CHARACTERIZATION OF *SALMONELLA* SPECIES AND DETERMINATION OF BASELINE ANTIBODY TITRE AMONG APPARENTLY HEALTHY INDIVIDUALS IN KADUNA STATE, NIGERIA

BY

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DECLARATION

I hereby declare that the work entitled "**Characterization of *Salmonella* species and Determination of Baseline Antibody Titre among Apparently Healthy Individuals in Kaduna State, Nigeria"** reported in this thesis was carried out solely by me under the supervision of **Professor E. D. Jatau and Prof. M. Aminu** of the Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria. The works of other researchers are duly acknowledged and referred to accordingly. I declare that no part of this work has been submitted elsewhere for a degree or diploma.

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CERTIFICATION

This dissertation titled **"Characterization of *Salmonella* species and Determination of Baseline Antibody Titre among Apparently Healthy Individuals in Kaduna State, Nigeria"** by Abdullahi Jaafaru Isah, meets the regulations governing the award of the degree of Master of Science in Microbiology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my father Late Alhaji Isah Abdullahi (R.A) and my beloved and caring mother Hajiya Hannatu Garba Rilwan, may Almighty Allah (SWA) be pleased with them all and increase her in health, Ameen.

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ABSTRACT

This study was conducted to determine the baseline antibody titre, isolate and characterize *Salmonella* species from apparently healthy individuals in Kaduna State, Nigeria. A total of 420 of each of stool and blood sample were collected from six Local Governments Areas (LGAs) of Kaduna State, with two LGAs from each Geopolitical Zones. All stool samples were cultured on Bismuth sulphite agar, and bacterial isolates where characterized. Widal slide agglutination and tube agglutination tests using Standard Salmonella "O" and "H" suspensions were carried out on the blood samples to detect the presence of antibodies to *Salmonella* species and their titres respectively. The result showed that a total of 338 out of the 420 participants had antibody titre ≥1:20 against *Salmonella* species giving a total prevalence of 80.5%. The remaining 82 (19.5%) had antibody titre <1:20. The result also showed that 29.5% (124/420) had antibody against *Salmonella* Typhi <1:20 while 70.5% (296/420) had antibody against *Salmonella* Typhi ≥ 1:20. Similarly, 53.3% (224/420) and 46.7% (196/420) had antibody levels against *Salmonella* Paratyphi A <1:20 and ≥ 1:20 respectively. Antibody titre <1:20 was seen in 54.1% (227/420) participants, while antibody titre ≥ 1:20 was seen in 45.9% (193/420) against *Salmonella* Paratyphi B. The antibody titre against *Salmonella* Paratyphi C of <1:20 was detected in 83.1% (349/420), while titre ≥ 1:20 was seen in 16.9% (71/420) of the participants. The frequency of occurrence of the antibodies to the different serotypes was generally higher in males with a range of 19-84% than in females with a range of 11-82%. *Salmonella* Typhi antibodies had the highest frequency of 87.6% for "H" antibodies and 84.0% for "O" antibodies, while *Salmonella* Paratyphi C antibodies had the least frequency with 18.6% and 21.0% for "C-O" and "C-H" agglutinins respectively. In this study, it was found out that participants in Giwa LGA which is a rural area from the Central Senatorial Zone had the highest prevalence (88.6%: 62/70) of *Salmonella* species antibodies while Zaria LGA which is an Urban area had the least prevalence of 67.1% (47/70). The result showed that the presence of antibody to *Salmonella* species varied significantly (χ^2 =9.398, df =1, p =0.002) with gender, insignificantly with age (χ^2=8.194, df =5, p =0.146), educational level (χ^2 =3.097, df =3, p =0.377), and occupation (χ^2 =5.612, df =3, p =0.132). *Salmonella* Typhi was isolated from a total of 3 stool samples with 1 positive sample each from Zaria, Giwa and Kagarko LGAs. None of *Salmonella* Paratyphi A, B or C was isolated in the study. The study found antibody titres against *Salmonella* Typhi, *Salmonella* Paratyphi B and *Salmonella* Paratyphi C up to 1:80 occurring in up to 5% of the study population, while antibody titres against *Salmonella* Paratyphi A up to 1:40 occurred in up to 5% of the study population. From the findings of the study, it is suggested that antibody titres greater than 1:80 against *Salmonella* Typhi, *Salmonella* Paratyphi B, *Salmonella* Paratyphi C, and antibody titres greater than 1:40 against *Salmonella* Paratyphi A should be the baseline antibody titres for Widal tests in Kaduna State.

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LIST OF ABBREVIATIONS

A-H	*Salmonella* Paratyphi A flagella antigen
A-O	*Salmonella* Paratyphi A somatic antigen
API	Analytical Profile Index
B-H	*Salmonella* Paratyphi B flagella antigen
B-O	*Salmonella* Paratyphi B somatic antigen
C-H	*Salmonella* Paratyphi C flagella antigen
C-O	*Salmonella* Paratyphi C somatic antigen
LGAs	Local Government Areas
PCR	Polymerase Chain Reaction

CHAPTER ONE

1.0 INTRODUCTION

Typhoid fever is a systemic infection caused by species of *Salmonella* (Eze *et al.,* 2011); *Salmonella enterica,* serotype Typhi which is a highly adapted human specific pathogen that has evolved remarkable mechanisms for its persistence in its host, ensuring its survival and transmission (Anagha *et al.,* 2012). Other species and strains of *Salmonella* that commonly cause typhoid fever in humans are *Salmonella* paratyphi A, *Salmonella* Paratyphi B and *Salmonella* Paratyphi C (Lerner and Lerner 2003; WHO, 2003). The different serotypes of *Salmonella* can co-infect an individual or cause infections differently (Samal and Sahu, 1991; WHO, 2003). Salmonellae are motile, ferment glucose with acid and sometimes with gas; and most of them produce H_2S, but not urease. They are not fastidious and grow rapidly on most laboratory media, and can survive outside the host in inhospitable environments, such as fresh water and freezing temperatures. These pathogens are resistant to chemicals such as bile and dyes (the basis for isolation on selective media) and do not lose virulence after long-term artificial cultivation (Patrick *et al.,* 2002).

Typhoid fever is so named because it bears a superficial resemblance to typhus, a rickettsial disease, even though the two diseases are otherwise very different. The typhoid bacillus usually enters the alimentary canal along with water or food contaminated by faeces, although it is occasionally spread by close personal contact (Patrick *et al.,* 2002). In some circumstances, other vehicles of transmission include ingestion of shellfish taken from sewage contaminated beds, unwashed raw fruits or vegetables fertilized by human waste, or milk contaminated by carriers (Heymann, 2004). Because humans are the exclusive hosts for *Salmonella* Typhi, asymptomatic carriers are important in perpetuating and spreading the organism. Once in the small intestine,

the incubation period is 10-14 days. Even six weeks after convalescence, the bacillus is still shed by about half of patients who have recovered from the illness. A small number of people chronically carry the bacilli for longer periods in the gallbladder. From this site, the bacilli are constantly released into the intestine and faeces (Patrick *et al.*, 2002).

The infective dose is between 1,000 and 10,000 bacilli (UDH, 2005). At the mucosa of the small intestine, the bacilli adhere and initiate a progressive, invasive infection that leads eventually to septicemia. Symptoms are fever, diarrhoea, constipation and abdominal pain. There may be headache, malaise, non productive cough, slow heart rate (bradycardia) and anorexia (UDH, 2005). The typhoid bacilli infiltrate the mesenteric lymph nodes and the phagocytes of the liver and spleen. In some people, the small intestine develops areas of ulceration that are vulnerable to hemorrhage, perforation and peritonitis. The presence of the organism in the general circulation may lead to nodules and abscesses in the liver or urinary tract. Prompt treatment greatly reduces the possibility of death (Patrick *et al.*, 2002).

The period of communicability is as long as the organism continues to be shed in excreta (i.e. stool or urine), ranging from the first week of illness throughout convalescence. About 10% of untreated patients will excrete the organism for 3 months after onset of signs and symptoms, and 2% to 5% become permanent carriers. A chronic carrier state is most common in persons infected during middle age or in persons with underlying biliary tract abnormalities such as gallstones (Heymann, 2004).

Typhoid fever is a global infection with a fatality rate of 10%. The disease is a cause for concern and a major public health problem in developing countries (Asia, Africa), especially in Nigeria due to poor sanitary conditions and lack of or inadequate potable water (Doughari, 2005). The

World Health Organization (WHO) estimated an annual infectious rate of 21.6 million and approximate death rate of 600 000 with the highest percentage in Africa and Asia (Ibekwe *et al.,* 2008, CDC, 2016).

An early and accurate diagnosis is necessary for a prompt and effective treatment. One has to rely on serological diagnosis, since many diagnostic laboratories in the developing countries, especially in rural areas do not have facilities for blood culture and thus the serological diagnosis becomes an important diagnostic tool (Anagha *et al.,* 2012).

The Widal test is a reaction involving agglutination of typhoid bacilli when mixed with serum containing typhoid antibodies from an infected individual (Srindar, 2009). The test detects the IgM and IgG antibodies to *S.* Typhi in the patient's serum from the second week of the onset of the symptoms of typhoid fever (Anagha *et al.,* 2012). Ever since its introduction about hundred years ago, the widal agglutination test is one of the over utilized diagnostic tests for typhoid fever in developing countries (Jeyakumari and Jaberlin, 2011). This test detects antibodies, O, H, AH, BH against *S.* Typhi and *S.* Paratyphi organisms. The test is considered positive if the antibody titres are higher than the cut-off value in a single test or a rising titre in paired sera collected at intervals of one or two weeks (Jeyakumari and Jaberlin, 2011). Random enquiry by the researcher from some diagnostic laboratories in Zaria, Kaduna State has shown that the laboratories do not have a uniform cut off antibody titre for the interpretation of widal tests. As some use an antibody titre of greater than or equal to 1: 80 as significant, others use greater than or equal to 1:40, which are both deduction made from the observation of an antigen-antibody reaction on a slide agglutination test and not tube agglutination tests.

In endemic areas, due to previous exposure and cross reacting antigen, the healthy population may have antibodies capable of reacting to variable titres in widal test and this is called the baseline antibody titre (Jeyakumari and Jaberlin, 2011). The antibody levels of individuals in a population of a given area give the base line titres which must be known before attaching significance to the test titres (Rao, 2009). This research work tried to find the baseline antibody titre to *Salmonella* Typhi in Kaduna state, Nigeria.

1.1 Statement of the Research Problem

The definitive diagnosis of typhoid fever requires isolation of the typhoid bacillus (Jeyakumari and Jaberlin, 2011). However, apart from being costly, the culture facilities are limited outside the tertiary hospitals and they are not employed on a routine basis (Pal *et al.*, 2012).

The diagnostic significance of serological tests depends among other factors on the positivity rate in the general population (Oyeyinka and Salimonu, 2002). Antibody build-up against specific antigens in a given community depends on the endemicity of the specific infecting agents of infection, and therefore their plasma antibody titres differ widely from place to place (Oyeyinka and Salimonu, 2002)

In endemic areas, due to previous exposure and cross reacting antigens, the healthy population may have antibodies capable of reacting to variable titre in widal test, and this is called baseline antibody titre (Jeyakumari and Jaberlin, 2011). Hence, in endemic areas, the interpretation of widal tests heavily depends on the baseline titres to *S.* Typhi and Paratyphi A, B and C among healthy population in the area (Pal *et al*., 2012). Other researchers have determined the baseline antibody titre to *Salmonella* Typhi in different places of the world and helped significantly in

improving the specificity and sensitivity of widal test in the diagnosis of typhoid fever in such places (Oyeyinka and Salimonu, 2002; Ibekwe *et al.*, 2008; Jeyakumari and Jaberlin, 2011).

The absence of a known baseline antibody titre to *S.* Typhi and *paratyphi* in Kaduna State therefore may lead to misdiagnosis of typhoid fever, with subsequent wrong drug administration that may lead to drug resistance.

1.2 Justification of the Study

Typhoid fever is a global infection with a fatality rate of 10% (Doughari, 2005). An early and accurate diagnosis, which can easily be achieved through serology (Widal test) is necessary for prompt and effective treatment (Anagha *et al.*, 2012). It is also evident that in endemic areas, the interpretation of widal tests depends on the baseline antibody titre to *S.* Typhi and *paratyphi* A, B and C among healthy individuals. To the best of our knowledge, there are little or no published study conducted to determine the baseline antibody titre to *Salmonella* Typhi and *paratyphi* in Kaduna State. It is therefore necessary to determine the level of antibody in the healthy individuals of Kaduna state so as to know what titres should be used as cut-off values for the diagnosis of typhoid fever. This will prevent over and under diagnoses of typhoid fever, and therefore prevent wrong drug administration and subsequent drug resistance.

1.3 Aim of the Study

The aim of this work was to determine the baseline antibody titre, isolate and characterize *Salmonella species,* from apparently healthy individuals in Kaduna State, Nigeria.

1.4 Objectives of the Study

The specific objectives of the study were to:

I. detect the presence of antibody to *Salmonella* Typhi and *S.* Paratyphi A, B and C in apparently healthy individuals using slide agglutination test;

II. determine the antibody levels to the O and H antigens of typhoid and paratyphoid organisms in apparently healthy individuals among the study population using tube agglutination test in order to determine the baseline antibody titre;

III. isolate and characterize *Salmonella species* from apparently healthy individuals using cultural and serological methods;

IV. determine some Socio-demographic factors associated with the presence of antibodies to *Salmonella species* in the study area.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Nomenclature of Salmonella

Salmonella is named after an American bacteriologist, D. E. Salmon, who first isolated *Salmonella choleraesuis* from porcine intestine in 1884 (Smith, 1994). Salmonellae are a group of bacterial organisms with a high genetic similarity and are differentiated by their serotyping results. The antigenic classification system of various *Salmonella* serotypes used today is a result accumulated from many years of studies on antibody interactions with surface antigens of *Salmonella* organisms established by Kauffman and White almost a century ago. All antigenic formulae of recognized *Salmonella* serotypes are listed in a document called the Kauffmann-White scheme (Popoff and Le Minor, 2001). The World Health Organization Collaborating Centre for Reference and Research on *Salmonella* at the Pasteur Institute, Paris, France (WHO Collaborating Centre) is responsible for the updating of the scheme.

Every year newly recognized serotypes are reported, and in the latest report, there are a total of 2,541 serotypes in the genus *Salmonella* (Popoff *et al.*, 2004). The terms "serotype" and "serovar" are both frequently used, but according to the Rules of the *Bacteriological Code* (1990 Revision) established by the Judicial Commission of the International Committee on the Systematics of Prokaryotes, the term serovar is preferred to the term serotype. Thus "serovar" is used in the Kauffmann-White scheme (Lim *et al.*, 1998).

Salmonella nomenclature is complex, and scientists use different systems to refer to and communicate about this genus. However, uniformity in *Salmonella* nomenclature is necessary for communication between scientists, health officials, and the public (WHO, 2011).

Unfortunately, current usage often combines several nomenclatural systems that inconsistently divide the genus into species, subspecies, subgenera, groups, subgroups, and serotypes (serovars), and this causes confusion. The CDC receives many inquiries concerning the appropriate *Salmonella* nomenclature for the reporting of results and for use in scientific publications (Brenner, 2000).

Currently, the nomenclature system used at the Centers for Disease Control and Prevention (CDC) for the genus *Salmonella* is based on recommendations from the WHO Collaborating Centre. According to the CDC system, the genus *Salmonella* contains two species, *S. enterica*, the type species, and *S. bongori*. On March 18, 2005, a new species, "*Salmonella subterranean*" was validly approved by the Judicial Commission (Shelobolina *et al.*, 2004); CDC may incorporate the species in their system in the near future. *Salmonella enterica* consists of six subspecies (Popoff and Le Minor, 2001; Brenner and McWhorter, 1998): I, *S. enterica* subsp. *enterica*; II, *S. enterica* subsp. *salamae*; IIIa, *S. enterica* subsp. *arizonae*; IIIb, *S. enterica* subsp. *diarizonae*; IV, *S. enterica* subsp. *houtenae*; and VI, *S.enterica* subsp. *indica* (Popoff *et al.*, 2004).

In subspecies I, serotypes (or serovars) are designated by a name usually indicative of the associated diseases, their geographic origins, or their usual habitats. In the remaining subspecies as well as those of *S. bongor*, antigenic formulae determined according to the Kauffmann-White scheme (Popoff and Le Minor, 2001) are used for those unnamed serotypes. Some members of these subspecies may have been named before 1966 and thus their names are retained and cited as those in the subspecies I.

To avoid confusion between serotypes and species, the serotype name is not italicized and starts with a capital letter. When cited at the first time in a report, the genus name is given followed by the word "serotype" (or the abbreviation "ser.") and then the serotype name, e.g., *Salmonella* serotype or ser. Choleraesuis, and *Salmonella* serotype or ser. Typhi. Afterward the name may be shortened with the genus name followed directly by the serotype name, e.g., *Salmonella* Choleraesuis or *S.* Choleraesuis, and *Salmonella* Typhi or *S.* Typhi (Popoff *et al.*, 2004). Because the type species name, *enterica*, has not been approved before 2005, serotype names are used directly after the genus name without the mention of the species. Following the officially approval of "*enterica*" as the type species name from January 2005 (JCICSP, 2005), further amendment to include species name in the *Salmonella* nomenclature by CDC may be expected (Popoff *et al.*, 2004).

For those designated by their antigenic formulae, the subspecies name is written in Roman letters (not italicized) followed by their antigenic formulae, including O (somatic) antigens, H (flagella) antigens (phase 1), and H antigens (phase 2, if present). A colon is used in between each antigen, e.g., *Salmonella* serotype II 39:z10: z6. For serotypes in *S. bongori* (previously belongs to subgenus V), V is still used for consistency, e.g., *S.* V 13,22:z35 (Popoff *et al.*, 2004).

In publications of the American Society for Microbiology (ASM), the *Salmonella* nomenclature used at CDC was accepted as the standard form by the Publications Board in the 1999 meeting and the Instruction to Authors has been updated to include this information since 2000. The 2006 ASM Instruction to Authors indicated that, for the species, "*Salmonella enterica*" is used at the first time, and "*S. enterica*" thereafter; for the subspecies, "*Salmonella enterica* subsp. *arizonae*" is used at first, and "*S. enterica* subsp. *arizonae*" thereafter. Serotype names should be in Roman type with the first letter capitalized, e.g., *Salmonella enterica* serotype Typhimurium.

After the first use, the serotype may be used without a species name, e.g., *Salmonella* serotype Typhimurium.

The nomenclature for these bacteria is confusing because the criteria for designating bacteria as individual species are not clear. Two main views on the nomenclature of the genus *Salmonella* have been discussed. Le Minor and Popoff (2001) suggested that two species should be recognized: *Salmonella bongori* and *Salmonella enterica*. *Salmonella enterica* included six subspecies, of which subspecies I (one) contained all the pathogens of warm-blooded animals. *S.* Typhi was a serotype within subspecies I: *Salmonella enterica* subspecies I serotype *typhi*. This proposal was rejected by the International Judicial Commission because the name was not well known to clinicians and its use might cause accidents, endangering health or life. The original rules therefore remain in force. Ezaki and colleagues have noted in the International Journal of Systematic and Evolutionary Microbiology that the correct nomenclature for the causal agent of typhoid fever is *Salmonella* Typhi and have requested that the current subspecific status of serotype Paratyphi A should be raised to specific status, i.e. *Salmonella* Paratyphi *A*. *Salmonella* Typhi has several unique features, the genetic basis of many of which is known as a result of early genetic studies and the recent sequencing of the whole genome.

2.2 The Typhoid Fever Disease

In severe infections, *S.* Typhi grows and multiplies in mononuclear phagocytic cells before being released into the bloodstream (WHO, 2003). After ingestion in food or water, typhoid organisms pass through the pylorus and reach the small intestine. They rapidly penetrate the mucosal epithelium via either microfold cells or enterocytes and arrive in the lamina propria, where they rapidly elicit an influx of macrophages (Mp) that ingest the bacilli but do not generally kill them. Some bacilli remain within Mp of the small intestinal lymphoid tissue. Other typhoid bacilli are

drained into mesenteric lymph nodes where there is further multiplication and ingestion by Mp. It is believed that typhoid bacilli reach the bloodstream principally by lymph drainage from mesenteric nodes, after which they enter the thoracic duct and then the general circulation. As a result of this silent primary bacteraemia the pathogen reaches an intracellular haven within 24 hours after ingestion throughout the organs of the reticuloendothelial system (spleen, liver, bone marrow, etc.), where it resides during the incubation period, usually of 8 to 14 days. The incubation period in a particular individual depends on the quantity of inoculum, i.e. it decreases as the quantity of inoculum increases, and on host factors (Wain *et al.*, 1998).

Incubation periods ranging from 3 days to more than 60 days have been reported. Clinical illness is accompanied by a fairly sustained but low level of secondary bacteraemia (~1-10 bacteria per ml of blood).

2.3 The Causative Organism of Typhoid Fever

Typhoid fever is caused by *Salmonella* Typhi, a Gram-negative bacterium (WHO, 2003). A very similar but often less severe disease is caused by *Salmonella* serotype Paratyphi A. Although many genes are shared with *Escherichia coli* and at least 90% with *S. typhimurium*, there are several unique clusters of genes known as pathogenicity islands and many more single genes that seem to have been acquired by *S.* Typhi during evolution. *S.* Typhi can be identified in the laboratory by several biochemical and serological tests. One of the most specific is that of polysaccharide capsule Vi, which is present in about 90% of all freshly isolated *S.* Typhi and has a protective effect against the bactericidal action of the serum of infected patients. This capsule provides the basis for one of the commercially available vaccines. Vi antigen is present in some other bacteria (*Citrobacter freundii*, *Salmonella* Paratyphi *C* and *Salmonella dublin*) but not in exactly the same genetic context. The ratio of disease caused by *S.* Typhi to that caused by *S.*

Paratyphi is about 10 to 1 in most of the countries where studies have been performed (WHO, 2011, CDC, 2016).

2.4 Symptoms of Typhoid Fever

The clinical presentation of typhoid fever varies from a mild illness with low-grade fever, malaise, and slight dry cough to a severe clinical picture with abdominal discomfort and multiple complications (CDC, 2016). Many factors influence the severity and overall clinical outcome of the infection (Wain *et al.,* 1998). They include the duration of illness before the initiation of appropriate therapy, the choice of antimicrobial treatment, age, the previous exposure or vaccination history, the virulence of the bacterial strain, the dose of inoculum ingested, host factors (e.g. HLA type, AIDS or other immunosuppression) and whether the individual was taking other medications such as H2 blockers or antacids to diminish gastric acid. Patients who are infected with HIV are at significantly increased risk of clinical infection with *S.* Typhi and *S.* Paratyphi (Gotuzzo *et al.,* 1991). Evidence of *Helicobacter pylori* infection also represents an increased risk of acquiring typhoid fever.

2.4.1 Acute Non-complicated Disease

Acute typhoid fever is characterized by prolonged fever, disturbances of bowel function (constipation in adults, diarrhoea in children), headache, malaise and anorexia. Bronchitic cough is common in the early stage of the illness. During the period of fever, up to 25% of patients show exanthem (rose spots) on the chest, abdomen and back.

2.4.2 Complicated Disease

Acute typhoid fever may be severe. Depending on the clinical setting and the quality of available medical care, up to 10% of typhoid patients may develop serious complications. Since the gut-associated lymphoid tissue exhibits prominent pathology, the presence of occult blood is a common finding in the stool of 10-20% of patients, and up to 3% may have melena. Intestinal perforation has also been reported in up to 3% of hospitalized cases (WHO, 2011). Abdominal discomfort develops and increases. It is often restricted to the right lower quadrant but may be diffuse. The symptoms and signs of intestinal perforation and peritonitis sometimes follow, accompanied by a sudden rise in pulse rate, hypotension, marked abdominal tenderness, rebound tenderness and guarding, and subsequent abdominal rigidity. A rising white blood cell count with a left shift and free air on abdominal radiographs are usually seen (WHO, 2011).

Altered mental status in typhoid patients has been associated with a high case-fatality rate. Such patients generally have delirium or obtundation, rarely with coma. Typhoid meningitis, encephalomyelitis, Guillain-Barré syndrome, cranial or peripheral neuritis, and psychotic symptoms, although rare, have been reported. Other serious complications documented with typhoid fever include haemorrhages (causing rapid death in some patients), hepatitis, myocarditis, pneumonia, disseminated intravascular coagulation, thrombocytopenia and haemolytic uraemic syndrome. In the pre-antibiotic era, which had a different clinical picture, if patients did not die with peritonitis or intestinal haemorrhage, 15% of typhoid fever cases died with prolonged persistent fever and diseases for no clear reason. Patients may also experience genitourinary tract manifestations or relapse, and/or a chronic carrier state may develop (WHO, 2011).

2.4.3 Carrier State

In About 1-5% of patients, depending on age, *S.* Typhi remains in the gallbladder. This makes them chronic carriers harbouring the *S.* Typhi and can shed the organism in excreta which may serves as source of infection for healthy individuals.

2.5 Magnitude of Typhoid Fever Disease

Typhoid fever is a global health problem. Its real impact is difficult to estimate because the clinical picture is confused with those of many other febrile infections. Additionally, the disease is underestimated because there are no bacteriology laboratories in most areas of developing countries. These factors are believed to result in many cases going undiagnosed. On the basis of the literature (NVD, 1986 cited in WHO 2011) and the incidence of typhoid fever recorded in control groups in large vaccine field trials with good laboratory support, it has been estimated that approximately 21 million cases of typhoid fever and 600 000 associated deaths occur annually (CDC, 2016). However, the estimates have been biased because study populations have usually been in areas of high incidence. Furthermore, these estimates of burden relate to the clinical syndrome of typhoid fever but not to *S.* Typhi exposure. Since the prevalence of bacteraemia in febrile children is quite high (2-3%) in areas of endemicity, it is suggested that exposure to the bacteria is higher than indicated by the figures that are based solely on the clinical syndrome of typhoid fever.

The incidence of the disease in areas of endemicity may resemble the incidences observed in control groups in large vaccine field trials, viz. between 45 per 100 000 per year and over 1000 per 100 000 per year. Preliminary results from recent studies conducted in Bangladesh by ICDDR,B show an incidence of approximately 2000 per 100 000 per year. Typhoid fever also has a very high social and economic impact because of the hospitalization of patients

with acute disease and the complications and loss of income attributable to the duration of the clinical illness (Ivanoff *et al.,* 1994; Punjabi, 1998). It is important to note that reports from some provinces in China and Pakistan have indicated more cases of paratyphoid fever caused by *S.* Paratyphi A than by *S.* Typhi. In areas of endemicity and in large outbreaks, most cases occur in persons aged between 3 and 19 years. In 1997, for example, this age range was reported during an epidemic of the disease in Tajikistan. Nevertheless, clinically apparent bacteraemic *S.* Typhi infection in children aged under three years has been described in Bangladesh, India, Jordan and Nigeria (Sinha *et a.,* 1999; Saha *et al.,* 2001). In Indonesia there is a mean of 900 000 cases per year with over 20 000 deaths. In Indonesia, people aged 3-19 years accounted for 91% of cases of typhoid fever and the attack rate of blood-culture-positive typhoid fever was 1026 per 100 000 per year. A similar situation was reported from Papua New Guinea (WHO, 2016).

When typhoid fever was highly endemic in certain countries in South America the incidence of clinical typhoid fever in children below 3 years of age was low. In Chile, however, single blood cultures for all children aged under 24 months who presented at health centres with fever, regardless of other clinical symptoms, showed that 3.5% had unrecognized bacteraemic infections caused by *S.* Typhi or *S.* Paratyphi (Ferrecio *et al.,* 1984).

2.6 Case Definition

Typhoid fever can be classified based on the signs, symptoms and diagnosis of the disease in an individual, these include:

2.6.1 Confirmed Case of Typhoid Fever

This is seen in a patient with fever (38°C and above) that has lasted for at least three days, with a laboratory-confirmed positive culture (blood, bone marrow, bowel fluid) of *S.* Typhi (WHO, 2011).

2.6.2 Probable Case of Typhoid Fever

This is when a patient has fever (38°C and above) that has lasted for at least three days, with a positive serodiagnosis or antigen detection test but without *S.* Typhi isolation (WHO 2011).

2.6.3 Chronic Carrier

This is when there is excretion of *S.* Typhi in stools or urine (or repeated positive bile or duodenal string cultures) for longer than one year after the onset of acute typhoid fever. Short-term carriers also exist but their epidemiological role is not as important as that of chronic carriers. Some patients excreting *S.* Typhi have no history of typhoid fever (WHO, 2011).

2.7 Contamination and Transmission of Typhoid Fever

Humans are the only natural host and reservoir. The infection is transmitted by ingestion of food or water contaminated with faeces. Ice cream is recognized as a significant risk factor for the transmission of typhoid fever. Shellfish taken from contaminated water, and raw fruit and vegetables fertilized with sewage, have been sources of past outbreaks.

The highest incidence occurs where water supplies serving large populations are contaminated with faeces. Epidemiological data suggest that waterborne transmission of *S.* Typhi usually involves small inocula, whereas foodborne transmission is associated with large inocula and high attack rates over short periods. The inoculum size and the type of

vehicle in which the organisms are ingested greatly influence both the attack rate and the incubation period. In volunteers who ingested 109 and 108 pathogenic *S.* Typhi in 45 ml of skimmed milk, clinical illness appeared in 98% and 89% respectively. Doses of 105 caused typhoid fever in 28% to 55% of volunteers, whereas none of 14 persons who ingested 103 organisms developed clinical illness. Although it is widely believed that *Salmonella* is transmitted via the oral route, the transmission of *S. typhimurium* via the respiratory route has been demonstrated in a mouse model (Ivanoff *et al.,* 1998; WHO, 2011).

Family studies were conducted in Santiago, Chile, during an era of high typhoid endemicity in order to ascertain whether chronic carriers were significantly more frequent in households where there were index cases of children with typhoid fever than in matched control households. Other epidemiological studies investigated whether risk factors could be identified for persons with typhoid fever in comparison with uninfected household members. It was concluded that chronic carriers in households did not play an important role in transmission. Subsequently, it was shown that the irrigation of salad with wastewater contaminated with sewage was the key factor responsible for maintaining the high endemicity of typhoid in Santiago. In developed countries, on the other hand, typhoid is transmitted when chronic carriers contaminate food as a consequence of unsatisfactory food-related hygiene practices (WHO, 2011).

2.8 Diagnosis of Typhoid Fever

The definitive diagnosis of typhoid fever depends on the isolation of *S.* Typhi from blood, bone marrow or a specific anatomical lesion. The presence of clinical symptoms characteristic of typhoid fever or the detection of a specific antibody response is suggestive of typhoid fever but not definitive. Blood culture is the mainstay of the diagnosis of this

disease (CDC, 2016). Although Ox bile medium (Oxgall) is recommended for enteric fever pathogens (*S.* Typhi and *S.* Paratyphi), only these pathogens can be grown on it. In a general diagnostic laboratory, therefore, where other pathogens are suspected, a general blood culture medium should be used. More than 80% of patients with typhoid fever have the causative organism in their blood. A failure to isolate the organism may be caused by several factors listed below:

(i) The limitations of laboratory media (Wain *et al.,* 1998 cited in WHO, 2011)

(ii) The presence of antibiotics (Gasem *et al.,* 1995 cited in WHO, 2011)

(iii)The volume of the specimen cultured (Wain *et al.,* 1998 cited WHO, 2011)

(iv)The time of collection (Soewandojo *et al.,* 1998).

Patients with a history of fever for 7 to 10 days being more likely than others to have a positive blood culture. Bone marrow aspirate culture is the gold standard for the diagnosis of typhoid fever (Hoffman *et al.,* 1986, Soewandojo *et al.,* 1998) and is particularly valuable for patients who have been previously treated, who have a long history of illness and for whom there has been a negative blood culture with the recommended volume of blood (Wain *et al.,* 2001). Duodenal aspirate culture has also proved highly satisfactory as a diagnostic test but has not found widespread acceptance because of poor tolerance of duodenal aspiration, particularly in children (Bopp *et al.,* 1999).

The confirmed diagnosis through blood or bone-marrow culture requires expensive and labor-intensive isolation and identification of the organism, which may take up to seven days. A cheap and rapid alternative laboratory test is desirable, especially for developing country settings where typhoid fever is a major public health burden. Various agglutination tests have

been developed (Olopoenia and King, 2000) of which the Widal method is the oldest and remains the most widely used. The test was first introduced by F.

Widal in 1896 (Olopoenia and King, 2000) and is based on a macroscopically visible serum - mediated agglutination reaction between *S.* Typhi somatic lipopolysacharide O antigens (TO) and flagellar H antigens (TH). Laboratories in industrialized countries have stopped performing the assay. In Africa the Widal test is still widely used because typhoid fever is perceived to be endemic in the area (Mweu and English, 2008) and the Widal test is the only rapid diagnostic assay that is available and affordable. The Widal test is commonly performed when children and adults present with fever to treatment centers, as few centers have the capacity to perform micro-bacterial culture (Chart *et al.,* 2008). Despite this widespread use, little has been published on its performance in Africa.

2.8.1 Specimen

If a bacteriology laboratory is not available on site, clinical specimens for culture can be transported to a main laboratory for processing. For blood culture it is essential to inoculate media at the time of drawing blood. For other specimens it is advisable to make the time of transportation to the laboratory as short as possible. It is more important to process the specimens quickly than to keep them cold. Once they have been inoculated, blood culture bottles should not be kept cold. They should be incubated at 37°C or, in tropical countries, left at room temperature, before being processed in the laboratory (WHO, 2011).

2.8.2 Blood

The volume of blood cultured is one of the most important factors in the isolation of *S.* Typhi from typhoid patients: 10-15 ml should be taken from school children and adults in order to

achieve optimal isolation rates; 2-4 ml are required from toddlers and preschool children (Wain *et al.,* 1998). This is because children have higher levels of bacteraemia than adults. In some regions it may be impossible to collect such large volumes of blood and so alternative diagnostic methods may be necessary for cases in which blood cultures are negative. Because reducing the blood volume reduces the sensitivity of the blood culture, however, an effort should be made to draw sufficient quantity of blood if at all possible. Blood should be drawn by means of a sterile technique of venous puncture and should be inoculated immediately into a blood culture bottle with the syringe that has been used for collection (WHO, 2011).

Several reports of pseudobacteraemia have been associated with the reinoculation of blood culture bottles after the collection of blood in contaminated vessels. The practice of inoculating blood culture bottles from specimens taken for biochemical or haematological analysis should therefore be avoided. The optimum ratio of the volume of blood to traditional culture broth should be 1 to 10 or more (e.g. 1:12). Some commercial blood culture systems have special resins in the media which allow higher volumes of blood to be used. The instructions with commercial blood culture systems should always be read and the recommended amounts should not be exceeded (WHO, 2011).

In general, if 5 ml of blood are drawn, they should be inoculated into 45 ml or more of broth. If 10-15 ml of blood are drawn, the specimen can be divided into equal aliquots and inoculated into two or more blood culture bottles. This allows the use of standard blood culture bottles of 50 ml. For small children the volume of blood drawn can be reduced but should still be inoculated into 45 ml of culture broth. In order to assist the interpretation of negative results the volume of blood collected should be carefully recorded. The blood culture bottle should then be transported to the main laboratory at ambient temperature (15°C

to 40°C) as indicated above. Blood cultures should not be stored or transported at low temperatures. If the ambient temperature is below 15°C it is advisable to transport blood cultures in an incubator. In the laboratory, blood culture bottles should be incubated at 37°C and checked for turbidity, gas formation and other evidence of growth after 1, 2, 3 and 7 days. For days 1, 2 and 3, only bottles showing signs of positive growth are cultured on agar plates. On day 7 all bottles should be subcultured before being discarded as negative.

2.8.3 Serum

For serological purposes, 1-3 ml of blood should be inoculated into a tube without anticoagulant. A second sample, if possible, should be collected at the convalescent stage, at least 5 days later. After clotting has occurred, the serum should be separated and stored in aliquots of 200 ml at +4°C. Testing can take place immediately or storage can continue for a week without affecting the antibody titre. The serum should be frozen at -20°C if longer-term storage is required (WHO, 2011).

2.8.4 Stool Samples

Stools can be collected from patients with acute infection and they are especially useful for the diagnosis of typhoid carriers. The isolation of *S.* Typhi from stools is suggestive of typhoid fever. However, the clinical condition of the patient should be considered. Stool specimens should be collected in a sterile wide-mouthed plastic container. The likelihood of obtaining positive results increases with the quantity of stools collected. Specimens should preferably be processed within two hours after collection. If there is a delay, the specimens should be stored in a refrigerator at 4°C or in a cool box with freezer packs, and should be transported to the laboratory in a cool box. Stool culture may increase the yield of culture-positive results by up to 5% in acute typhoid fever. If a stool sample cannot be obtained,

rectal swabs inoculated into Carry Blair transport medium can be used but these are less successful (WHO, 2011).

2.9 Microbiological Procedures

The different samples used in the diagnosis of Typhoid fever are analyzed in different ways in the laboratory as follows:

2.9.1 Blood Culture

A typical blood culture bottle contains 45 ml of tryptic soy broth or brain heart infusion broth. These are inoculated with 5 ml of fresh blood and incubated at 37°C. Negatives should be kept for at least seven days. Because *S.* Typhi is not the only bacterial pathogen found in blood, subculturing is performed on days 1, 2, 3 and 7 on non-selective agar. The best agar is blood agar (horse or sheep blood) as this allows the growth of most bacterial pathogens. If blood agar is not available, nutrient agar can be used in combination with MacKonkey agar. In some laboratories the use of MacConkey agar alone is preferred as this allows the growth of only bile-tolerant bacteria such as *S.* Typhi and does not allow the growth of many Gram-positive contaminants (WHO, 2011).

The contamination of blood cultures reduces isolation rates for *S.* Typhi and should be prevented as far as possible. It is important to identify contaminating bacteria that come from the skin of patients or the air of the laboratory so that measures can be taken to prevent further problems. MacKonkey agar should therefore not be used as the only agar for the sampling of blood cultures in a diagnostic microbiology laboratory (WHO, 2011).

Furthermore, because it is selective, MacKonkey agar does not permit the growth of Gram-positive pathogens or even all *E. coli.* For suspected tyhoid fever, subculture plates should be incubated at 37°C for 18-24 hours in an aerobic incubator.

2.9.2 Stool or Rectal Swab Culture

This involves inoculating 1g of stool into 10 ml of selenite F broth and incubating at 37°C for 18-48 hours. Because selenite broth is very sensitive to heat the manufacturer's instructions should be carefully followed during preparation and overheating of the broth during sterilization should be avoided. Once a batch is prepared it should be stored at 4°C. Selenite broth inhibits the motility of *E. coli* found in stools but does not kill this bacterium. A subculture of selenite broth on a selective agar is therefore made from the surface of the broth without disturbing the sediment. The choice of agar media includes Mac Conkey agar, desoxycholate citrate agar, xylose-lysinedesoxycholate agar, and hektoen enteric agar or SS (*Salmonella_Shigella*). The plate is incubated at 37°C for 24 hours. Different batches of agar plates can give slightly different colonies of *S.* Typhi and it is therefore important to keep one strain of *S.* Typhi for use in quality control for each batch of agar plates and selenite broth. New batches of media are inoculated with the control strain and the amount of growth and the appearance of the colonies are recorded. If *S.* Typhi does not grow as well as usual in any batch of medium, discard the medium and make a fresh one (WHO, 2011).

The identification of colonies as *S.* Typhi is straight forward if reagents of satisfactory quality are available. Colonies from solid media can be used for agglutination with specific antisera. Several salmonellae may share the same antigenic structure. Consequently, confirmation by means of biochemical tests is always necessary (WHO, 2011).

2.9.3 Colonial Characteristics of *S.* Typhi and *S.* Paratyphi

The colonies of *S.* Typhi and *S.* Paratyphi have different appearances on different growth media which aid in their identification. These characteristic appearances are described below.

Blood Agar: On blood agar, *S.* Typhi and *S.* Paratyphi usually produce non-haemolytic smooth white colonies.

MacConkey Agar: On MacConkey agar, salmonellae produce lactose non-fermenting smooth colonies.

Salmonella-Shigella Agar: On SS agar, salmonellae produce lactose non-fermenting colonies with black centres (except *S.* Paratyphi A colonies which do not have black centres).

Desoxycholate Agar: On desoxycholate agar, salmonellae produce lactose non-fermenting colonies with black centres (except *S.* Paratyphi A colonies which do not have black centres).

Xylose-lysine-desoxycholate Agar: On xylose-desoxycholate agar, salmonellae produce transparent red colonies with black centres (except *S.* Paratyphi A, whose colonies do not have black centres).

Hektoen enteric Agar: On hektoen enteric agar, salmonellae produce transparent green colonies with black centres (except *S.* Paratyphi A, whose colonies do not have black centres).

Bismuth sulfite Agar: On this medium, salmonellae produce black colonies.

2.9.4 Biochemical Identification of *Salmonella species*

The suspected colonies with characteristic appearance typical of *Salmonella* species obtained on the any of the growth media are screened by means of the biochemical tests.

The biochemical profile of the suspected organism should tally with the standard biochemical profile of *Salmonella species* (Table 2.1).

2.10 Serological Procedures

The use of serology in the diagnosis of Typhoid fever is very important especially in developing countries where the facilities for the Isolation of the organism are limited or even unavailable. This as stated earlier, makes the widal test the a necessary option due to its simplicity, affordability and relative accuracy.

2.10.1 Serological Identification of *Salmonella*

Salmonellae can be characterized by their somatic (O) and flagellar (H) antigens, the latter existing in some serotypes in phases 1 and 2. Some salmonellae also have an envelope antigen called Vi (virulence). The O antigen is usually determined by means of the slide agglutination test with group-specific antiserum followed by agglutination with factor antiserum. Growth from non-selective agar or Kliger's iron agar can be used for the determination of O antigen. Strains of *S.* Typhi and *S.* Paratyphi C may possess Vi antigen that render the strains non-agglutinable in O antisera. These cultures agglutinate in Vi antiserum. They will agglutinate in O antiserum, however, after destruction of the Vi antigen by boiling the culture for 10 minutes. The specific O antigen is confirmed by slide agglutination with factor antiserum (WHO, 2011).

The H antigen is usually determined by means of the tube agglutination test. The organisms should be motile and from a liquid culture. The motility of weakly motile organisms can be enhanced by repeated passage in liquid cultures. Determination of the O antigen and the phase 1 H antigen only is usually sufficient for the identification of typhoid fever organisms and paratyphoid fever organisms. The specific phase 1 antigens for typhoid fever organisms are described below. Antisera against these antigens are used, usually in the tube agglutination test (WHO, 2011).

Table 2.1: Biochemical Profile of *Salmonella species* and Related Organisms

Organism	Kliger's Iron agar							Citrate
	Slant	Butt	H_2S	Gas	Motility	Indol	Urea	Utilization
S. Typhi	Alk	Acid	Wk+	-	+	-	-	-
S. Paratyphi *A*	Alk	Acid	-	+	+	-	-	-
Others	Alk	Acid	V	V	+	-	-	V
Salmonella spp.								
E. coli	Acid	Acid	-	+	+	+	-	-
Klebsiella spp.	Acid	Acid	-	++	-	V	+	+
Citrpbacter spp.	V	Acid	+++	+	+	V	-	+
Proteus spp.	Alk	Acid	+	+	+	V	++	V

Alk = alkaline, Wk = weak, V= variable result (WHO, 2003). S. Typhi = *Salmonella* Typhi

In some cultures, only some organisms express phase 1 H antigens and others express phase 1 and 2 H antigens at the same time. Such cultures agglutinate with both phase 1 and phase 2 antisera. In cultures where a single phase antigen is expressed the antigen in the other phase can be induced by incubating the culture with antiserum to the phase antigen being expressed (Papoff *et al.*, 1997).

Salmonella Typhi with flagella variant Hj and phase 2 antigen Z66 have been reported but they are rare. *Salmonella java* can be misidentified as *S. paratyyphi* B because these two serotypes have identical antigens. However, the serotypes can be differentiated biochemically. The former is tartrate-positive and produces non-typhoid salmonellosis, and the latter is tartrate-negative and produces typhoid salmonellosis. *S. java* is now considered to be a tartrate-positive variant of *S.* Paratyphi B. *S. dublin* and *Citrobacter freundii* possess Vi antigen. However, the phase 1 H antigens of *S. dublin* are g, p as against d in *S.* Typhi. Unlike *C. freundii*, *S.* Typhi does not grow in KCN broth (Bopp *et al.*, 1999).

Some of the non-typhoidal salmonellae can cause febrile illness that mimics enteric fever. However, these strains can be differentiated biochemically.

2.10.2 Felix-Widal Test

This test measures agglutinating antibody levels against O and H antigens. The levels are measured by using doubling dilutions of sera in large test tubes. Usually, O antibodies appear on days 6-8 and H antibodies on days 10-12 after the onset of the disease. The test is usually performed on an acute serum (at first contact with the patient) (WHO, 2011).

A convalescent serum should preferably also be collected so that paired titrations can be performed. In practice, however, this is often difficult.

At least 1 ml of blood should be collected each time in order to have a sufficient amount of serum. In exceptional circumstances the test can be performed on plasma without any adverse effect on the result.

The test has only moderate sensitivity and specificity. It can be negative in up to 30% of culture-proven cases of typhoid fever. This may be because of prior antibiotic therapy that has blunted the antibody response. On the other hand, *S.* Typhi shares O and H antigens with other *Salmonella* serotypes and has cross-reacting epitopes with other Enterobacteriacae, and this can lead to false-positive results. Such results may also occur in other clinical conditions, e.g. malaria, typhus, bacteraemia caused by other organisms, and cirrhosis. In areas of endemicity there is often a low background level of antibodies in the normal population. Determining an appropriate cut-off for a positive result can be difficult since it varies between areas and between times in given areas (WHO, 2011).

It is therefore important to establish the antibody level in the normal population in a particular locality in order to determine a threshold above which the antibody titre is considered significant. This is particularly important if, as usually happens, a single acute sample is available for testing. If paired sera are available a fourfold rise in the antibody titre between convalescent and acute sera is diagnostic. Quality control of the test is achieved by running a standard serum with a known antibody titre in parallel in each batch of assays. The variations in the standard serum should not exceed one tube, i.e. double dilution.

Despite these limitations the test may be useful, particularly in areas that cannot afford the more expensive diagnostic methods (Pang, 1989). This is acceptable so long as the results are interpreted with care in accordance with appropriate local cut-off values for the

determination of positivity. This test is unnecessary if the diagnosis has already been confirmed by the isolation of *S.* Typhi from a sterile site. New diagnostic tests are being developed.

2.10.3 New Diagnostic Tests: Current Status and Usefulness

There is a need for a quick and reliable diagnostic test for typhoid fever as an alternative to the Widal test. Recent advances include the IDL Tubex® test marketed by a Swedish company, which reportedly can detect IgM O9 antibodies from patients within a few minutes. Another rapid serological test, Typhidot®, takes three hours to perform (Bopp *et al.*, 1999).

It was developed in Malaysia for the detection of specific IgM and IgG antibodies against a 50 kD antigen of *S.* Typhi. A newer version of the test, Typhidot-M®, was also developed to detect specific IgM antibodies only. The dipstick test, developed in the Netherlands, is based on the binding of *S.* Typhi-specific IgM antibodies in samples to *S.* Typhi lipopolysaccharide (LPS) antigen and the staining of bound antibodies by an anti-human IgM antibody conjugated to colloidal dye particles (House *et al.*, 2001; Hatta *et al.*, 2002).

2.10.4 IDL Tubex Test

The Tubex test is simple (essentially a one-step test) and rapid (taking approximately two minutes). It exploits the simplicity and user-friendliness of the Widal and the slide latex agglutination tests but uses the separation of coloured particles in solution to improve resolution and sensitivity. Specificity is improved by means of an inhibition assay format and by detecting antibodies to a single antigen in *S.* Typhi only (Bopp *et al.*, 1999).

The O9 antigen used in the test is extremely specific because its immunodominant epitope is a very rare dideoxyhexose sugar that occurs in nature. This antigen has been found in serogroup D *salmonellae* but not in other microorganisms. The closest to it is the tyvelose antigen found in *Trichinella spiralis* but antibodies to these two antigens do not cross-react with each other. A positive result given by Tubex invariably suggests a *Salmonella* infection, although the test cannot tell which group D *Salmonella* is responsible. Infections caused by other serotypes, including *S.* Paratyphi A, give negative results (House *et al.,* 2001; Hatta *et al.,* 2002).

Immunogenically, the O9 antigen is immunodominant and robust. Unlike the capsular (Vi) and flagellar antigens that are thymus-independent type II in nature and poorly immunogenic in infants, the O9 antigen (or LPS in general) is thymus-independent type I, immunogenic in infants, and a potent B cell mitogen. It can stimulate B cells without the help of T cells (unlike protein antigens) and, consequently, anti-O9 responses are rapid. This is important teleologically, as they form the first line of host defence. For reasons yet to be elucidated, Tubex® detects IgM antibodies but not IgG. This makes it invaluable as an aid in the diagnosis of current infections. The test pack includes:

i) Sets of specially-designed V-shaped tubes that allow six samples per set to be examined simultaneously;

ii) Reagent A, comprising magnetic particles coated with *S.* Typhi LPS;

iii) Reagent B, comprising blue-coloured latex particles coated with a monoclonal antibody specific for the O9 antigen (Hatta *et al.,* 2002).

The reagents are stable for over a year at 4°C, and for at least some weeks at ambient temperature (Hatta *et al.,* 2002). A drop of test serum is mixed for about one minute with a

drop of reagent A in the tube. Two drops of reagent B are then added and the contents are mixed thoroughly for 1-2 minutes. The set of tubes is then placed on a magnet-embedded stand, across which they are slid several times. The result, which can be read immediately or up to many hours later, is based on the colour of the reaction mixture. A range of colours involving varying proportions of redness and blueness can be expected, and a colour chart is provided for the purpose of scoring. Red indicates negativity while increasing blueness denotes increasing positivity (Lim *et al.,* 1998).

The rationale of the test is as follows. If the serum is negative for O9 antibodies the antibody-coated indicator particles bind to the antigen-coated magnetic beads. When a magnet is applied, the magnetic particles settle to the bottom of the tube together with any blue indicator particles associated with these. Consequently a background red colour is left in the solution. This background colour is actually exploited to camouflage the sample colour of haemolysed sera. If, on the other hand, the patient's serum contains O9 antibodies, these bind to the magnetic particles and prevent the indicator particles from binding to them. The indicator particles thus remain suspended and the resultant colour of the solution is blue. Tubex® has not been evaluated extensively but several trials are being planned. In a preliminary study involving stored sera the test performed better than the Widal test in both sensitivity and specificity (Lim *et al.,* 1998).

2.10.5 Typhidot Test

This test makes use of the 50 kD antigen to detect specific IgM and IgG antibodies to *S.* Typhi (Ismail *et al.,* 1991). It has undergone full-scale multinational clinical evaluation of its diagnostic value (Jackson *et al.,* 1995; Choo *et al.,* 1997; Lu-*Fong et al.,* 1999). This dot EIA

test offers simplicity, speed, specificity (75%), economy, early diagnosis, sensitivity (95%) and high negative and positive predictive values.

The detection of IgM reveals acute typhoid in the early phase of infection, while the detection of both IgG and IgM suggests acute typhoid in the middle phase of infection. In areas of high endemicity where the rate of typhoid transmission is high the detection of specific IgG increases. Since IgG can persist for more than two years after typhoid infection (Choo *et al.,* 1999), the detection of specific IgG cannot differentiate between acute and convalescent cases. Furthermore, false-positive results attributable to previous infection may occur. On the other hand, IgG positivity may also occur in the event of current reinfection. In cases of reinfection there is a secondary immune response with a significant boosting of IgG over IgM, such that the latter cannot be detected and its effect is masked. A possible strategy for solving these problems is to enable the detection of IgM by ensuring that it is unmasked (Bhutta and Munsurali, 1999). In order to increase diagnostic accuracy in these situations the original Typhidot® test was modified by inactivating total IgG in the serum sample. Studies with the modified test, Typhidot-M®, have shown that inactivation of IgG removes competitive binding and allows access of the antigen to the specific IgM when it is present. The detection of specific IgM within three hours suggests acute typhoid infection. Evaluations of Typhidot® and Typhidot-M® in clinical settings showed that they performed better than the Widal test and the culture method (Bhutta and Mansurali, 1999).

In laboratory diagnoses of typhoid fever the method used as the gold standard should approach 100% in sensitivity, specificity and positive and negative predictive values. Evaluation studies have shown that Typhidot-M® is superior to the culture method. Although culture remains the gold standard it cannot match Typhidot-M® in sensitivity (>93%),

negative predictive value and speed (Choo *et al.*, 1997). Typhidot-M® can replace the Widal test when used in conjunction with the culture method for the rapid and accurate diagnosis of typhoid fever. The high negative predictive value of the test suggests that Typhidot-M® would be useful in areas of high endemicity.

2.10.6 IgM Dipstick Test

The typhoid IgM dipstick assay is designed for the serodiagnosis of typhoid fever through the detection of *S.* Typhi-specific IgM antibodies in serum or whole blood samples. The assay consists of a dipstick, a lyophilized non-enzymatic detection reagent, liquid to reconstitute the detection reagent, liquid to wet the test strip of the dipstick before incubation with serum and detection reagent, and test tubes. The components are stable for two years if stored in the temperature range 4-25°C in a dry place and protected from direct exposure to sunlight. The assay is based on the binding of *S. typhi*-specific IgM antibodies to *S.* Typhi LPS antigen and the staining of bound antibodies by an anti-human IgM antibody conjugated to colloidal dye particles. The white test strip of the dipstick contains the antigen immobilized in a distinct line. The strip also has a control line with anti-human IgM antibodies (WHO, 2011).

The assay is performed by incubation of the wetted test strip in a mixture of serum and detection reagent, the serum being diluted at 1:50 in the detection reagent. Whole blood may be tested at a 1:25 dilution in detection reagent. The incubation period is three hours at room temperature. When incubation is complete the test strip is rinsed thoroughly with water and then allowed to dry. The result is read by visual inspection of the test strip for staining of the antigen and control lines. The test result is scored negative if no staining of the antigen line occurs and is graded 1+, 2+, 3+ or 4+ if there is weak, moderate strong or very strong staining as indicated by comparison with a coloured reference strip.

The control line should stain in all runs. Evaluations of the dipstick test in laboratory-based studies in Indonesia (House *et al.,* 2001; Hatta *et al.,* 2002), Kenya, Viet Nam (Gasem *et al.,* 2002) and Egypt (Ismail *et al.,* 2002) have shown consistent results.

These studies indicated sensitivities of 65% to 77% for samples collected at the time of first consultation from culture-confirmed patients and specificities of 95% to 100%. The results of culture and serological investigation may be influenced by various factors, among them the time of sample collection and the use of antibiotics before consultation and sample collection. In a study conducted in Makassar, Indonesia, the sensitivity of the blood culture method was estimated to be 66%, and that of the dipstick test calculated for the combined group of culture-confirmed and culture-negative patients with a final clinical diagnosis of typhoid fever was 48%. The sensitivity ranged from 29% for samples collected during the first week of illness to 96% for samples collected at a later stage. Tests on follow-up samples showed seroconversion in the majority of the dipstick-negative typhoid patients (Gasem *et al.,* 2002).

The dipstick test provides a rapid and simple alternative for the diagnosis of typhoid fever, particularly in situations where culture facilities are not available. The assay can be performed by people without formal training and in the absence of specialized equipment. Electricity is not required, as the components can be stored without cooling.

The results of the dipstick test can be obtained on the day when patients present. This makes prompt treatment possible. Specific antibodies usually only appear a week after the onset of symptoms and signs. This should be kept in mind when a negative serological test result is being interpreted (Ismail *et al.,* 2002).

2.10.7 Antimicrobial Susceptibility Test for Typhoid Fever Organisms

Antimicrobial susceptibility testing is crucial for the guidance of clinical management. Isolates from many parts of the world are now multidrug-resistant (MDR) (Gupta, 1994; Bhutta, 1996; Rowe *et al.*, 1997).

Isolates are usually resistant to ampicillin, chloramphenicol, sulfonamide, trimethoprim, streptomycin and tetracycline. Alternative drugs that are used for treatment include: fluoroquinolones (e.g. ciprofloxacin), third-generation cephalosporins (e.g. ceftriaxone, cefotaxime), a monobactum beta-lactam (aztreonam) and a macrolide (azithromycin). Even though resistance to the first two has been noted they nevertheless remain useful (Saha *et al.*, 1999). Reduced susceptibility to fluoroquinolones is indicated by in vitro resistance to nalidixic acid (Murdoch *et al.*, 1998).

In vitro susceptibility testing usually involves disc diffusion. The choice of antimicrobial agents for the test is dictated by the agents that are currently being used for treatment and the desire to determine the prevalence of MDR strains. After the previous first line drugs were discontinued for the treatment of typhoid fever in Bangladesh because of the emergence of MDR strains, the prevalence of multidrug resistance decreased and the possibility arose of using these drugs again (Saha *et al.*, 1997). It is therefore recommended that susceptibility tests be performed against the following antimicrobial agents: a fluoroquinolone, a third-generation cephalosporin and any other drug currently used for treatment, nalidixic acid (for determining reduced susceptibility to fluoroquinolones because of the possibility of false in vitro susceptibility against the fluoroquinolone used for treatment), and the previous first-line antimicrobials to which the strains could be resistant (chloramphenicol, ampicillin, trimethoprim/sulfamethoxazole, streptomycin and tetracycline). Azithromycin disc test

results should be interpreted with caution. The appropriate break-point recommendations for azithromycin against *S.* Typhi are still not clear. Patients may respond satisfactorily to azithromycin even if isolates are intermediate according to current guidelines.

2.10.8 Storage of Typhoid Fever Organisms

The isolates can be stored for up to two to three years on a nutrient agar, e.g. trypticase soy agar. The butt is stabbed and the slant is streaked and incubation takes place at 37°C for 18-24 hours. The tube is corked and made airtight by either covering the cork with parafilm or dipping the cork in melted paraffin. Alternatively, sterile mineral oil is poured on to cover the growth in the slant and the tube is corked. The tube is stored at room temperature, preferably between 20°C and 22°C, away from light in a closed cupboard. There is a risk that plasmids encoding antimicrobial resistance or other properties can be lost from isolates stored in this way (WHO, 2011).

Isolates can be stored for several years by freeze-drying, inoculating a thick suspension of growth in a nutrient broth (e.g. trypticase soy broth) with 15% glycerol in a cryovial, 10% skim milk in a cryovial, or a cryovial with beads, and freezing the vial at -70°C. Plasmids in isolates stored by these methods are stable. The use of a cryovial with beads has the advantage that beads can be removed for subculture without thawing the culture.

2.10.9 Quality Control

The steps involved in the accurate laboratory diagnosis of typhoid fever include specimen collection and transport, the performance of laboratory procedures, and reporting. It is important that the correct specimen is collected in the correct volume, that it is transported to the laboratory in the right condition, that correct laboratory procedures are followed and that

reporting is accurate. These steps should therefore be monitored at all levels and correction should take place if unacceptable performance is identified. Quality assurance is vital to the success of such investigations. Quality control programmes ensure that the information generated by laboratories is accurate, reliable and reproducible. This is accomplished by assessing the quality of specimens and monitoring the performance of test procedures, reagents, media, instruments, and personnel. Laboratories should have internal quality control programmes (WHO, 2015).

A panel of reference isolates consisting of typhoid and non-typhoid salmonellae and other Enterobacteriaceae should be maintained. At periodic intervals, e.g. monthly, the laboratory supervisor should submit a random selection of the reference isolates under code to laboratory technologists for evaluation. Quality control of the disc susceptibility test should take place. *Escherichia coli* ATCC 25922 should be run in parallel with the test strains. The susceptibility zones for the reference strain against various antimicrobials should be within the acceptable ranges. The results of these evaluations should be entered in a quality control monitoring book. Appropriate measures should be taken to solve any problems that are encountered. It is desirable to participate in external quality control programmes whenever possible. It should be noted that this is relatively expensive and that there could be problems relating to the timely transport of quality control specimens to certain countries because of uncertainties about carriers and customs. It is important to confirm *Salmonella* isolates in a reference laboratory because of the possibility of their misidentification (WHO, 2015).

2.11 Treatment of Typhoid Fever

The general management of Typhoid fever is aimed at, managing active infections, complicated cases and carrier states.

2.11.1 General Management

Supportive measures are important in the management of typhoid fever, such as oral or intravenous hydration, the use of antipyretics, and appropriate nutrition and blood transfusions if indicated. More than 90% of patients can be managed at home with oral antibiotics, reliable care and close medical follow-up for complications or failure to respond to therapy (WHO, 2011). However, patients with persistent vomiting, severe diarrhea and abdominal distension may require hospitalization and parenteral antibiotic therapy.

2.11.2 Antimicrobial Therapy

This involves the use of antibiotics. The most important criteria for the selection of first-line antibiotics to be used in developing countries are Efficacy, availability and cost (WHO, 2011). It should be noted, however, that therapeutic strategies for children, e.g. the choice of antibiotics, the dosage regimen and the duration of therapy, may differ from those for adults.

The fluoroquinolones are widely regarded as optimal for the treatment of typhoid fever in adults (Chinh *et al.,* 2000), as they are relatively inexpensive, well tolerated and more rapidly and reliably effective than the former first-line drugs, viz. chloramphenicol, ampicillin, amoxicillin and trimethoprim-sulfamethoxazole. The majority of isolates are still sensitive. The fluoroquinolones attain excellent tissue penetration, kill *S.* Typhi in its intracellular stationary stage in monocytes/macrophages and achieve higher active drug levels in the gall bladder than other drugs. They produce a rapid therapeutic response, i.e. clearance of fever and symptoms in three to five days, and very low rates of post-treatment carriage (Arnold *et al.,* 1993; Cristiano *et al.,* 1995). Evidence from various settings in Asia indicates that the fluoroquinolones are equally effective in the treatment of typhoid fever in Children.

However, the emergence of MDR strains has reduced the choice of antibiotics in many areas. There are two categories of drug resistance: resistance to antibiotics such as chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole (MDR strains) and resistance to the fluoroquinolone drugs. Resistance to the fluoroquinolones may be total or partial. The so-called nalidixic-acid-resistant *S.* Typhi (NARST) is a marker of reduced susceptibility to fluoroquinolones compared with nalidixic-acid-sensitive strains. Nalidixic acid itself is never used for the treatment of typhoid. These isolates are susceptible to fluoroquinolones in disc sensitivity testing according to current guidelines. However, the clinical response to treatment with fluoroquinolones of nalidixic-acid-resistant strains is significantly worse than with nalidixic-acid-senstive strains. There is a significant number of MDR strains from the Indian subcontinent and some other Asian countries (not Indonesia). *S.* Typhi has recently emerged as a problem in Kenya. Nalidixic-acid-resistant strains are now endemic in many areas of Viet Nam and have also been reported from the Indian subcontinent and Tajikistan.

There are disturbing recent reports of the emergence of fluorquinolone-resistant isolates in various parts of Asia (Das and Bhattacharya, 2000; Dutta *et al.*, 2001; Gupta *et al.*, 2001) and there have been a few reports of resistance to third-generation cephalopsorins in the same region. Reassuringly, however, many of these reports are coupled with evidence of the re-emergence of sensitive isolates in the same regions.

2.11.3 Management of Complications

Both outpatients and inpatients with typhoid fever should be closely monitored for the development of complications. Timely intervention can prevent or reduce morbidity and mortality.

The parenteral fluoroquinolones are probably the antibiotics of choice for severe infections but there have been no randomized antibiotic trials (Dutta *et al.,* 2001).

In severe typhoid the fluoroquinolones are given for a minimum of 10 days. Typhoid fever patients with changes in mental status, characterized by delirium, obtundation and stupor, should be immediately evaluated for meningitis by examination of the cerebrospinal fluid. If the findings are normal and typhoid meningitis is suspected, adults and children should immediately be treated with high-dose intravenous dexamethasone in addition to antimicrobials (Punjabi, 1998). If dexamethasone is given in an initial dose of 3 mg/kg by slow I.V. infusion over 30 minutes and if, after six hours, 1 mg/kg is administered and subsequently repeated at six-hourly intervals on seven further occasions, mortality can be reduced by some 80-90% in these high-risk patients. Hydrocortisone in a lower dose is not effective (Rao, 2009). High-dose steroid treatment can be given before the results of typhoid blood cultures are available if other causes of severe disease are unlikely.

2.11.4 Management of Carriers

An individual is considered to be a chronic carrier if he or she is asymptomatic and continues to have positive stool or rectal swab cultures for *S.* Typhi a year following recovery from acute illness. Overall, some 1-5% of typhoid fever patients become chronic carriers. The rate of carriage is slightly higher among female patients, patients older than 50 years, and patients with cholelithiasis or schistosomiasis. If cholelithiasis or schistosomiasis is present the patient probably requires cholecystectomy or antiparasitic medication in addition to antibiotics in order to achieve bacteriological cure.

In order to eradicate *S.* Typhi carriage, amoxicillin or ampicillin (100 mg per kg per day) plus probenecid (Benemid®) (1 g orally or 23 mg per kg for children) or TMP-SMZ (160 to 800 mg twice daily) is administered for six weeks; about 60% of persons treated with either regimen can be expected to have negative cultures on follow-up. Clearance of up to 80% of chronic carriers can be achieved with the administration of 750 mg of ciprofloxacin twice daily for 28 days or 400 mg of norfloxacin. Other quinolone drugs may yield similar results. Carriers should be excluded from any activities involving food preparation and serving, as should convalescent patients and any persons with possible symptoms of typhoid fever (Ferreccio, 1988; Gotuzzo, 1991).

Although it would be difficult for typhoid carriers in developing countries to follow this recommendation, food handlers should not resume their duties until they have had three negative stool cultures at least one month apart. Vi antibody determination has been used as a screening technique to identify carriers among food handlers and in outbreak investigations. The Vi antibodies are very high in chronic *S.* Typhi carriers (Kubin, 1993).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study area for the research was Kaduna State, Nigeria. The State is situated in the southern part of the high plains northern Nigeria. To the South-West, the state shares a border with the Federal Capital Territory, Abuja, Nassarawa State to its South, Bauchi and Plateau States to the East, Niger to the west, while Zamfara, Katsina and Kano States take up its Northern boundaries positioning it as a communication and economic hub. It has a total population of 6,113,503 with 3,090,438 males and females 3,023,065 (NPC, 2006). The State is divided into three senatorial zones with a total of Twenty three (23) Local Government Areas (LGAs). The Northern senatorial zone includes Zaria,Sabon-gari, Makarfi, Kudan, Ikara, Kubau, Soba and Lere LGAs. The Central zone includes Igabi, Birnin-gwari, Chikun, Kaduna North, Kaduna South and Giwa LGAs, while Sanga, Zangon-kataf, Kachia, Kagarko, Jema'a, Kaura, Kauru and Jaba LGAs constitute the Southern zone.

The Study covered both rural and urban areas of the State. Two LGAs (one rural and one urban) were selected randomly from each of the senatorial zones. Zaria and Soba LGAs were selected from the Northern zone, Kaduna North and Giwa LGAs from the Central zone with Kachia and Kagarko LGAs from the Southern zone. The rural LGAs selected were Soba, Giwa and Kagarko, while Zaria, Kaduna North and Kachia were selected as Urban LGAs.

3.2 Study Design

The research was a community based, cross sectional study that employed the use of Questionnaires and experiments. Two LGAs were selected from each of the three senatorial

zones, with one rural and the other urban LGA. Seventy of each of the study samples (blood and stool) were collected randomly from each of the selected LGAs.

3.3 Study Population

The study populations were individuals of all ages, sex and from all works and activities of life including Bankers, Commercial motorcycle riders, blood donors, secondary school students, primary school pupils, farmers and civil servants in the general population of the selected Local Government Areas.

3.4 Inclusion Criteria

Participants in the selected LGAs who gave their consents, regardless of being previously infected or not and without active fever were included in the study.

3.5 Exclusion Criteria

Participants in the selected LGAs who did not give their consents or had active fever were excluded from the study.

3.6 Ethical Approval and Consent

An approval was obtained from Kaduna State Ministry of Health to carry out the research (Appendix I). All the procedures for sample collection were explained to the participants to get their consents (Appendix II) before they participated in the study.

3.7 Data Collection

Questionnaires (Appendix III) were used to obtain demographic data and also to assess the risk factors associated with typhoid fever in the study area.

3.8 Sample Size Determination

The sample size was determined using the following equation as described by Naing *et al.* (2006) and 50% prevalence was used due to absence of exact figures from literature.

$$n = \frac{Z^2P(1 - P)}{d^2}$$

where n= sample size

Z = standard normal distribution at 95% confidence interval usually 1.96

P = Prevalence (50%)

d= permissible error of estimate at 0.05 (5%)

$$n = \frac{(1.96)^2 \times 50\% \times (1- 50\%)}{(0.05)^2}$$

$$n = \frac{3.8416 \times 0.5 \times 0.5}{0.0025}$$

$$n = \frac{0.9604}{0.0025} \qquad n = 384$$

The sample size calculated was 384, which was the least number of samples to be used for this research. Therefore, 420 blood samples and 420 stool samples were collected from all the participants and assayed in this research.

3.9 Sample Collection

Samples collected were blood and stool from each participant. Non repetitive samples (n= 420) were collected randomly from individuals in the general population of the study area between September 2014 and August 2015, with 70 each of blood and stool samples from each of the selected LGAs of the three senatorial zones.

3.9.1 Blood Collection

Five milliliters of blood samples were collected by vena puncture in plain sample bottles. The blood samples were allowed to clot, centrifuged at 5000 rpm for 1 minute, and the sera separated into clean sample bottles using pipettes and used for slide and tube agglutination tests to detect the presence and determine the levels of antibodies to *Salmonella* Typhi and *S.* Paratyphi.

3.9.2 Collection of Faecal Sample

Faecal samples were collected in sterile universal bottles and transported to Department of Microbiology, A.B.U, Zaria laboratory on cool pack.

3.9.3 Analysis of Blood Sample

The sera obtained from the blood samples were used to detect the presence and levels of antibodies to *Salmonella* Typhi and *S.* Paratyphi.

3.9.4 Slide Agglutination Test

This was carried out first as a screening test to detect the presence of the antibody in the serum using a commercial test kits (ANTEC Diagnostic Products, 2015) containing *Salmonella enterica serovar* Typhi O and H antigens, *Salmonella enterica serovar* Paratyphi AH antigen and BH antigen. The antigen in the test kit was mixed with the serum on a slide for visible agglutination reactions indicating a positive serum.

3.9.5 Tube Agglutination Test

This was carried out to determine the antibody titre in the serum using commercial test kits (ANTEC Diagnostic Products, 2015) containing *Salmonella enterica serovar* Typhi O and H antigens, *Salmonella enterica serovar* Paratyphi AH antigen and BH antigen. Positive sera from the slide test were further titrated to determine the antibody titre. Eight (8) plastic tubes were

labeled accordingly, and with the aid of a pipette, 1.9 ml of 0.85% saline was dispensed into the first well and 1ml into the remaining seven tubes. Then 0.1 ml of the subject's undiluted serum was dispensed into the first tube and thoroughly mixed using large pipette volume tip set to 0.1 ml, after which 1 ml was pipetted from the first tube and dispensed into the second tube and thoroughly mixed. This double dilution technique was continued serially up to the seventh tube. 1 ml from the seventh tube was discarded and the eight tube containing only saline was used as a control without any serum. The antigen suspension in the test kit was shaken vigorously and a drop of the appropriate antigen suspension was added into each tube, thoroughly mixed and incubated at 50^{o}C for 2 hours. It was then left overnight in the fridge after which it was allowed to reach room temperature before the tubes were examined for agglutination. The titre was taken as the last tube that showed agglutination (Cheesbrough, 2015).

3.9.6 Analysis of Faecal Sample

The faecal samples were used in detecting the presence of *Salmonella* Typhi and *S.* Paratyphi using standard methods (Cheesbrough, 2015; WHO, 2015).

3.9.7 Pre-enrichment and Culture

Twenty five grams of the faeces was transferred into 225ml of Selenite F broth, incubated at 37^{o}C for 8 hours and sub cultured by streaking onto Bismuth sulfite agar plates. The cultured plates were incubated at 37^{o}C for 24 hours and examined daily for characteristic colonies of *S.* Typhi and Paratyphi (Cheesbrough, 2015).

3.9.8 Purification of Isolates

The suspected colonies were sub cultured onto fresh Bismuth sulfite agar plates and nutrient agar for isolation of pure culture to be used for biochemical characterization.

3.9.9 Biochemical Characterization

Characterization of the isolates was achieved using biochemical test methods described by Cheesebrough (2015).

A 24 hours pure culture of each isolate was used to determine their Gram reaction, after which the following biochemical tests were carried out: indole, triple sugar iron, citrate utilization, methyl-red, Voges-Proskauer, lysine decarboxylation, ornithine decarboxylation, urease, sugar (galactose, sorbitol, maltose, mannitol, trehalose and dulcitol) fermentation and motility tests.

3.9.10 Gram's Staining Technique

A drop of normal saline was placed on a clean grease-free microscope slide and a 24 hour colony was picked with a sterile inoculating loop. The colony was emulsified in the normal saline, and spread to form a smear. The slide was allowed to air dry and heat fixed over a burning flame. Crystal violet stain was applied for 60 seconds, washed with water after which Lugol's iodine solution was applied for 60 seconds and decolorized rapidly with acetone. The slide was washed immediately with water and counter stained with 0.1% neutral red for 30 seconds, followed by washing with water and drying. Each slide was examined under the microscope using x100 objective.

3.9.11 Motility Test: Hanging Drop Method

A ring of plasticine was made on a clean grease-free slide. A loopful of 24 hour bacteria broth culture was placed on a cover slip. The ring of plasticine was carefully pressed onto the cover slip with the drop of culture in the center of the ring and not touching the slide. With a quick movement, the slide was inverted so that the cover slip is uppermost. It was then examined under the microscope using ×10 and ×40 objectives.

3.9.12 Indole Test

The isolate was grown in 5ml peptone water for 24 hours and five drops of Kovac's reagent were added and the mixture was shaken gently. It then was observed for the development of pink ring in the reagent layer above the medium.

3.9.13 Citrate Utilization Test

Simmon's citrate agar slants were prepared and the surface inoculated with each of the isolates. They were incubated for 24 hours at 37^{o}C. Positive results were indicated by a deep blue colour, while the original green colour of the medium remained unchanged in negative results.

3.9.14 Methyl Red Voges-Proskauer Test

Five milliliter of Methyl red Voges-Proskauer broth was inoculated with each of the isolates and incubated at 37^{o}C for 72 hours, 1ml of the broth was transferred to a smaller tube and 3 drops of methyl red were added and then observed for red colouration.

To the rest of the broth in the original tube, 15 drops of 5% α-naphthole followed by 5 drops of 40% potassium hydroxide solution were added. The tube was shaken, its cap loosened and placed in a sloping position. It was observed for the development of a red colour at the liquid-air interface within an hour.

3.9.15 Triple Sugar Iron Agar (TSI agar)

The TSI agar slant was streaked with the isolates using a sterile wire loop and the butt stabbed using a straight wire and incubated at 37^{o}C for 24 hours. The positive results for *Salmonella* cultures showed alkaline (red) slants and acid (yellow) butts with gas formation (bubbles) and mostly with formation of hydrogen sulfide (blackening of the agar).

3.9.16 Oxidase Test: Filter Paper Method

A piece of filter paper was soaked in the reagent solution. A fresh growth from the cultured plate was picked with a disposable loop and smeared onto the filter paper. It was observed for purple colour development within 10 seconds.

3.9.17 Lysine Decarboxylase Test

The organism was inoculated just below the surface of the lysine decarboxylase broth and overlayed with sterile mineral oil and incubated at 37^{o}C for 24 hours. Turbidity and purple colour after incubation indicated a positive reaction. A yellow coloration indicated a negative reaction.

3.9.18 Ornithine Decarboxylase Test

The organism was inoculated just below the surface of the medium and overlayed with sterile mineral oil, incubated at 37^{o}C for 24 hours. Turbidity and a purple colour after incubation indicate a positive reaction. A yellow colour indicates a negative reaction.

3.9.19 Urease Test

The urea agar slant surface was inoculated with the organism and incubated at 37^{o}C for 24 hours. The slants changed colour to red in positive tests.

3.9.20 Sugar Fermentation Test

Sugars were prepared at 1% concentration and 2ml capacity dispensed into separate sterile Bijou bottles. Pure isolates were picked and inoculated into each sugar and incubated at 37^{o}C for 24 hours. Development of a pink colour indicated a positive result.

3.9.21 Analytical Profile Index (API 20E)

Procedure: Biochemical identification of the Isolates was also carried out using API 20E strip kit (bioMe´rieux®, Inc., France). The reagents used included API NaCl 0.85% medium, API 20 E reagent kit, Zn reagent, oxidase, mineral oil, and API 20E Analytical Profile Index. To prepare the strips, an incubation box (tray and lid) was used and 5ml of distilled water were distributed into the honey-combed wells of the tray to create humid atmosphere. To prepare the inoculum, single well isolated colony was removed from the isolation plate and emulsified in 5ml of API 0.85% NaCl in order to achieve a homogeneous bacterial suspension. Anaerobiosis in the tests arginine dihydrolase (ADH), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), H_2S and URE was maintained by overlaying with mineral oil. The incubation box was closed and incubated at 37°C for 24 hours as described by the manufacturer, and the results were determined according to API 20E.

3.9.22 Serotyping

Serological identification of the *Salmonella* Typhi and Paratyphi were carried out using polyvalent and monovalent antisera (OXOID, UK). Culture of organisms derived from pure culture and identified as *Salmonella* by biochemical tests were serotyped.

3.9.23 Procedure for Serotyping (Slide Agglutination Test Method)

Five colonies of the presumptive *Salmonella species* were suspended in 0.5ml physiological saline used as antigen suspension. A drop of polyvalent antiserum and physiological saline each were placed on a clean glass slide partitioned into several parts with glass pencil, which served as control. Then a drop of the antigenic suspension was placed onto the serum and physiological saline on another part of the glass slide. The suspension was mixed by tilting the glass slide back and forth for one minute. A gross agglutination within one minute in the antigen-antiserum

mixture was observed and recorded as positive. The procedure above was repeated using monovalent antisera to identify *Salmonella species.*

CHAPTER FOUR

4.0 RESULT

The presence of antibody to *S.* Typhi and *S.* Paratyphi A, B and C and its levels were determined among 420 apparently healthy individuals in Kaduna State. The result showed that 338 out of the 420 participants had antibody titre ≥1:20 against *Salmonella species* giving a total seroprevalence of 80.5%. The remaining 82 (19.5%) had antibody titre <1:20 (Figure 1).

Further analysis of the result showed that 29.5% (124/420) had antibody against *Salmonella* Typhi <1:20 while 70.5% (296/420) had antibody against *Salmonella* Typhi ≥ 1:20. Similarly, 53.3% (224/420) and 46.7% (196/420) had antibody levels against *Salmonella* Paratyphi A <1:20 and ≥ 1:20 respectively. Antibody titre <1:20 against *Salmonella* Paratyphi B was seen in 54.1% (227/420), while antibody titre ≥ 1:20 was seen in 45.9% (193/420) of the participants. The antibody titre of <1:20 against *Salmonella* Paratyphi C was detected in 83.1% (349/420), while titre ≥ 1:20 was seen in 16.9% (71/420) of the participants (Table 4.1).

The result also showed that participants in Giwa LGA which is a rural area from the Central Senatorial Zone had the highest prevalence (88.6%: 62/70) of *Salmonella species* antibodies while Zaria LGA which is an Urban area in the North senatorial district had the least prevalence of 67.1% (47/70) (Table 4.2).

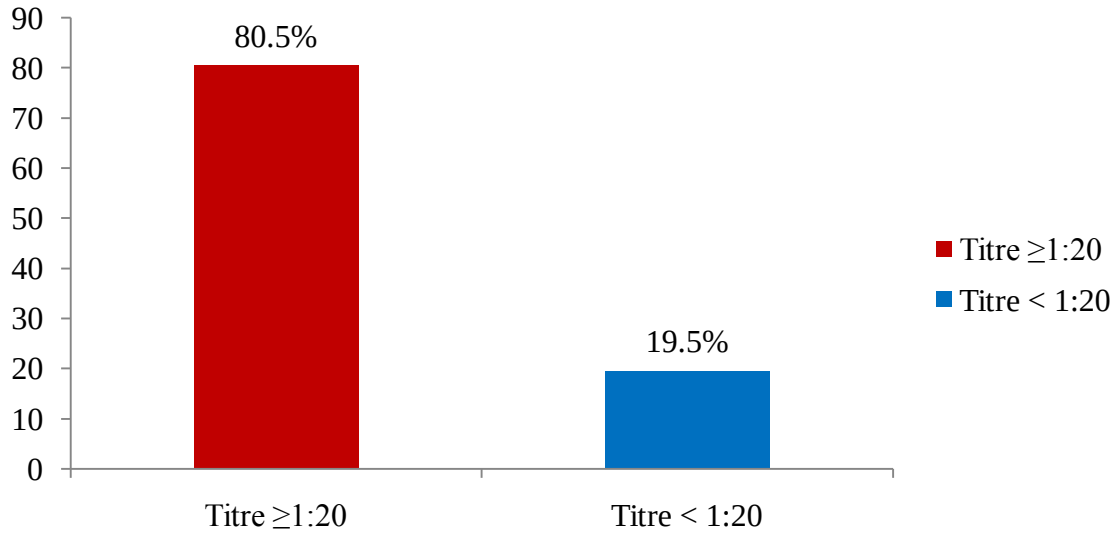


Fig. 4.1: Prevalence of *Salmonella species* Antibodies in Kaduna State

Table 4.1: Prevalence of *Salmonella species* Antibody Serotypes in Kaduna State (N=420)

Serotype	No. Positive (%)	No. Negative (%)
S. Typhi	296 (70.5)	124 (29.5)
S. Paratyphi A	196 (46.7)	224 (53.3)
S. Paratyphi B	193 (45.9)	227 (54.1)
S. Paratyphi C	71 (16.9)	349 (83.1)

Table 4.2: Prevalence of *Salmonella* species Antibodies Based on Senatorial Zones in Kaduna State

Senatorial Zone	LGA	Total (N=70)		Male		Female
		No. Positive (%)	No. Tested	No. Positive (%)	No. Tested	No. Positive (%)
North	Zaria	47 (67.1)	27	21 (77.8)	43	26 (60.5)
	Soba	57 (81.4)	52	46 (88.5)	18	11 (61.6)
Central	Kaduna North	56 (80.0)	39	34 (87.2)	31	22 (70.9)
	Giwa	62 (88.6)	49	46 (93.8)	21	16 (76.2)
South	Kachia	57 (81.4)	51	48 (94.1)	19	9 (47.4)
	Kagarko	59 (84.3)	58	52 (89.7)	12	7 (58.3)

Table 4.3 shows the distribution of 338 (80.5%) individuals with antibody levels ≥ 1:20 against the different serotypes of *Salmonella* species. The results showed that more males had *Salmonella* antibody titres for *S.* Typhi O (83.3%: 207/247) and *S.* Typhi H (89.1%: 220/247). More so, 59.5% (147/247) males had *Salmonella* antibody titres for *S.* Paratyphi A-O, 59.9% (148/247) for *S.* Paratyphi B-O, 19.0% (47/247) for *S.* Paratyphi C-O, 63.9% (158/247) for *S.* Paratyphi A-H, 61.1% (151/247) for *S.* Paratyphi B-H, and 21.9 (54/247) for *S.* Paratyphi C-H.

The result showed that more females had *Salmonella* antibody titres for *S.* Typhi H (83.5%: 76/91) followed by *S.* Typhi O (81.3%: 74/91), *S.* Paratyphi B-H (46.2%: 42/91), *S.* Paratyphi B-O (41.8%: 38/91), *S.* Paratyphi A-H (41.8%: 38/91), *S.* Paratyphi A-O (37.5%: 34/91), *S.* Paratyphi C-H (18.7%: 17/91), and *S.* Paratyphi C-O (17.6%: 16/91). The frequency of occurrence of the antibodies to the different serotypes was generally higher in males with a range of (19-84%) than in females with a range of 11-82%. *Salmonella* Typhi antibodies had the highest frequency of 87.6% (296/338) for "H" antibodies and 83.1% (281/338) for "O" agglutinins, while *Salmonella* Paratyphi C antibodies had the least frequency of 18.6% (63/338) and 21.0% (71/338) for "C-O" and "C-H" antibodies respectively.

Further analysis of the 281 individuals with anti-O antibody levels ≥ 1:20 against *Salmonella* Typhi showed that 15.3% (43/281) individuals had antibody titre of 1:80 while the most frequent titre was 1:20 in 54.1% (152/281) individuals (Table 4.4).

Table 4.5 shows the distribution of the 296 individuals with anti-"H" antibody titre ≥ 1:20 against *Salmonella* Typhi. Antibody titres 1:20, 1:40, 1:80, 1:160 and 1:320 were observed in 159 (53.7%), 85 (28.7%), 49 (16.6%), 2 (0.7%) and 1 (0.3%) individuals respectively.

Table 4.3: Distribution of different *Salmonella species* Antibodies in Samples with titre ≥1:20 in relation to Sex of Study Subjects

Serotype	Antibody type	No. Positive (%)		
		Total (N=338)	Male (N=247)	Female (N=91)
S. Typhi	O	281 (83.1)	207 (83.8)	74 (81.3)
S. Typhi	H	296 (87.6)	220 (89.1)	76 (83.5)
S. Paratyphi A	A-O	181 (53.6)	147 (59.5)	34 (37.4)
S. Paratyphi A	A-H	196 (57.9)	158 (63.9)	38 (41.8)
S. Paratyphi B	B-O	186 (55.0)	148 (59.9)	38 (41.8)
S. Paratyphi B	B-H	193 (57.1)	151 (61.1)	42 (46.2)
S. Paratyphi C	C-O	63 (18.6)	47 (19.0)	16 (17.6)
S. Paratyphi C	C-H	71 (21.0)	54 (21.9)	17 (18.7)

Table 4.4: Distribution of Antibody Titres in Samples with anti-O titre ≥1:20 against *Salmonella* Typhi in relation to Sex of Study Subjects

Antibody titre	No. Positive (%)		
	Total (N=281)	Male (N=207)	Female (N=74)
1 in 20	152 (54.1)	98 (47.3)	54 (72.9)
1 in 40	81 (28.8)	69 (33.3)	12 (16.2)
1 in 80	43 (15.3)	35 (16.9)	8 (10.8)
1 in 160	3 (1.1)	3 (1.4)	0 (0.0)
1 in 320	2 (0.7)	2 (0.9)	0 (0.0)

Table 4.5: Distribution of Antibody Titres in samples with anti-H titre ≥1:20 against *Salmonella* Typhi in Relation to Sex of Study Subjects

Antibody titre	No. Positive (%)		
	Total (N=296)	Male (N=220)	Female (N=76)
1 in 20	159 (53.7)	101 (45.9)	58 (76.3)
1 in 40	85 (28.7)	73 (33.1)	12 (15.8)
1 in 80	49 (16.6)	43 (19.5)	6 (7.9)
1 in 160	2 (0.7)	2 (0.9)	0 (0.0)
1 in 320	1 (0.3)	1 (0.5)	0 (0.0)

Tables 4.6 and 4.7 show the distribution of 181 and 196 individuals with anti-O and anti-H titres ≥ 1:20 respectively, against *Salmonella* Paratyphi A. The highest titre was 1:160 for "A-H" with a frequency of 1(0.5%) and 1:80 for "A-O" with a frequency of 6 (3.3%), while the highest titres with frequency of up to 5% was 1:40 for A-O and 1:80 for A-H agglutinins with frequencies of 47 (26.0%) and 48 (24.5%) respectively.

Table 4.8 shows the distribution of 186 samples with anti-O titres ≥ 1:20 against *Salmonella* Paratyphi B. The highest antibody titre that occurred in up to 5% of the study population was 1:80 with a frequency of 29 (15.6%). The distribution of 193 samples with anti-H antibody titre ≥ 1:20 against *Salmonella* Paratyphi B is given in Table 4.9. The titre 1:80 is the highest titre that occurred in up to 5% of the study population and occurred in 25 (13.0%) individuals with frequencies of 14 (9.3%) in males and 11 (26.2%) in females.

Table 4.10 shows the distribution of 63 samples with anti-O titre ≥ 1:20 against *Salmonella* Paratyphi C. Titre 1:40 occurred in 19 (30.2%) of the study population and 1:80 occurred in only 2 (3.2%) individuals.

Antibody titre 1:80 that occurred in 5 (7.0%) of the 71 samples with anti-H titre ≥ 1:20 against *Salmonella* Paratyphi C was the highest titre with a significant frequency while the highest titre observed was 1:160 but occurred in only 2 (2.8%) of the study population (Table 4.11) .

Table 4.12 shows the distribution of *Salmonella* Typhi isolated from the different LGAs of the three senatorial zones. *Salmonella* Typhi was isolated from a total of 3 stool samples out of the 338 that had antibody titres ≥ 1:20, with 1 positive sample each from Zaria, Giwa and Kagarko LGAs. None of *Salmonella* Paratyphi A, B or C was isolated in the study.

Table 4.6: Distribution of Antibody Titres in samples with anti-O titre ≥1:20 against *S.* Paratyphi A in Relation to Sex of Study Subjects

Antibody titre	No. Positive (%)		
	Total (N=181)	Male (N=147)	Female (N=34)
1 in 20	128 (70.7)	110 (74.8)	18 (52.9)
1 in 40	47 (26.0)	33 (22.4)	14 (41.2)
1 in 80	6 (3.3)	4 (2.7)	2 (5.9)
1 in 160	0 (0.0)	0 (0.0)	0 (0.0)
1 in 320	0 (0.0)	0 (0.0)	0 (0.0)

Table 4.7: Distribution of Antibody Titres in Samples with anti-H titre ≥1:20 against *S.* Paratyphi A in Relation to Sex of Study Subjects

Antibody titre	No. Positive (%)		
	Total (N=196)	Male (N=158)	Female (N=38)
1 in 20	139 (70.9)	117 (74.1)	22 (57.9)
1 in 40	48 (24.5)	33 (20.9)	15 (39.5)
1 in 80	8 (4.1)	7 (4.4)	1 (2.6)
1 in 160	1 (0.5)	1 (0.6)	0 (0.0)
1 in 320	0 (0.0)	0 (0.0)	0 (0.0)

Table 4.8: Distribution of Antibody Titres in Samples with anti-O titre ≥1:20 against *S.* Paratyphi B in Relation to Sex of Study Subjects

Antibody titre	No. Positive (%)		
	Total (N=186)	**Male (N=148)**	**Female (N=38)**
1 in 20	108 (58.1)	94 (63.5)	14 (36.8)
1 in 40	44 (23.7)	29 (19.6)	15 (39.5)
1 in 80	29 (15.6)	21 (14.2)	8 (21.1)
1 in 160	5 (2.7)	4 (2.7)	1 (2.6)
1 in 320	0 (0.0)	0 (0.0)	0 (0.0)

Table 4.9: Distribution of Antibody Titres in samples with anti-H titre ≥1:20 against *S.* Paratyphi B in Relation to Sex of Study Subjects

Antibody titre	No. Positive (%)		
	Total (N=193)	Male (N=151)	Female (N=42)
1 in 20	121 (62.7)	104 (68.9)	17 (40.5)
1 in 40	46 (23.8)	32 (21.2)	14 (33.3)
1 in 80	25 (13.0)	14 (9.3)	11 (26.2)
1 in 160	1 (0.5)	1 (0.7)	0 (0.0)
1 in 320	0 (0.0)	0 (0.0)	0 (0.0)

Table 4.10: Distribution of Antibody Titres in samples with anti-O titre ≥1:20 against *S.* Paratyphi C in Relation to Sex of Study Subjects

Agglutinating titre	No. Positive (%)		
	Total (N=63)	**Male (N=47)**	**Female (N=16)**
1 in 20	41 (65.1)	32 (68.1)	9 (56.3)
1 in 40	19 (30.2)	12 (25.5)	7 (43.8)
1 in 80	2 (3.2)	2 (4.3)	0 (0.0)
1 in 160	1 (1.6)	1 (0.2)	0 (0.0)
1 in 320	0 (0.0)	0 (0.0)	0 (0.0)

Table 4.11: Distribution Antibody Titres in samples with anti-H titre ≥1:20 against *S.* Paratyphi C in Relation to Sex of Study Subjects

Agglutinating titre	No. Positive (%)		
	Total (N=71)	**Male (N=54)**	**Female (N=17)**
1 in 20	43 (60.6)	31 (57.4)	12 (70.6)
1 in 40	21 (29.6)	17 (31.5)	4 (23.5)
1 in 80	5 (7.0)	4 (7.4)	1 (5.9)
1 in 160	2 (2.8)	2 (3.7)	0 (0.0)
1 in 320	0 (0.0)	0 (0.0)	0 (0.0)

Table 4.12: Distribution of *Salmonella* Typhi Isolates With Respect to Senatorial Zones in Kaduna State

Senatorial Zone	LGA	Number of Isolates	
		Biochemically Confirmed	Serologically Confirmed
North	Zaria	3	1
	Soba	0	0
Central	K/North	0	0
	Giwa	2	1
South	Kachia	0	0
	Kagarko	1	1

Out of the individuals enrolled in the study, 276 (65.7%) were males and 144 (34.3%) were females. Most of the participants 152 (36.2%) were in the age group 20-29 years old, while the age group 0-9 years had the least number; 6 (1.4%). With respect to educational level, 56 (13.3%) had tertiary education while 182 (43.3%) had secondary education, with 97(23.1%) participants having no formal education. Similarly, 155 (36.9%) participants were farmers, 85 (20.2%) business men and women, 75 (17.9%) civil servants and 106 (25.2%) engaged in other occupations (Table 4.13). The result showed that out of the 82 (19.5%) that had antibody levels <1:20 to *Salmonella* species and were considered as negative, 29 (35.4%) were males and 53(64.6%) were females.

The result also showed that out of the 338 individuals that had antibody levels $\geq$ 1:20 against *S.* Typhi and *S.* Paratyphi A, B and C, 247 (73.1%) were males and 91(26.9%) were females giving a prevalence of 89.5% (247/276) in males and 63.2% (91/144) in females. Analyses of the result further showed that the presence of antibody to *Salmonella* Typhi and *Salmonella* Paratyphi A, B and C varied significantly (χ^2 =9.398, df =1, p =0.002) with gender, insignificantly with Age group (χ^2=8.194, df =5, p =0.146), Educational Level (χ^2 =3.097, df =3, p =0.377), and Occupation (χ^2 =5.612, df =3, p =0.132) (Table 4.13).

Table 4.13: Prevalence of *Salmonella* species Antibodies Based on Demographic Data (N=420)

Parameter	Number Tested	No. Positive (Titre $\geq$ 1:20)	Percentage	P Value
Gender				
Male	276	247	89.5	0.0022
Female	144	91	63.2	
Age group (years)				
0 – 9	6	0	0.0	0.1459
10 – 19	83	62	74.7	
20 – 29	152	127	83.6	
30 – 39	122	109	89.3	
40 – 49	52	38	73.1	
50 – 59	5	2	40.0	
Educational Level				
Tertiary	56	37	66.1	0.3769
Secondary	182	143	78.6	
Primary	85	69	81.2	
None	97	89	91.8	
Occupation				
Business	85	76	89.4	0.1321
Civil Service	74	44	59.5	
Farming	155	132	85.2	
Others	106	86	81.1	

CHAPTER FIVE

5.0 DISCUSSION

Antibodies for typhoid and paratyphoid groups tested in this study were evidently very frequently found in the blood of the subjects.

The overall prevalence of antibody to *Salmonella* species was found to be 80.5%, with higher prevalence in rural areas compared to urban areas. The higher prevalence in the rural areas could be due to lack of portable drinking water, nearness of wells to pits and improper defecation along streams and water bodies especially on market days as observed during sample collection. This collaborates the data obtained from analysis of the questionnaire and the observations made during data collection in the rural areas. This finding is in agreement with the findings of (WHO, 2009) that related Typhoid fever and paratyphoid fever to less industrialized countries and communities, and in contrast with the findings of Allen and Honest (2010). The low prevalence observed in the urban areas, with the least prevalence in Zaria LGA could be due to availability of potable drinking water and most of the respondents were seen treating their drinking water before consumption or consumed already treated water. The implication of having a high prevalence of *Salmonella* species antibodies in the healthy population is that more and more individuals could easily be diagnosed wrongly with typhoid fever especially when having infections that have similar symptoms with typhoid fever such as malaria.

For practical purposes, titres occurring in more than 5% of subjects under study should not be diagnostically significant and should be regarded as normal in that population (Ibekwe *et al.,* 2008; Okonko *et al.,* 2010). Based on this premise, it would seem that *Salmonella* titres of

1:80 occurred in a significant proportion of the samples as well as in the males and females. Antibody titres against *Salmonella* Typhi, *Salmonella* Paratyphi B and *Salmonella* Paratyphi C up to 1:80 occurred in between 7% -16.6% of the study population. Antibody titre against *Salmonella* Paratyphi A up to 1:80 occurred in 3.3-4.1% of the study population while antibody titres of 1:40 occurred in between 24.5%-26.0%. Therefore, antibody titres above 1:80 against *Salmonella* Typhi, *Salmonella* Paratyphi B and *Salmonella* Paratyphi C could be used in the presumptive diagnosis of typhoid fever in Kaduna State. However, antibody titres above 1:40 against *Salmonella* Paratyphi A should be used in the presumptive diagnosis of typhoid fever in Kaduna State, but should be confirmed if a second sample is possible. These findings agree with the findings of Shukla *et al.* (1997), Ibekwe *et al.* (2008) and Okonko *et al.* (2010) cited in Jeyakumari and Jaberlin, (2011), who determined the baseline antibody titre to *Salmonella* species to be greater than 1:80 in Abeokuta South-Western Nigeria, Akwa South-Eastern Nigeria and Central India respectively. The finding is however, in contrast with the findings of Punia (2003) who determined a higher antibody titre of 1:160 for *Salmonella* Typhi and a lower titre of 1:20 for *Salmonella* Paratyphi A and B in Chandigarh, India. Anand *et al.* (2007) determined the antibody titre to *Salmonella* Typhi to be 1:80 and that of *Salmonella* Paratyphi A and B to be 1:40 among children in Karnataka, India.

This result also showed that the presence of antibody to *Salmonella* species was higher in males than in females. This could be attributed to their way of life as the male tend to eat and drink outside their homes, which makes them more vulnerable to *Salmonella* infections. This is in agreement with the findings of Samreen *et al.* (2014) who also found a higher prevalence of antibody to *Salmonella* species in males than females in Pakistan, and in

contrast with the findings of Okonko *et al.* (2010) that found higher prevalence of antibody to *Salmonella species* in females.

Although all ages were affected in this study with no significant difference with respect to age, the higher prevalence of antibody to *Salmonella* species were found in the age groups 20-29 and 30-39 years. This could be due to previous symptomatic or asymptomatic infections as these age groups are more active and more engaged in day to day activities, which predisposes them to the infection. This observation agrees with the findings of Samreen *et al.* (2014) who found a higher prevalence of antibody to *Salmonella* species in participants with mean age of 29 years. The result however contrasts the findings of Lillian *et al.* (2015) who found a higher prevalence of antibody to *Salmonella* species in participants with mean age of 15 years.

Similarly, the highest prevalence of *Salmonella* species antibodies seen in subjects without formal education could be attributed to lack of basic knowledge on the causes, transmission and control of typhoid fever. The participants in this group were mostly the same subjects involved in business activities and other forms of occupations that also showed a high prevalence possibly due to likelihood of eating and drinking freely from public sources. This agrees with the observation of Allen and Honest (2010), who related higher prevalence of typhoid fever to limited formal education.

Among the *Salmonella* species, *S.* Typhi antibodies had the highest prevalence. This could be due to the fact that *S.* Typhi has a unique cluster of genes known as the pathogenicity island and polysaccharide capsule Vi that have protective effect against the bacteriocidal action of serum of infected patients (WHO, 2011). This is in agreement with the findings of Ibekwe *et*

al. (2008) who found a higher prevalence of *S.* Typhi antibodies among healthy individuals in Akwa, South-Eastern Nigeria.

In this study, the prevalence of *Salmonella* "H" antibodies was generally higher than that of *Salmonella* "O" antibodies in both male and female subjects, and for all the *Salmonella* serotypes. This contrasts the findings of Ibekwe *et al.* (2008) and Okonko *et al.* (2010), who independently found the prevalence of *Salmonella* "O" antibodies to be higher than that of *Salmonella* "H" antibodies in males.

Antibody levels for the typhoid and paratyphoid groups tested in this study were evidently very frequently found in the blood of the subjects, and in high quantities. Although this order of typhoid prevalence is by far higher and calls for the need to have a strong control protocol implemented in Kaduna State, these results require careful interpretation because typhoid fever in the tropics presents a huge diagnostic challenge. In most parts of Nigeria, typhoid fever diagnosis is done based on mainly clinical presentation and on serological (widal) test, which is associated with numerous limitations (Uneke, 2008).

Notwithstanding that widal test is inexpensive and readily available in most health care settings in the tropics, serious doubts have been raised regarding its validity. There are currently numerous reports suggesting it is inaccurate and of limited diagnostic value, especially if the baseline antibody titre to *Salmonella* species in the general population in question is not known (Buck *et al.*,1987; Agwu *et al.*, 2009).

Although there are controversies surrounding the increase in titre beyond the first week of illness in some endemic areas, it is generally accepted by clinicians that, towards the end of the first week of illness, titres of either O and H antibodies may rise to as high as 1:160.

However, the lack of paired sera may lead to an erroneous interpretation of test results (Hoffman *et al.,* 1986; Olopoenia *et al.,* 2000).

The isolation of *S.* Typhi in only three samples showed that the presence of the antibody to *Salmonella* species in most of the participants was due to previous exposure to the organisms and not active infection. However, the three participants that were harboring the organism had very high antibody titres up to 1:320. The three isolates confirmed by API20E and serology were isolated from some of the 32 participants with known previous infections with typhoid fever. This finding is in conformity with the 10% carrier status in typhoid fever reported by WHO (2015).

The high antibody titre observed in this study may be unrelated to antigenic stimulation, or due to infections with cross-reacting organisms other than *Salmonella*. For instance, cross reaction between a normally harmless gut bacterium *Citrobacter fruendi* can confuse the serological diagnosis of typhoid bacilli sharing antigenic properties (Ibekwe *et al.,* 2008). Moreover, a number of coliform bacteria share antigens with *Salmonella*. This could lead to confusion in the serological diagnosis of typhoid fever. Therefore, serological findings have to be interpreted with a lot of caution particularly in Kaduna State where there are yet to be laid down standards baseline titres.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The result of this study showed that *Salmonella* antibodies occurred at high frequency among apparently healthy individuals in Kaduna State. The antibodies exist at different concentrations among the healthy population that do not harbor the antigen. These titres are therefore due to previous exposure to the organisms. The findings of this research, further confirms that an infected person may continue to produce antibodies to an infection even after recovery. These "post-recovery" antibodies can be confused with the ones produced in active infections during serological diagnosis of typhoid fever and therefore lead to false positive results in the diagnosis of typhoid fever. A solution to this problem is of course a proper diagnostic procedure that can differentiate the IgG and IgM antibodies synthesized by the body at different periods of contact with the organism. Unfortunately, this cannot be achieved with the Widal test which is the norm in the diagnosis of typhoid fever in Nigeria. It is therefore pertinent to have a cut-off antibody titre for the interpretations of Widal tests.

Since the positive sera with antibody titres up to 1:80 against *Salmonella* Typhi, *Salmonella* Paratyphi B and *Salmonella* Paratyphi C occurred in up to 5% of the samples tested, this study therefore suggests that such titres be regarded as normal among the populations in Kaduna State. There should be a high index of suspicion of clinical infections in titres above 1:80 when a second serum is impractical. However, for *Salmonella* Paratyphi A, the normal titre should be 1:40 and titre greater should be diagnostically significant.

It can also be concluded that a small proportion of the study population harbors *Salmonella* Typhi, and can be contacted by healthy individuals and may cause infections. These individuals had a known history of typhoid fever and are therefore typhoid carriers. This problem represents that typhoid fever is still a problem of developing countries like Nigeria.

Moreover, this study showed that the presence of antibody to *Salmonella* species varied significantly with gender and insignificantly with Age group, Educational level and Occupation

6.2 RECOMMENDATIONS

Based on the result of this study, it is recommended that:

(i) There should be an improvement on public sanitation and control of water pollution which could be achieved through organized public enlightenment programmes by the State Government on Public health education specifically on good eating habits, hand hygiene and sewage and refuse disposal;

(ii) There should be periodic evaluation of baseline titres of Salmonella serotypes agglutinins in Kaduna State to avoid false positive results;

(iii) The significant value for the diagnosis of typhoid fever should be greater than or equal to 1:160 for "O" and "H" agglutinins for *Salmonella* Typhi and *Salmonella* Paratyphi B, *Salmonella* Paratyphi C, and greater than or equal to 1:80 for "O" and "H" agglutinins for *Salmonella* Paratyphi A in Kaduna State;

(iv) A proper diagnostic procedure that can differentiate the IgG and IgM antibodies synthesized by the body at different periods of contact with the organism should be used in the diagnosis of typhoid fever since the study the antibodies occurred in individuals that do not harbor the organisms;

(v) Serological findings have to be interpreted with a lot of caution particularly in Kaduna State where there are yet to be laid down standards baseline titres;

(vi) Known *Salmonella* Typhi carriers should be persuaded to eliminate the organism from their systems through proper medications so as to prevent accidental contamination and subsequent infection of healthy individuals.

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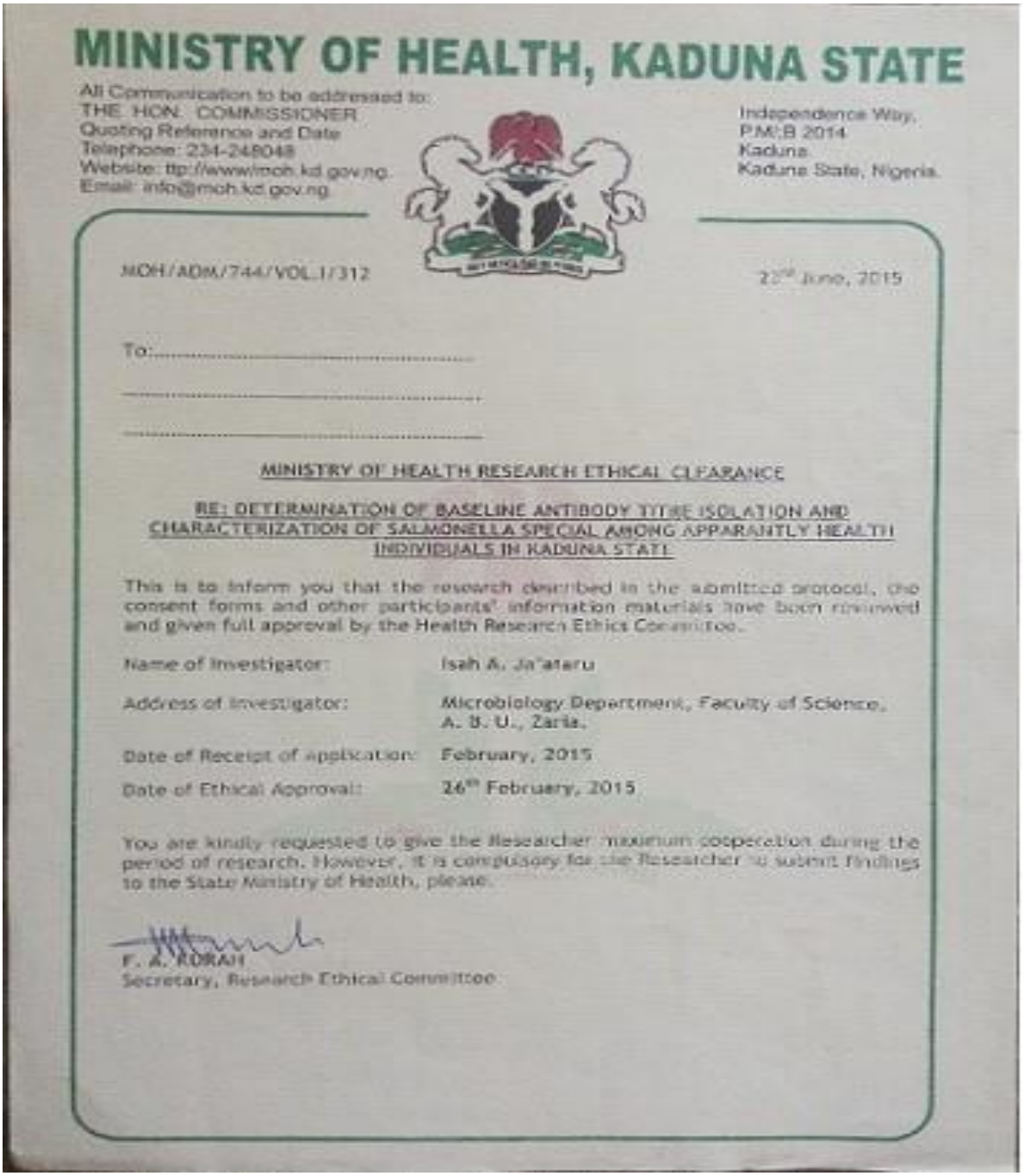
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APPENDICES

APPENDIX I: ETHICAL APPROVAL



MINISTRY OF HEALTH, KADUNA STATE

All Communication to be addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Telephone: 234-248048
Website: ttp://www.moh.kd.gov.ng
Email: info@moh.kd.gov.ng

Independence Way,
P.M.B 2014
Kaduna
Kaduna State, Nigeria.

MOH/ADM/744/VOL.1/312

22nd June, 2015

To:.....

.....

.....

MINISTRY OF HEALTH RESEARCH ETHICAL CLEARANCE

RE: DETERMINATION OF BASELINE ANTIBODY TITRE ISOLATION AND CHARACTERIZATION OF SALMONELLA SPECIAL AMONG APPARANTLY HEALTH INDIVIDUALS IN KADUNA STATE

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

Name of Investigator:	Isah A. Ja'afaru
Address of Investigator:	Microbiology Department, Faculty of Science, A. B. U., Zaria.
Date of Receipt of Application:	February, 2015
Date of Ethical Approval:	26th February, 2015

You are kindly requested to give the Researcher maximum cooperation during the period of research. However, it is compulsory for the Researcher to submit findings to the State Ministry of Health, please.

F. A. KURAH
Secretary, Research Ethical Committee

APPENDIX II: CONSENT FORM

I, Isah Abdullahi Jaafaru a postgraduate student of Microbiology Department in Ahmadu Bello University, Zaria will be undertaking a research work on the topic: **CHARACTERIZATION OF *SALMONELLA* SPECIES AND DETERMINATION OF BASELINE ANTIBODY TITRE AMONG APPARENTLY HEALTHY INDIVIDUALS IN KADUNA STATE, NIGERIA.**

The purpose of the research is to determine the baseline antibody titre to *Salmonella species* and detect the Antigen among apparently healthy individuals in Kaduna State. This study will provide information to aid medical practitioners in the diagnosis and management of typhoid fever, provide baseline data for further researches and recommendations that will be helpful to the public on the prevention and control of the typhoid fever.

Your participation will involve your cooperation, answering questionnaires, providing stool sample and 5mls of blood that will be taken from you using a needle which will cause a little pain. I appreciate your participation in this study.

Also, all information obtained will be treated as confidential and used only for the research purpose. Participation is voluntary. You can withdraw from the research exercise at any point in time. All investigation will be free.

Kindly fill the space below if you have given your consent to participate.

I ..... give my consent for me or my wards to participate in this study. I have been duly informed of the process and have given my free will not under any pressure.

.....	.....
Signature/ thumbprint of subject/ Guardian	Date
.....	.....
Signature of witness	Date
.....	.....
Signature of the investigator	Date

APPENDIX III: QUESTIONNAIRE

I am Isah Abdullahi Jaafaru, a Postgraduate student of Microbiology Department, Ahmadu Bello University Zaria. I am carrying out a research on the topic "CHARACTERIZATION OF *SALMONELLA* SPECIES AND DETERMINATION OF BASELINE ANTIBODY TITRE AMONG APPARENTLY HEALTHY INDIVIDUALS IN KADUNA STATE, NIGERIA". I am soliciting for your cooperation to collect samples for the success of the study. All information will the treated with privacy. Thank you very much

SECTION A: BIODATA

1. Local Government Area……
2. Senatorial Zone…….
3. Age……..
4. Gender Male [ ] Female [ ]
5. Educational Level Tertiary [ ] Secondary [ ] Primary [ ] None [ ]
6. Occupation…….

SECTION B: CLINICAL DATA

7. Have you had fever in the last six months? Yes [ ] No [ ]
8. If Yes, did you visit any hospital or take any treatment? Yes [ ] No [ ]
9. Have you had any form of Gastroenteritis in the last six months? Yes [ ]No [ ]
10. If Yes, did you visit any hospital or take any treatment? Yes [ ] No [ ]
11. What is your source of drinking water?

Tap [ ] Well [ ] Stream [ ] River [ ] Pond [ ] Borehole [ ]

12. Do you treat your water before consumption? Yes [ ] No [ ]
13. If Yes, what type of treatment? Heat[ ] Filtration [ ]Chemicals[ ]
14. Have you ever been diagnosed with Typhoid fever? Yes [ ] No [ ]
15. Did you take any treatment? Yes [ ] No [ ]
16. What type of treatment? Hospital Treatment [ ] Traditional [ ]
17. Has any member of your family come down with typhoid fever in the last six months?

    Yes [ ] No [ ]

18. If Yes, did he/she visit any hospital for treatment? Yes [ ] No [ ]