.) Degree in Immunology of the Department of Medicine, Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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**(Signature)** **Date**

## DEDICATION

Dedicated to the memory of my late father, Mr Damian Ugwoke

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Finally, I give glory to God who granted me strength and wisdom for this great accomplishment. To Him is glory forever.

## ABSTRACT

Typhoid fever is endemic in the tropics and sub-tropical regions of the world. It is a major public health problem in most developing countries including Nigeria. Efforts are ongoing to develop new test techniques and improve existing ones in order to mitigate the morbidities and mortalities of typhoid and paratyphoid infections. As such 180 serum samples from apparently healthy blood donors of the Department of Hematology, Ahmadu Bello University Teaching Hospital Zaria were evaluated for the levels of *Salmonella typhi* and *Salmonella paratyphi* A somatic O and flagella H antibodies using the quick tile technique and the Widal microtitire plate method to determine the baseline antibody titres. The standard *Salmonella* O and H (Oxoid/Remmel diagnostic) antigens were used. The presence of specific IgM/IgG antibodies was also tested among the subjects using the Typhidot IgM/IgG test cassettes. The occurrence of specific IgM in the study population was found in 3.9% of the samples studied while IgG occurred in only 1.1% of the population. The distributions of the antibody titres of *S. typhi and S. paratyphi A* among the subjects were determined. Since titres of ≤ 1:20, 1:20, 1:40, were found in a significant proportion (>80%) of the population tested for *S. typhi* O and H, the cut off titre for *Salmonella typhi* was found to be 1:80 for both O and H antibodies. Titres above 1:80 are therefore significant for the diagnosis of typhoid in our locality. For *Salmonella paratyphi* A, the cut off titre was found to be 1:40 for both anti O and anti H antibodies. Titres ≥ 1:80 are therefore significant titres for *S. paratyphi* A infections when using the Widal technique. A comparison of results from the tile method with the microtitre plate method showed a significant difference (p value 0.0001), indicating negative correlation between the quick tile method and the Widal microtitre plate method. The study has established the baseline titers, the cut off titre and the significant titres of

Widal test for *S. typhi* and *S. paratyphi A* in Zaria. This study clearly demonstrates that there are deficiencies with the use of the quick tile technique in Widal testing and that the microtitre plate (MTP) method would be more appropriate and beneficial for Widal testing in this setting.

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## LIST OF ABBREVIATIONS

**AIDS** = Acquired Immunodeficiency Syndrome

**APC** = Antigen-Presenting Cells;

**ASC** = Antibody Secreting Cells;

**BCR** = B-cell Receptor;

**CD** = Cluster Differentiation

**CMI** = Cell Mediated Immunity

**DC** = Dendritic Cells;

**DNA** = Deoxyribonucleic Acid

**EIA** = Enzyme Immuno-Assay

**GRA** = Government Reserved Area

**H** = Flagella Antibodies

**HIV** = Human Immunodeficiency Virus

**HLA** = Human Leukocytes Antigen;

**LPS** = Lipopolysaccharide Structures

**MAIT** = Mucosal Associated Invariant T-cells;

**M$\varphi$** = Macrophages;

**NK** = Natural Killer Cells;

**NPC** = National Population Commission

**O** = Somatic Antibodies

**OMP** = Outer Membrane Protein

**PCR** = Polymerase Chain Reaction

**PMN** = Polymorphonuclear Neutrophil;

**TCM** = Central Memory T-cells;

**TCR** = T-cell Receptor;

**TEM =** Effector Memory T-cells;

**TEMRA** = Effector Memory Expressing

**TM** = Memory T-cells;

**$T_{reg}$,** = Regulatory T-cells;

**WHO** = World Health Organization

**AH** = *Salmonella paratyphi A* Flagella Antibody

**AO** = *Salmonella paratyphi A* Somatic Antibod

**IgG** = Immunoglobulins *G*

**IgM** = Immunoglobulins *M*

**MTP** = Microtitre Plate Method

**QCT** = Quick Tile Method

**TH** = *Salmonella typhi* Flagella Antibody

**TO** = *Salmonella typhi* Somatic Antibody

# CHAPTER ONE

## 1.0 INTRODUCTION

Every year, thousands of cases of typhoid and paratyphoid infections are reported worldwide (Hurley *et al.*, 2014). *Salmonella enterica serovar typhi* (*S. typhi*) and *Salmonella paratyphi A,B,C* are the causative agents of enteric fevers. Typhoid fever is an acute, life threatening febrile illness caused by *S. typhi* which results in about 200,000 deaths worldwide each year largely in developing countries (Crump and Mintz, 2010). *Salmonella typhi* infections exact a heavy toll on human populations particularly among children. Despite the explosion of knowledge on the pathogenesis of enteric diseases experienced during the past decade, the number of diarrheal episodes and human deaths reported worldwide remain remarkably high (Fiorentino *et al.*, 2013).

Due to the importance of *Salmonella* in the clinical and public health setting, there has been a significant effort to improve on the laboratory diagnosis of typhoid and paratyphoid fever. The definitive diagnosis of typhoid fever requires the isolation of *Salmonella typhi* from blood, feces, urine, or other body fluids (Kataria *et al.*, 2013). In developing countries facilities for isolation and culture are often not available especially in primary and secondary health institutions. However, where facilities are available, easy access to across the counter drugs by patients especially antibiotics make isolation of the organism difficult. Diagnosis relies greatly upon the clinical features and the detection of agglutinating antibodies to *S. typhi* by the Widal test.

Various investigators have given varying views on the diagnostic value of a single pretreatment Widal test (Frimpong *et al.*, 1999; Ibekwe *et al.*, 2008). While some found

the test valuable, others found it of limited use. However, all the authors arrived at the consensus that for a reliable interpretation of the Widal test, the circulating *Salmonella* antibody titre profiles (baseline values) of healthy individuals in all the geographical areas concerned must be determined.

Therefore, this study was undertaken to determine the baseline *Salmonella* antibody levels in healthy population in Zaria and eventually establish the significant Widal anti-O and anti- H titres for a more accurate and reliable diagnosis of typhoid fever using a single pretreatment Widal test in Zaria, Kaduna State.

## 1.2 Statement of the Research Problem

Culture of the causative organism, *Salmonella enterica* serotype *typhi* is the most definitive for the diagnosis of typhoid fever(Sreenath *et al.,* 2014). However, it is difficult to isolate *Salmonella typhi* from bone marrow, blood, urine and stool specimens in most developing countries. This may be due to lack of culture media, expertise and/or previous exposure to inadequate doses of antibiotics. In addition, blood culture is relatively expensive. Costs of between three and five thousand (3,000- 5,000) naira are reported in most centers. It takes up to a week and it may not yield the growth of *Salmonella* even when the clinical features are highly suggestive. Bone marrow is more useful in yielding isolates but is rarely used because of the invasive nature of the diagnosis.

Efforts have been made to develop other techniques in the diagnosis of typhoid. These include the use of nested PCR (polymerase chain reaction) ,real time PCR for the detection of *Salmonella* DNA(deoxyribonucleic acid)(Farrell *et al.,* 2005; Prakash *et al.*, 2005); use of rapid diagnostic kits (Tubex, Typhidot ,etc) for the detection of IgM,

IgM and/or IgG (Olsen *et al.*, 2004; Tam *et al.*, 2008; Siba *et al.*, 2012). DNA test for *Salmonella* cost between ten to twelve thousand naira (10,000 - 12,000) per test in private commercial centers. The rapid diagnostic tests cost three to five thousand (3,000 – 5000) naira. Widal test costs about five hundred to one thousand naira per test.

In terms of turnaround time, the rapid diagnostic test takes within five (5) minutes to three (3) hours. Widal has a turnaround time of two (2) days while techniques involving PCR takes 4 days. Considering complexity of test and equipment requirement, PCR technique comes first followed by blood culture, then Widal and lastly, the rapid diagnostic test.

Another problem relating to serological diagnosis of typhoid is the abuse of the Widal test. Some clinicians will not treat or suspect the disease unless the test is positive, while others treat with a positive result even in low titres in an endemic zone of typhoid. Interference with cross-reacting antigens affects serological diagnosis of typhoid leading to over diagnosis (Otegbayo, 2005). Typhoid fever in most developing countries is thus a disease of over and under diagnosis. There is no single test among the new techniques that has proven to be sufficiently sensitive, specific and practical for use in low income countries and as such these techniques are not often used on a large scale (Siba *et al.*, 2012). It is therefore likely that the Widal test will remain in use in Nigeria and many other low income countries for a foreseeable future despite its known limitations. For these reasons, the study of the baseline value for typhoid agglutinins in our locality, Zaria to improve the diagnostic utility of the Widal's test and typhoid diagnosis is necessary.

## 1.3 Justification of the Study

Population growth, urbanization and poor social amenities such as housing, water supply, waste and sewage management has a negative effect on public health. Zaria which houses the Ahmadu Bello University and many other Federal institutions has many students, civil servants and other immigrant population who have come to provide services and to obtain their livelihood. The population of Zaria has therefore increased rapidly without a corresponding improvement in amenities. This has led to negative health profile. The prevalence (6.3%) of typhoid fever in Zaria is high (Mbuh *et al.*, 2003).

In Nigeria and other developing nations Widal agglutination test is the sole laboratory diagnostic tool employed to buttress clinical diagnosis of typhoid fever for the purpose of directing therapeutic measures against this disease. Blood culture is the definitive diagnosis for typhoid. Stool or urine may also yield a positive result (Kataria *et al.*, 2013; Mittal *et al.*, 2014). However, access to blood culture is poor or limited due to technical difficulties and the cost involved in isolation and identification of causative organism. There is therefore over reliance on Widal test and a need to standardize the test.

Moreover, Widal test requires acute and convalescent phase serum samples but usually only one sample at time of clinical presentation is used. In a situation where a second sample is not feasible, knowledge of the levels in the sera of normal subjects from the patient's community can form the baseline on which a diagnosis can be made (Ibekwe *et al.*, 2008).

In addition, high titre of Widal may be obtained which may be as a result of cross reacting antibodies of other *Salmonella* species, malaria and or other

*enterobacteriaceae* and not due to *S. typhi or paratyphi*. A high prevalence of antibodies is found among healthy individuals in areas of endemicity. In a given population therefore, the interpretation of a single Widal test result is based on the determined average baseline titre of *Salmonella* agglutinins among the healthy individuals.

Circulating antibodies to *Salmonella typhi* vary by geographical location due to predisposing factors such as poor environmental sanitation, lack of potable water, health education, personal and communal hygiene, drug resistance, fake and counterfeit drugs, etc (Kataria *et al.*,2013). Hence, there is a need to determine the baseline titre of *Salmonella* antibodies in our environment, Zaria in order to improve the use of Widal test as a diagnostic tool for typhoid fever.

One other controversy surrounding the Widal test is the technique of performance of test. Most of the laboratories use the quick slide technique; applying equal volume of sample to equal volume of reagent and subjectively determining the titre of the test. This leads to erroneous Widal reports. This research intends to use the standard tube dilution method in comparison with the quick slide technique for a more scientifically based evaluation.

This research therefore, sets out to determine the baseline Widal titre of healthy individuals in Zaria; to establish the cut off titre for a positive Widal for the diagnosis of typhoid fever in Zaria with a view to improving clinical interpretation and application of the Widal test.

## 1.4 Aim

To determine baseline titres of *Salmonella* agglutinins among apparently healthy individuals in Zaria, Kaduna State, Nigeria.

## 1.5 Objectives

The objectives of this study were to:

1. determine the baseline titres of *Salmonella* antibodies among apparently healthy individuals in Zaria using Widal tube (microtitre plate) agglutination method.
2. establish the cut off titre and the significant titres for a positive Widal for the diagnosis of typhoid in Zaria
3. determine the relationship between the Widal microtitre plate method and the quick tile method
4. determine the prevalence of specific *Salmonella typhi* IgM/IgG antibodies among apparently healthy individuals in Zaria

## 1.6 Research Question

What is the baseline Widal antibody titre to *Salmonella typhi* and *Salmonella paratyphi A* among apparently healthy individuals in Zaria?

.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 *Salmonella enterica*

*Salmonella enterica* are Gram-negative facultative intracellular anaerobes. The genus *Salmonella* comprises of two species, *S. enterica* and *S. bongori. Salmonella enterica spp* is further divided into six sub-species which are biochemically differentiated into serovar based on the composition of their carbohydrate, flagella and lipopolysaccharide (LPS) structures. All *Salmonella enterica* serotypes can be designated by an antigenic formula or somatic O and flagella H antigens in addition to capsular $V_I$ antigens (Hurley, *et al.,* 2014). *Salmonella typhi* is characterized by the presence of somatic O antigens 9, 12, and flagella (H), d. *Salmonella paratyphi A* is characterized by somatic O antigen, 1, 2, 12 and (H) a; *S. paratyphi B* has somatic O antigen 1, 4,5,12 and H antigen b, 1, 2. *S. paratyphi* C possess somatic antigen 6, 7 and H antigens c, 1 and 5 (Cheesbrough, 2006). The diagram below demonstrates the typical bacilli of S. typhi showing their flagellae (Fig. 2.1)

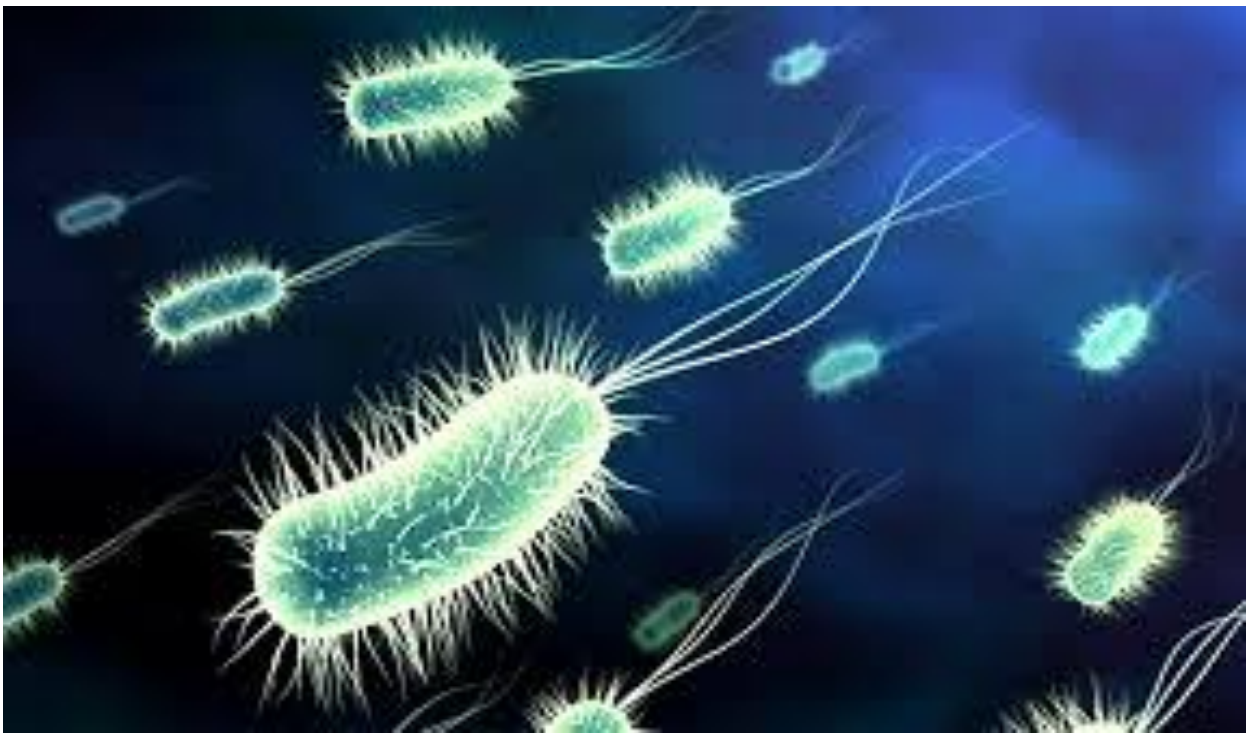


**Figure 2.1: Bacilli of *Salmonella typhi (***Maza *et al.*, 2013)

## 2.2 Typhoid and Paratyphoid Fever

Typhoid (enteric) fever is one of the major health problems in developing countries. In endemic areas, typhoid fever afflicts the local population as well as travelers visiting the region. *Salmonella enterica* serotypes *typhi* and *Salmonella enterica* serotype *paratyphi A* are causative organisms for typhoid and paratyphoid fevers respectively. Serotype *paratyphi B* is rare (Pokharel *et al.*, 2006; Maskey *et al.*, 2008; Pokhrel *et al.*, 2009).Salmonella typhi and Salmonella paratyphi A is a gram negative bacterium which is motile though non flagellate variants exist. The bacteria do not form capsule and are intestinal pathogens (Philip, 2000). *Salmonella* is pathogenic to both man and animals and is associated with inflammatory reactions in the intestinal tract (Okonko *et al.*, 2010).

Typhoid fever is among the water borne infections prevalent in slums and in environments with poor sanitation and hygiene. Typhoid and paratyphoid bacteria are passed in faeces and urine of infected people. Human infection with *Salmonella* is mainly by the oral route through ingestion of fecal contaminated food and water, unclean hands, flies and meat from infected animals (Okonko *et al.*, 2010). The incidence of typhoid fever is higher in the rainy season as a result of flooding and water pollution with fecal materials (Chakraborty, 2005).Children are the most likely group of individuals to present with Salmonellosis. The rate of diagnosed infections in children less than five (5) years old is higher than the rate diagnosed in all other persons. Other groups at risk such as the elderly and immunocompromised individuals are the most likely to present with severe forms of the disease (Hurley *et al.*, 2014).

## 2.3 *Salmonella enterica* Pathogenesis

People become infected by the pathogen 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 bacteria. *Salmonella* species are highly invasive pathogens. The first significant cellular contact typhoid pathogens have occurs at the level of intestinal epithelium. This invasive bacteria have evolved an array of mechanisms to breach the integrity of intestinal barrier, either by targeting tight junction proteins directly or by altering transduction signals regulating their assemblage (Simonovic *et al.*, 2000; Fiorentino *et al.*, 2013). On entrance of bacteria into the person's gut they multiply and spread from the intestines into the bloodstream. *Salmonella* organisms are able to survive and multiply within the mononuclear phagocytic cells of the lymphoid follicles, the liver and spleen. At a critical point that is probably determined by the number of bacteria, their virulence and the host response, bacteria are released from this sequestrated intracellular habitat into the blood stream. The incubation period is usually 7 to 14 days. In the bacteraemic phase, the organism is widely disseminated. The most common sites of secondary infection are the liver, spleen, bone marrow, gall bladder and Peyers patches of the terminal ileum. Gallbladder invasion occurs either directly from the blood or by retrograde spread from the bile. Organisms excreted in the bile re-invade the intestinal wall or are excreted in feces (Parry and Threlfall, 2008).

## 2.4 Signs and Symptoms of *Salmonella enterica* Infection

Infection with *Salmonella enterica* manifest through a broad range of clinical symptoms and can result in asymptomatic carriage, gastroenteritis, systemic disease such as typhoid fever and in severe cases death (Gunn *et al.*, 2014; Hurley *et al.*, 2014).

Typhoid fever is characterized by fever, headache, abdominal pain, relative bradycardia, splenomegaly and leucopenia (Pearson and Guerrant, 2000; Willke *et al.*, 2002). In 5-10% of infected people, neuropsychiatric manifestations occur. Complications such as gastrointestinal bleeding, intestinal perforation and typhoid encephalopathy occur in 10-15% of patients (Fraser *et al.*, 2007). Typhoid fever induces systemic and local humoral and cellular immune responses but confer incomplete protection against relapse and re-infection. The interaction of the immunologic mediators and bacterial factors in infected tissue may contribute to the necrosis of Peyer's patches in severe disease (Parry and Threlfall, 2008). Persons with diarrhea usually recover completely after a few days of the initial infection, although it may be several months before their bowel habits return to normal. Contrary to this could be a small number of persons with Salmonella infections that develop pain in their joints, irritation of the eyes and painful urination. These symptoms indicate a disease called reactive arthritis. This can last for months or years and can lead to chronic arthritis, which is extremely difficult to treat. Antibiotic treatment does not make a difference in whether or not the person develops arthritis (Batz *et al.*, 2013). Even after recovery from typhoid or paratyphoid fever, a small number of individuals (carriers) continue to harbor the bacteria. These people are usually a source of infection for others. 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).

Typhoid fever caused by *Salmonella typhi* is often encountered in tropical countries of the world including Nigeria where they constitute serious morbidity and mortality. It is a major health problem in the developing countries of the world with an estimated annual incidence of 540 per 100,000 (Ibekwe *et al.*, 2008). In Nigeria, studies have

estimated over 33 million cases and 50,000 deaths due to typhoid fever per year (Otegbayo, 2005).

## 2.5 Immunity to *Salmonella typhi* in Humans

Immunity to *S. typhi* is extremely complex involving multiple antigen-presenting cells (e.g. macrophages, dendritic cells, B cells) and effector cells (e.g., various effector and regulatory T-cell subsets, Bcells, NK, and MAIT cells).APC, antigen-presenting cells; ASC, antibody secreting cells; DC, dendritic cells; CD8, $CD8^+$ T-cells; CD4, $CD4^+$T-cells; MAIT, mucosal associated invariant T-cells; M$\varphi$, macrophages; NK, natural killer cells; PMN, polymorphonuclear neutrophil; TM, memory T-cells; TCM, central memory T-cells; TEM, effector memory T-cells; TEMRA, effector memory expressing CD45RA; $T_{reg}$, regulatory T-cells; HLA, human leukocytes antigen; HLA-I,HLA class I; HLA-II, HLA class II; BCR, B cell receptor; TCR, T-cell receptor; MR1, HLA-I non-classical (b) molecule MR1; Ig, immunoglobulin (Sztein, *et al.*, 2014).

*S. typhi* is a facultative intracellular bacterium, both antibodies and CMI might play complementary roles in protection from infection. While antibodies are likely to play an important role in defense against extracellular bacteria, CMI is expected to be essential in eliminating *S. typhi*- infected cells. However, the relative contribution of each main arm of the effector immune response, i.e., humoral and cellular, and the antigen specificity of the responses remain largely unknown (Kilhamn *et al.*, 2003).

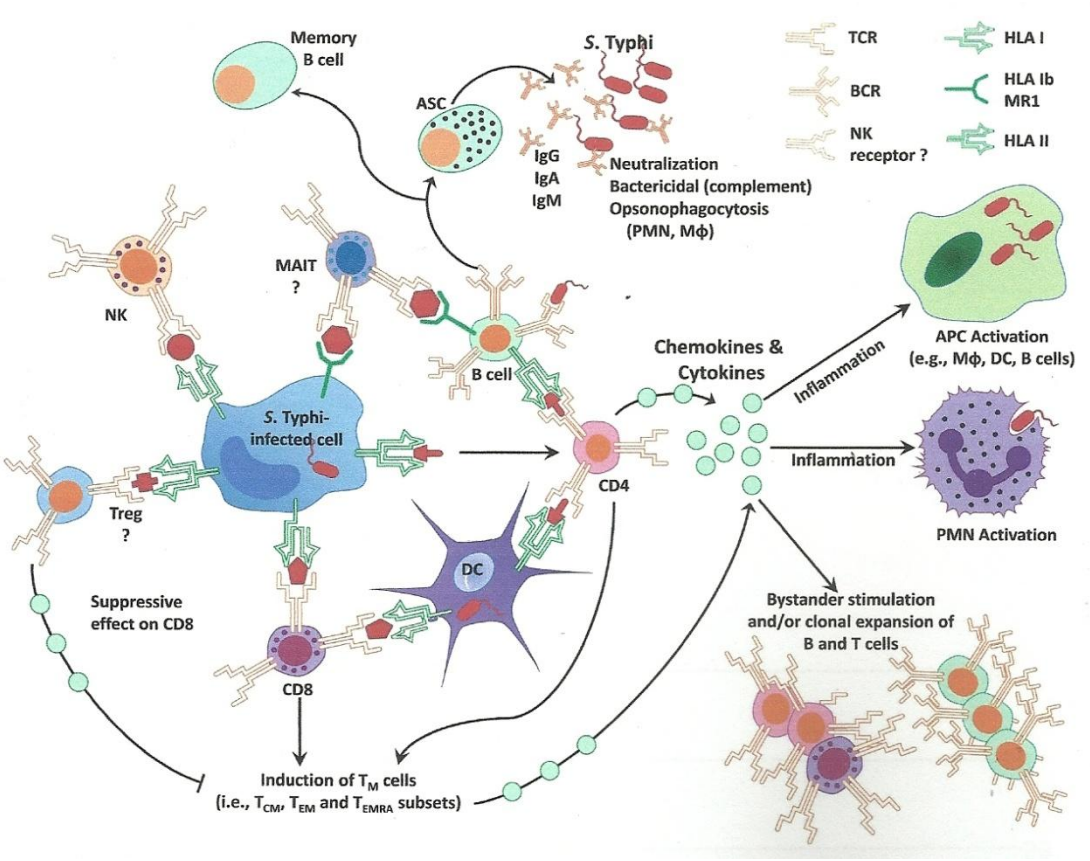



**Figure 2.2: Simplified diagram of immunity to *S. typhi* in humans (Sztein *et al.*, 2014).**

## 2.6 Diagnosis of *Salmonella enterica* Infection

Definitive diagnosis of typhoid fever is based on the isolation of *Salmonella* from blood, bone marrow, stool, urine, bile and other body fluids (Easmon, 2003; Ananthanarayan, 2005; Pokharel *et al.*, 2006; Aftab and Khurshid, 2009). Bacteriologic culture method is relatively costly and is not always available in less developed countries such as Nigeria. For this reason, an alternative laboratory test widely used for serological diagnosis of typhoid fever known as Widal agglutination test is readily used (Ibekwe *et al.*, 2008; Jog *et al.*, 2008). In recent years, various alternative diagnostic tests for typhoid fever have been developed and evaluated. Previous studies have shown that various prototypes and commercially available diagnostic tests may have advantages over the Widal tests (Olsen *et al.*, 2004; Kawano *et al.*, 2007; Ley *et al.*, 2011). However, there remains no single test that has proven to be sufficiently sensitive, specific and practical for use in low income countries and as such these diagnostic kits are seldom used on a large scale (Siba *et al.*, 2012).

Widal agglutination test was developed by Georges Ferdinand Isidore Widal in 1896 to aid in the diagnosis of typhoid fever. It utilizes a suspension of killed *Salmonella enterica* as antigen to detect typhoid fever in serum of patients with suspected typhoid fever(House *et al.*, 2001; Katz *et al.*, 2002). The Widal test is a presumptive serological test for typhoid fever. It is a demonstration of agglutinating antibodies against antigens O – somatic and H – Flagella in the serum during the acute and convalescence period of infection (Everest *et al.*, 2001). About 70% of adults show an early rise of antibody titre in the first week of infection (Cheesbrough, 2006). The earliest serological response in acute typhoid fever is the rise in the O- antibody, with a gradual elevation of the H- antibody titre, which persists longer than the O- antibody cut off titre (Hurley *et al.*, 2014; Mittal *et al.*, 2014). The frequency of H- agglutinins in a given population

reflects the latent infection. While the concentration of O- agglutinins on the other hand do not vary frequently in different regions and reflect the recent infection with *Salmonella* species (Mittal *et al.*, 2014).

However, high antibody titre may be seen in healthy individuals in the presence of cross reacting antigens such as brucellosis, malaria, dengue fever, chronic liver diseases, healthy carrier state, endocarditis or other enterobacterial infections (Okonko *et al.*, 2010). There are more than forty (40) cross reacting antigens between *S. typhi* and other enterobacteriaceae (WHO, 2008). Individuals who had past typhoid fever infection or who were vaccinated with typhoid vaccine (TAB) may develop transient anamnestic reaction in the event of unrelated febrile illness such as malaria (Ananthanarayan, 2005).

The baseline titre of Widal test is determined by the epidemiology of cross-reacting antigens because antibodies produced in these diseases may cross react with *Salmonella* antigens. A fourfold increase in antibody titers between acute and convalescent phases is considered as a significant change in a given individual. This kind of comparison is not practically feasible in establishing diagnosis of an acutely ill person. A single cut off value is mostly 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 exceeding a cut off value should be regarded as significantly elevated titers which may be used for diagnosis in an appropriate clinical setting (Ibekwe *et al.*, 2008; Pokhrel *et al.*, 2009; Saxena *et al.*, 2013; Sreenath *et al.*, 2014).

## 2.7 Tile Agglutination Test, Widal tube technique and Typhidot Test

The quick tile agglutination test is a screening test for *Salmonella* antibodies. The presence or absence of visible agglutination indicates the presence or absence of the corresponding antibody to the O and H antigens of the *Salmonella* serotypes. The titre is determined subjectively and by training and experience (Okonko *et al.,* 2010). The slide agglutination test is a screening test, however, it is abused by many laboratories who employ the method as their sole standard testing technique. The Widal tube agglutination test is a semi-quantitative test used to determine the antibody titre in suspected patients having typhoid and paratyphoid infections. The tube agglutination test requires much more technical work. It serves as a means of confirming the result of the slide technique. It involves serial dilutions of the patient serum 1:20, 1:40, 1:80, 1:160, 1:320 etc in microtitre tubes and the addition of specific antigens of *Salmonella* serotypes. The mixtures are incubated for 16 to 20 hours. The agglutinations are observed and titres recorded (Ley *et al.,* 2011).

The Typhidot is a qualitative antibody test. The dot-enzyme immunoassay (EIA) is a relatively newer serologic test based upon the presence of specific IgG and IgM antibodies to a specific 50-KD outer membrane protein (OMP) antigen on *S. typhi* strains and has been commercially marketed as a dot-EIA (Typhidot®, Malaysia Bio diagnostic, Research SDN BHD, Kuala Lumpur, Malaysia). The test incorporates micro cellulose strips impregnated with OMP antigen and separately identifies IgM and IgG antibodies. A positive Typhidot result (IgM and IgG) is defined as a visible reaction of an intensity equal or greater than that of the control reaction on the nitrocellulose strips (Kawano *et al.,* 2007).

## 2.8 Baseline Titres for *Salmonella typhi and Salmonella paratpyhi*

In a study in Abeokuta, south-western Nigeria, blood samples were collected from 840 apparently healthy people; 460(54.8%) females and 380(45.2%) males (Okonko *et al.*, 2010). The samples were examined for the presence and the levels of *Salmonella typhi* antibodies by Widal agglutination technique. The positive sera with titres of less than 1:80 occurred in more than 5% of the samples tested. The study suggests that such titres, be regarded as normal among the communities studied while there should be a high index of suspicion of clinical infections in titres above 1:80 when a second serum is impractical(Okonko *et al.*, 2010).

In another study in Awka, south-eastern Nigeria, blood samples were collected from 200 subjects; 82 males and 118 females; and examined for the presence and levels of *Salmonella* antibodies by Widal agglutination technique (Ibekwe *et al.*, 2008). Of the 200 sera tested, agglutinins to *S. typhi* were more prevalent in male subjects accounting for 39% of the "O" antigens and 41.5% of the "H" antigens at the various dilutions, while in the female subjects 10.7% accounts for "O" and 29.5% for "H" antigens. There was a male preponderance (M/F 2:1).The study suggests that the titre of 1:80 be regarded as normal among the communities studied since the positive sera with titres of $\geq$1:80 occurred in more than 5% of the samples(Ibekwe *et al.*, 2008).

In Kumasi, Ghana, Frimpong *et al.* (1999), in their research using 307 healthy individuals and 34 blood culture positive patients as controls, suggested that titres of $\geq$ 1:160 and $\geq$ 1:320 for anti –O and H respectively are significant for the diagnosis of typhoid and paratyphoid fever in their locality. The adoption of these values were based on the findings that only 3(1%) of the healthy population had anti-O titres of $\geq$ 1:160 while 8 (2.6) had anti-H, titres of $\geq$ 1:320 for *S. typhi*. In the majority, 214

(69.7%) persons and 149 (48.5%) persons of the healthy study population, titres of ≤ 1:20 for both anti-O and anti-H were observed.

Pokhrel *et al.* (2009) in their study worked on 100 blood samples collected from healthy volunteers. 62 individuals had significant antibody titres (≥ 1:20) against one of the four antigens against *S. enterica*. Among 54 samples with an anti- O titre against serotype *typhi*, 15 and 36 samples had titres of ≥1:60 and ≥1:40 respectively. A significant proportion (12% of all) had anti- O titre of ≥1:80. Similarly, among the 59 samples demonstrating anti-H titres of ≥ 1:20 to *S. enterica* serotype *typhi*, 29 had a titre of ≥1:80 and 12 had 1:160. For *S. enterica* serotype *paratyphi A* and B, anti-H titres of ≥1:20 were found only in 12% and 3% respectively, of all samples tested. The researchers concluded that when a single Widal agglutination titre is used for the diagnosis of typhoid fever , it will be more appropriate to change the currently used cut off levels against *S. enterica* serotype *typhi* to ≥1:80 for anti –O and ≥ 1:160 for anti H titres for Nepal (Pokhrel *et al.*, 2009).

There has been work that established baseline titre for Widal agglutination test for Borno and Plateau States in northern Nigeria (Mohammed *et al.*, 1992), Ile Ife (Zailani *et al.*, 2003) and Awka (Ibekwe *et al.*, 2008) in the south. *Bala and Auty* in Zaria, suggested that a Widal agglutination titre for salmonella 'O' antibody exceeding 1:64 or a twofold rise in Widal agglutination during the disease was accepted as diagnostic. This is based on the unpublished work of Greenwood and Whittle which showed low antibody titres in Zaria area despite the endemicity of typhoid infection (Bala and Auty, 1977).However, normal baseline titre of Widal agglutination test for healthy individuals and cut-off values for diagnosis of typhoid fever in Zaria has not been recently established.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Study Area

The study was carried out in Zaria, Kaduna State. Zaria is a major city in Kaduna State in Northern Nigeria as well as being a Local Government Area. The population of Zaria according to National Population Commission (NPC, 2006) was 408,198.The old part of the city is known as Birnin Zaria or Zaria City. Adjacent to Zaria City is Tudun Wada. The two (2) neighbourhoods are occupied predominantly by the indigenous Hausa but due to the location of many Federal institutions in Zaria, other populations are well represented. Other neighbourhoods include Samaru, Sabon Gari, Danmagaji/Wusasa, PZ, Kongo, GRA (Government Reserved Area) Zaria, Hanwa, Bassawa and Shika. The inhabitants are civil servants, students, traders and famers. There is public water supply. However, this is not constant or wide spread. Many homes use water from wells. Most homes have pit toilets or modern toilets

but there is poor drainage system in the streets in most of the neighborhoods. There are also low cost restaurants, street food vendors and many workers and students buy their meals from these sources (Mbuh *et al.,* 2003)

### 3.2 Study Design/Study Population

The study is a descriptive cross sectional study. Samples of blood were collected from apparently healthy volunteers (blood donors of Hematology department ABUTH) who lived in Zaria for one year and above. Distributions of titres in the study population were analyzed and positive and negative kit controls were also included in test. All titres above the cut off titre to be determined would be ascribed as abnormal titres. The

samples from volunteers were also tested for the presence of specific *Salmonella typhi* IgM/IgG antibodies.

### 3.3 Inclusion Criteria

i. Apparently healthy volunteers (18-59years) who had lived in Zaria for one (1) year and above at the time of the survey who consented.

ii. Volunteers who had not taken antibiotics within the past one (1) month

### 3.4 Exclusion Criteria .

i. Persons who were with active infection, febrile or had been treated recently of malaria, typhoid fever, tuberculosis, hepatitis or HIV/AIDS were excluded from the study.

ii. Individuals who had received typhoid vaccine were also excluded from the study.

iii. Individuals less than eighteen (18) years and those above 59 years of age were excluded from the study.

### 3.5 Sample Size

Sample size was obtained using the following formulas (*Araoye,2004*)

$$n = \frac{Z^2 pq}{d^2}$$

And

$$q = 1 - p$$

Where

$n$= Sample Size

$p$ = Prevalence

$Z$ = desired normal deviate usually at 1.96 which corresponds to 95%confidence level.

$d$ = degree of accuracy = 5% i.e. 0.05

The prevalence of typhoid, 6.3% reported in Zaria (Mbuh *et al.*, 2003).

Therefore

$$n = \frac{Z^2pq}{d^2}$$

$$n = \frac{1{\cdot}96^2 \times 0{\cdot}063 \times (1 - 0{\cdot}063)}{0{\cdot}05^2}$$

$n$ = 90 minimum sample size. The sample size calculated was 90, which is the minimum sample size to be used for the study. However, 100% increase (180) were used for the study in order to increase the power of the study.

### 3.7 Sampling Technique

Convenience sampling technique was applied. Individuals who came to donate blood in the Hematology Department of ABUTH and were qualified to donate blood following the department standard operating procedures were enrolled for the research after obtaining their consent.

### 3.7 Ethical Approval/Consent

Ethical approval was obtained from ABUTH-Research and Ethical Committee (Appendix I) Informed consent of volunteers for this study was also obtained (Appendix II). Confidentiality of Subjects and their data were maintained throughout the study.

## 3.8 Data Collection

A standardized questionnaire was administered to volunteers to capture the age, sex, district in Zaria, antibiotic use, occupation, sources of drinking water, recent ailment and other socio demographic information (Appendix III)

## 3.9 Sample Collection and Processing

Five (5) milliliters of blood was collected by venepuncture from consenting volunteers in a plain bottle. The blood was transported to the laboratory and centrifuged at 3,000 rpm for 5 minutes in order to separate the serum from blood cells. All sera were stored at -20$^0$C until analysis.

## 3.10 Serology

### *3.10.1 Widal Agglutination Test*

Widal agglutination test was run on each serum sample collected using Commercial Febrile antigen kit Oxoid (United Kingdom). First, the rapid slide screening test was carried out, followed by tube agglutination test to determine the titre, according to manufacturer's protocol.

### *3.10.2 Slide Screening (quick tile) Test*

The test reagents and the samples were allowed to attain room temperature by bringing them out from the fridge and freezers. Antigen vial was shaken gently to re-suspend the antigen. 50ul (1drop) of the serum was dropped on a circle on the testing card/tile. One drop of kit positive control serum and 1 drop of kit negative control serum was placed on circles on the test card. A drop of the appropriate well shaken antigen suspension was added to each serum containing circle. The contents of each circle was mixed with a disposable stirrer and spread over the area enclosed by the circle. Separate applicators

were used for each mixture. The slide was rocked gently by means of a mechanical rotator for 3 minutes. The reaction was observed under a light source for any agglutination. Smooth suspension with no visible agglutination as shown by negative control was recorded as negative (1:20). Any degree of agglutination that was visible macroscopically was recorded as Positive and graded as 1:40, 1:80, 1:160, 1:320 etc depending on the size of clumps formed and the speed of clump formation(Cheesbrough, 2006).

*3.10.3 Tube Agglutination Test*

Results that were obtained using the rapid slide agglutination screening method were confirmed using the microtitre tube agglutination method. Serum samples, normal saline and Widal antigens were brought out from the fridge and allowed to attain room temperature. The microtitre plates were labeled with identification numbers. The multichannel pipette was set at 285ul. Two hundred and eighty five (285ul) of normal saline was dispensed into the wells of line A of the microtitre plate(s).The multichannel pipette was set to 150ul. Then 150ul of saline was dispensed into wells of line B-H. Single channel pipette was used to dispense 15ul of individual samples into specific wells (line A 1-12) containing 285ul of saline. Wells 11 and 12 are negative and positive controls respectively. Contents of the wells of line A was mixed well using a multichannel pipette set at 150ul. 150ul was aspirated and moved to the next wells of line B. The doubling dilution technique was continued till the end (line H). At the end of the dilution 150µl of mixture at wells line H was aspirated and discarded. The multichannel pipette was set to 15µl. 15µl of well mixed antigen was added into all the wells. The plate was tapped gently to mix and sealed with a sealer. The plate was incubated at 37ċ for 24 hours. The plate was examined for agglutination. Formation of

agglutination (carpets) and the disappearance of colour (blue or red) from the initial colloidal mixtures depicted agglutination reactions after incubation (Cheesbrough, 2006; Ibekwe *et al.*, 2008; Pokhrel *et al.*, 2009).

### 3.11 Typhidot (IgM/IgG) Test

Serum samples, test cassettes and chase buffer were brought out and allowed to attain room temperature. If precipitates were noted in the chase buffer reagent, the bottles were shaken vigorously and allowed to warm up further at room temperature. The pouches of test cassettes were torn open and the cassettes were labeled with sample numbers. Forty-five microliters (45ul) of test serum was added to each sample well. One drop of buffer was added to each sample well. The cassette was tapped gently to facilitate the flow of sample up the membrane. The results were read after 20 minutes (Fig. 2.3). Samples positive for *Salmonella typhi* specific antibodies showed coloured bands at the control lines(C) and the test lines (T). Samples negative for *Salmonella typhi* specific antibodies showed only coloured bands on the control lines. If the control line was absent the test was invalid and was repeated (Ismail *et al.*, 1991; Jesudason and Sivakumar, 2006).




**Figure 2.3: Showing Typhidot Test Cassettes used in the Research.**

Cassette 83 shows positive for IgM antibodies.

### 3.12 Data Analysis

Data was presented using descriptive statistical programmes e.g. tables, bar charts. Statistical analysis was done using Statistical Package for Social Science IBM- (SPSS) version 20.0. Titres of antibodies against Salmonella antigens ≤ 1:20 were regarded as negative while titres > 1:20 are positive (WHO, 2004; Pokhrel *et al.*, 2009) The baseline (normal range) is a range of values (titres) that is regarded as normal for a given population. For practical purposes, titres occurring in more than 5% of the subjects under study are considered not diagnostically significant and were regarded as normal in the population (Collard *et al.*, 1959; Ibekwe *et al.*, 2008; Okonko *et al.*, 2010). After the establishment of the normal, baseline titre for healthy individuals, the titre immediately above the baseline was regarded as the cut off titre. The cut off titre is the absolute value (titre) that designates the point below which is negative and above which is positive. Comparison of titres was done using the student t-test. A p value $\underline{\leq}$ 0.05 was accepted as significant. In comparing the microtitire plate method with the quick tile method, the titre values in reciprocal were converted to decimal values. The titre values were then converted to logarithms. The Geometric Mean of the values were calculated. The Standard Deviation of the Mean were also calculated and the p value determined using the student t-test. Correlation between the microtitre plate method and the quick tile method was done to find whether there is a relationship between the two methods.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Socio Demographic Characteristics of Study Population

One hundred and eighty (180) apparently healthy blood donors of the Department of Hematology of Ahmadu Bello University Teaching Hospital were evaluated for levels of *Salmonella typhi* and *Salmonella paratyphi A* antibodies using Widal test method. The presence of specific IgM and IgG antibodies against *S. typhi* was also tested for among the subjects. The subjects were predominantly males (98.9%). The median class age of the volunteers was 31- 35years. Subjects between 46-50 years and above were only 3.4% of the population. More than half of the populations of donors were between 21 to 35 years age group. The study population was made up of 61.1% married people. Among the participants; 8.9% had Islamic education; 4.4% primary schools; 31.1% attended secondary schools and 53.3% attended tertiary institutions. Eighty (80%) of the volunteers often eat outside their homes. The occupation of the subjects was also captured in the demographic table (Table 4.1). It shows that 30% of the subjects were businessmen, 25% civil servants and 24.4% were students. The places of residence of the volunteers were distributed throughout the neighborhoods of Zaria which includes Zaria City (35.6%), Sabon Gari (12.8%), Samaru (28.3%), Shika (14.4%) and Tudun Wada (8.9%) (Table 4.1).

The source of water used by the subjects is shown in Figure 4.1. Well water was the main source of water supply in the communities. Approximately, forty eight (48.3%) of the subjects use well water, 21.1% have access to municipal tap water while 12.2% use both well water and tap water. Some of the subjects (14.4%) have boreholes as their source of water. Figure 4.2 depicts the human excretal waste disposal system in Zaria. Approximately forty six percent (46.1%) of the volunteer population use the water

closet toilet system, 53.3% have pit toilet in their homes while just one volunteer (0.6%) practices open defecation in the fields.

**Table 4.1: Demographic Data of Subjects (n=180)**

| Parameter | Frequency | % |
|---|---|---|
| **Sex** | | |
| Male | 178 | 98.9 |
| Female | 2 | 1.1 |
| **Age Group** | | |
| < = 20yrs | 11 | 6.1 |
| 21yrs - 25yrs | 44 | 24.4 |
| 26yrs - 30yrs | 28 | 15.6 |
| 31yrs - 35yrs | 42 | 23.3 |
| 36yrs - 40yrs | 35 | 19.4 |
| 41yrs - 45yrs | 14 | 7.8 |
| 46yrs - 50yrs | 3 | 1.7 |
| > 50yrs | 3 | 1.7 |
| **Marital Status** | | |
| Single | 70 | 38.9 |
| Married | 110 | 61.1 |
| **Level Education** | | |
| Illiterate | 4 | 2.2 |
| Islamic School | 16 | 8.9 |
| Primary | 8 | 4.4 |
| Secondary | 56 | 31.1 |
| Tertiary | 96 | 53.3 |
| **Eating Habits** | | |
| No | 36 | 20.0 |
| Yes | 144 | 80.0 |
| **Occupation** | | |
| Civil Servant/ teacher | 45 | 25.0 |
| Industry Worker | 10 | 5.6 |
| Famer/ Fisher Man | 15 | 8.3 |
| Health Worker | 8 | 4.4 |
| Vendor in Restaurant | 1 | .6 |
| Student | 44 | 24.4 |
| Unemployed | 3 | 1.7 |
| Business man | 54 | 30.0 |
| **Dwelling Place in Zaria** | | |
| Zaria City | 64 | 35.6 |
| Sabon Gari | 23 | 12.8 |
| Samaru | 51 | 28.3 |
| Shika | 26 | 14.4 |
| Tudun-wada | 16 | 8.9 |

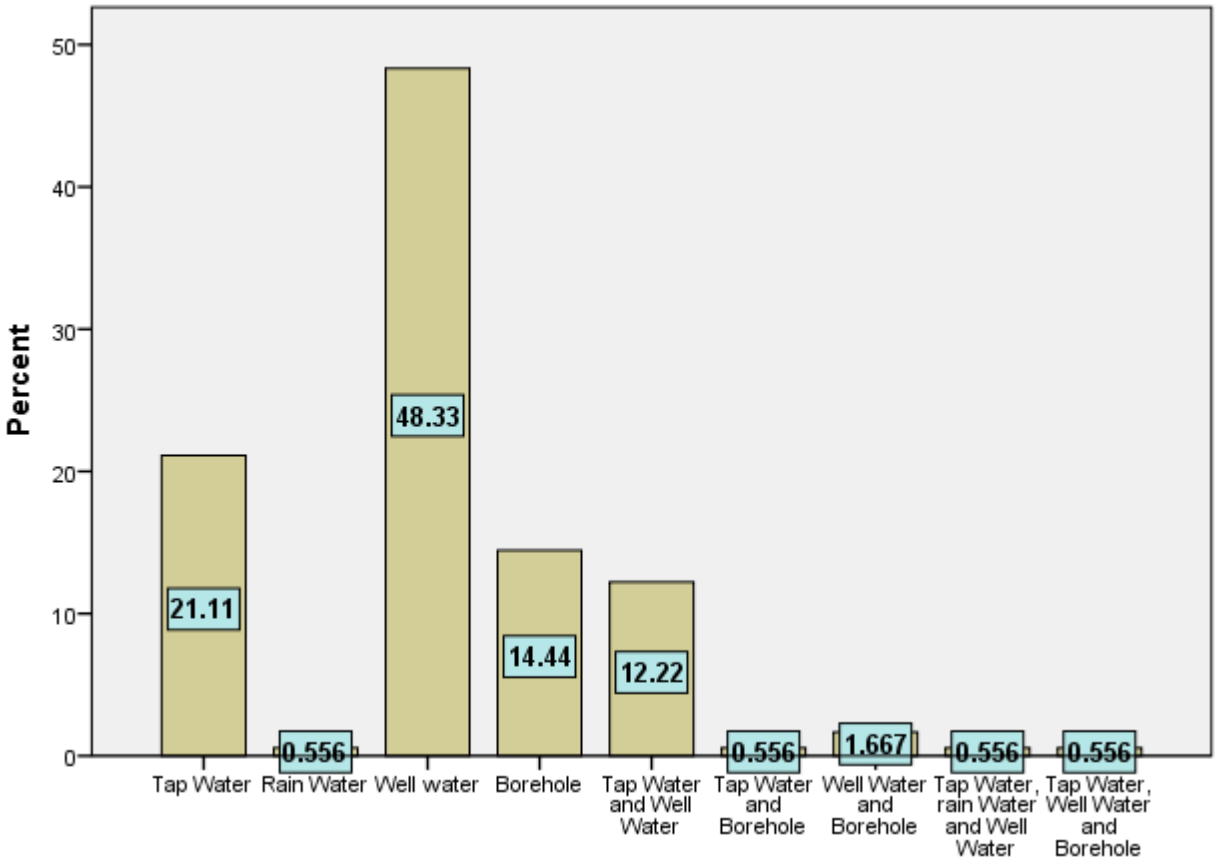



**Figure 4.1 Sources of Water used by the Participants**

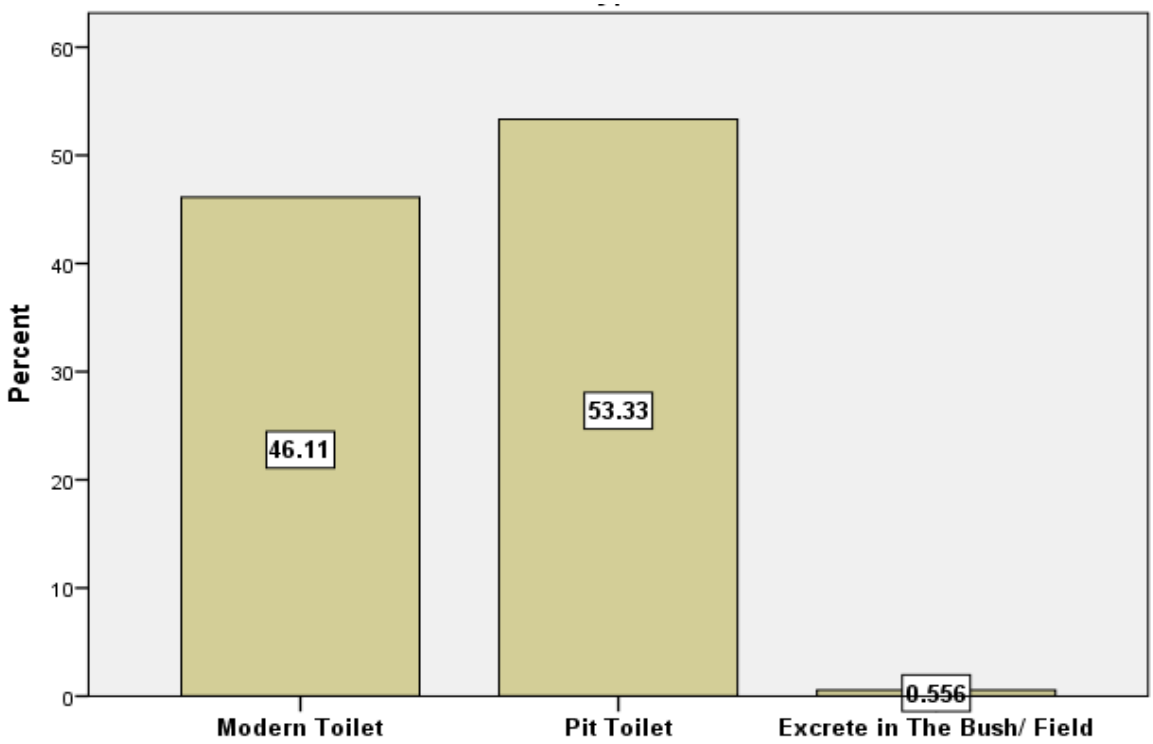



**Figure 4.2: Types of Toilet Facilities used by Subjects**

The subjects were tested for the presence of *Salmonella typhi* and *Salmonella paratyphi A,* O and H agglutinins using the microtitre tube dilution method. Eleven 19 (11%) of the subjects tested positive for *S. typhi* O and161 (89%) tested negative. For *S. typhi* H flagella antibodies, 45 (25%) showed positive reaction while 135 (75%) were negative. Antibodies against S. *paratyphi A O* were low in the population. Two 4 (2%) were positive, 176 (98%) tested negative. For *S. paratyphi* A H antibodies, 5 (3%) were positive while 175 (97%) tested negative (Table 4.2).

The breakdown of the distribution of titres for *S. typhi* somatic antibodies O is as follows: ≤ 1:20; 30(16.7%), 1:20; 131(72.8%), 1:40; 19(10.5%), 1:80; (00%) and 1:160; (00%) (Table 4.3). A total of 75% of the subjects were negative for *S. typhi* H antibodies while 25% tested positive. The distribution of the titers areas follows: ≤ 1:20; 12(6.7%), 1:20; 123(68.3%), 1:40; 34(18.9%), 1:80; 8(4.4%) and 1:160; 3(1.7%) (Table4.3). The distribution of antibody titres against *Salmonella paratyphi* A O among the subjects is as follows: Ninety eight (98%) 176 of the population tested negative while 4 (02%) tested positive. Titers of ≤ 1:20 occurred in 165(92%), 1:20; 11(06%), 1:40; 4 (0.2%) (Table 4.3). Table 4.2 shows that 175 (97%) of the study population were negative for *S. paratyphi* H antibodies. Only 5 (03%) showed agglutination reaction. The distribution of antibodies occurred as follows:

≤ 1:20; (00%), 1:20; 175(97.2%), 1:40; 2(1.1%) and 1:80; 3(1.7%). There were no reactions at titres of 1:160 and 1:320.

**Table 4.2: Distribution of *Salmonella typhi* and *Salmonella paratyphi A* Positive Antibody Titres Using Microtitre Plate Method (MTP) (n=180)**

| ***Salmonella* Antibodies** | **No. tested** | **No. Positive (%) >1:20** | **No. Negative (%)≤1:20** |
|---|---|---|---|
| *S. typhi* [O] | 180 | 19(11) | 161(89) |
| *S. typhi* [H] | 180 | 45(25) | 135(75) |
| *S. paratyphi* A [O] | 180 | 4(2) | 176(98) |
| *S. paratyphi* A [H] | 180 | 5(3) | 175((97) |

**Table 4.3: Distribution of *Salmonella typhi* O, H And *Salmonella paratyphi* A O,H Antibody Titres Among the Subjects Using Microtitre Plate Method (MTP) (n=180)**

| MTP TO Titre | Frequency | % | MTP TH Titre | Frequency | % |
|---|---|---|---|---|---|
| <1:20 | 30 | 16.7 | <1:20 | 12 | 6.7 |
| 1:20 | 131 | 72.8 | 1:20 | 123 | 68.3 |
| 1:40 | 19 | 10.5 | 1:40 | 34 | 18.9 |
| 1:80 | 0 | 0 | 1:80 | 8 | 4.4 |
| 1:160 | 0 | 0 | 1:160 | 3 | 1.7 |
| 1:320 | 0 | 0 | 1:320 | 0 | 0 |
| **MTP AO** | | | **MTP AH** | | |
| <1:20 | 165 | 91.7 | <1:20 | 0 | 0 |
| 1:20 | 11 | 6.1 | 1:20 | 175 | 97.2 |
| 1:40 | 4 | 2.2 | 1:40 | 2 | 1.1 |
| 1:80 | 0 | 0 | 1:80 | 3 | 1.7 |
| 1:160 | 0 | 0 | 1:160 | 0 | 0 |
| 1:320 | 0 | 0 | 1:320 | 0 | 0 |

**KEY:**

**TO= *S. typhi* O Antibodies,TH= *S. typhi* H Antibodies AO= *S. paratyphi* O Antibodies, AH= *S. paratyphi* H Antibodies**

**MTP = Microtitre Plate**

**Cut-off titre**
**S. typhi TO = 1:80, S. typhi TH = 1:80, S. paratyphi AO = 1:40,**
**S. paratyphi AH = 1:40**

Results from the quick tile method shows that 104 (58%) of the population tested were positive for *S. typhi* O antibody while 76 (42%) were negative. For *S. typhi,* H antibodies, 124 (69%) were positive while 56 (31%) tested negative. One hundred and seventy five 175 (97%) of the volunteers tested negative for *S. paratyphi A* O antibody. For S. paratyhi A H antibody 52 (29%) of the study population tested positive, while 128 (71%) were negative (Table 4.4).

The distribution of *S. typhi* O antibody titres among the population tested using the quick tile method is as follows: 1:20; 76(42.2%), 1:40; 30(16.7%), 1:80; 35(19.4%), 1:160; 35(19.4%) and 1:320; 4(2.2%) (Table 4.5). There was a raised antibody titre level among the volunteers when quick tile method was used compared with the microtitre plate (MTP) method (Table 4.3). The distribution of antibody titres of *S. typhi* H using the quick tile method (QCT) is shown in Table 4.5. The titres are as follows: 1:20; 56(31.%), 1:40; 44(24.4%), 1:80; 52(28.9%), 1:160; 25(13.9%) and 1:320; 3(1.7%). When the distribution of antibody titres of the MTP method, ≤ 1:20 (6.7%), 1:20 (68.3%), 1:40 (18.9%), 1:80 (4.4%), 1:160 (1.7%) and 1:320 (00%) is compared with the tile method an increased titre distribution value was also observed.

**Table 4.4: Distribution of *Salmonella typhi* and *Salmonella paratyphi A* Positive Antibody Titres Using Quick Tile Method (n=180)**

| ***Salmonella* Antibodies** | **No. tested** | **No. Positive (%) >1:20** | **No. Negative (%) ≤1:20** |
|---|---|---|---|
| *S. typhi* [O] | 180 | 104(58) | 76(42) |
| *S. typhi* [H] | 180 | 124(69) | 56(31) |
| *S. paratyphi* A [O] | 180 | 5(3) | 175(97) |
| *S. paratyphi* A [H] | 180 | 52(29) | 128(71) |

**Table 4.5: Distribution of *Salmonella typhi* O, H And *Salmonella paratyphi A* O,H Antibody Titres Among the Subjects Using Quick Tile Method (QCT) (n=180)**

| QCT TO Titre | Frequency | % | QCT TH Titre | Frequency | % |
|---|---|---|---|---|---|
| <1:20 | 0 | 0 | <1:20 | 0 | 0 |
| 1:20 | 76 | 42.2 | 1:20 | 56 | 31.1 |
| 1:40 | 30 | 16.7 | 1:40 | 44 | 24.4 |
| 1:80 | 35 | 19.4 | 1:80 | 52 | 28.9 |
| 1:160 | 35 | 19.4 | 1:160 | 25 | 13.9 |
| 1:320 | 4 | 2.2 | 1:320 | 3 | 1.7 |
| **QCT AO** | | | **QCT AH** | | |
| <1:20 | 0 | 0 | <1:20 | 0 | 0 |
| 1:20 | 175 | 97.2 | 1:20 | 128 | 71.1 |
| 1:40 | 3 | 1.7 | 1:40 | 15 | 8.3 |
| 1:80 | 2 | 1.1 | 1:80 | 22 | 12.2 |
| 1:160 | 0 | 0 | 1:160 | 7 | 4.0 |
| 1:320 | 0 | 0 | 1:320 | 8 | 4.4 |

The comparison of the results of the quick tile method with the Widal microtitre plate method is shown in table 4.6. A significant difference was found between the two tests methods using the student t test. A p value < 0.0001* was found in all the groups compared. Furthermore, correlation tests done to assess the relationship between the quick tile method (QCT) and the standard microtitre plate method (MTP) revealed that QCT TO titre and the MTP TO titre had a correlation coefficient (r) of 0.091 and p value of 0.225. QCT TH titre and MTP TH titre gave r = 0.314 and p value of 0.0001. For QCT AO titres and the MTP AH titres, a value of -0.051 for r and a p value of 0.497 while QCT AH titres and MTP AH titres had r = 0.192 and p= 0.010.No relationship was found between the two methods (Table 4.6).

Immunoglobulin G was present only in 1.1 % of the study population tested (Figure 4.3). IgM was observed among 3.9 % of the subjects tested. Approximately ninety six percent (96.1%) of the subjects were negative for IgM (Figure 4.4).

**Table 4.6: Correlation of Widal Microtitre Plate Method with Quick Tile Method (n=180)**

| PARAMETER | MTP TO Titre | | MTP TH Titre | | MTP AO Titre | | MTP AH Titre | |
|---|---|---|---|---|---|---|---|---|
| | **r** | **p – Value** | **r** | **p-Value** | **r** | **p-Value** | **r** | **p-Value** |
| QCT TO Titre | 0.091 | 0.225 | -------- | -------- | -------- | -------- | -------- | -------- |
| QCT TH Tire | -------- | -------- | 0.314 | 0.001 | -------- | -------- | -------- | -------- |
| QCT AO Titre | -------- | -------- | -------- | -------- | -0.051 | 0.497 | -------- | -------- |
| QCT AH Titre | -------- | -------- | -------- | -------- | -------- | -------- | 0.192 | 0.010 |

r = Correlation coefficient, MTP =Microtitre plate method, QCT = Quick Tile Method, TO= *S. typhi* O Antibodies, TH= *S. typhi* H Antibodies AO= *S. paratyphi* O Antibodies, AH= *S. paratyphi* H Antibodies

**Table 4.7: Comparison of Quick Tile (QCT) Method with Microtitre Plate (MTP) Method (n= 180)**

| 1/Titre value | **Geometric Mean ± SD** | | **test statistics (t)** | ***p*-value** |
|---|---|---|---|---|
| | **QCT (n = 180)** | **MTP (n = 180)** | | |
| *S. typhi* [O] | 3.02 ± 0.18 | 1.86 ± 0.13 | 13.43 | <0.0001* |
| *S. typhi* [H] | 3.09 ± 0.15 | 2.13 ± 0.13 | 12.71 | <0.0001* |
| *S. paratyphi* A [O] | 2.04 ± 0.04 | 1.07 ± 0.10 | 27.67 | <0.0001* |
| *S. paratyphi* A [H] | 2.45 ± 0.15 | 2.04 ± 0.04 | 7.03 | <0.0001* |

*Significant difference by paired student t-test

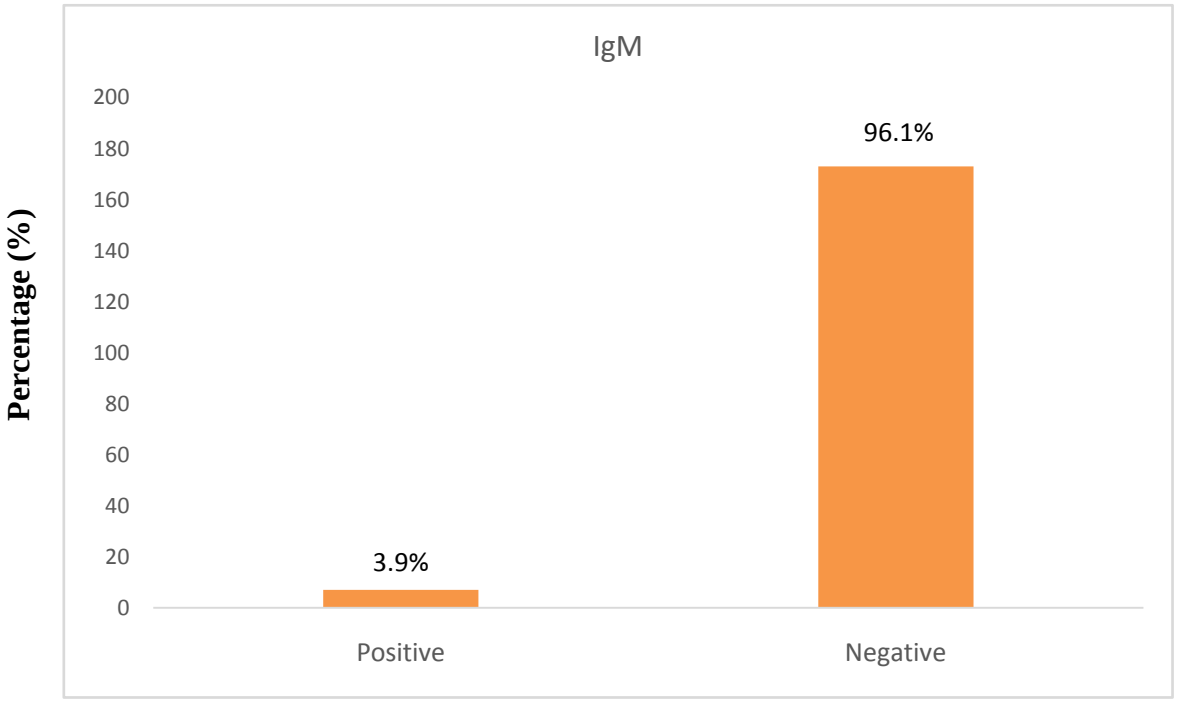



**Figure 4.3: A Chart Showing Prevalence of *S. typhi* Specific IgM among Apparently Healthy Individuals in Zaria**

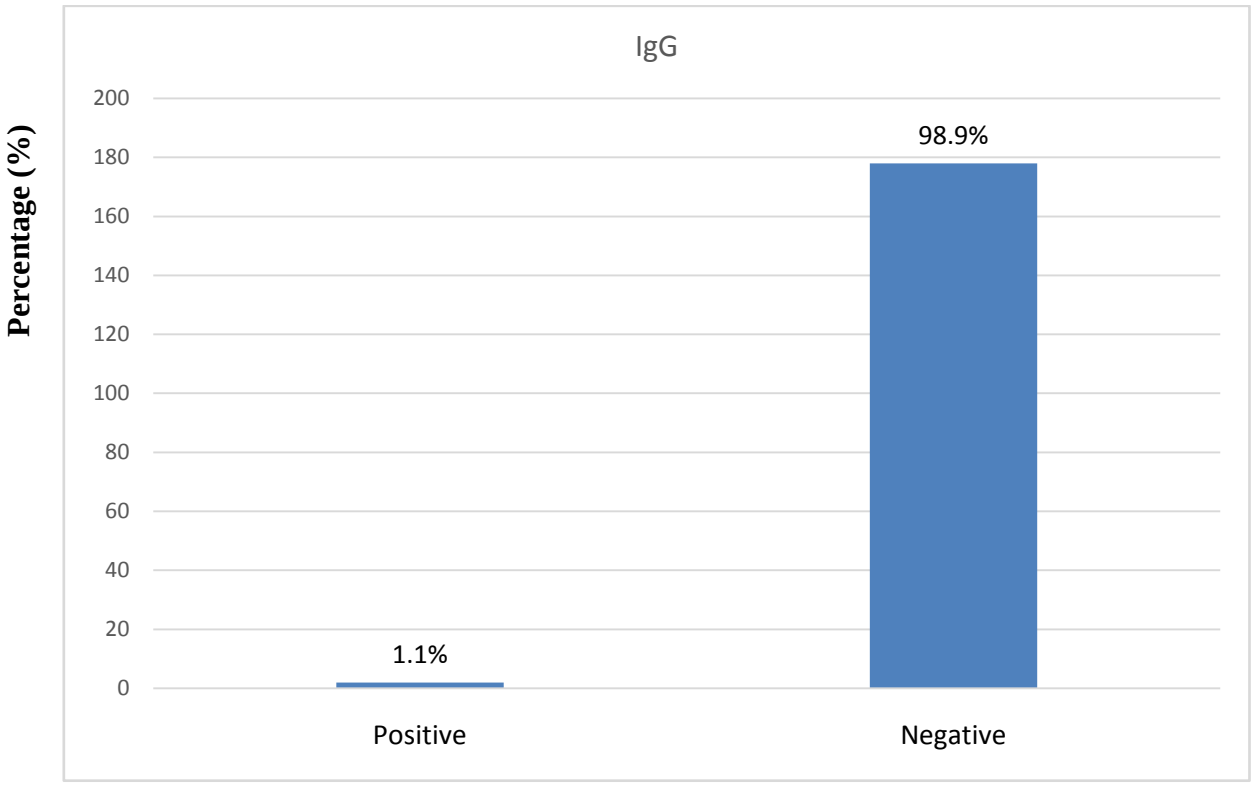



**Figure 4.4: A Chart Showing Prevalence of *S. typhi* Specific IgG among Apparently Healthy Individuals in Zaria**

# CHAPTER FIVE

## 5.1 DISCUSSION

In this study involving 180 healthy blood donors, 98.9% were males. This male preponderance agrees with the reports from other donor centers (Nsutebu *et al.*, 2002; Adias *et al.*, 2010) Traditionally, females do not feature prominently as donors because of the demand placed on them by menstruation. They are at the risk of iron deficiency if they donate blood of more than one unit in a year. (Gordeuk *et al.*, 1990; Adias *et al.*, 2010)

It was observed that about 80% of the subjects in this study have the habit of eating outside their homes at restaurant and road side food vendors. However, these volunteers were mostly businessmen, civil servants and students who by nature of their daily routine operate outside their homes and therefore have no choice than to patronize these restaurants and roadside food vendors. This predisposes one to typhoid infections due to the risk of food contamination by carriers or by contamination during the processing of food products. Socio demographic factors are believed to be associated with the prevalence of typhoid fever and typhoid agglutinins among the communities. While education may have positive effect on personal hygiene and public health, some behavioral patterns may encourage the acquisition of typhoid fever (Frimpong *et al.*, 1999; WHO, 2003).

The World Health Organization reported the benefit of proper sanitation in reducing the risk of all diarrheal pathogens including *Salmonella typhi* (WHO, 2003).In this study it was observed that the volunteers who lived in different parts of Zaria depended largely on well water with a few having access to tap water and borehole. Access to good quality water is relatively high in this study area. This should reduce the burden of

water borne diseases. Another observation in this study was the quality in the handling of human waste disposal systems.While46.1% of the subjects use water closet toilet system, 53.3% use pit latrines in their homes. Open field defecation was reported only in 0.6 % of the study population.

Evaluation of the distribution of *Salmonella typhi* O antibodies using the microtitre tube method reveals that 11% of the population studied had positive antibody titres (> 1:20) while 89% had negative antibody titre (≤ 1:20). No reactions were observed at titres ≥ 1:80. Furthermore, the distribution of flagella antibodies *Salmonella typhi* H reveals that 75% of the population was negative for the antibodies while 25% showed positive reactions.

For application in practice, titres occurring in more than 5% of the population under study are not considered diagnostically significant and are regarded as normal in the population (Collard *et al.*, 1959; Ibekwe *et al.*, 2008). From this study, it then implies that titres of ≤ 1:20, 1:20 and 1:40 which occurred in a significant proportion of the study population should all be regarded as normal (baseline) titres in the population. Titres greater than > 1:80 could be used in presumptive diagnosis of typhoid fever in the study area, Zaria, for a single sera Widal test. The result agrees with the findings of Ibekwe *et al.* (2008), in South Eastern Nigeria and Mohammed *et al.* (1992), in Borno northern Nigeria. However, it differs from the findings in Kumasi Ghana where titres ≥ 1:160 and ≥ 1:320 were found diagnostic for S. *typhi* antibodies-O and H respectively (Frimpong *et al.*, 1999). The levels of *Salmonella typhi* antibodies titres in Zaria are still low as established by Greenwood and Whittle (Bala and Auty, 1977). The researchers had found that *Salmonella* O antibodies exceeding 1:80 is diagnostic for Zaria. This may be due to the maintained quality of public health standards such as

good quality water source supply and human sewage management systems as observed from the respondents of the questionnaire. Similarly, *Salmonella paratyphi A* antibodies in the population studied in Zaria is very low. Two (2%) of the population had positive results for *Salmonella paratyphi A* O (somatic antibodies) while a total of 98% of the population were negative. Only 3% of the population were positive for *S. paratyphi A* H (flagella) antibodies.

From the study therefore, it was established that the cut-off titres of *Salmonella paratyphi* A O and AH is 1:40. Titres greater than 1:40 should be regarded as significant for diagnosis of *Salmonella paratyphi A* infections. The low baseline titires for *S. paratyphi A* O and H found from this study of healthy population tested, suggest that paratyphoid fever caused by *S. paratyphi A* is less prevalent in this region. This finding also agrees with the work of Greenwood and Whittle who reported very low occurrence of *Salmonella paratyphi* antibodies in the population (Bala and Auty, 1977). Other researchers have found low levels of *Salmonella paratyphi A* antibodies among populations they studied (Zailani *et al.*, 2003; Pokhrel *et al.*, 2009). However there have been reports of increased outbreaks of *Salmonella paratyphi A* epidemics in some countries (Tankhiwale *et al.*, 2003; WHO, 2008)

A comparison between the quick slide method and the microtitre plate showed no relationship between the two methods. Also, there was no relationship between the quick tile and Typhidot 1gM/1gG kit results but this was corroborated by the microtitre plate/tube dilution technique. This clearly shows that the quick slide method which is used in the diagnosis of typhoid in many laboratories and hospitals is probably inappropriate.

A comparison of the two methods using their geometric mean and standard deviation gave a p value of < 0.0001 for all groups compared. This also shows that there is a significant difference between the microtitre plate method and the quick slide method.

Ideally, quick slide technique is a screening test for *Salmonella* agglutinins and all positive samples from the slide test must be confirmed using the microtitre tube method (Ibekwe *et al.*, 2008; Okonko *et al.*, 2010). Moreover, in this study it was observed that a few samples that proved to be negative (1:20) using the quick tile method became positive (1:40) after incubation at optimum temperature. The result obtained in the Widal test is dependent upon the techniques of testing applied. Many laboratories are using the quick tile method as a standard operating technique in their laboratories. The comparison of distribution pattern of the antibody titres seen in the microtitre plate method with the quick tile method clearly reveals the reasons for the over-reported cases of typhoid in many centers. Widal microtitre plate method and the quick tile technique using the student t- test shows a statistical difference.

As such where, the use of paired sera sample is possible for the confirmation of typhoid diagnosis, it is advisable to confirm the result of the Widal test by observing a rise in the titre of the second sample. The second Widal has a sensitivity and specificity of over 90% (Willke *et al.*, 2002). The application of Typhidot IgM/IgG may become an alternative in cases of conflicting diagnosis and to buttress typhoid diagnosis in absence of paired sera. In this study the occurrence of IgM/IgG *Salmonella typhi* specific antibodies using Typhidot 1gM/1gG kit among healthy individual was 3.9% for IgM and 1.1% for IgG

The advantages of Typhidot over the Widal and the gold standard blood culture is the short time it requires to obtain a result and that it does not require establishing a local

cut-off value as with Widal. It has also a high sensitivity (> 93%) and specificity (75%). Evaluation of Typhidot in clinical settings has shown that it performed better than Widal and culture(WHO, 2003; Siba *et al.*, 2012). In settings that can afford the relatively high cost of Typhidot and that require instant individual diagnosis to support the clinical diagnosis of typhoid, Typhidot IgM/IgG is superior to Widal tube agglutination. Nonetheless, for settings with limited financial and technical resources, the Widal microtitre plate agglutination test can produce a similar performance as Typhidot at a lower cost, though it requires more time (Ley *et al.*, 2011).

The occurrence of *Salmonella typhi* specific IgM antibodies in apparently healthy blood donors highlights the issue of subclinical infections of typhoid among donors. Agglutinins of IgM class indicate current infection while those of IgG point to cumulative antibody from previous exposure or vaccination(Greenwood and Whittle, 1981; Oyeyinka, 2002).

This study thus raises the issue of safe blood transfusion as positive antibody titres to *S. typhi* were obtained in blood donors. The implications of this need to be explored. The awareness about the safety of blood and blood products in Nigeria is growing. In many blood banks, testing for human immune virus (HIV), hepatitis B virus (HBV) and hepatitis C is being done. The possibility of transmitting *Salmonella* via blood transfusion is real. No blood or blood product is safe until it is free of all agents that can create undesirable consequences after transfusion. The detection of specific *Salmonella typhi* IgM in 3.9% of the population of blood donors studied further demonstrates the need to screen blood for Salmonella organism especially *Salmonella typhi.* The IgM/IgG Typhidot may be useful in solving this challenge. Nsutebu *et al.* (2002) and Adias *et al.* (2010)suggested using the Widal test in screening for *Salmonella* organism

in blood donors. However, this may be cumbersome, time wasting and may not achieve the set goal as blood transfusion is often an emergency service.

Comparison of data from other researchers in other centers revealed differences and relationships. There is similarity in the distribution of data presented in the quick tile method in this study and the distribution presented byOkonko *et al.* (2010)who studied the Widal baseline titre in Abeokuta south western Nigeria. The values and distribution agree with the distribution obtained in the quick tile methods of this study. The picture becomes clearer when the titre distribution patterns of *S. typhi* O and H and *S. paratyphi A* H from this study are compared with the result of their study. From the methodology used by Okonko *et al.* (2010), it is observed that a modified tile method was used. However, the distribution in this study agrees with the study by Frimpong *et al.* (1999) in Ghana which demonstrated the paucity of *Salmonella paratyphi A* O and H antigens. The distribution from their study compares with the result of this study. The standard microtiter plate method was used by the researcher.

From this study, it was observed that cross reactions are most present among the *S. typhi O, H and S. paratyphi H* antibodies. The antibodies which occur in a much greater titre than would be expected from the antigen pattern of the indigenous *Salmonella* may be natural antibodies unrelated to antigen stimulation or this may arise as a result of infection with cross reactive organisms (Oyeyinka, 2002). Most cross reactions are often pseudo agglutinations which may be formed by other weak bonds such as hydrogen bonds, plasma proteins, and Vanderwals forces. Effects of such weak bonds are eased off by titrations and dilutions using the standard microtitre tube dilution method for Widal testing.

Strikingly, few samples that gave negative results in quick slide method produced slightly positive (1:40) results after titrations and dilution in this study. When the ratios of antigens to antibodies are not optimal, false negative results may occur. The effects of plasma proteins can also give false positive results. Time and optimal temperatures also are factors that affect antigen antibody reaction. In general, dilution with saline improves the specificity of antibody and antigen reactions. These factors play effective roles in the results produced by the microtitre plate method. Another important factor in achieving good result in diagnosis of typhoid and paratyphoid infections using the Widal technique is the quality of antigens. This study obtained the antigen used for the research from Oxoid Diagnostics. The need for centers to use standardized antigens for Widal testing cannot be over emphasized. The purity of antigens employed in the assay helps in reducing cross reactions.

# CHAPTER SIX

## 6.0 CONCLUSION AND RECOMMENDATION

## 6.1 CONCLUSION

The agglutinins for *Salmonella typhi* and *S. paratyphi A* in apparently healthy individuals in Zaria are low. The baseline titre of *Salmonella typhi* O and H Widal antibody titre is 1:40. The cut off titre for *S typhi* O and H is 1:80. The significant titre for the presumptive diagnosis of typhoid in our locality, Zaria is > 1:80 for somatic O and flagella H antibodies, while the significant titre for *S. paratyphi A* antibodies for presumptive diagnosis of paratyphoid using Widal technique is > 1:40 for both O and H agglutinins. The baseline titre for *S. paratyphi A* antibodies is 1:20. Cut off titre for *S. paratyphi* is 1:40. The prevalence of specific *Salmonella typhi* IgM and IgG antibodies among healthy individuals in Zaria is 3.9% and 1.1% respectively. There is a significant difference in results obtained between the quick tile method and the Widal microtitre plate method.

## 6.2 RECOMMENDATIONS

1. This study recommends the use of microtitre plate method in Widal testing of all clinical samples and in research for Widal baseline determinations. The use of the quick tile method in the Laboratories should be discontinued.

2. Significant titres for *Salmonella typhi* O and H should be > 1:80 and significant titres for *Salmonella paratyphi A* should be > 1:40 for both A O and A H. While clinicians are advised that titres at the cutoff point in cases of suspected typhoid infections should be further investigated using the Typhidot IgM/IgG

3. Institutions should adapt and encourage the use of Typhidot specific antibodies Kits as a means to buttress typhoid diagnosis. The introduction and use of the Typhidot

by many institutions will bring down the cost off the test kit and reduce the cost burden of typhoid diagnosis and treatment.

4. Blood transfusion services should consider the inclusion of screening for *Salmonella typhi* using the Typhidot test kits in order to increase the safety of blood.

## 4.3 LIMITATION

1. Inability to carryout multicenter study with a very larger sample size in order to validate the baseline titres determined in this work.

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## APPENDIX 1

## ETHICAL APPROVAL


# HEALTH RESEARCH ETHICS COMMITTEE

**AHMADU BELLO UNIVERSITY TEACHING HOSPITAL**

**SHIKA - ZARIA, NIGERIA.**

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*Our Ref:* ABUTH/HREC/TRG /36

*Your Ref:* _____

*Date:* 16th Sept, 2014

***ABUTH HREC FULL ETHICAL CLEARANCE CERTIFICATE***

**Baseline Antibody Titre to Salmonella Typhi/Paratyphi among Apparently Healthy Individuals in Zaria, Kaduna State Nigeria.**

| | | |
|---|---|---|
| **ABUTH Ethics Committee assigned number:** | - | **ABUTHZ/HREC/L04/2014** |
| **Name of the principal Investigator:** | - | **Mr Odugu Jude Amakaeze** |
| **Address of the Principal Investigator:** | - | **Immunology Unit, Dept of Medicine Faculty of Medicine ABU Zaria** |
| **Date of receipt of valid application:** | - | **10/5/14** |
| **Date of meeting when final determination on ethical approval was made:** | - | **3rd & 4th Sept, 2014** |

This is to inform you that the research described in the submitted protocol, the consent forms and other participant information materials have been reviewed and ***given full approval by the Health Research Ethics Committee.***

Please note: this approval dates from **17th September, 2014– 17th September, 2015**

No participant recruitment into this research may be conducted outside these dates.

All informed consent forms in this study must carry the ABUTH HREC number assigned to this research and the duration of ABUTH HREC approval of the study.

This HREC expects that you submit your application as well as an annual report for ethical clearance renewal 3 months prior to expiration of study dates. This is to enable you obtain renewal of your approval and avoid interruption of your research.

If there is delay in starting the research, please inform the ABUTH HREC so that starting dates can be adjusted accordingly.

No changes are permitted in the research without prior approval by ABUTH HREC, except in circumstances outlined in national code for Health Research Ethics: http://www.nhrec.net.

ABUTH HREC reserves the right to conduct compliance assessment visits to your research site without prior notification.

**Prof. A. I. Mamman**
**Chairman, ABUTH HREC**

## APPENDIX I1

## INFORMED CONSENT

My name is ODUGU JUDE.I am an M.Sc. student from the Department of Immunology of the Ahmadu Bello University Zaria. This study is designed for Academic Research purpose as well as the benefit of the community. The study aims at determining the baseline titre of *Salmonella* antibodies among healthy volunteers in Zaria. This will help in setting the cut off titre for the treatment of typhoid using Widal test for patients in Zaria. Five(5) mls of blood will be taken from each of the subjects. The confidentiality of the study will be assured to the subjects and the results of the study will be communicated to the subjects. The study is voluntary and with no cost/burden on the subject(s).

**INFORMED CONSENT FORM**

I……..……..of…….……has voluntarily agreed to be one of the subjects in this study - Baseline Titre of *Salmonella* antibodies among healthy individuals in Zaria .The full procedure was explained to me. I have had the opportunity to ask questions about it and the questions that I have asked have been answered to my satisfaction.

Participants Name…… Sign…….

Researcher's Name……..... Sign…….

## APPENDIX III

## QUESTIONNAIRE

**Section 1: Demographic Information** **Date: ……**

1. Sex: (a) Male [ ] (b) Female [ ]

2. Age:_____

3. Marital Status: Tick **(√)**

   (a) Single [ ]

   (b) Married [ ]

   (c) Divorced [ ]

   (d) Widowed [ ]

   (e) Separated [ ]

4. Educational qualification

   (a) Illiterate [ ]

   (b) Arabic school [ ]

   (c) Primary [ ]

   (d) Secondary [ ]

   (e) Tertiary [ ]

   (f) Others specify_____

**Section II: Clinical Data**

5. Have you had fever within last one month?

   (a) Yes [ ] (b). No [ ](c) Unknown [ ]

6. Are you taking any treatment?

   (a) Yes [ ] (b). No [ ](c) Unknown [ ]

7. If yes what are you taking? _____

8. Have you ever taken typhoid vaccine?

   (a) Yes [ ] (b). No [ ](c) Unknown [ ]

**Section IV: Possible Source of Infection during Exposure Period**

9. Have you typhoid in less than (<) 1 (one) year?

   (a) Yes [ ](b) No [ ] (c) May be but not confirmed [ ] (d) No [ ]

   (e) Don't know [ ]

10. Have you contact (work colleagues, School mates relatives, Neighbours etc) with typhoid within one year. (a) Yes [ ] (b) No [ ]

11. If Yes where?

   (a) In household [ ] (b) Neighbour [ ] (c) Working place [ ]

   (d) In the School [ ] (e) In hospital [ ]

12. What is your Occupation?

   (a) Civil servant, teacher [ ]

   (b) Industry worker [ ]

   (c) Farmer, Fisherman [ ]

   (d) Health worker [ ]

   (e) Vendor in restaurant [ ]

   (f) Student [ ]

   (g) Unemployed [ ]

   (h) Others specify _____

13. Did you travel outside your community recently (three months ago)?

   (a). Yes [ ](b) No [ ]

14. If yes, for how

   (a) Long? _____(b) Where? _____

15. Do you often eat outside?

   (a) Never [ ] (b) Yes [ ]

16. If yes, how many times per month? _____

17. If yes where?

(a) In the restaurant [ ](b) In the streets [ ]

18. How often do you eat these types of food?

(Tick one box each line) (t = times)

| | Never | 1-2 t/month | 1-2 t/week | 3-4 t/week | Everyday |
|---|---|---|---|---|---|
| (a) Shell fish | [ ] | [ ] | [ ] | [ ] | [ ] |
| (b) Fresh/Raw fish | [ ] | [ ] | [ ] | [ ] | [ ] |
| (c) Meat | [ ] | [ ] | [ ] | [ ] | [ ] |
| (d) Salad | [ ] | [ ] | [ ] | [ ] | [ ] |
| (e) Local Beer (*Burukutu*) | [ ] | [ ] | [ ] | [ ] | [ ] |
| (f) Yorghurt, *Fura/Nunu* | [ ] | [ ] | [ ] | [ ] | [ ] |

19. How do you drink water?

(a) Drinking boiled water [ ]

(b) Drinking un-boiled water from city taps [ ]

(c) Drinking un-boiled water from wells [ ]

(e) Drinking stream water [ ]

20. Do you often wash your hands?

(a) Before eating [ ]

(b) After using the toilet [ ]

21. What types of water supply do you have in your house/family?

(a) Municipal tap water supply [ ](b) Rainfall water [ ] (c) Wells [ ]

(d) Stream [ ](e) Others [ ]

22. What type of Latrines do you have?

(a) Modern (water cistern) toilet [ ]

(b) Pit toilet [ ]

(c) Excrete into Rivers [ ]

(d) Excrete directly in the field/bush [ ]

23. Which of the following domestic animals do you keep

(a) Cow [ ] (b) Sheep/goat [ ](c) Pig [ ] (d) Chicken/Poultry [ ]

(e) Dogs [ ] (f) No [ ]

*Thank you for your assistance.*