

TITLE

Clinical and Environmental Distribution of *Salmonella enterica* Var *typhi*/
paratyphi with Concomitant Endemic Malaria in Enugu Urban and Peri-urban
Areas of Nigeria.

By

Eleazar, Clara Idara. B.Sc (Unical), M.Sc (UNN)

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DECLARATION

I declare that the Ph. D project titled ‘**Clinical and Environmental Distribution of *Salmonella enterica* Var *typhi/paratyphi* with Concomitant Endemic Malaria in Enugu Urban and Peri-urban Areas of Nigeria**’ is an original research work produced by Eleazar, Clara Idara under my supervision. I have examined and found it acceptable for the award of Ph. D in Microbiology (Public Health).

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Professor C.U. Iroegbu

Date

Supervisor

.....

.....

Professor J.A. Akinyanju

Date

External Examiner

DEDICATION

To God Almighty, the Father of my Lord and Saviour Jesus Christ and the Holy Spirit, who strengthened and inspired me during this work.

ACKNOWLEDGMENT

My sincere appreciation goes to my supervisor, Professor C. U. Iroegbu for his tireless and selfless effort in reading and correcting this work. His useful criticisms, suggestions and contributions, no doubt, made this work a success. I do also acknowledge the encouragement of Professor B.O Ogeneh and Professor U. C Ozumba, Head of Department of Medical Microbiology, College of Medicine, UNEC. My colleague and beloved sister Mrs. I. P Udoh, was of immense help both in sampling and bench work throughout the course of this research. The staffs of the Department of Microbiology Nsukka did also offer some assistance. The technical staffs, Mr. Ele, Mr. G. Onyechi, Mrs. E. Ezike, Mr. J. Obi and others, offered no small help in going to the various areas to motivate subjects and obtain samples. The Kenyatta market leader Mr. Aniagu and the executive forum were of immense help in creating awareness and mobilizing the trader's populace. I do appreciate Bertha Uzodima, the Chief Medical Laboratory Scientist in my Department, for her understanding and support during the whole period. My husband also, a Chief Medical Laboratory Scientist, was particularly, of immense support to me throughout the tedious

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ABSTRACT

Evaluation of distribution rates of *Salmonella enterica typhi/paratyphi* in subjects at acute and convalescent stages of typhoid fever was carried out using Widal test, stool and blood cultures. Incidences of the concurrent enteric and malaria fever illnesses were determined. Environmental distributions of the salmonella organisms in domestic water sources were also investigated. Two hundred and sixty nine (48.3%) symptomatic and 23 (11.1%) asymptomatic subjects were positive for enteric fever bacilli by Widal test (titre $\geq 1/160$) and blood culture methods. The specificity rate of Widal test, stool and blood cultures techniques were 72.4%, 70.8% and 96.0% while sensitivity rates were 88.4%, 75.1% and 57.5%, respectively. There was no significant difference ($P=0.103$.) in total incidence rate of typhoid fever in male (32.8%) and female (42.8%) subjects in the whole population. The age range with the greatest risk of enteric fever infection was 21 – 30 years, with mean age was of 32.8 years. The rate of infection was not significantly high in peri-urban area ($P = 0.464$). There was 11.4% rate of typhoid/malaria co-existent in the population. The rate of typhoid infection was highest among students (40.0%) while malaria was more prevalent among petty traders/artisans (51.4%). The rate of carriers in male and female subject was 49.0% and 44.7%, respectively ($P > 0.05$). The total rate of salmonella isolates from water samples was 12.6%, while the rate from pipe-borne water was 8.5%. High rate of the isolates (95.7%) were susceptible to ciprofloxacin (5mcg). The sensitivity rate to other

antibiotics were as follows: ofloxacin (5mcg) 92.0%, pefloxacin (5mcg) 75.3%, gentamicin (10mcg) 47.8%, chloramphenicol (30mcg) 40.7%, ceftriazone (10mcg) 36.0% amoxicillin (30mcg) 30.0% and cefuroxime (10mcg) 30.0%. Ampicilin (10mcg) and nalidixic acid (30mcg) showed no sensitivity zones.

INTRODUCTION

Typhoid and paratyphoid fevers are vastly disseminated systemic diseases commonly known as enteric fever. In most endemic areas, approximately 90%

of the enteric fever cases are actually typhoid fever caused by *Salmonella enterica enterica serovar typhi* (Parry, 2008). *Salmonella enterica enterica serovar paratyphi* A, B, and C, respectively, cause paratyphoid fever, a disease which is indistinguishable from typhoid except for their varied severities. These bacterial organisms belong to the family, *Enterobacteriaceae* (Crump *et al.*, 2004). *Salmonella* consists of facultative anaerobe anaerobic, does not possess capsule non-encapsulated; is a non-spore forming Gram-negative rod shaped bacterium measuring 1-3 μ m by 0.5-0.7 μ m (Huckstep and Wright, 2002; Crum, 2003). Most strains are peritrichously flagellated and hence motile. Compared to other Gram negative rods, the salmonellae are relatively more resistant to various environmental conditions, including drying, salting, smoking and freezing, for varying lengths of time and, hence, survive in many foods as well as frozen water. The pathogen would survive for days in groundwater, pondwater, or seawater, and for months in contaminated eggs and frozen oysters (Bhan *et al.*, 2005); but would be killed when exposed to temperatures of 60 $^{\circ}$ -70 $^{\circ}$ C for 15 minutes (Huckstep *et al.*, 2002).

Salmonella enterica enterica serotypes or subspecies are the only *Salmonella* that cause disseminated human disease; pathogenicity of others in humans remains uncertain. Several of these other serovars have been isolated particularly from cold-blooded animals. Ubiquitous (non-host adapted) *Salmonella* strains such as *S. typhimurium* are known to cause clinical typhoid-like syndromes in infants but in adults they produce food borne toxic syndromes

(Le Minor, 2006). *Salmonella enterica* serovars *typhi*, *S. paratyphi* A and *S. Sendai* are human adapted pathogens that cause typhoid fever (Dobryan *et al.*, 2006). *Salmonella paratyphi* B and C serovars are antigenically and genetically distinct from *S. typhi* and *S. paratyphi* A (Selender 1990; Porwollik *et al.*, 2002); but they would also cause typhoid-like illnesses in humans. Typhoid and paratyphoid fevers which are diseases associated with poor personal and environmental hygiene have largely been eliminated in many parts of the world by improved sanitation, but remains a significant health threat in developing nations (Bhan *et al.*, 2005). Enteric fever incidence can conveniently be a barometer for gauging the environmental sanitation condition in a region or country. Incidence of typhoid decreases when the level of development of a country (i.e. controlled water sewage system, proper waste disposal system, pasteurization of milk and diary products) increases. Where these hygienic conditions are lacking, the probability of fecal contamination of water and food remains high and so is the incidence of typhoid (Santillana, 1991; Singh *et al.*, 1995).

Although the extent of laboratory proven typhoid is undetermined in Nigeria (Ameh and Opara, 2004), it is believed that diagnosis of the disease is largely confounded by endemic malaria (WHO, 2003). Malaria is caused by a protozoan parasite of the genus, *Plasmodium*, and is transmitted by *Anopheles* mosquito. The various species are *P. falciparum*, *P. ovale*, *P. vivax* and *P. malariae*. Individuals in the endemic regions, such as Nigeria, are at substantial

risk of both typhoid and malaria either concomitantly or singly, one as acute secondary infection to the other, which is occurring as a primary disease (Keong and Sulaiman, 2006). The confounding situation has given rise to two opinions in Nigeria; one, that most of what is treated as typhoid is actually malaria and the other, that typhoid is as rampant as malaria. For application of proper and adequate measures and public health planning it has become necessary to determine the prevalence of typhoid occurring singly or concomitantly with malaria in the region.

AIMS AND OBJECTIVES

- To determine the distribution of the *Salmonella enterica* bacilli in symptomatic and asymptomatic subjects using serological test, stool and blood cultures.
- To determine the prevalence of the enteric and malaria fever illnesses singly and concurrently.
- To evaluate the distribution rate of *Salmonella enterica* var *typhi/paratyphi* in carrier sources, domestic water sources and subjects exposed to the water sources.

CHAPTER TWO

LITERATURE REVIEW

2.1 HISTORICAL REVIEW

Typhoid fever, as a disease, was recognized long before the bacteria were discovered and recognized as aetiological agents. By then the pathology of typhoid fever was only defined using clinical signs, symptoms and anatomical changes. Petit and Serres (1813) described intestinal ulcerations near the caecum and Bretonneau (1822) was able to distinguish enteric fever from enteritic tuberculosis. Louis (1829) proposed the name typhoid and described some of the clinical features that characterized the disease. The term “typhoid” comes from the Greek word *typhus* meaning “cloud” or “mist” and Hippocrates used it to describe the clouded mental status of patients. Typhoid fever was confused with typhus fever until 1850 when Jenner, having examined 66 fatal cases of the two diseases clearly differentiated the clinical symptoms before a distinct aetiological agent could be defined for each disease. It was not until the middle of the 19th century that physicians began to distinguish between typhoid, typhus and malaria (Cunha and Cunha, 2004).

One of the first large studies carried out on the disease dealt with mental changes in patients and was reported by a contemporary of Budd (1856), who analyzed cases in patients with typhoid fever seen in Massachusetts General Hospital between 1821 and 1835. They presented with delirium and somnolence, deafness or what they described as “watchfulness”. After this development, typhoid had been clearly defined, the study of its epidemic transmission and its pathogenesis was rigorously pursued; and this resulted in the definition of the preventive measures and treatment. Budd (1856) noted that

each case of typhoid was epidemiologically linked to a previous one and suggested that a specific “toxin” was disseminated with the patient’s faeces. This postulation informed the early concept of mode of transmission and dissemination of the disease. Pettenkofer (1868) speculated the role of contaminated water supply and sea foods in epidemic transmission.

The first observation of typhoid bacillus was made by Eberth (1880) in spleen sections and mesenteric lymph nodes from a patient who died from the disease. In 1881 Koch confirmed the findings and in 1884 Gaffky succeeded in cultivating the microorganism (Le Minor, 2006). However, it could not be distinctly differentiated from other bacilli at that time hence the findings were taken with skepticism until Pfeiffer and Kolle in 1896 and Gruber and Durham, in the same year, also demonstrated that the serum from an animal immunized with the typhoid bacillus agglutinated the same typhoid bacillus. Independently, in London, Widal demonstrated that the serum of suspected typhoid cases agglutinated the typhoid bacillus (Le Minor, 2006). Widal (1897), in Paris, adapted the agglutination reaction in serodiagnosis of the disease and this gave rise to Widal test used till this date. Immunization against typhoid by using heat killed bacteria was initiated by Chantemesse and Widal (1888) and was carried further by Wright and Semple (1897). In 1896, also, two strains were isolated from two patients with clinical typhoid but with negative Widal test result (Achard and Bensaude 1896). Consequently, the latter disease was branded paratyphoid and the organism called “*bacilli paratyphique*”. When a

similar case was encountered by Gwyn (1898), the bacillus was called “paracoli bacillus”. In 1901 Schottmuller confirmed that although “paracoli bacilles and bacilli paratyphique were responsible for clinical cases of typhoid they were distinct from the typhoid bacillus both serologically and culturally (Le Minor, 2006). The first organism was, therefore named “*paratyphus A*” and the second “*paratyphus B*”. This discovery brought the concept of a disease caused by a cluster of related bacteria.

Antigenic analysis began when Castellani in 1902 described a method for absorbing antisera. Somatic and flagellar antigens were differentiated (Smith and Reagh, 1903). The O and H antigens were described by Weil and Felix (1918) and Weil and Felix (1920), respectively, and the diphasic nature of flagellar antigens discovered by Andrewes (1922, 1925) and Le Minor (2006). Felix and Pettit (1934) found out that a surface antigen Vi could prevent agglutination of the typhoid bacillus. White (1926) had developed the first antigenic scheme; and this was subsequently expanded by Kauffmann (1966a). The Kauffmann-White scheme contained 100 serotypes in 1941 (Kauffmann 1941).

2.2 NOMENCLATURE

The genus *Salmonella* was named after the pathologist Salmon, who first isolated the organism from the intestines of animal (Crump *et al.*, 2004).

It contains two principal species, namely, *Salmonella bongori* and *S. enterica*. The later species contains six subspecies, namely are *S. enterica*, *S. salamae*, *S. arizonae*, *S. diarizonae*, *S. indica*, and *S. houtenae*, sometimes designated as I, II, IIIa, IIIb, IV, V and VI, respectively. Of these six subspecies, only subspecies I is associated with disease in warm-blooded animals. Within subspecies I there are over 2,300 known serovars that differ in their prevalence, host range and pathogenesis. Only a few of these serovars account for most *Salmonella* infections in humans and domestic animals (Porwollik *et al.*, 2002). *Salmonellae* are divided into distinct serologic groups (A-E) on the basis of their somatic O antigens. *Salmonella enterica* serovars are defined by antigenic variation of the lipopolysaccharide moieties (O antigen), flagellar antigens (H antigen), and capsular polysaccharides (Vi antigen), (Porwollik *et al.*, 2002). The agents that cause enteric fever are, therefore, *Salmonella enterica* subspecies *enterica* serovar typhi and paratyphi; commonly referred to as *Salmonella typhi* and *Salmonella paratyphi* A, B, and C (Parry *et al.*, 2006).

Prior to introduction of serological characterization of *Salmonella*, sugar fermentation and phage typing were the main techniques used in bacteriological characterization. At that time each of what is now regarded as *Salmonella* serovar was designated as a species; and strains were named according to host specificity and geographical origin. Hence such binomial nomenclature as *Salmonella typhi*, *Salmonella typhimurium*, etc dominated the old literature. This, of course, did nothing to separate serovars of subgenera I, II, IV, which

were lumped together as species. Various propositions were made to reduce the increasing numbers of species (Popoff and Le Minor, 1997). The problem got solved by the use of DNA-DNA hybridization technique; and now all salmonella serovars are known to cluster in one hybridization group. With this arrangement a single species is composed of seven subspecies representing seven evolutionary groups (Jawetz *et al.*, 2004).

2.3 TAXONOMY

At first criteria for classification of Salmonella, as it was for other pathogenic organisms, were weighted in favour of clinical signs and symptom, host range, biochemical reactions and antigenicity (Jawetz *et al.*, 2004). The complexity of the taxonomy and classification of salmonella is appreciated when it is realized that the organisms are a continuum rather than a defined species. *Salmonella enterica* serotype paratyphi A was first named *Salmonella choleraesuis* serovar *Paratyphi* A, (Castellani, 1902), then *Salmonella paratyphi-A* and subsequently *Salmonella choleraesuis choleraesuis* (serotype *paratyphi* A), *Salmonella paratyphi* A, *Bacterium paratyphi typhus* A, *Bacterium paratyphi* or "*Salmonella paratyphi-A*". A new strain of *Salmonella enterica* subspecies *enterica* serovar *paratyphi* B strain SPB7 is the documented aetiology of paratyphoid fever and was isolated from a female patient in Malaysia in 2002 (NCBI Taxonomy). The serotype was at first variously designated *Bacterium paratyphi Typus B*, *Bacillus paratyphosus B*, *Salmonella*

paratyphi B, *Bacillus paratyphosus B* Schottmuller type, Group IV, *Salmonella paratyphosus B*, *Salmonella schottmuelleri*, or *Typus Schottmuller* (Kelterborn *et al.*, 1967). The classification of *Salmonella enterica enterica* species was later established as follows: Kingdom - Eubacteria, Phylum - Enterobacteria, Class - Gamma Proteobacteria, Order - Enterobacteriales, Family -Enterobacteriaceae and Genus -Salmonella. The genus Salmonella is composed of bacteria related to each other both phenotypically and genotypically. The Salmonella DNA base composition is similar to those of *Escherichia*, *Shigella* and *Citrobacter* (Crosa *et al.*, 1973). The bacteria of the genus Salmonella are, therefore, related to each other by DNA sequence, similar to the relationships determined by numerical taxonomy (Johnson *et al.*, 1975). Kauffmann (1963, 1966) subdivided the genus Salmonella into four sub-genera. The Kauffmann-White classification scheme, first developed in 1934, classified the *Salmonella* into different O serogroups, each containing a number of serotypes possessing a common O antigen not found in other groups. The O serogroups were first defined and designated by capital letters A to Z and those discovered later by the numbers (57-67). It was later considered more correct to designate each O serogroup by its characteristic O factor (Ewing 1986, Popoff and Le Minor 1997). Within each O serogroup the different serotypes were distinguished by their particular A antigen or combination of H antigens. A scheme for characterization of *Salmonellae* of sub-species IIIa and IIIb (the *arizonae*) was developed independent of the Kauffmann-White scheme (Farmer *et al.*, 1984;

Ewing, 1986). This was because the five hybridization subgroups corresponded to the former sub-genera of Kauffmann except that of subgenus III which was then divided into IIIa and IIIb. However, it is possible to integrate all *arizonae* serotypes in the Kauffmann-White scheme by assigning to them the equivalent *salmonella* O and A antigenic structures (Popoff and Le Minor 1997).

Another taxonomic system was proposed by Edwards and Ewing (1972), in which the genus *Salmonella* was limited to Kauffmann's sub-genera. In this system, bacteria referable to the genus *Arizona* were classified in another genus and strains of the Kauffmann's subgenera II and IV were considered as atypical strains of either *Salmonella* or *Arizona*. As has been said earlier, all these taxonomic problems have been solved by DNA hybridization data. The DNA studies (Crosa *et al.*, 1973) showed that the so called subgenera I-IV constituted a single hybridization group with 5 subgroups (Humbert, 2006). Later in 1982 an additional subgroup VI designated the Bongor group, comprising of a few serovars was found to constitute a second DNA-hybridization group. The level of DNA relatedness among DNA subgroups is consistent with that of the subspecies within a single *Salmonella* species. Subgroup V (sub species *bongori*) is somewhat less related to the I-IV and represents a second species (Le minor *et al.*, 1982). These designations may be abridged to read; *Salmonella* I, II and III. *Salmonella* IIIa and IIIb correspond to Kauffmann's subgenera III, which are monophasic and diphasic *Arizona*, respectively. Groups IV, V and VI are the recently delineated groups. To avoid confusion

with the familiar names of serovars, the species name *Salmonella enterica* have been proposed with the following names for the subspecies *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), *indica* (V) and *bongori* (VI).

Applying these guidelines, two species, *S. enterica* and *S. bongori* are recognized in line with the proposition of Le Minor *et al.* (1982). In summary the genus *Salmonella* is made up of only two genomic species, *S. enterica* and *S. bongori*. Jawetz *et al.* (2004) recognized more than 2500 serotypes within DNA hybridization group I (Sub-species I).

2.3.1 Antigenic Classification of Salmonella

There are over 2500 serotypes of salmonellae including more than 1400 in DNA hybridization group I that can infect humans. However, the nomenclature *Salmonella typhi* and *Salmonella paratyphi* have persisted fortuitously in literature. The four serotypes that cause enteric (typhoid and paratyphoid) fever can be identified by biochemical and serological tests. (Jawetz *et al.*, 2004) They are *Salmonella paratyphi A* (serogroup A), *Salmonella paratyphi B* (serogroup B), *Salmonella choleraesuis* (serogroup C₁) and *Salmonella typhi* (serogroup D). The other serotypes belonging to the group I sub-species can also be isolated and serogrouped by their O antigens. Still other sub species of salmonella belong to group II to IV. These are uncommon pathogens in man.

Nearly all of the salmonella serotypes that infect humans are in DNA hybridization group I (Jatwetz *et al.*, 2004)

The serotypes can be characterized by the somatic (O) antigen, flagella (H) antigen and capsular (Vi) antigen associated with virulence. The somatic antigens represent the side chains of repeating sugar moieties projecting outwards from the lipopolysaccharide layer on the surface of the bacterial cell wall, which bears the antigenic specificity (Lindberg and Le minor, 1984).

Somatic antigens are heat stable and alcohol resistant. Somatic (O) factors labeled with the same number are closely related but may not be antigenically identical (Le Minor, 2006). Lipopolysaccharide cell wall consist of specific polysaccharide chains lipid A core (common to all salmonella serovars) and the polysaccharide chain is associated with the O antigenic specificity. Major O antigens are determined by gene locus *rfb*, located at 42 min on the Tyhimurium LT2 (Le Minor, 2006).

Flagella antigens are heat-labile proteins. The presence of flagella and motility are controlled by a number of genes located at 23 and 40 min on the *typhimurium* serovar chromosomal map. *Salmonella enteritidis* and *Salmonella typhi* serovars produce flagella which have the same antigenic specificity (similar to non salmonella bacteria). This H antigen is then termed monophasic. However most salmonella serovars can, alternatively produce flagella with two different H antigenic specificity, such H antigen is, therefore, said to be diphasic. Antigenic specificity is associated with the

lipopolysaccharide (Lindberg and Le minor, 1984). Loss of the ability to build complete polysaccharide chains results in the rough (R) mutant state. Change from smooth (S) to R form may result in loss of pathogenicity and autoagglutinability of strains.

2.3.2 Genetic Characterization.

The gene responsible for the production of phase 1 flagella antigen is called H1 gene and is located at 40 min on the *Salmonella typhimurium* chromosomal map. The H2 gene responsible for the production of phase 2 flagella antigen and the gene Vh2 responsible for the phase variation are both located 56 min on the chromosomal map of *Salmonella paratyphi*. A serovar is phenotypically monophasic when the Vh2 gene is not functional (Iino and Kutsukake, 1983).

Serovars *paratyphi* B and C are antigenically and genetically distinct from serovars *typhi* and *paratyphi* A (Selender *et al.*, 1990; Porwollik *et al.*, 2002).

The genetic determinants responsible for the systemic dissemination of organism during human infection have not been identified.

Table 1: Antigens of some representatives of the genus of Salmonella (Kaufmann- white classification)

SEROTYPE	O AND Vi ANTIGENS	H ANTIGEN PHASE 1	HANTIGEN PHASE 2
<i>S. paratyphi A</i>	1, 2, 12	a	-
<i>S. paratyphi B</i>	1, 4, 5, 12	b	1, 2
<i>S. paratyphi C</i>	6, 7, Vi	c	1, 5
<i>S. typhi D</i>	9,12, Vi	d	-

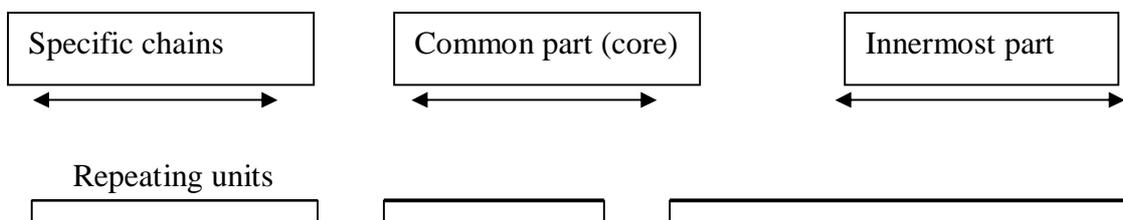


FIG.1**Structure of the lipopolysaccharide of *Salmonella enterica*
Serotype paratyphi B (0:4, 12).**

Key: KDO: keto deoxyoctonate' Hep: heptose, Glc, glucose;
GLcNac: N-acetylglucosamic, Rha: rhamnose; Man: mannose. Abe
abequose

PCR-based techniques have been developed to distinguish *S. typhi* serovar from other salmonella serovars; these techniques target the Vi antigen-encoding gene and the flagellin antigen (Li C-d gene) (Hirose *et al.*, 2002; Massi *et al.*, 2005;

Farrell *et al.*, 2005). These targets are not entirely specific for serovar *typhi* (Baker *et al.*, 2005). Lateral transfer of serogroup genes has been observed, and so it is possible that *Paratyphi A* arose by transfer of serogroup A genes into a strain very similar to *S. typhi* (McClelland *et al.*, 2004). Comparative genomic analyses with DNA microarrays have also shown that serovars *typhi*, *paratyphi A* and *sendai* have similar genetic contents but are diverse from the other *S. enterica* serovars (Ohanu *et al.*, 2003, Porwollik *et al.*, 2002). Protocols and methodology that can successfully identify *typhi*, *paratyphi A* and *sendai* would help to accurately diagnose the incidence of enteric fever, thus enhancing the worldwide surveillance of the pathogen (Dobryan *et al.*, 2006).

The genus *Salmonella enterica enterica* is composed of bacteria related to each other both phenotypically and genotypically. Crossa *et al.* (1973) observed that genera with DNA most related to that of *Salmonella* are *Escherichia*, *Shigella* and *Citrobacter*. The DNA base composition of salmonella was reported to be 50-52 GC mol% similar to that of *Escherichia*, *Shigella* and *Citrobacter*.

Several genes have been identified in association with the complex mechanism of *S. enterica* (Darwin and Miller 1990, 2001; Lostroth *et al.*, 2000; Detwsiler *et al.*, 2001; Lucas and Lee, 2001). The hyper invasive locus A (HiIA) gene promoters, a component regulation of type III secretion apparatus of the *Salmonella typhi/paratyphi*, have been found in salmonella serovar *typhimurium*. Cardona *et al.* (2002) detected HiIA gene sequences in salmonella

serovars *typhi*, *enteritidis*, *cholerasuis*, *paratyphi A* and *B* and *pullorum*, using amplification by polymerase chain reaction (PCR) and hybridization techniques.

An adherent and invasive phenotype of *S. enterica* is activated under conditions similar to those found in the human small intestine (high osmolarity, low oxygen). The invasive phenotype is mediated, in part, by salmonella pathogenicity island (SPI)-1, a 40 kb region of the chromosome which encodes regulator proteins (e.g. HilA), a type III secretion system (TTSS) that delivers bacterial proteins from the salmonella cytosol into the host cell, and several effector proteins which induce changes within the host cell and promote bacterial uptake (House *et al.*, 2001).

The genetic determinants responsible for the systemic nature during human infection with the *Salmonella enterica* species have not been identified. Interestingly PCR based techniques have been developed to distinguish serovar *typhi* from other *salmonella* serovars; these techniques target Vi antigen-encoding gene and the flagellin antigen (Hirose *et al.*, 2005; Massi *et al.*, 2005; Patel *et al.*, 2005). These targets are not entirely specific for serovar *typhi* but may also be found in some Vi negative strains of the serovar which are endemic (Baker *et al.*, 2005): These loci are not encoded by serovar *paratyphi A*. However, the use of these loci along with O and H antigen encoding genes in five locus multiplex PCR assay distinguishes both serovars *typhi* and *paratyphi A* from a large panel of *S. enterica* serovars (Hirose *et al.*, 2002). Serovar *typhi*, though genetically distinct, is one of the most homogenous serovars of *S.*

enterica as observed by multilocus enzyme electrophoresis (Reeves *et al.*, 1989, Selander *et al.*, 1990). Multilocus sequence typing (Kidgell *et al.*, 2002), protein profiling (Franco *et al.*, 1992) and plasmid analysis (Maher *et al.*, 1986). These assays, in targeting STY 4220 and STY 4221, provide the means for the molecular detection of serovar *typhi* and indeed of all human-adapted typhoidal serovars.

Molecular biology-based techniques have recently been adopted in the detection of *Salmonella enterica*. Although the method has the advantage of being less laborious and less time-consuming, it requires expensive sophisticated equipment affordable only by advanced laboratories (Zhang *et al.*, 2006). Accordingly, reference and research laboratories in developing countries, as in industrialized countries, are turning to multiplex PCR methods as a consistent, high-throughput approach to typing etiologic agents (Abdissa *et al.*, 2006, Berkley *et al.*, 2005). The development of a simple dot blot enzyme-linked immunoabsorbent assay (ELISA) for the identification of *S. enterica* serovar *choleraesuis*, a phase 1-C S from cultures was reported by Choo *et al.* (1999). Furthermore, cultures of blood obtained from septicemia were used to evaluate the potential applications of the assay. With this, biochemical identification of pure serovar *choleraesuis* is no more necessary. The identification is finished within a day instead of 2 to 3 days required by conventional biochemical methods.

Salmonella hybridomas were used to produce the Mab using the Barber protein of *S. enterica* serovar *paratyphi C* as the immunogen. The ELISA and immunoblot results were positive for salmonella strains expressing the phase 1-c flagellin including strains of serovars *paratyphi C* and *choleraesuis* protein with a molecular mass of 16kDa.

2.4 DIAGNOSIS OF TYPHOID FEVER

The classical symptoms of typhoid fever have changed over the years. Atypical presentations have more often been encountered in recent times and this could delay the clinical suspicion of the disease. Presumably the contributory factor to this is that most patients who visit hospital in an endemic area come after receiving self treatments; and this could alter the presentation of the disease significantly.

Clinical evidence of enteric fever consists of the presence of at least six of the following features – history of fever of at least 38° C documented in a hospital (essential), residence in a known typhoid-endemic area, family history of enteric fever, hepatomegaly, doughy abdomen on palpation, elevated serum C-reactive protein concentration, leucocytosis or leucopenia, failure of fever to resolve after exclusion of other causes, response to chloramphenicol therapy and development of complications including intestinal haemorrhage and perforation (Choo *et al.*, 1999). Clinical features of paratyphoid fever are similar to those of typhoid fever but with less severity and a shorter incubation period. *Salmonella*

paratyphi A and *S. paratyphi* B infections, each can manifest with jaundice, thrombosis, and systemic infections. *Salmonella paratyphi* B might occasionally have an onset similar to non-specific salmonella gastroenteritis. Gastrointestinal symptoms are usually not present with *S. paratyphi* C but there have been cases with systemic complications such as septicaemia and arthritis. In such cases, clinical diagnosis of typhoid may be difficult. There are, however, some implications for diagnostic and treatment protocols in endemic areas, particularly, diagnosis and treatment of typhoid among febrile children, which, at present, largely focus on malaria as a cause of fever without localizing signs (Bhutta, 2006). Blood leucocyte counts are often low in relation to the fever and toxicity, but the range is wide; in younger children leucocytosis is a common association and may reach 20000-25000/mm³. Thrombocytopenia may be a marker of severe illness and may accompany disseminated intravascular coagulation. Other haematological parameters are non-specific. Liver function test results may be deranged, but significant hepatic dysfunction is rare. Electrocardiographic changes suggestive of myocarditis are rather common in typhoid fever but the incidence of true myocarditis is low both in adult patients and children (Malik, 2002). Despite these new developments, the diagnosis of typhoid in much of the developing world is made based on clinical criteria. This poses problems, since typhoid fever may mimic many common febrile illnesses in children with multisystem features. The early stages of enteric fever may be confused with conditions such as acute gastroenteritis, bronchitis, and

bronchopneumonia. Subsequently, the differential diagnosis includes malaria, sepsis with other bacterial pathogens, infections caused by intra-cellular organisms such as tuberculosis, brucellosis, tularaemia, leptospirosis, rickettsial diseases and viral infections such as dengue fever, acute hepatitis, and infectious mononucleosis (Bhutta, 2006).

2.4.1 Diagnosis Using Culture and Isolation Method

Most *Salmonella* serotypes/strains are able to grow on minimal medium; however, they are usually isolated on complex media. The strategy for isolation adopted would depend on the source of specimen - sterile area (blood) or an unsterile area with bacterial flora, such as the digestive tract, environmental samples and animal carcasses (Le Minor, 2006).

Enrichment and selective isolation are usually required when the sample is from a site usually associated with bacterial flora. This could be achieved in a number of liquid media including modified tetrathionate and selenite broths used universally (Edwards and Ewing, 1986). The selectivity of brilliant-green tetrathionate broth and selenite F broth may be improved by incubation at 42-43°C instead of 37°C, since many salmonellae can multiply at higher temperatures than common contaminants (Post, 1993). Rappaport malachite green magnesium chloride broth has been reported to be more efficient than other enrichment media for the isolation of *Salmonella* from faeces, water and food samples (Frierker, 1987). Strontium selenite broth was found to be superior

to ordinary selenite broth for the isolation of *S. typhi* especially when relatively few typhoid bacilli are present in faeces (Singh, 1997). Bromocresol purple lactose agar medium has also been devised for the isolation of Salmonella. It is differential but non-selective containing lactose with pH indicators but does not contain any inhibitor for other organisms. The slightly selective and differential media containing inhibitor to other organisms are MacConkey, Drigalski, Eosin-methylene blue agar and Deoxycholate bile salt agar (DCA), (Le Minor, 2006). The most commonly used selective media for Salmonella are salmonella-shigella agar (SSA), Wilson-Blair bismuth sulphite agar and Hektoen enteric medium.

Blood culture for Salmonella isolation is most appropriately carried out using streptokinase clot culture technique (Wain *et al.*, 2001). Although the blood culture is highly specific, the sensitivity varies from 48-78% and the yield depends on whether or not the patient has been on antibiotic therapy (Tsang and Chau, 1991). In mild typhoid, the bacterial count may be as low as one colony-forming unit (CFU) per ml of blood (Wain *et al.*, 2001). Singh (2001) reported a significant increase in isolation rate of *S. typhi* in 210 cases of enteric fever tested using streptocase bile salt. Whole blood is usually in citrate solution, and clotted blood in a plain sterile bottle (Huckstep and Wright, 2002). The concentration of typhoid bacilli in the buffy coat (presumably in monocytes and polymorphonuclear leukocytes) means that direct plating of this cell layer provides an alternative and more rapid method of diagnosis than whole-blood

broth culture. Colonies were usually evident after an overnight incubation (Wain *et al.*, 2002). To determine the number of intraleukocytic colony forming units in blood samples, quantitative cultural assays are performed on peripheral blood buffy coats. The determination of the concentration of typhoid bacilli in the buffy coat is performed on a randomly chosen subgroup of patients. Heparinized whole blood (2.5 or 5 ml) is taken and centrifuged at 2,700 revolutions per minute for 10 minutes. The plasma is removed carefully with a sterile plastic pipette, and 0.1 ml including the buffy coat layer is aspirated with a sterile 1 ml syringe. Quantitative assays are performed on this 0.1ml sample by mixing it with 19 ml of molten Columbia agar, pouring the mixture as an agar plate, and incubating it for up to 4 days. Peripheral blood phagocytes containing more than one bacterium will produce a single colony in solid culture medium. To determine precisely the number of intracellular bacteria per infected phagocyte, a second 0.1 ml buffy coat sample is taken and the leukocytes are lysed by incubation with 0.1 ml of 0.1 % digitonin for 10 min at 37°C. This treatment is expected to release the intracellular bacteria. Quantitative cultural assays are then performed on this sample in the same way as for the first sample above. Blood samples from a healthy volunteer are usually included as control. In this way Wain *et al.* (2002) estimated the number of *S. typhi* bacteria per milliliter of blood from the number of CFU observed on each pour plate.

Salmonella organism is obtained from blood during the first week of the illness and persists as late as the fifth week and from stool during the second and third weeks; but from urine it is less frequently obtained (Bhutta, 2006). However, the organism can be cultured from stool throughout the duration of disease but it will require a selective medium. This is not as specific as blood culture. It is, however, a valuable diagnostic procedure. Contrary to the commonly held view, stool culture is often positive before the third week of illness, and may be positive at any stage of the disease (Huckstep and Wright, 2002). Stool samples are processed using *Salmonella* isolation protocol; first enriching in selenite - F-broth incubated at 37°C for 24 - 48 hours and then subculturing onto brilliant green MacConkey agar incubated at 37°C for 18 - 24 hours. The emergent non-lactose fermenting colonies on this medium are further subcultured onto Wilson and Blair's bismuth sulphite agar incubated at 37°C for 18-24 hours. Growth showing central sheen, indicating *Salmonella* organism, is subcultured into nutrient agar for further characterization (Ngwu and Agbo, 2003). Specific identification in positive cultures is by a panel of biochemical and agglutination tests (Choo *et al.*, 1999).

The definitive diagnosis of typhoid fever is by the isolation of *S. typhi* from blood, bone marrow or a specific anatomical lesion. Thus, blood and bone marrow aspirate cultures are the gold standards for the diagnosis of typhoid fever. Although bone marrow cultures are more sensitive, they are difficult to

obtain, relatively invasive, and of little use in Public Health settings (Bhutta, 2006). Duodenal aspirate culture has also proved highly satisfactory in diagnosis of typhoid, but it has not found widespread acceptance because of the invasive nature of the aspiration protocol, particularly in children (Ismail, 2006). Culture of bone marrow or bone snips, on the other hand, yield growth where it cannot be obtained from blood, stool and urine. The only known flaw of blood culture as the gold standard is its low sensitivity, particularly in endemic areas. This low sensitivity has sometimes been blamed on the high level use of antibiotics in these regions. This makes true specificity rather difficult to determine (Bhutta, 2006). Besides, blood culture is positive in only 40-60% of cases. The outcome of blood cultures in patients with typhoid fever depends on many factors including the volume of blood samples used, the bacteremic level of *S. typhi*, the type of culture medium used, and the duration of incubation of the culture (Massi *et al.*, 2005). In a comparative study by Singh (1997) *S. typhi* was isolated from 40% of blood, 37% of stool, 7% of urine but 63% of rose spots skin snips. In the case of paratyphoid fever, bone marrow culture yielded growth even from patients on antibiotic therapy (Gianella, 1996). Wain *et al.* (2001) noted that bone marrow is the best specimen for culture with sensitivity rate of 80-95%, followed by blood cultures (60-80%). The chance of isolation from the later is improved by increasing the volume of blood sample inoculated. Pearson and Guerrant (2000) also noted that bacteria could be isolated from blood cultures in 73 to 97% of cases before antibiotic use. Escamilla *et al.* (1986) reported 52%

isolation of *S. paratyphi* from blood-TSB, 67% from blood clot-bile, and 68% from blood clot-streptokinase; and for *S. typhi*, 69% from blood-TSB, 84% from blood-bile and 70% from blood clot-bile. Blood culture is not always feasible, particularly when small children are involved (Vaishnavi *et al.*, 2006). However, Nizami *et al.*, (2006) observed that conventional methods for the isolation and identification of salmonella in blood are laborious and time consuming; and suggested a rapid molecular technique for detecting presence of the micro-organisms. Detection of *S. typhi* DNA by polymerase chain reaction (PCR) amplification has recently been shown to be very sensitive indicator of infection (Massi *et al.*, 2005).

2.4.2 Diagnosis Using Serological Methods

(A) Widal Agglutination Test

The classic Widal test detects/measures antibodies against O and H antigens of *S. typhi/paratyphi* and has been in use for more than 100 years. Although robust and simple to perform, this test is low in both sensitivity and specificity; and reliance on it alone in typhoid endemic areas may lead to overdiagnosis or misdiagnosis due to either false positive or false negative results (Parry *et al.* 1999; Bhutta, 2006). Widal test is apparently the only typhoid diagnostic technique available in most tropical regions because it is relatively cheap (Ismail, 2006). All the same the mixed results in endemic areas notwithstanding, this method is still considered valuable for screening large number of samples;

but it requires standardization of reagents and quality assurance (Bhutta, 2006). Thus, Widal test has gained widespread acceptance as an adjunct to clinical assessment and bacterial isolation method (Choo *et al.*, 1999).

The reagents for the Widal test are bacterial suspensions of *S typhi* and *S. paratyphi* 'A' and 'B', prepared such as to retain only the 'O' and 'H' antigens. These antigens are reacted with homologous antibodies in the serum to agglutinate the bacterial cells in the antigen suspension. The IgM somatic O antibody appears first and represents the initial serologic response in acute typhoid fever, while the IgG flagella H antibody usually develops more slowly but persist far longer (Olopoenia and King, 2000). Two types of agglutination techniques are available - the slide and the tube tests, respectively. The slide test is rapid and is used as a screening procedure. An initial positive screening test is followed by the determination of the titre of the antibody in the serum (Olopoenia and King, 2000). For the tube test a mixture of suspended antigen and antibody is incubated for up to 20 hours at 37° C in a water bath. Serum is considered positive for Widal test when anti-O or anti-H titer is ≥ 160 (Mekara *et al.*, 1990). Usually, O antibodies appear around days 6-8 and H antibodies around days 10-12 after the onset of the disease. The test is usually performed on acute and convalescent sera with the aim of observing a rising titer. The Widal test does not provide accurate results after two days storage of blood samples (Choo *et al.*, 1999). The usefulness of the Widal test in diagnosing childhood typhoid fever in endemic areas has been investigated. Valid

information of Widal test result in diagnosis of typhoid in children in endemic areas requires knowledge of agglutination levels in apparently normal children in the region (Chow *et al.*, 1987). Specification for storage (2-8 degrees C), reaction time (5 minutes) and amount of serum needed (\pm 300 ul for two dilutions) were given by Olsen *et al.* (2003).

The outcome of Widal reaction for patients with suspected typhoid and malaria depends on the individual immune responses, which becomes stimulated in febrile conditions associated with malaria fever. This memory response could cause positive Widal reactions in previously sensitized patients and accounts for up to 35% of reported false positive Widal test results (Mbuh *et al.*, 2003). This may account for the demonstration of high prevalence Salmonella antibodies in healthy individuals. A positive widal test may, therefore, be seen in apparently healthy persons from malaria/typhoid endemic areas as a result of previous subclinical infection. However, the geometric mean titres reported in this group of healthy subjects are about 1:62 for O and 1:75 for H agglutinins (Thelma *et al.*, 1991). In Malaysia, up to 61% of healthy subjects are reported positive for O and H antibodies at titres $>1/200$ for typhi D serotype. In Turkey the titre was around 1/200 and in Vietnam it was 1/200 and 1/100 for O and H, respectively (Ayse *et al.*, 2002). In Rhodesia 1/480 titre was obtained for both O and H antibodies (Parry *et al.*, 1999). Base-line titre is always high in endemic regions (Ayse *et al.*, 2002). Shukla *et al.* (1997) estimated the base-line titre for Singapore, Ceylon, Malaysia and Philipines to

be about 1/480. Cheesbrough (2006), however, reported that in the endemic area only 24.8% patients with titre 1:160 were positive in cultures while 75.2% of those with titres above 1: 160 were culture-positive. In a study conducted by Ayse *et al* (2002) to evaluate the efficiency of the Widal test in the diagnosis of typhoid fever, 52% of 31 cases positive for blood culture singly were positive by Widal test at $\geq 1/200$ titre. After 7-10 days the rate of positivity positive increased to 90%, showing that Widal test may not effectively detect infections at early acute phase. The test detected 5(12%) and 2(5%) of O and H antibodies, respectively, among the asymptomatic individuals at the early stage of infection (Ayse *et al.*, 2002). Widal test is, therefore, used in demonstrating the presence of typhoid fever cases during acute and convalescent period of the infection, provided there is a four-fold rise of antibody between the two periods (Olopaenia and King, 2000). Invariably, baseline titres in the general population must be determined for each geographic region (Shukla *et al.*, 1997; Frimpong *et al.*, 2000; Ibadin and Ogbomi, 2002). However, Widal test is only positive in 50% at the beginning of hospitalization, the presence of typhoid can still be suspected on clinical groups if malaria is ruled out and the titre is high. Using a cutoff titre of $> 1/200$ for the O antigen test performed on acute-phase serum, the sensitivity and specificity of Widal test was calculated to be 52% and 88%, respectively: with positive predictive values(PPV) and negative predictive values(NPV) of 71% and 93%, respectively (Ayse *et al.*, 2000). A four-fold rise in O, H or Vi antibodies titre provides a validity for the serologic diagnosis of

the disease. Although effective treatment early in the disease appears to prevent further antibody rise, which would diminish the likelihood of demonstrating the diagnostic fourfold rise, there is conflicting data on the effect of antimicrobial therapy on the Widal test. Indefinite persistent rise of the titre may follow an illness despite treatment; hence, the Widal test cannot be used to gauge therapeutic response (Thelma *et al.*, 1991). In statistically comparing Widal analysis and isolation methods at 5% probability level, it has been shown that Widal test alone is not significant and sufficient for diagnosis (Itah and Akpan, 2004).

Numerous other serologic tests have been developed, namely ELISA (Nguyen *et al.*, 1997; Jesudason *et al.*, 1998 and House *et al.*, 2005), Salivary IgA (Herath *et al.*, 2003), a modified Widal test to detect IgM (Pai *et al.*, 2003) and dipstick assay (Hatta *et al.*, 2002). Among the advances in serodiagnosis are the development of Typhidot and Tubex, which directly detect IgM antibodies against a host of specific *S. typhi* antigens. None of these, however, has proved sufficiently robust in large scale evaluations in community settings.

(B) Typhidot Test

Typhidot test is recently developed dot enzyme immunosorbent assay. The test uses 50 KD outer membrane protein (OMP) antigens from *Salmonella typhi*. This has been found to be a cost-effective and a more rapid alternative to the Widal test with at least comparable sensitivity and specificity. It was developed in Malaysia for the detection of specific IgM and IgG antibodies against the 50

KD antigen of *S. typhi* (Ismail, 2006). The Typhidot allows separate evaluation of the presence of specific serum immunoglobulin (IgM antibodies) against the OMP (Choo *et al.*, 1999). To increase diagnostic accuracy, a modification of the original Typhidot test has been developed in which serum IgG is inactivated in order to enhance detection of specific IgM (the Typhidot-M test) (Choo *et al.*, 1999). Typhidot takes three hours to perform. The protocol of the test involves applying 1:100 dilutions of patient serum to standard aliquots of 0.3 µg purified OMP dotted on to nitrocellulose strips. After 1 hour, horseradish peroxidase-conjugated antiserum to human IgG or IgM is added. The presence of antigen-antibody complex is assessed visually from the resultant colour change in comparison to that from positive control sera. This dot enzyme immunoassay (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, the detection of specific IgG cannot differentiate between acute and convalescent cases. Inactivation of IgG in Typhidot protocol enhances IgM detection thus helping the detection of IgM and this also differentiates current from previous infections (Gopalakrishnan *et al.*, 2002). Ismail (2006) observed that evaluations of Typhidot and Typhidot-

M, in clinical settings, were better diagnostic tools than the Widal test and the culture methods. Both Typhidot and Typhidot-M tests can be completed within three hours of venesection and does not need special training or equipment (Choo *et al.*, 1999).

(C) Dipstick Test

A newer version of Typhidot-M was recently developed in the Netherlands as dipstick. It is based on the binding of *S. typhi* specific IgM antibodies in serum samples to *S. typhi* lipopolysaccharide (LPS) antigen absorbed on nitrocellulose strip and the staining of bound antibodies by colloidal dye particles conjugated with anti-serum IgM antibody (Ismail, 2006). The nitrocellulose membrane strip is dotted with the 50 kDa specific protein and a control antigen. Ten microlitres of patient serum and controls are pre-absorbed for at least one minute with 90 μ l of IgG inactivation reagent. A 900 μ l of sample diluent is then added into the reaction wells and the mixture incubated at room temperature on a rocker platform for one hour. The strips are washed thrice for a total of five minutes, and 1 ml of anti-human IgM conjugate is added and incubated for one hour. The strips are washed thrice again, then 1 ml of colour development solution is added and test samples incubated for 15 minutes. The reaction is stopped by washing the strips in distilled water. The strips are then washed and dried at room temperature. The sera were graded (0 to 4) according to the staining intensity of the coloured band corresponding to

the antigen (House *et al.*, 2001). If both dots on the test strip appear as dark as or darker than the corresponding dots on the positive control strip, the test is reported as positive (Gopalakrishnan *et al.*, 2002).

The dipstick version seems to be a practical alternative in the field and in small hospitals with fewer facilities. It has higher sensitivity and specificity than classic Typhidot, but other reports suggest that the performance may not be as robust in community settings as in hospital (Gopalakrishnan *et al.*, 2002). The sensitivity and specificity rates were found to be 73-95 % and 75-91 %, respectively (Bhutta, 2006). House *et al.* (2001) recorded 77% sensitivity and 95% specificity rates, respectively. Positive predictive value of 19% and negative predictive value 93.6% have been reported for dipstick (Nizami *et al.*, 2006). Oslen *et al.* (2003) standardized the amount of serum needed to be 10 µl, reaction time, 90 minutes and the storage temperature, 2-8°C.

(D) Tubex Test

test detects anti-salmonella O9 (IgM and IgG) antibodies in patients by inhibiting the binding between an anti-O9 IgM monoclonal antibody (MAb) conjugated to coloured latex particles and *S. typhi* lipopolysaccharide (LPS) conjugated to magnetic latex particles (Lim *et al.*, 1998). Like the Widal test, Tubex protocol consists of a single step and the result is read visually based on the appearance of the liquid contents in the tube. A specially designed set of tubes is used in in the test, and, instead of whole bacterial cells, *S. typhi* LPS

adsorbed to magnetic particles is used as the detecting reagent. Use of a monoclonal antibody (MAb) which recognizes the immunodominant O9 determinant in *S. typhi* LPS makes the test more specific (Lim *et al.*, 1998). When the antibody-conjugated particles bind to the antigen-sensitized magnetic particles and the latter are sedimented by use of a magnet, the colour of the liquid (supernatant) in the tube changes. The magnetic particles coated with antigen (*S. typhi* LPS) are mixed with blue latex particles coated with anti-*S. typhi* LPS (O9) antibody, the two types of particles will bind to each other. On the other hand, if the magnetic particles are sedimented to the bottom of the tube by use of a magnet at the end of the experiment, the blue latex particles are also brought down. This leaves behind a clear supernatant known as bovine serum albumin (BSA) coated red latex particles which are also added to the reaction mixture and remain suspended in the solution throughout the experiment. This makes the supernatant red when the blue particles are sedimented, which is easier to see than a colorless supernatant. If a patient's anti-O9 antibodies are present in the reaction mixture, they will inhibit the binding of the blue particles to the magnetic particles. Consequently, the supernatant remains purplish blue (unchanged from the beginning) due to the presence of blue particles and also, in a lower concentration, of red particles (Lim *et al.*, 1998). The infection-specific anti-O9 antibodies in typhoid patients are detected by their ability to block the binding between the two types of particles, hence, no change in colour. Bhutta, (2006) recorded sensitivity of 65-88% and specificity of 63-89%, respectively.

In the examination of 16 stored sera obtained from 14 patients with proven cases of typhoid fever and 78 serum samples from 75 subjects without typhoid fever, Tubex test was found to be 100% sensitive and 100% specific (Lim *et al.*, 1998). For 210 samples tested, sensitivity was 26.9% and specificity 88% (Nizami *et al.*, 2006). False Positive/Positive predictive value (PPV) was 24.1% while false Negative/Negative predictive value (NPV) was 89.5% (Nizami *et al.*, 2006).

(E) ELISA Test

This utilizes a direct ELISA format. The test is performed by coating Immulon 1b flat-bottom 96-well microtiter plates overnight with 100 µl of either 1 µg of antigen/ml in buffer of 0.1 M carbonate (pH 9.4), or coating buffer alone (antigen negative) at 4°C. The plates are blocked for 1 hour at 37°C with 200 µl of phosphate-buffered saline containing 1% bovine serum albumin (BSA). Sera are either assayed at a single dilution or serially diluted (starting at a dilution of 1/50). The plates are incubated for 4 hours at room temperature. Bound antibodies (IgA, IgG, or IgM) are detected using heavy-chain-specific goat antibodies directly conjugated to alkaline phosphatase and are diluted in Tris-buffered saline containing 0.1% BSA and 0.05% Tween 20. One hundred microliters of the diluted antibodies are added to each well, and the plates are incubated overnight at 4°C. One hundred microliters of p-nitrophenyl phosphate are added to each well and incubated at ambient temperature in the dark for 30

to 40 min. For the titration assays, sera are assayed in triplicate (two wells antigen positive and one well antigen negative), and the titer are taken as the highest dilution giving a net OD (mean OD of antigen-positive wells minus OD of antigen-negative well) of ≥ 0.3 (anti-LPS IgG) or ≥ 0.2 (all other antibodies). Samples are scored 0 to 10 according to intensity of the colour. Reaction scoring of >2 are considered positive (House *et al.*, 2001). At a specificity of 0.93, the estimated sensitivities of the different tests were 75%, 55%, and 52% for the anti-LPS IgM, IgG, and IgA ELISAs, respectively; and 28% for the antiflagellum IgG ELISA (House *et al.*, 2001).

Among subjects with typhoid fever who had positive blood cultures ELISA tests were reported positive in 89-100%, whereas the Widal test was positive in only 61-83% (Mekara *et al.*, 1990). The PanBio ELISA kits give 78% sensitivity and 80% specificity. When comparing PanBio ELISA with Typhidot and Typhidot M tests, PanBio ELISA showed lower sensitivity, PPV, NPV but higher specificity. This observation is a reflection on the antigen used which seemed to be more specific for *S. typhi* (Gopalakrishnan *et al.*, 2002).

(F) Polymerase Chain Reaction (Nucleic Acid Detection Test)

Polymerase chain reaction (PCR) based detection of *Salmonella enterica* serovar *typhi* was used as an aid for diagnosis of typhoid fever in addition to other diagnostic tests (Nizami *et al.*, 2006). Of several indirect methods used for the detection of infective organisms, the polymerase chain reaction (PCR) has

emerged as a reliable method for detection of their genome. It is thought that as few as 10 cells could be detected using specific PCR. Thus PCR may be helpful in the early diagnosis of infectious diseases, especially typhoid fever, the morbidity and mortality of which are likely to be reduced significantly if they are treated early and promptly (Nizami *et al.*, 2006). Most investigators have used either flagellar or ViaB gene for PCR detection of *S. enterica typhi* (Nizami *et al.*, 2006).

(G) Serological detection of *Salmonella enterica* serovar Typhi in faecal specimens:

Laboratory diagnosis requires isolation and identification of the organism from the patient's blood or faeces and this is the most reliable means of confirming an infection (Vaishnavi *et al.*, 2006). During the incubation phase of the disease, the bacilli may be occasionally cultivated from the faeces and rarely from the blood of the so called 'precocious carrier' and subclinical cases (Vaishnavi *et al.*, 2006). Faecal specimens are inoculated into selenite F broth and incubated at 37°C for 6 hours or overnight. After incubation, the broth culture is centrifuged at 1000 rpm for 10 minutes. The supernatant obtained is checked for the presence of Vi antigen with the respective antisera. The supernatants testing positive with Vi antigen are heated for 1 hour and then checked for O9 and H-d antigen. Samples which test negative for Vi antigen are checked (without heating) for O9 and H-d antigens. Strong agglutination occurring within 1 minute is taken as positive for the applied test. Latex beads

coated with normal rabbit antiserum are included as negative control. All samples that give strong positive reactions with at least two tests, among the O9, H-d or Vi serological tests, were considered to have *S. typhi* (Vaishnavi *et al.*, 2006). Diagnostic tests for typhoid fever employing antibody responses may not correctly detect a current disease because antibody formation may be delayed or antibodies may be present due to vaccination or subclinical infection (Vaishnavi *et al.*, 2006).

2.5 Malaria parasites Identification.

The laboratory diagnosis of malaria infection employs a more simple and direct diagnostic procedure commonly used in the tropics. This involves a careful examination of a well prepared and stained thin and or thick blood film by a trained microbiologist (USAID and WHO, 2000). The rapid diagnostic tests (RDTs) for malaria which uses immunochromatographic methods to detect Plasmodium specific antigens in a finger prick blood sample, can be performed in 15 minutes using test kits, requires no electricity and no special equipment (Payne, 1988). The main disadvantages of this method, compared to microscopy, are lack of sensitivity at low level of parasitaemia, inability to quantify parasite level, inability to differentiate among the various species, persistently positive tests in spite of parasite elimination following chemotherapy and relatively high cost per test (USAID and WHO, 2000;

Moody, 2002). Other diagnostic tests like fluorochromes microscopy, PCR and serology antibody detection are available but are neither suitable for wider application nor for routine use (USAID and WHO, 2000).

2.6 Virulence Factors in *Salmonella enterica*

Active attachment promoted by the bacterium is usually necessary before invasion can occur and this most likely involves unidentified adhesion molecules on the bacterium interacting with receptors on the host cell (House *et al.*, 2001). The organism produces infection in the host and penetrates the epithelial cells in the membrane associated vacuoles (Kubori *et al.*, 1998, Lostroh *et al.*, 2004). It also induces apoptosis and kills macrophages by caspase-I-dependent mechanism that also releases proinflammatory cytokines (Zhou *et al.*, 2000, Santos *et al.*, 2001). Caspase-I-activation correlates with the ability to colonize the lymph nodes and liver in mice (Detweiler *et al.*, 2001). Salmonella pathogenicity island 1 (SCSP I1) is composed of several genes *hilA* (hyper invasive locus A). This is one of the virulence factors (Lee 1994, 1996; Lee *et al.*, 1992; Bajaj *et al.*, 1995, Lostroh *et al.*, 2000; Milrod *et al.*, 2001). This gene is required for the regulation of the type 111 secretion genes (Lostroh *et al.*, 2000, Lesnick *et al.*, 2001). Virulence genes like type 111 secretion apparatus produce proteins that are probably related to cell invasion (Kubori *et al.*, 1998; Darwin and Miller 2001, Lesnick *et al.*, 2001). The development of additional horizontal genes in the SPI represents the separation of the *E. coli*

and salmonella lineages and allows the targeting of intestinal epithelial cells by the salmonella organism (Fierer and Guiney, 2001).

Salmonella invades epithelial cells *in vitro* by a process of bacteria-mediated endocytosis, involving cytoskeletal re-arrangement, disruption of the epithelial cell brush border and the subsequent formation of membrane ruffles. The ability of *S. typhi* to survive in human macrophages *in vitro* suggests that this pathogen resides within an intracellular compartment during growth in liver and spleen. All these mechanisms described enable the organism to cross the intestinal epithelial barriers and cause systemic infection (Miras *et al.*, 1955; Kubori *et al.*, 1998; Lostroh *et al.*, 2004; Zhou *et al.*, 2000; Detweiler *et al.*, 2001; Santos *et al.*, 2001).

In mice, *S. enterica* serovar *typhimurium* replicates in macrophages, it is thought that either the macrophage or dendritic cells carry the bacteria from the Peyer's patches to the lymph nodes, spleen and liver and produces systemic, typhoid-like disease (Miller *et al.*, 1995; Darwin and Miller 1999; Detweiler *et al.*, 2001). In immune-competent humans, *S. enterica* serovar *typhimurium* does not cause systemic disease unlike serovars *typhi* and *paratyphi* A, B, C, and bacterial replication is limited to the intestine where it produces gastroenteritis. It is, however, unclear why *S. enterica* serovar *typhimurium* causes various diseases in mice while other serovars would not (Detweiler *et al.*, 2001; Fierer and Guiney, 2001). In humans other *Salmonella* species produce various clinical syndromes including typhoid fever by serovars *typhi* and *paratyphi* A, B, C,

gastroenteritis by *typhimurium*, *newport*, *enteritidis* and *pollorum*, exceptional bacteraemia as well as localized tissue infections by serovar *cholerasuis* (Miller *et al.*, 1995). Indeed the entire pathogenesis of Salmonella is considered complex by experts (Pang *et al.*, 1995). The pathogenicity of typhoid fever is a complex process which proceeds through several stages (House *et al.*, 2001; Wain *et al.*, 2002; Andrade and Andrade 2003), however the pathogenicity of the different serovars have not been fully studied.

2.7 Pathogenicity and Pathology of *Salmonella enterica* var *typhi*/ *paratyphi*

The pathogenicity of salmonellae appears primarily to depend upon the ability of the bacilli to survive and multiply inside phagocytes and on the toxicity of their lipopolysaccharide endotoxin, which is associated with their O antigen. However, the *typhi* and *paratyphoid C* bacilli have additional aggressive factor, a microcapsule consisting of a glycolipid which is the virulence (Vi) antigen. The Vi substance appears to protect the bacilli against the lytic action of antibody and complement and also prevents phagocytosis to some extent (Duguid *et al.*, 1996). The small intestine and, particularly, the Peyer's patches of the terminal ileum provide the portal of entry facilitating dissemination to systemic sites (Bhan *et al.*, 2002). After the ingestion the bacteria survives the acidic environment of the stomach and attaches to the epithelial cells of the intestinal villi to induce their intake into the cells by a process akin to phagocytosis. Salmonella organism avoids encapsulation in

lysosomes by diverting normal cellular mechanisms (Patel *et al.*, 2005). *Salmonella typhi* survives and replicates in the underlying lymphoid tissue of the Peyer's patches. Through this route bacteria may proceed to disseminate via the lymphatics to the regional lymph nodes and pass into the blood via the thoracic duct resulting in transient primary bacteremia (Tsolis *et al.*, 1999). Shortly following invasion of the gut epithelium, invasive *Salmonella* species encounter macrophages within the gut-associated lymphoid tissue. However, the macrophages do not kill the organism. The ability of *S. typhi* to survive in human macrophages *in vitro* suggests that this pathogen resides within an intracellular compartment during growth in liver and spleen (Tsolis *et al.*, 1999). Within a period of 24 hours they penetrate the lamina propria and submucosa where they proliferate. This initial proliferation actually takes place in the Peyer's patches of the lower small intestine. The invading organisms pass through the intestinal epithelial cells and come in the Peyer's patches of the intestinal wall. Here phagocytosis actually takes place, but it is fascinating to note that the organisms actually remain alive and multiply within the phagocytes, which they kill and escape. From there, they pass via the lymphatics to the mesenteric lymph nodes, where after a period of multiplication again, they invade the blood stream via the thoracic duct. Release of the bacilli is responsible for the septicaemic phase of the typhoid and for infectious metastasis. From here it spreads to the liver, spleen, gall bladder, kidney, bone marrow and reticuloendothelial system. For a period of 1 to 3

weeks the organisms multiply within these organs (House *et al.*, 2001). During the asymptomatic incubation period which may range from 7 to 14 days, the organism proliferates and spreads through the blood stream to other cells in the reticuloendothelial system in the liver, spleen, bone marrow and gallbladder. The gall bladder is suspected to be a significant site for ongoing exposure of the intestinal epithelial cells (Shetty *et al.*, 1998). Rupture of the infected cells (especially that of the gall bladder) occur liberating the organisms into the bile for a second time, causing infection of the lymphoid tissue of the small intestine, particularly in the ileum. Although the ileum is the classical seat of typhoid pathology, some other parts of the intestinal tracts, such as jejunum and ascending colon may also be affected. The ileum usually contains larger and more numerous Peyer patches than the jejunum and so is mostly affected (Bhan *et al.*, 2002). The Peyer patches and the lymphoid follicles are particularly involved in the inflammatory reaction. It is this phase of heavy infection that brings the classical bowel pathology of the lymphoid organ. Infiltration with mononuclear cells is followed by necrosis, sloughing and the formation of lymphoid ulcers. Haemorrhage of varying degree may occur and the perforation through a necrotic Peyer patch may complicate the illness. (Everest *et al.*, 2001). Invasion of the mucosa causes the epithelial cells to initiate the release of various pro-inflammatory cytokines including interleukin-1 (IL-1), interleukin - 6 (IL-6), interleukin-8 (IL-8) tumor necrosis factor - B (TNF-B), interferon and GM-CSF (Giannella, 1996; Hensel, 2000). The release of

cytokines is principally an innate immune response of macrophages. The inflammatory mediators elicit changes in the local blood vessels. The reaction involves the dilation of local arterioles and capillaries, from which plasma escapes. Edema fluid accumulates and fibrin forms a network and occludes the lymphatic channels, limiting the spread of the organisms. While various activators act upon mononuclear phagocytes inducing them to release IL-1, interleukin-8 is synthesized both by macrophages and endothelial cells. Endotoxin of the salmonella organisms causes release of Interlukin-1, which in turn induces fever (Giannella, 1996). Fever is the most common systemic manifestation of the inflammatory response elicited as a result of salmonella infection. The body temperature is controlled at the thermoregulatory centre of the hypothalamus, which is subject to stimuli. Antigen-antibody complex can also serve as an activator. This acts to induce macrophage to release the IL-1. The IL-1 is then carried by the blood stream to the thermoregulatory center in the hypothalamus where physiologic responses are initiated resulting in fever manifestation. High fever complicated by other symptoms has remained a typical manifestation of typhoid/paratyphoid fever illness (Dutta *et al.*, 2001). In the untreated cases the temperature shows a step ladder rise over the first week of the illness. It remains at 39.5°C to 40°C for 7 to 10 days than falls during the third or fourth week. A fever exhibiting a stepladder rise over the first week, with a plateau in the second week and a gradual fall in the third or fourth week is typical of typhoid fever (Bahl *et al.*, 2004). Confirmed case of typhoid fever

is defined, according to the World Health Organization (WHO), as a patient with fever ($>38^{\circ}\text{C}$) that has lasted for at least three days, with a laboratory confirmed positive culture of *S. typhi*. However, Ismail, (2006) documented a probable case of typhoid fever as patients with fever ($>38^{\circ}\text{C}$) that lasted for >3 days, with a positive serodiagnosis test without *S. typhi* isolation.

Caumes *et al.* (2001) noted that the clinical features of typhoid fever usually begin with the onset of a remitting diurnal fever, anorexia, headache, lethargy, confusion, cough, abdominal pains and constipation. They also reported that diarrhea and vomiting is mostly recognized in young children and those with acquired immune deficiency syndrome. Vaishnavi *et al.* (2006) reported that a short episode of diarrhea and vomiting sometimes occurs in the first day or two of an attack of typhoid fever though constipation rather than diarrhea characterizes the later stages of the disease. Manifestation of typhoid fever ranges from asymptomatic infection to fatal illness (Levine and Kaper, 1983). Typhoid fever and paratyphoid fever are clinically similar illnesses, but paratyphoid fever is often less severe. Paratyphoid disease is not only milder, but is also of shorter duration (WHO, 2001) and often presents with acute gastroenteritis (Crump *et al.*, 2004). The *S. paratyphi* A disease can be particularly virulent in some outbreaks its acclaimed mildness notwithstanding (McClelland *et al.*, 2004). The severity of the disease is proportional to the inoculum size of the bacteria. Connor *et al.* (2005) and Parry *et al.* (2006) documented an incubation period of *S. typhi*, lasting 7-14 days; the range may

sometimes be wider (3-60 days). *Salmonella enterica* serovar *typhi* infections result in a clinical syndrome that varies in severity. The incubation period is dependent on the host immunity status and inoculum size. Following the incubation period is a classical onset of the disease which manifests as daily remittent fever pattern with the body temperature fluctuating between of 40°C and 41°C. The fever is usually associated with chills, headache and malaise. The most common clinical manifestations seen in patients were prolonged fevers and headaches, followed by abdominal pain and diarrhea (Hoffner *et al.*, 2000). Sometimes afebrile period may occur even when the blood culture is positive. The fever is remittent during the first week and rises in a step wise fashion becoming persistent in the later phase of the illness (Otogbayo *et al.*, 2002).

The clinical symptoms of the intestinal infections vary from asymptomatic to a most severe diarrhea with fever and nausea. Prolonged fever does not occur with infections other than those caused by *Salmonella typhi*, which is classified as enteric fever (Gutherie, 1991). Not all patients experience classic symptoms, however, some stool and blood cultures should be performed on patients with persistent high fevers who have recently traveled to a developing country (Snow, 2006). Patients typically present with influenza-like symptoms with chills after the onset of fever but rigors are rare. A dull frontal headache, malaise, anorexia, and nausea are also present (Connor and Schwartz, 2005). High fever, toxemia, constipation during first week of fever, diarrhoea during

second week of fever, mild splenomegaly and leucopenia/neutropenia, complicated by encephalopathy, intestinal haemorrhage and perforation during third week of fever are the typical manifestations of typhoid fever (Pearson and Guerrant, 2000; Dutta *et al.*, 2001). Constipation usually occurs in older children and adults whereas diarrhea may occur in younger children. A few patients may have non-specific symptoms like cough and conjunctivitis (WHO, 2001). Relative bradycardia was noted as feature of enteric fever and increase heart rate at the later stage correlating with mortality (Otoegbayo *et al.*, 2002). Hypotension is suggestive of severe condition (Giannella, 1996). Blood leucocyte counts are often low in relation to the fever and toxicity, but the range is wide; in younger children leucocytosis is a common association and may reach 20,000-25,000/mm³ especially with infections of *S. typhi* or *S. paratyphi A* (Bhutta, 2006; Parry, 2008).

Physical examination in early phase of the disease does not reveal any specific or characteristic finding except raised temperature and relative bradycardia in few patients. Coated tongue may also be of a diagnostic value at this stage. Small pale, slightly raised blanching macules indicate the presence of rose spots. These are occasionally detected around the umbilical region in some few patients during the first week of the illness (Connor *et al.*, 2005). Hoffner *et al.* (2000) described these spots as blanching 2 to 4 mm diameter papules are classically found on the upper abdomen and lower chest; reported to occur in 30 to 50% of light-skinned patients. Rose spots are reported in 5-30% of cases but

are easily missed in dark-skinned patients (Parry, 2008). Otoegbayo *et al.* (2002) observed it on the thorax and abdomen of 60% of light skinned patients. Assessment of homodynamic and mental status was important as they correlated with the severity of the illness (Hag *et al.*, 1997). Soft tender hepatomegaly is frequently present by the second week of the illness. Physical examination in the third week of continuous febrile illness usually reveals a typical “typhoid face” appearance, here the face looks thin, pale, with wide bright eyes with a pathetic staring expression (Connor *et al.*, 2005).

Respiratory signs may be quite prominent in a few patients. In an untreated case there is anorexia that is associated with change in sensorium (Joshi, 2003). In a research conducted by Chowta and Chowta (2005) diarrhoea was seen in 20.4% patients. In a study by Melzer *et al.* (2006) splenomegaly was seen to persist in all 26 patients even at the follow up scan done on the 15th day. Chowta and Chowta (2005) observed splenomegaly in 34.09% patients and myocarditis in two patients. Out of these 21 (47.7%) were males and 23 (52.3%) were females. Burning micturition was observed in 5 (15.6%). Thirty-two (age >13 years, 19 males and 13 females) culture-positive typhoid fever patients were admitted during the period. The mean age of the patients was 23.9 years (range 13-55 years), 15 (46.9%) of 32 patients had atypical manifestations (Dutta *et al.*, 2001).

Signs and symptoms presentations of typhoid infections have been challenging the professional competence of well experience medical personnel

(Lai *et al.*, 2006). The disease sometimes presents with unusual signs and symptoms and are being encountered by medical expert's world over (Kacpizak *et al.*, 2002). A proper understanding of the disease with its unusual presentations will help in better and prompt management with better outcome (Chandel and Chaundry, 2001). In Poland typhoid fever was found to manifest as a respiratory tract infection in a five year old boy (Rozkie wicz *et al.*, 2005). De Ramos, (2004), Bansal *et al.* (1995) observed, in Jamaica, an unusual aphasia symptom in a 20 years old adult female with typhoid fever. Infections with *Salmonella enterica* serotype *paratyphi A* are occasionally associated with acute renal failure (Nakaya *et al.*, 1998). Sakamoto *et al.* (1998) also reported cases of diarrhoea caused by the same serotype. A case of *Salmonella enterica* serotype *paratyphi A* inducing acute diarrhea in a 45 year male was reported in the Indian capital metropolis of New Delhi (Pawan *et al.*, 2004). In south East Asia *Salmonella paratyphi A* has been associated with replication at unusual locations causing multiple liver abscesses in a 28 year old male patient (Rajagopal *et al.*, 2002). *Salmonella enterica* serotype *paratyphi A* was also isolated from the urine of a 37 year old Saudi patient who was a known case of nephrolithiasis and hyaronephrosis with frequent admission for management of renal stones (Al-otaibi, 2003). The commonest symptoms encountered among the subjects were fever 72.4%, headache 45%, and weakness 40.7%. Other symptoms encountered include unusual nausea 10.8%, fatigue 12.8%, Chills 21.9%, coughing 3.1% and anorexia 19.5%. Evaluation of the signs of the

enteric fever among the subjects recorded bradycardia 10.7%, low blood pressure 13.4%, splenomegaly 7.8% and hepatomegaly 3.7% as the commonest signs. Other signs encountered were weight loss 0.6%, coated tongue 2.3%, icterus 6.1%, rose spots 3.1%, psychosis 1.8%, and night sweats 1%. (Jombo *et al.*, 2007) Anad (1993) and Hoge *et al.* (1998) recorded 100% fever symptoms each in their respective studies. Crump *et al.* (2003) recorded 23.0% fever in their study. Secmeer *et al.* (1995) and Anadd (1993) recorded 40% and 25% respectively on the varied reports of headache symptoms on patients studied. Caumes *et al.* (2001) also made varied reports on other symptoms like diarrhea, constipation anorexia and chills. In a research conducted in a teaching unit of a hospital in south India 15(46.5%) patients presented with atypical manifestations. Dutta *et al.* (2001) observed burning micturation (15.6%), diarrhea in first week (6.2%), encephalopathy in the first week (3.1%) and bone marrow depression (6.2%). Neutropenia was observed in 3(9.4%) patients. One (3.1%) patient had severe anaemia with Hb of 5g/dl without any apparent cause. Two (6.2%) patients had eosinophilia and two others developed bone marrow depression. Pneumonitis was observed in (3.1%) patient. Richens (2004), however, noted that case fatality rate rarely exceeds one percent if promptly treated.

Malaria and typhoid fevers often present with similar symptoms especially in the early stages of typhoid fever (Ammah *et al.*, 1999; Ohanu *et al.*, 2003). The situation often presents a diagnostic problem and could lead to misdiagnosis. Thielman (2005) gave a very good differential diagnosis of other

infections which may mimic typhoid fever. In Africa, malaria is probably the most important disease from which typhoid must be distinguished (Nsutebe *et al.*, 2003; Cunha, 2006). In Cameroun a cross-sectional study of 200 subjects was carried out to determine the prevalence of typhoid fever in patients with confounding clinical symptoms. Typhoid fever was confirmed in 2.5% against 47% malaria (Nsutebe *et al.*, 2002). Although the signs and symptoms of malaria and typhoid could overlap, it was observed in Pakistan that subjects with dual infection had significantly higher rates of nausea, vomiting, abdominal pain, and diarrhoea. It was also noted that, unlike, the intermittent fever pattern generally seen with malaria, patients with dual infection tended to exhibit a continuous fever more typical of enteric fever (Khan *et al.*, 2005).

2.8 Complications and Mortality

Deaths resulting from enteric diseases are most often due to complications, which occur in about 10-15% of cases (WHO, 2006). The most common and most important complications among many others that have been described are gastrointestinal bleeding, intestinal perforation and typhoid encephalopathy (Hoffner *et al.*, 2000; Parry, 2008). Perforations are found usually in the ileum but may also occur in the caecum and proximal large bowel (Parry, 2008). Perforations affecting the ileal portion of the small intestine often manifests as small bowel typhoid ulcer. Such cases are usually surgical emergency and any

delay in diagnosis results in high mortality. Intestinal perforation is common with paralytic ileus (Malik, 2002). However, complication such as ileal perforation with or without peritonitis is a serious life threatening clinical problem that often results in high case fatality. Intestinal perforation occurs in 1-3% of hospitalized patients. Gastrointestinal bleeding occurs in up to 10% of patients and results from erosion of a necrotic Peyer's patch through the wall of an enteric vessel (Parry, 2008). Early intervention is crucial, as mortality rates increase if there is a long delay between perforation and surgery. This may present as an acute abdominal disturbances or more commonly as worsening in an already sick patient with increasing abdominal signs, rising pulse and falling blood pressure (Ugwu *et al.*, 2005). Hoffner *et al.* (2000) and Khan *et al.*, (2000) assessed the risk factors for transmission. The important finding was the significant association between short duration of symptoms and perforation. The patients seem to have had a more fulminant course, with early perforation, and because of the severity of their illness they may have been more likely to receive hospital care than patients who did not present with signs of perforation. They also reported that the mean duration of symptoms was lower in patients confirmed by blood cultures with enteric perforation than in those without it. In contrast, Everest *et al.* (2001) reported an association between longer duration of symptoms and perforation.

Typhoid ileal perforation is still frequently reported (Van Der Werf and Cameron, 1990; MacConkey, 2002). In accordance with most reported series in the tropical environment, especially West Africa, the prognosis of typhoid ileal perforation remains poor, with an over all mortality of 28% (Mauryas *et al.*, 1984; Parry *et al.*, 2003). Many factors such as late presentation, inadequate pre-operative resuscitation, delayed operation, the number of perforations and the extent of facial peritonitis, have been found to have a significant effect on the prognosis (Adeniran *et al.*, 2005).

The clinical course of typhoid ileal perforation may be different for the very young. The typically high rate of complications can be reduced if operation is undertaken earlier. Solitary ileal perforations can be managed safely with simple closure. Intestinal perforation associated with typhoid in children has a high mortality and its surgical treatment leads to other complications (Malik, 2002). Symptoms and signs of typhoid ileal perforation in Nigerian children are not different from those in other geographical areas, (Atamanalp *et al.*, 2007) with diarrhoea and fever more prominent in those below five years of age. Children less than five years old also have atypical features of generalised peritonitis and it may not be easy to make a diagnosis of peritonitis in them with certainty. Therefore, a high index of suspicion is needed for a diagnosis in this age group as demonstrated by Ekenze and Ikefuna (2008). Reports from Nigeria had it that the paediatric age group accounted for more than half the cases of typhoid

intestinal perforation (Ameh, 1999; Uba *et al.*, 2007). Children as young as 15 months could be affected by typhoid ileal perforation. A report from Zaria, (Ameh *et al.*, 1997) North Central Nigeria, recorded an incidence in a two-month-old infant. This is an unusual finding and may be due to contaminated expressed breast milk among other possibilities. The older children exhibit classical features of peritonitis in over 90% of cases, supporting the diagnosis (Ekenze and Ikefuna, 2008). Ileal perforations occurs within the first week of typhoid fever in over 50%, with reference to earlier reports from Northern Nigeria (Akoh, 1992) and other parts of tropical Africa (Archampong *et al.*, 2000). In the endemic areas, children below the age of 15 years account for more than 50% of the intestinal perforation cases, with higher mortality in them than the adult population (Ugwu *et al.*, 2005; Uba *et al.*, 2007). The reasons for these high mortality rates and postoperative complications are, continuing severe peritonitis, septicaemia, malnutrition, fluid, and electrolyte derangements. Prompt surgery after adequate resuscitation, is the treatment of choice for typhoid perforation; this has considerably reduced mortality from 30–60% to approximately 6.8% in a recent series (Karmacharya and Sharma, 2006). Mortality rate of ileal perforation is usually between 10 and 32% (Parry, 2006). Adesunakanimi and Ajao (1997) recorded up to 43% mortality rate. Keenan and Halley (1984), Gibney (1988) and Meier *et al.* (1989), however, observed that the survivors of ileal perforation were often faced with overwhelming wound infection and high incidence of dehiscence. Butler *et al.* (1985), Bitar and

Tarpley (1985) also reported on survivors being presented with severe wound infection and a history of long hospital stays. Mortality rates typhoid perforation vary from 14% in Nigeria (Ugwu *et al.*, 2005) to 34% in Cote d'ivoire (Koume *et al.*, 2004). Single perforations are most common (70%) in the terminal ileum, but multiple perforations may occur (Agbakwuru *et al.*, 2003). Many cases of typhoid perforations have been reported in West African regions (Ven Der Werf and Cameron, 1990, Adeloje, 1987). Parry (1984) and Butler *et al.* (1985) attributed this to the hypersensitivity in the Peyers's patches in West African patients.

Cases of typhoid perforation not properly treated may result in relapses at a rate of 5 to 10 percent. Although such cases are usually of short duration and of milder character than the initial illness they can be severe and may end fatally. Untreated and inadequately treated cases also usually lead to involvement of all the organs leading to prolonged illness, systemic complications and high mortality. *Salmonella paratyphi* A has been associated with replication at unusual locations. This was seen to cause multiple liver abscesses in a 28 year old male patient (Rajagopal *et al.*, 2002). This serotype was also isolated from the urine of 37 year old Saudi patient (Al-Otaibi, 2003).

Other sequelae and complications of enteric fever include hepatitis, meningitis, nephritis, toxic myocarditis, bronchitis, arthritis, osteomyelitis, parotitis, orchitis, periosteitis, osteitis, splenomegaly, hepatomegaly,

choleccystitis, bronchopneumonia, empyema, endocarditis encephalopathy and seizures (Duguid *et al.*, 1996). Complications could also include jaundice and psychosis which is considered for enteric fever on patients with suspected psychiatric or viral hepatitis ailments (Akande and Musa, 2005). James *et al.*, (1997) observed some forms of bone marrow suppression in 16(44.5%) of 36 patients of typhoid fever. Other complications apart from hepatic or splenic abscess (Chaudhry *et al.*, 2003) and osteomyelitis (Laloum *et al.*, 2005), include encephalomyelitis (Krishna *et al.*, 1999), splenic rupture and pancreatitis (Julia *et al.*, 2000). Glomerulonephritis and renal failure may also occur (Khan *et al.*, 1998). The presentation of enteric fever in the form of psoas abscess, myocarditis hepatic abscess, meningitis, palatal palsy and acute glomerulonephritis among others makes prompt diagnosis difficult (Lambottle *et al.*, 2000; Pacharoen *et al.*, 2001, Wang *et al.*, 2005). In a review study of 676 subjects with unusual presentation of enteric fever carried out by Jombo *et al.* (2007), the following common presentations were recorded ; meningitis 27.7%, splenic abscess 12.4%, hepatic abscess 10% and acalculous acute cholecystitis 11.1%, pneumonia 8.7%, neonatal typhoid 7% dysentery 5.8% and palatal 0.1% were also encountered. Acute acalculus cholecystitis is also presented as a rare complication of typhoid fever (Inian *et al.*, 2006). Myocarditis is a common cause of circulatory collapse. One third of patients developed complications, such as hepatitis, bone marrow suppression, paralytic ileus, myocarditis, psychosis and osteomyelitis (Malik, 2002). Typhoid encephalopathy, often

accompanied by shock, is associated with high mortality. Encephalopathy or typhoid state is a well-recognised entity in typhoid fever. This occurs typically in third week of illness. Early encephalopathy (encephalopathy during the first week of illness), is one of the grave complications observed in one (3.1%) of the patients (Dutta *et al.*, 2001). Mental apathy may progress to an agitated delirium (Parry, 2006). During pregnancy typhoid fever may be complicated by miscarriage. Vertical intra-uterine transmission from a typhoid-infected mother may lead to neonatal typhoid, a rare but severe and life-threatening complication (Parry, 2006).

2.9 PROGNOSIS

The duration of untreated illness prior to the initiation of therapy influences the severity of the enteric disease (Parry, 2006). House *et al.* (2001) noted that the median duration of illness for the typhoid patients was 8 (5 to 14) days. Patients may be hospitalized for a period of nine days (Hoffner *et al.*, 2000). If untreated, the fever persists for two weeks or more and defervescence occurs slowly over the following 2-3 weeks. Convalescence may last for 3-4 months. If an appropriate antibiotic is given the fever gradually falls over 3-4 days. A chronic carrier state, defined as a persistence of *S. typhi* in the stool for more than 1 year, may occur in up to 3% of treated patients (Hoffner *et al.*, 2000). About 10% of people recovering from untreated typhoid fever may excrete *S. typhi* in the stool for at least 3 months and the rate is higher for women, those

older than 50 years, and patients with schistosomiasis, cholelithiasis, carcinoma of the gall bladder, and other gastrointestinal malignancies. Most chronic carriers are asymptomatic and almost a quarter may have had no history of typhoid fever (Bhan *et al.*, 2005). Connor *et al.* (2005), however, reported that chronic biliary carriage may occur in 2-5% of cases, even after treatment.

2.10 EPIDEMIOLOGY

The annual global incidence of typhoid fever was estimated at 0.3% corresponding to above 16 million cases (WHO, 1996; Mermin *et al.*, 1998; Kariuki *et al.*, 2004). Issack (2005) further confirmed that *Salmonella typhi* and *Salmonella paratyphi*, causing enteric fever globally, accounts for at least 16 million infections with 600,000 deaths each year. The estimate was based on a small numbers of incidence studies and extrapolations were made to cover countries not included in the studies, particularly in Africa. The annual incidence varies from 100 to 1000 cases per 100 000 population (Connor *et al.*, 2005). With extrapolated studies the global annual incidence of paratyphoid fever has been put at 5.4 million (Crump *et al.*, 2004). This estimate was based on the global population of the year 2000 and was accounted for by improvements in water safety and sanitation, changes to data collection methods, and population changes. Incidentally, also the growth of the global population by approximately 20%, from 4.8 billion to 6.1 billion had contributed to the larger contain contemporary global typhoid fever burden

(WHO, 2000). The current population of 6.4 billion equated the figure to 19.2 million cases (Population Division, 2004). A more recent estimate places the total number of cases at 21.7 million with 216,510 deaths per year, accounting for a case fatality rate of 1% or approximately 22 million with 200,000 deaths (Crump *et al.*, 2004; WHO, 2004). Several of the studies that contributed to the estimate of the global typhoid fever burden were conducted during the 1950s, 1970s, (Wehden *et al.*, 1982). Changes in the determinants of typhoid fever incidence such as improvements of water supply and sanitary conditions have given validity for the 2000 estimate against these historical data (WHO/UNICEF, 2000). A variety of changes have taken place since 1984 to update the estimate of the global data of typhoid fever. This include the availabilty disease surveillance data (Martinez, 2000), the initiation of population-based typhoid fever incidence studies (Linf, 2000; Sinha *et al.*, 1999) and the publication of vaccine studies from new regions (Yang *et al.*, 2001). Sinha *et al.* (1999) also noted that the population based studies of incidence rate opened new advances in understanding of the age distribution of the typhoid fevers, these measured among narrow age cohorts could more accurately be extrapolated to the general population.

Developed countries have brought the incidence of typhoid fever to very low levels. Europe and North America being developed regions recorded low incidence of <10/100,000 cases/year (Crump *et al.*, 2004). In United Kingdom, the incidence of the disease was reported to be just one per 100,000 populations.

The disease is currently rare in the United States and continental Europe (Santos *et al.*, 2001). In the USA the annual incidence dropped from 7.5 per 100,000 in 1940 to 0.2 per 100,000 in the 1990s, and the proportion of cases related to foreign travel increased from 33% in 1967-72 to 81% in 1996-97 (Connor *et al.*, 2005). Prescott *et al.* (2002) reported that only 400 to 500 cases of typhoid fever occur annually in the United States of America. Salmonella is also one of the most common causes of enteric infection the U.S.A and more common in Georgia. In Maryland, reported incidence of salmonella illnesses are about 17 cases per 100,000 persons (CDC, 2000). Although incidence rate of typhoid fever is low in United States, illness due to other salmonella (salmonellosis) is common. Approximately 500 to 1000 persons or 31% of all food related illnesses are caused by salmonella in United States daily (CDC, <http://www.cdc.gov/health/diseases/htm>). The annual report of the Centers for Disease Control and Prevention (CDC, 2001), registered 360 different serovars in human infections in the United States. In 2000 there was an outbreak of domestically acquired typhoid fever that involved seven persons in the U. S. A. (Yoon *et al.*, 2004). According to Olsen *et al.* (2003), there were a total of 60 outbreaks of *S. typhi* from 1960-1999. In fifty-four outbreaks the exposure occurred inside the U.S.A and involved a total of 957 cases. An asymptomatic carrier was the cause in 21 of the 60 outbreaks and in 16 of them food preparation had been provided by the asymptomatic carriers. Each year the Centre for Disease Control receives approximately 250-445 reported cases,

most of which are acquired outside the U.S.A. In 1986 an asymptomatic food handler was implicated in an outbreak of 10 cases in Maryland. She had emigrated to the U.S.A from an endemic area. Over 40,000 such cases are reported and confirmed yearly in the U.S.A (CDC, 2001). In Scotland meat handlers spread the infection to others. In, England and Wales typhoid and paratyphoid illnesses have been associated with foreign travel, with exception of few indigenous transmissions occurring through contact with carriers and cases in family settings (Presscott *et al.*, 2002).

In contrast to that of developed countries typhoid fever remains an important health problem in the developing world (Kariuki, 2004; Issack, 2005), where annual incidence in Papua New Guinea and Indonesia may as high as 1200/100,000 populations (Crump *et al.*, 2004). Approximately, 10,000 patients are hospitalized annually for enteric fever in Turkey and more than 60% of these are reported from the south-east region of the country; the incidence ranges from 210 to 320 cases/100,000 populations (Ceylan *et al.*, 2003; Hosoglu *et al.*, 2003).

Annual incidence of 1% in the developing countries of Asia and Africa with case fatality rate of 10% was recorded. Countries with high incidence (South central and south eastern Asia) had 1000/100,000 cases/year (Graham, 2002; Crump *et al.*, 2004; Brooks *et al.*, 2005; Siddiqui *et al.*, 2006); those of medium incidence (Asia, Africa, Latin American, Caribbean and Oceania) had 10-100/100,000 cases/year. Similarly the World Health Organization (2003)

recorded typhoid fever cases in high, intermediate and low endemic areas as 1000-2000 per 100,000, 150 per 100,000 and 15 per 100,000, respectively. In 1990-1998 a large out break of typhoid fever occurred in Tajikistan causing more than 24000 cases. An increasing number was reported from neighboring Uzbekistan (WHO, 2000) Enteric fever is endemic in many developing countries of Africa, Asia and Latin America (ISID 2001). In Asia, the incidence of enteric fever caused by *S. paratyphi* is about 15-20%; however such incidence is becoming increasingly widespread in India (Tankhiwale *et al.*, 2003) and China (Ochiai *et al.*, 2005).

There is evidence that typhoid fever incidence have been declining over the past several decades for Chile (Levine *et al.*, 1990; Black *et al.*, 1990; Levine *et al.*, 1990; WHO/UNICEF, 2000), Egypt (Wahdan *et al.*, 1980), India (Wain *et al.*, 2001), former Soviet Union (Hejfer *et al.*, 1969) and viet Nam (Lin *et al.*, 2000; Ying *et al.*, 2001). The annual typhoid incidence rate in Egypt was estimated at 13 per 100,000 (Crump *et al.*, 2003). Multiple data available over time from each country indicates trends towards declining typhoid fever incidence for these countries except Viet Nam; this trend was consistent with improvements in sanitary conditions (WHO/UNICEF, 2000). This lower incidence may have been consistent with reductions of other enteric diseases resulting from improved management of the diarrhoeal and enteric diseases. WHO/ UNICEF (2000) also observed that the reductions could be attributed to the fact that large and growing proportions of persons living in both rural and

urban areas have access to safe water. In Pakistan, the incidence of culture proven and serological diagnoses of enteric fever in children was estimated at 170 per 100,000 populations (Siddiqui *et al.*, 2006).

To further define the estimate of the global burden of typhoid fever, improvement was needed in the quality and quantity of data collection. The regions lacked either eligible population-based studies of the disease incidence or surveillance system that could measure the rate at the population level. The lack of data is most notable for eastern, central and western Africa. While population-based studies from Egypt in the Northern Africa region are in the middle incidence range, a single study from South Africa placed the Southern African in the high incidence range (Crump *et al.*, 2004). This may suggest that the typhoid situation in the rest of the African continent might reflect more closely, the data obtained in Egypt than that in South Africa. However, population based studies of typhoid fever incidence are needed elsewhere in Africa to clarify the typhoid fever situation for the continent. This is necessitated by the fact that the incidence of the enteric fever disease is still high in the under-developed and developing world compared to that of the western world. Data are yet lacking in Africa and other developing regions of the world (Akinyemi *et al.*, 2007).

2.10.1 Distributions of the *Salmonella* Infections by Serovars

Salmonella paratyphi A and *typhi* are the deadliest human restricted serovars, responsible for 600,000 deaths per annum (Didelot *et al.*, 2007). *Salmonella paratyphi* A is the second most prevalent cause of typhoid, responsible for one third of cases or more in southern and eastern Asia. During the surveillance period, 285 *S. typhi* episodes and 84 *S. paratyphi* A episodes were detected at the 4 sites. In Indonesia, 14% of enteric fever episodes were caused by *S. paratyphi* A, in Pakistan 15%, in India 24%, and in China 64%. The highest *S. typhi* incidence was observed in Pakistan (394/100,000/year) (Butt *et al.*, 2005). The lowest *S. typhi* incidence was found in China (15.2/100,000/year). The highest *S. paratyphi* A incidence was also seen in Pakistan. (72/100,000/year), and the lowest *S. paratyphi* A incidence was seen in Indonesia (13.7/100,000/year) (Ochiai *et al.*, 2005).

TABLE 2: Estimated Numbers of Typhoid Cases and Deaths (WHO, 2000)

CONTINENTS	TOTAL CASES	INCIDENCE (100000/YEAR)	DEATHS
Africa	2655,000	0.5%	4.8%
Asia	13310,000	0.5%	3.3%
Latin America	595,500	1.5%	1.6%
Oceania	7,500	1.5%	1.6%
Europe/N.America	22,620	0.002%	0.32%

During the 1997 global survey of salmonella serotyping response rate of 34%-70% was obtained, worldwide, 3572 *Salmonella typhi* isolates and 888 paratyphoid isolates were reported (Hesikstad *et al.*, 2002). This corresponds to 0.25 paratyphoid fever illnesses for every typhoid fever illness. The ratio was validated by review of the 8(36%) of 22 population-based typhoid fever. By applying the proportion of 0.25 to the global typhoid fever estimate an estimate of 5412744 paratyphoid fever cases was obtained (Simanjuntak *et al.*, 1991; Ying *et al.*, 2001). Ngwu and Agbo (2003) isolated 135 enteric bacilli from 260 samples (51.5%) and the distributions were as follows; *S. typhi* 46(34.3%); *S. paratyphi B* 34(25.4%); *S. paratyphi C* 20 (14.9%); other salmonella species 10(25%). Approximately 50% of these infections were caused by only three salmonella serovars, specifically *typhimurium*, *enteritidis*, and *Newport*. About 12 most prevalent salmonella serovars were responsible for >70% of all human salmonella infections. Similarly, 41.8% of all veterinary infections were attributed to only two salmonella serovars, namely, *typhimurium* and *Newport* (Porwollik *et al.*, 2002). Studies from India and Nepal suggest that, in some settings, paratyphoid fever caused by *S. paratyphi A* can contribute up to half of all cases of typhoid fever (Bhan *et al.*, 2005). In France, an environmental investigation at a processing plant that produced a raw goats' milk cheese was incriminated in the outbreak of typhoid fever illness. Using Phage typing and genotyping of food and human samples *S. paratyphi B* was implicated as the aetiologic factor (Desenclos *et al.*, 1996).

2.10.2: Distributions of *Salmonella typhi/paratyphi* Infections in Nigeria

Typhoid fever is, at present, a disease of major public health importance in Nigeria and indeed the rest of Africa (Wasfy *et al.*, 2002; Akinyemi *et al.*, 2007; Nkemngu *et al.*, 2005). Some studies have been conducted recently within some regions of Nigeria to determine the distribution of *S. typhi* and *S. paratyphi* in patients' samples. Generally, detailed data of incidence studies are lacking at present. A research conducted at University of Nigeria recorded 16% (128/809) isolates of enteric fever bacilli from stool culture. A higher proportion of paratyphoid infection over typhoid infection was however obtained in this study (Oboegbulam *et al.*, 1995). In Lagos Smith *et al.*, 2004 reported 26% positive for *Salmonella typhi*. Akinyemi *et al.*, (2002) recorded 16.2% prevalence rate in Lagos also. Ameh and Opara (2004) conducted study at Sokoto with 168 patients treated with typhoid. The result showed that 58.9% males and 41.1% females were infected. Incidence of typhoid fever in this part of the world is difficult because of lack of accurate population data and the population-based approaches can only capture only a fraction of patients (Crump *et al.*, 2003). Moreover researches in the public health sector of Nigeria poses a great challenge, sample collection, being greatly hampered by uncooperative attitude of the populace.

2.10.3: Incidence of Typhoid Disease with Concomitant Malaria.

Malaria and typhoid are the most endemic diseases in the tropics. However, malaria remains the most overwhelming health problem facing humanity in the vast majority of the tropical and sub-tropical regions, with 300 to 500 million cases and up to 3 million deaths annually (WHO, 2000). About 90% of all malaria deaths in the world today occur in the sub-Saharan Africa mostly as a result of majority of infection being caused by most dangerous species of parasite, *Plasmodium falciparum*: accounting for an estimated 1.4 to 2.6 million deaths per year in the region (WHO, 2006).

In the last two decades the relationship and co-existent prevalence of typhoid and malaria diseases have been substantiated by studies from Africa and Asia (Ohanu *et al.*, 2003; Sur *et al.*, 2006; Kanjilal *et al.*, 2006). The prevalence of malaria and typhoid co-infection using Widal test ranged from 4.4% to 70% (Tanyigna *et al.*, 2001; Ibadin and Ogbimi, 2004), on the other hand the prevalence of concurrent malaria and typhoid fever using cultural technique alone ranged from 11.1% to 26.6% (Smith *et al.*, 2004; Khan *et al.*, 2005; Akinyemi *et al.*, 2007). Mbuh *et al.*, (2003) also observed that the two studies that used both techniques reported higher rates of co-infection compared with that using bacteriological culture technique. Nwuzo *et al.*, (2009), analysed 250 blood samples from symptomatic subjects for malaria parasite and typhoid fever using parasitological strip, Widal screening and bacteriological cultural techniques respectively. The result showed that 33 (13.2%) samples were

positive for malaria parasite, 53 (21.2%) were positive for typhoid fever by the Widal test and 2 (0.8%) were positive by culture method. Fourteen patients (5.6%) had co-infection which was significantly high when diagnosed by Widal test than by cultural method (0.8%). In a research carried out by Florence *et al.*, (2004). Sixty samples were positive for malaria parasites, 22 of which were positive for typhoid by the Widal test and only one by the culture method. The rate of co-infection was significantly high when typhoid was diagnosed by Widal (10.1%) than by blood culture method (0.5%). The correlation analysis showed no specific relationship between malaria parasite load and the level of Salmonella antibody titres in malaria patients. Florence *et al.*, (2004) noted that the incidence of typhoid and malaria co-infection will greatly reduce if the diagnosis of typhoid fever in malaria endemic areas is based blood culture.

2.11 PREDISPOSING/RISK FACTORS OF INFECTION AND TRANSMISSION

2.11.1 Environmental Factors

The main reservoir for *S. typhi* is the human being, principally in the intestinal tract and the blood stream. The transmission of *S. typhi* most often occurs through food and water, which have been contaminated by faeces or urine from infected humans (Reller *et al.*, 2003). Salmonella organism is, therefore, transmitted by fecal-oral route or urine-oral route. This occurs either directly through hands soiled with feces or urine of the cases of carriers or indirectly by ingestion of contaminated water, milk, food or other food items

(Xavier, 2006). An infected individual continues to exhibit the clinical signs and symptoms as well as excreting the bacilli in the stool and urine. During this period such reservoir remains infectious to others. Carriers may be temporary or chronic. Temporary (convalescent or incubatory) carriers usually excrete bacilli up to 6 to 8 weeks. Individuals at their sub-clinical stage are more important source of contamination than clinical cases (Brooks *et al.*, 2004). Persons who excrete the bacilli for more than a year after clinical attack are termed chronic carriers. A chronic carrier may excrete bacilli for several years either continuously or intermittently (Levine *et al.*, 1990). Both ill people and carriers shed *S. typhi* in their faeces (Xavier, 2006). Infected persons shed *S. typhi* in their faeces from the first week of illness through convalescence. About 30% of those infected may become transient carriers who excrete *S. typhi* in their faeces or urine for weeks or months; 5% become long-term carriers. Although the infective dose is thought to be small, chronic carriers shed from 10^6 to 10^{11} microorganisms per gram of feces. The longest duration of the carrier state reported in the literature was 52 years (Snyder and Matthews, 1996). Many people become carriers as a result of inadequate medication following diagnosis or self-medication generally practised in Nigeria. About 1-5% of people who are infected with *S. typhi* become asymptomatic chronic carriers (Levine, 1999; APHA *et al.*, 2002). About 10% of untreated typhoid fever patients excrete the bacteria for 3 months after onset of symptoms (APHA *et al.*, 2002). However, some patients excreting *S. typhi* have no history of

typhoid fever (Ismail, 2006). Typhoid is mainly transmitted when chronic carriers contaminate food through break down in proper practices of personal and food hygiene. They themselves pose a high risk to others (Levine, 1999). High prevalence of carriers is, therefore, a factor in transmission and spread of enteric fever. In the USA, up to 30% of infections are due to exposure from previously or newly diagnosed chronic carriers (Bhan *et al.*, 2005). In endemic areas, peaks of transmission occur in dry weather or at the onset of rains. Epidemics originating from water contamination are particularly explosive being that infection ensues easily, since the gastric acid that would have inactivated the pathogen is diluted by water during drinking. Explosive epidemics are also made possible by large population using the same water source (WHO, 2003). Poverty, uncontrolled urbanization and inadequate infrastructure do contribute to the contamination of water supplies ((Luxemburger *et al.*, 2001; Kumar *et al.*, 2002; Tran *et al.*, 2005). Epidemiological data records that water borne transmission of *S. typhi* usually involves small inocula, whereas food borne transmission is associated with large inoculum (Ismail, 2006). Although the source of infection may vary, person to person transmission through poor hygiene and sewage contamination of water supply are the most important (Bhutta, 2006). In the many part of Nigeria safe drinking water and proper sanitation measures are still lacking giving rise to increase incidence cases of enteric fever. In a study conducted by Ameh and Opara (2004) at Sokoto in Nigeria it was observed that drinking water was most

likely the main source of typhoid fever among the affected patients irrespective of the source. A greater population of this locality under study lack access to clean and safe water, the populace resort to untreated shallow well water from the ground, which may possibly have been contaminated by sewage.

Recently, there has been a great surge and demand of commercially packaged sachet water to supplement the scarce water supply. This invariably results in high rate of consumption of such products. This too poses a threat and danger to health; as the hygienic condition and safety of such water cannot be attested for. The scarce supply of pipe-borne water which has remained the only source of drinking water is likely to be contaminated too through rusted and perforated pipes.

High prevalence of carriers, in turn, is a factor to transmission and spread of the illness. This is particularly so in developed countries where infections are most likely to occur from a source of food contaminated by a carrier (WHO, 2003). Direct faecal-oral transmission may occur if vegetables fertilised with human waste are eaten raw, while shellfish that have been harvested from sewage-contaminated beds, and milk products contaminated by worker's hands all may result in typhoid infection (Xavier, 2006.) Some out breaks of enteric fever have been traced to inadequate pasteurization and improper handling of milk and dairy products. Also infections have ensued from frozen eggs, infected poultry and rodents (Jawetz *et al.*, 2004). *Salmonella* infection may also result from contamination of other types of meat (cattle, pigs, goats) mostly

contamination of the muscles and intestinal contents. However, surface contamination of meat is usually of little consequence, as proper cooking will sterilize it (Le Minor, 2006). Infection may occur if cooking is superficial. Toxic infection may follow ingestion of any food that supports multiplication of the organism especially creamed foods. Kapil *et al.* (1997) reported a case study in New Delhi, India, where there was an outbreak of infection during a Muslim festival. Though the first suspected source of the infection was contaminated food, it was later found out the source of the infection was contaminated water source. According to Mermin *et al.* (1998), from 1985 to 1994 there were 4408 cases recorded; the most sizable outbreak during this time frame was due to a carrier who worked as a food handler. Luby *et al.*, (1998) reported inadequate food and personal hygiene as well as close contact with recent typhoid fever, poor housing with and recent consumption of antimicrobials as being risk factors. Parry (2008) noted that eating food prepared outside the home, such as ice creams or flavoured iced drinks from street vendors, drinking contaminated water and eating vegetables and salad that has been grown in human waste/fertilizer could all pose as risk of typhoid infection. Other reported risk factors include a history of contact with other patients before illness, not using soap for washing hands and poor housing (Bhan *et al.*, 2005).

Housefly vector has also been found to be involved with mechanical transmission of typhoid fever. The cause of the typhoid epidemics in the U.S.A

Army camps was investigated and concluded that, next to human contact, the housefly was the most active agent in the spread of the disease (Parry, 2006). British medical officers in South Africa, facing even worse typhoid epidemics, reached the same conclusion. The experiences of the American and British armies finally convinced the medical profession and public health authorities that these insects conveyed typhoid. Military and civilian sanitarians waged fly-eradication campaigns that prevented the housefly's access to breeding places (especially human excrement), and that protected food and drink from contamination. Fly control is still an important public health measure in the 21st century, especially in developing countries (Cirillo, 2006).

2.11.2 Socioeconomic Factors

The socioeconomic factors, therefore, affecting the prevalence and distribution of typhoid/paratyphoid bacilli include indiscriminate disposal of waste, sewage spillage, lack of good toilet facilities, inadequate sanitary facilities in homes and general poor sanitation culture (WHO, 2003). The disease condition continues to be unabated in the developing countries such as Nigeria and other African countries. The situation is rendered more complex by the web of social, cultural and economic factors which determine the quality of life of the people. The socio-economic factors amongst others play a major role in the transmission and distribution of typhoid/paratyphoid fever illnesses. The disease occurs in all parts of the world where water supply is unsafe and

sanitation is sub-standard. These factors are prevalent in most parts of Nigeria and Enugu locality.

Other likely sources of the disease included food and meat sold to the public by vendors at the road side. Improperly cooked and hygienically handled food and food stuff are being consumed indiscriminately due to deteriorating socio-economic statuses coupled with lack of health education. The alarming menace of typhoid fever fatality, therefore, may not be unconnected with these factors.

2.11.3 Sexual Transmission:

In this outbreak, onset of typhoid fever after oral-anal or oral-penile sex documented that the illness occurred after receptive anal sex only suggests that the mode of transmission through sexual-intestinal inoculation can also occur. The plausibility of anal intercourse as a risk factor for typhoid fever is corroborated by reports of transmission of *S. typhi* infection via an unsterilized endoscope, polyvinyl duodenal tube, or rectal tube (Reller *et al.*, 2003).

2.11.4 Risk of Travelers and Tourists.

People who travel to high-endemic areas from low endemic areas are at risk of typhoid infection (Reller *et al.*, 2003). Travelers to South-East Asia were at three times higher risk of typhoid than those visiting South America and eight times higher than those visiting the Caribbean (Ackers *et al.*, 2000). Typhoid is predominantly a disease of the returning traveler however; malaria remains the

most febrile disease requiring hospitalization (Anatinori *et al.*, 2004).

Another report by Steften *et al.*, (2003) suggested that travelers to the Indian sub-continent and parts of West Africa are at considerable risk with incidence rate of 30 cases per 100,000 travelers per month. The risk of contacting typhoid fever is, therefore, highest for travelers to the Indian sub-continent (India, Pakistan and Bangladesh), South East Asia and parts of Latin America and Africa. The attack rate of travelers in these regions was estimated at 10 per 100,000 travelers. In other developing countries the risk was estimated to be 3 cases per 100,000 travelers per month. The risk of contacting typhoid and paratyphoid fever in resource rich countries such as Europe, North America and Australia is less than one case per million visits (Steffen *et al.*, 2003).

2.11.5 Occupational Risk

Microbiology laboratory scientists and technicians do constitute a high risk group for the development of typhoid fever. Typhoid fever is a demonstrated hazard to laboratory personnel. Ingestion or parenteral inoculation of the organism represents the primary laboratory hazards. The importance of aerosol exposure is not known. A review from the Centers for Disease Control and Prevention revealed that 11.2% of the reported sporadic cases of typhoid fever in the United States occurred in these occupational groups (CDC, 2001).

2.11.6 Host Factors

(A) Age and Sex Gender

Typhoid fever occurs at any age but occurs more commonly in children and young adults. The highest attack rate was found to occur in children 8-13 years. Recently, contrary to these popular findings the disease was found to affect even children aged 1-5 years in Delhi, India (Walia *et al.*, 2006). In France also, the isolation rate was highest among infants and children aged 1 to 5 years (Desenclos *et al.*, 1996). Ashebolt and Ashebolt (2004) reported that 3.1% of all deaths and amongst these nine out of ten deaths occurred in children. In developing countries the incidence of enteric fever peaks in children between 5 to 12 years, with relatively high incidence in young adults. The age range considered to be at greatest risk is 5 to 25 years (Saha *et al.*, 2001). World Health Organization (2000) noted that in South America the highest incidence occurred in children aged 5-19 years and in adults over 35 years. A higher frequency of *S. typhi* and *S. paratyphi A* isolates were reported in children in Pakistan, showing a greater burden of enteric fever in children than in the adults (Graham, 2002; Brooks *et al.*, 2005; Siddiqui *et al.*, 2006). Recent population based studies from South Asia suggest that the incidence is highest in children aged less than 5 years, with higher rates of complications and hospitalisation, and may indicate risk of early exposure to relatively large infecting doses of the organisms in these populations (Bhutta, 2006). Comparison of strains distribution between adults and children, on the contrary, showed that children

bore 79.3% of the *S. typhi* burden as compared to 59.9% of *S. paratyphi A* (Walia *et al.*, 2006; Sur *et al.*, 2007). The adult prevalence of *Salmonella typhi* and *paratyphi* per 100,000 detected through blood culture was estimated at 4.7, 3.8, 29.20, 66.00 and 52.7 for Viet Nam, China, Indonesia, India and Pakistan respectively. Wahdan *et al* (1980) and Wahdan *et al* (1982) have previously documented an annual typhoid fever incidence of 209 per 100,000 persons in 1972-1973 and 48 per 100,000 persons in 1978-1981 among the school-aged children in Alexandria, Egypt. Vaccine studies conducted of high risk groups may have accounted for the overestimate.

Kapil *et al* (1997) recorded high incidence of the disease in young adults also, mean age of infection was 20.1years. At India (Kapil *et al.*, 1997) reported the infection with *Salmonella paratyphi A* to be more prevalent in young adults and the mean age being 20.1 years. At Egypt the median age of patients with typhoid fever is 22 years (range 5- 60 years), 5 (26.3%) patients were female (Crump *et al.*, 2003). Crump *et al* (2003) recorded that the median age of patients with typhoid fever is 22 years with range of 5-60 years. At Sokoto young adults of 11-20 years and adolescents of 21-30 years age were mostly affected (Ameh and Opara, 2004). Jombo *et al.*, (2007) however, reported that age distribution pattern was inconclusive; the age range was as vast as 6 days to over 75 years. Excellent reviews, however, shows that typhoid ileal perforation affects both adults and children (Graham, 2002; Parry *et al.*, 2003; WHO, 2003; Richens, 2004; Kamel, 2004).

Studies have suggested that male sexes are more prone to typhoid perforation (Chatterjee *et al.*, 2001). At all India University, the male to female ratio was 2:1 (Kapil *et al.*, 1997). In the study of clinical presentations of enteric fever, 676 subjects out of 3992 presenting with unusual features, the male female ratio was 1:18. The study conducted at Sokoto by Ameh and Opara (2004) showed that more males (313 or 58.9%) than females (218 or 41.1%) were infected with the enteric fever illness. Age related difference has also been found in antimicrobial resistance posing as a risk (Luxemburger *et al.*, 2001; Sur *et al.*, 2007).

(B) Other Host Factors.

For typhoid infection to occur, the organism must achieve an infectious dose. Though the Vi negative strains of *S. enterica* are less virulent than the Vi positive strains, they must all survive the gastric acid barrier en route to the small intestine. Achlorhydria, due to ageing, previous gastrectomy, and treatment with H₂ receptor antagonists, proton pump inhibitors, and large amounts of antacids or *Helicobacter pylori* infection increase susceptibility to typhoid fever (Bhan *et al.*, 2002). Concomitant *H. pylori* infection may express itself via the hypochlorhydria (Frenck *et al.*, 2003).

Leukopenia was found to be an independent risk factor for enteric perforation (Hosoglu *et al.*, 2004). Yaramis *et al.*, (2001) recorded a white cell count of 4.5 x10⁹/ liter in 18% of the children in one study. In another study

white blood cells were found to be normal in 12 typhoid fever patients and elevated in 2 patients and leucopenia (4,000 white cells / μ l) was noted in seven patients (Hoffner *et al.*, 2000). In contrast, Khan *et al.*, (2000) reported that the rate of leucopenia was higher among patients with no complications in their study result of 5 out of 21 patients with intestinal perforation. Hosloglu *et al.*, (2004) further enumerated other potential risk for typhoid enteric perforation as follows: age (>40 years), gender, inadequate treatment, durations of symptoms, high fever (>38.5°C), elevated transaminase levels (>1.5 times the normal values), hepatosplenomegaly, leucopenia (>3,000 white blood cells/ μ l), anaemia (hemoglobin level <8g/dl), and elevated erythrocyte sedimentation rate. Prior antibiotic usage was also found to be a risk factor for typhoid (Luby *et al.*, 1998; Srikantiah *et al.*, 2007). Carrier state of enteric fever was also associated with urinary pathology such as urinary schistosomiasis which is usually evidenced by urinary excretion (Kamel, 2004). Duta *et al.*, (2000) also discovered that carrier state was associated with cholelithiasis

2.12 CHEMOTHERAPY

Chloramphenicol (daily dose 50-75 mg/kg) was used to treat uncomplicated typhoid at home within 14-21 days. For severe typhoid at home fully susceptible second-line oral drug was chloramphenicol (daily dose 100 mg/kg) for 14 - 21 (Bhan *et al.*, 2005). Historically, typhoid fever was treated with chloram-

phenicol, ampicillin, or trimethoprim-sulfamethoxazole (co-trimoxazole) (Connor and Schwatz, 2005). However, outbreaks of chloramphenicol-resistant typhoid were reported in 1972. At this time, the isolates were still sensitive to co-trimoxazole and ampicillin or amoxicillin. Chloramphenicol resistance soon became widespread and, since 1989, resistance to all three has been noted in strains in India, Pakistan, China, and the Persian. Resistance appears to be plasmid mediated, allowing the simultaneous acquisition of resistance to multiple drugs and the emergence of multidrug-resistant strains. About 50-80% of isolates from China and the Indian subcontinent are now multidrug resistant, and 93% of isolates in an outbreak in Tajikistan were multidrug resistant (Connor and Schwatz, 2005). In the late 1980s and 1990s, outbreaks of typhoid caused by organisms resistant to chloramphenicol, co-trimoxazole, ampicillin, and amoxicillin were reported (Bhan *et al.*, 2005). Such multidrug resistant typhoid is now reported from many parts of the world (Chandel *et al.*, 2000). The emergence of strains of *Salmonella typhi* resistant to multiple antibiotics poses a serious problem.

Although ampicillin sulfonamides and co-trimoxazole have been the antibiotics of choice, multi-drug resistant (MDR) organisms strains had developed (Mills-Robertson *et al.*, 2002; Kariuki *et al.*, 2004; Wain and Kidgell, 2004). Some epidemiologic studies have shown a rise of the MDR organisms (Walia *et al.*, 2005). Emergence of multi-drug resistance to chloramphenicol,

ampicillin and co-trimoxazole has greatly complicated management of typhoid fever disease (Okeke *et al.*, 2005). Tsonyo *et al.*, (2007), noted that MDR isolates of *S. typhi* and *S. paratyphi A* were those resistant to all three first line antityphoid drugs (ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole). In some regions with high MDR prevalence, sensitivity to chloramphenicol has re-emerged (Wasfy *et al.*, 2002). Chandel *et al.*, (2000) recorded that 7 (32%) of 22 isolates tested were resistant to both chloramphenicol and cotrimoxazole. Chowta and Chowta (2005) recorded 63.6% sensitivity rate with Cotrimoxazole.

When treated early, typhoid fever usually responds well to appropriate antibiotics. Increasing antibiotic resistance has prompted wider use of third-generation cephalosporins and quinolone derivatives, according to the World Health Organization. The uses of chloramphenicol, ampicillin and co-trimoxazole have, however, become infrequent and quinolones have become the first line of treatment of typhoid fever. However, over the last few years there has been increase in the defervescence period in patients treated with quinolones (Chowta and Chowta, 2005). Standard treatment with chloramphenicol or amoxicillin is associated with a relapse rate of 5-15% or 4-8% respectively, whereas the newer quinolones and third generation cephalosporins are associated with higher cure rates (Bhutta, 2006). Chloramphenicol, the traditional first-line drug of choice, is less effective than fluoroquinolones in all these respects and in

terms of persistence of the organism in bone marrow, even for treatment of patients with fully sensitive isolates (Bhan *et al.*, 2005). Also chloramphenicol does not prevent relapse unless given for 2-3 weeks; the carrier state is not also eradicated nor is it useful against MDR strains (Thaver, 2006). Today due to its changing modes of presentation, as well as the development of multidrug resistance, typhoid fever is becoming increasingly difficult to diagnose and treat

Sulfonamides, such as sulfadiazine and sulfamethoxazole, used together with trimethoprim, produce synergistic antibacterial activity (MICROMEDEX, 2001). Almost 80% of carriers were cured by 750 mg of ciprofloxacin twice daily for 28 days, and 11 out of 12 carriers treated with 400 mg norfloxacin twice daily had negative stool and bile cultures for *S. typhi* after 28 days of treatment. In patients with gallstones, cholecystectomy along with antibiotic therapy might be required (Bhan *et al.*, 2005).

Azithromycin, and cefixime, an oral third-generation cephalosporin, have a clinical cure rate of over 90% with a fever clearance time of 5-7 days and relapse and faecal carriage rates of less than 4% in typhoid fever (Bhan *et al.*, 2005). Third generation cephalosporins and azithromycin have proved effective in endemic areas (Parry *et al.*, 2003). Furthermore, oral azithromycin was comparable to intravenous ceftriaxone in uncomplicated typhoid fever in children and adolescents (Connor and Schwatz, 2005).

The introduction of fluoroquinolones was a major advance. The drugs were found to be highly effective, well-tolerated, and could be administered orally. Antibiotics such as ciprofloxacin quickly became first-line agents (Ciprofloxacin (500 mg twice daily) was found to be effective (Connor and Schwatz, 2005). The results of ciprofloxacin and ofloxacin were similar to that of azithromycin. Fluorquinolones have been the drug of choice for treatment of typhoid and paratyphoid fever since the beginning of the 1990s. In recent years, however, strains with decreased susceptibility to quinolones have emerged (Parkhill *et al.*, 2001), and clinical treatment failure is a serious concern *S. typhi* and *S. Paratyphi A* with decreased susceptibility to fluoroquinolones emerged on the Indian subcontinent, Southeast Asia and Central Asia in the mid-1990s. Reports, however, suggested that the multi-drug resistant (MDR) rate amongst *S. typhi* in Pakistan decreased from 50% in 1995 to 20% in 2001 (Okeke *et al.*, 2005) The quinolone-resistant strains were noted to be sensitive to ceftriaxone, cefixime, and azithromycin but the clinical response was slower, with fever clearance taking 7 days or more and failure rates of >20% (Connor and Schwatz, 2005).

Findings strongly suggest that high-level of quinolone resistance was induced through the long-term carrier state of *S. paratyphi A* under selective pressure of frequent quinolone administration (Adachi *et al.*, 2005). Quinolone resistant isolates of *Salmonella enterica* serovar Typhi have reduced susceptibility to ciprofloxacin, with minimum inhibitory concentrations between

0.1 mg/l and 1 mg/l compared with wild type strains (<0.1 mg/l). The currently agreed definition for resistance is a minimum inhibitory concentration >1 mg/l, and such isolates are therefore reported as being susceptible to ciprofloxacin by disc sensitivity testing although systemic infections with these isolates responded poorly to ciprofloxacin and ofloxacin (Parry *et al.*, 2003). Resistance to quinolones is independent of resistance to other drugs that are mainly plasmid mediated, it may occur in otherwise sensitive strains (Chandel *et al.*, 2000). Although multidrug-resistant outbreaks of *S. typhi* with an increase in numbers of strains with decreased susceptibility to ciprofloxacin have occurred, cases of drug-resistant *S. paratyphi A* have been relatively uncommon. Adachi *et al.* (2005) reported a sudden increase in drug-resistant by *S. paratyphi A*. The emergence of bacterial resistance to fluoroquinolones, and to cross-resistance within this class of antimicrobial agents, has become a significant concern. The last two decades have seen a change in the pattern of enteric fever with the emergence of multidrug-resistant strains (MDRS), particularly strains resistant to nalidixic acid (Walia *et al.*, 2005). Multi-drug resistant strains are noted to be more virulent and associated with increased mortality. Such strains of *S. typhi* were first reported in Bangladesh in 1986 and from Pakistan in 1988 and are currently endemic in South Asia (Chau *et al.*, 2007). Isolates with-level resistance to fluoroquinolones have become common in Asia (Dutta *et al.*, 2001). These strains are causing increasing number of infection in travelers returning from Asia (Kapil *et al.*, 1997; Ackers *et al.*, 2000; Aarestrup *et al.*,

2003). Strains were interpreted as ciprofloxacin susceptible but showed a gradual increase in the MIC although the MIC values were still below the breakpoint of resistance (Kapil *et al.*, 2002).

Increase resistance of *S. enterica* serotype *typhi* and *paratyphi A* to quinolones, mainly ciprofloxacin, has been widely reported (Chandel *et al.*, 2000; Threlfall and Ward, 2001). Vinh *et al.*, (2005) and Thaver (2006) in comparing fluoroquinolones with other antibiotics concluded that there was little to recommend them over first line drugs (chloramphenicol, ampicillin and co-trimazole). However, fluoroquinolones reduced failure rates when compared to third generation cephalosporins; Thaver (2006) admitted their conclusion differ from those of Parry (2004) and standard textbooks which recommend fluoroquinolones as modern first line therapy (Richens, 2004; Thielman, 2005). The study recommended multi-center outpatients trials comparing fluoroquinolones and first line therapy to settle this disparity.

2.13 PREVENTION AND CONTROL.

2.13.1 Quarantine of Carriers.

Infected and humans carriers are the reservoir for *S. typhi*. Therefore identification and treatment of these individuals represent one strategy for interruption of transmission. Measures taken to prevent transmission were restricted to industrialized countries and localized epidemics (Katz *et al.*, 2002;

Yoon *et al.*, 2004). Preventive measures were also mainly provided to travelers returning from endemic areas (Mermin, 1998). Trends in source and transmission were during the recognition of fecal contamination of food and water supplies as the main mode of transmission (Casner, 2001).

According to Heymann (2004), “typhoid carriers should be excluded from food handling and from patient care. They should not be released from restriction from occupation until local or state regulations are met. According to Cruickshank (1990), “a food handler who is symptomatically ill with a gastrointestinal illness with *S. typhi* and *S. paratyphi* presents a real hazard and should be excluded from work. Also six consecutive negative fecal cultures are required for clearance for those whose work involves preparing or serving unwrapped foods or food not subject to further heating (Public Health Laboratory Service, 2004). Each stool must be obtained one week apart starting three weeks after treatment is completed. For case contacts, two negative stools 48 hours apart after a case has commenced treatment are required. According to Heymann (2004), “household contacts should not be employed until two consecutive negative stool and urine cultures taken 24 hours apart have been obtained. Furthermore stool specimen for culture should be collected for all contacts to a case and from anyone assessed to have had similar exposure to the case in the month preceding the onset of disease in the case (Public Health Laboratory Service, 2004).

When a child or staff member of child care setting is identified to be infected with *S. typhi* he or she should be excluded from attendance until three consecutive stool cultures are negative. Specimens from all other attendees and staff should be cultured and if positive, exclude until three consecutive negative results post treatment (Stephens and Levine, 2002). It has been suggested by a joint collaborative project of ‘The American Academy of Pediatrics, American Public Health Association and National Resource Center for Health and Safety (2002) that in child care settings, caregivers with “diarrhea defined as three or more stools in 24 hour shall be excluded from childcare. Exclusion for acute diarrhea shall continue until diarrhea stops or stools are deemed non-infectious by appropriate health official. Children who develop diarrhea should be isolated from other children pending arrival of parent who should remove them from the facility. In the case of *S. typhi*, three negative stool cultures are required before return to school setting. The American Academy of Pediatrics (2005) recommended “temporary exclusion” when a child has diarrhea. Exclusion is necessary until diarrhea resolves, cases of *S. typhi* requires three negative stool cultures and clearance from health professional or health department. Return to school is recommended 24 hours after first normal stool (Richardson *et al.*, 2001). According to American Academy of Pediatrics (2005), “when *S. typhi* serotype infection is identified in a symptomatic child care attendee or staff member, stool cultures should be collected from other attendees and staff members, and all infected people should be excluded”. The recommended

length of exclusion varies with the infected person's age; for children younger than 5 years of age, three negative stool specimens are recommended for return. For persons of 5 years of age and older, 24 hours without a diarrheal stool is recommended before return to a group setting. Knowledge of carrier status and other likely sources of transmission will help, also, set up proper preventive measures. This will also hopefully prompt and implement an effective control measures in this locality.

2.13.2 Food and Water Hygiene.

Filtration and chlorination of water are effective methods of interrupting water-borne diseases such as typhoid (Shoenen, 2002; Tulchinsky *et al.*, 2000). The control of typhoid fever was greatly enhanced by the treatment of water supplies for general consumption; however, this was not an absolute control since the organism does readily establish a carrier condition in recovering patients, and in some of these individuals the condition may be permanent (Gutherie, 1991). The most important safeguards are good food handling practices and water sanitation. Snow, (2006) recommended the following precautional measures for travelers to typhoid endemic area: drinking of bottled water, boiling of tap water for at least 5 minutes before drinking, cooking, or brushing teeth with it, avoiding ice in beverages and in desserts and treats that contain ice, eating well-cooked food that is still steaming hot. They also advised against eating raw foods, including garden or fruit salads but rather vigorously

wash the outside of the fruit then peeling fresh fruits before eating them after washing hands with soap and water. Foods sold by street vendors are also to be avoided. In Sokoto, Nigeria Ameh and Okpara (2004) attributed high prevalence of typhoid fever disease to ignorance, low standard of personal hygiene and insanitary environment and therefore, advocated Public Health education campaign as a necessity for the control of the disease. Sur *et al.*, (2006) also identified improved personal hygiene, among others, as public health measures that could help to prevent and control typhoid fever. An accurate picture of the distribution and epidemiology of the disease will also be necessary as a tool for organizing, managing and utilizing the scarce health care resources for the control of the infection. Data on the prevalence and distribution rate will help to determine the magnitude of the disease and potential high risk, respectively. For application of proper and adequate curative measures, it has become necessary to accurately diagnose and determine the prevalence of typhoid disease with the confounding symptoms and within the concomitant endemic malaria setting.

2.13.3 Biosafety Level.

Laboratory Hazards may be caused by agent present in feces, blood, gallbladder (bile), and urine. The importance of aerosol exposure is not known (CDC: Bacterial Agents, <http://wwwCdc.gov/OD/ohs/biofly>). Biosafety equipment and facilities are recommended for all activities utilizing known or

potentially infectious clinical materials and cultures. These procedures are recommended for activities likely to generate aerosols or for activities involving production of large quantities of organisms (CDC: Bacterial Agents, <http://wwwCdc.gov/OD/ohs/biofly>). Licensed vaccines have been shown to protect 70-90% of recipients. This may be a valuable adjunct to good safety practices in personnel regularly working with cultures or clinical materials which may contain *S. typhi* (CDC, 2001).

2.13.4: Vaccination

Vaccination is another approach to control and eradication of typhoid fever. There are moderately effective vaccines for *Salmonella typhi*, with weak cross-protection against *S. paratyphi* A. Connor and Schwatz, (2005) recommended the use of vaccines for travelers to endemic areas: even if the travel is for short periods (Steinberg *et al.*, 2004). Mass vaccination campaigns have been used to lower the risk of disease in India and Thailand, but their use in the rest of the developing world is otherwise limited or non-existent. A report from the then epidemic in Tajikistan advocated mass vaccination (Tarr *et al.*, 1999). A recent report from an urban slum community in Delhi, India showed the high prevalence of typhoid fever and therefore recommended more widespread vaccination exercise (Bahl *et al.*, 2004). Centre for Disease Control (2001) recommends typhoid vaccination for people traveling to developing countries in Africa, Asia, the Indian subcontinent, Central and South America, and the

Caribbean. A vaccine for *S. paratyphi A* is under development (McClelland *et al.*, 2004). Acquired immunity to *S. typhi* infection is both humoral and cellular but is incomplete, allowing for re-infections and this restricts the efficacy of the vaccines (Levine *et al.*, 2001; Mastroeni and Manoger, 2003). The humoral immunity vaccine requires one injection with booster in three years and confers protection within 7-10 days of inoculation. Parenteral whole cell vaccines, formally used, resulted in significant local and systemic reactions (Garmory *et al.*, 2002). However, two new vaccines are in current usage: parental capsule polysaccharide based on vi antigen and oral live attenuated vaccine containing strain TY21a. The Two vaccines are available in the United States: a live attenuated (Ty21a strain of *S. typhi*) vaccine given orally and an Intra-muscular vaccine (typhi Vi). Although the immunogenic properties of wild type *S. typhi* are maintained when Ty21a is grown under appropriate conditions, the galE phenotype contributes to strain attenuation *in vivo*. As a result of the mutagenesis method used during the generation of the vaccine strain, further spontaneous mutations were generated. These mutations included the *via* and *ilvD* genes, leading to the loss of the Vi capsular polysaccharide and an auxotrophic phenotype for isoleucine and valine, respectively, and a mutation precluding H₂S utilization. An additional mutation in the *rpoS* gene, which also contributes to the avirulence of the Ty21a strain, was inherited from wild type parental strain Ty2. It is likely that the poor capacity of Ty21a to survive starvation conditions and resist various environmental stresses results, at least in

part, from the *rpoS* mutation (Guzman *et al.*, 2006). On the other hand, the TY21a requires several doses and is only moderately immunogenic and its efficacy is reduced by simultaneous anti-malarial therapy, though reports from Gabon contradict this effect (Faucher *et al.*, 2002). The oral vaccine is taken in multiple doses (Snow, 2006). The current Vi and TY21a vaccines are not licensed for use in children less than 2 years, in whom its efficacy is not proved, and therefore are deemed unsuitable for expanded immunization programs which target infants in their first year of life (Levine, 2000). The one-dose parenteral vaccine is an option for children of ages 2-6, immunocompromised patients, and those who may not adhere to the oral dosage regimen (Snow, 2006). TY21a vaccine is, currently, the only licensed live oral vaccine against typhoid fever (Guzman *et al.*, 2006). The additional mutations present in Ty21a are instrumental in the added safety level of Ty21a. Clinical trials have also shown either a limited and transient level or a complete lack of shedding in the stools of volunteers depending on the administered dose of Ty21a. The inability to culture Ty21a from the small intestine suggests that the strain has a limited ability to proliferate *in vivo*. Neither person-to-person transmission nor invasion of the bloodstream has been observed in vaccinees. The very low excretion rate of Ty21a combined with its genetic attenuation significantly reduces its ability to survive in humans and the environment (Guzman *et al.*, 2006).

The oral vaccination is generally associated with lower rates of side-effects and higher acceptance. A systematic review for the Database showed that the

new vaccine has significantly reduced side effects and efficacy: the efficacy rate being only 50% in comparison to the older whole cell vaccines (Engels and Lau, 2000). The efficacy of Ty21a was assessed in a large number of clinical trials, with over 500,000 vaccinated adults and children. Excellent tolerability and an overall protective efficacy of 67-80% have been demonstrated. The field studies conducted in Santiago (Chile) confirmed the efficacy and tolerability of Ty21a, and provided evidence of indirect protection (herd immunity). The incidence of typhoid fever fell in the placebo control group in the first field trial during the years in which Ty21a field trials were performed and started to rise again when the vaccination was not carried out (Guzman *et al.*, 2006).

Two possible mechanisms have been suggested for the herd immunity effect of Ty21a. Firstly, individuals vaccinated with Ty21a have significantly reduced excretion of virulent *Salmonella* in comparison with the non-vaccinated population, thereby resulting in reduced contamination of water supplies. Secondly, fewer temporary carriers (i.e., children with sub-clinical or incubating acute infections) may reduce the transmission of the disease. The optimum booster schedule for Vivotif vaccine has not been determined. Efficacy has been shown to persist for at least 7 years. Further, there is no experience with Vivotif vaccine as a booster in persons previously immunised with parenteral typhoid vaccine. The excellent safety and tolerability profile of Ty21a was further confirmed in more than 200 million vaccinees during its over 20 years use worldwide (Guzman *et al.*, 2006). (Kirkpatrick *et al.*, 2005) noted that these

current vaccines do not also afford protection against paratyphoid strains and this has thereby evoked the search for better vaccines.

The Disease of the Most Improved (DOMI) project is undertaking a randomized cluster vaccination program in Asia which should help to clarify the effects of mass typhoid vaccination (Acosta *et al.*, 2005). The World Health Organization appears to advocate mass vaccination in endemic areas (WHO, 2003; WHO and UNICEF, 2006). However, it is sad to note that most measures taken to prevent transmission has been restricted to industrialized countries and localized epidemics (Katz *et al.*, 2002). Preventive measures were also mainly provided to travelers returning from endemic areas (Mermin, 1998). Vaccination against typhoid fever is not the main preventative measure. Indeed, avoidance of potentially contaminated food and drink should be the basic advice given to travellers, because the ingestion of a large inoculum of *S. enterica typhi* may result in infection despite immunization. Vaccination protects only 50% to 80% of travelers (Papadimitropoulos *et al.*, 2004).

CHAPTER THREE

MATERIAL AND METHODS

3.1 STUDY AREA

The study took place in Enugu, capital city of Enugu State, south east of Nigeria. Enugu State is made up of mainly rural, urban and some peri-urban units with a population density of about 248 persons/m² and a total population of persons 2,452,996 (1991 population). The state has an area of 8,022.95km³. The urban units of the state capital are zoned into Enugu east, west, North and south. Majority of the urban and the peri-urban dwellers lack access to portable and safe drinking water.

3.2 SUBJECTS AND SAMPLE POPULATIONS SELECTION

The representative population was defined in terms of clinical features and risk of exposure (population at risk), which included subjects who have been previously exposed (James and Schaffer, 1974). The population was also defined in terms of sex, age and occupational characteristics. The occurrence and distribution of the disease was described by place and persons and eventually identifying those characteristics associated with presence or absence of the enteric fever disease in the individuals. The place characteristics were the urban and the sub-urban demographic area.

Awareness campaigns were made in the market, schools and churches. Subjects were sensitized and voluntarily enrolled in the studies having met the eligibility criteria for the clinical and the non-clinical studies. The subjects were, therefore, drawn from hospital clinic attendees, private laboratory clients, public markets, schools and churches. Each subject completed a questionnaire for personal data and information on clinical status. For the illiterate, information was obtained verbally wherever applicable. The information on the form was used to establish a demographic link wherever necessary. These were subsequently enrolled in the studies.

Criteria for selection of subjects for clinical studies included the provisional diagnosis of typhoid by the clinician and the symptoms expressed by the subjects in narrating the history of illness. The common symptoms considered in the selection were persistent fever, headache and abdominal disturbances. The subjects included in the research, therefore, were those at the acute or convalescent stage of the disease. The individuals enrolled as control were apparently asymptomatic. Thus a total of 765 subjects were enrolled in the studies.

Subjects enrolled to be screened for carrier status included those who had been diagnosed of or had suffered symptoms of typhoid in the past, about six months back, and had recovered; and were no more experiencing the clinical symptoms as of the time of enrollment. A total of 1060 subjects were selected for the studies.

For the case study population, participants for this study were recruited through demographic linkage. Reports of some incessant typhoid fever outbreaks were obtained for some area (peri-urban settlements) and within some families. All the respondents who lived within the vicinity/household and fed from the similar source were included in the study. These included 7 subjects living at Udi sidding. They complained of having several episode of typhoid fever every year since 1991 and 2000, respectively. Most of them were apparently ill during sthe time of this survey. Five subjects manifested severe and typical symptoms, while 2 subjects were asymptomatic as of the time of sampling. Most subjects complained of previously having more than one episode within the one year period. Their common source of drinking water was pipe-borne water. Two other subjects were also invoved in the cohort follow-up studies. Both rarely had their meals at home but rather at the market, from commercial food vendors (“*mama put*”).

The cohort surveilliance studies took place for a period of about 2 years. During the period 2-5 samples each were obtained from subjects within 1 year and tests were repeated each time. Subjects that were positive for the typhoid fever infections had susceptibility test carried out, and were referred to the physicians/clinician for appropriate medication. After the full course of treatment, a period of 3 months was allowed to elapse before the test was repeated. Those that had relapse or reoccurrence of symptoms had the test repeated all over before being placed on another course of treatment. Suspected

water samples consumed by the subjects during the study period were also subjected to antibiotics sensitivity testing.

3.3 COLLECTION OF SAMPLES

3.3.1 Blood and Stool Samples

Blood were drawn aseptically using 10 ml syringe and needle (Weintein, 1996). About 8 ml of blood were collected from adult and 1-3 ml from each child into vacutainer (plain sterile 15 ml volume stoppered glass tubes). The blood was allowed to clot and after centrifugation the serum was separated from the clot. The serum was used for serological screening while the clot was poured into sterile universal containers and minced with scissors. About 2 ml of bile salt broth was added to the clotted blood and incubated. About 3 ml of the blood of patients were also introduced into EDTA anticoagulant bottles. Each tube was carefully labeled with the patients number already indicated in the laboratory request forms.

For collection of stool samples the subjects were provided with clean wide-mouthed containers with screw covers or sterile containers with wooden spatular. Peanut-sized stool specimens were introduced into these and taken to the laboratory without delay.

3.3.2 Water Samples

(A) Tap Water

The outside nozzle of the tap was cleaned with 70% alcohol to remove any grease or dirt. The tap was turned on full and allowed to run to waste for about a minute. The tap nozzle was sterilized using the flame from spirit lamp and allowed to cool. The sample bottles were filled by a gentle flow of water and the cap replaced.

(B) Stream and Shallow Surface Water

The cap and cover of the sterile sample bottles were aseptically removed, and the mouths of the bottle faced towards the flow of water. The necks of the bottles were plunged downwards 30 cm below the water surface and then tilted slightly sideways to let them fill completely before carefully replacing the caps. Where there was no current the bottles were pushed horizontally until they were filled (Colle *et al.*, 1996)

3.4 LABORATORY ANALYSIS:

3.4.1 Serological Test

(A) Widal (Qualitative) Slide agglutination Test Method.

Commercially prepared stocks of the widal agglutination kits were stored at 2-8°C in the refrigerator. Prior to use, the antigens and the controls were brought out and stood on the bench to attain a steady room temp. The vials were resuspended by aspirating dropper several times to obtain a thorough mixing. A

drop of serum was placed into each row of circles on paper cards. One drop of each of the negative and positive control sera were dispensed into two additional circles. One drop of the appropriate antigen suspension was added to each circle next to the sample to be tested. Disposable stirrers or applicator sticks were used to mix and spread over the entire area enclosed by the ring, using separate stick for each mixture. Next, the mixtures were rocked gently on the card for a period of two minutes. The reactions were observed immediately under a suitable light source for any degree of agglutination as positive reaction.

(B) Quantitative Widal (Tube Agglutination) Test

Six clean plain tubes were placed in racks in two rows and 0.9 ml of normal saline (0.85% sodium chloride) was delivered into the first tube in each row and 0.5ml into others. Next, 0.1 ml of the test sera were delivered into the first tubes and mixed. Quantities of 0.5 ml volume of the diluted sera from the first tubes were delivered into the second tubes to obtain two-fold dilutions. This procedure was repeated up till the last tube (tube 6) and 0.5 ml of the diluted sera was discarded from tube 6. (At this stage the serial doubling dilutions of 1 in 20 to 1 in 640 was obtained). Similar set-up was prepared for the positive and negative control sera. Then, 0.5 of appropriate antigen suspension was added to each (containing serum dilution). The two racks were shaken carefully to mix the antigens and the sera. Experiments with somatic O antigen were incubated at 50°C for 4 hours in water bath while those containing the flagella H antigen

were incubated for 2 hours in the same temperature. After incubation the result of control tubes were read first by examining the pattern of the sediment and then shaking the tubes gently. Negative reactions showed no visible clumping. Suspension also showed typical swirl when the tubes were flicked. The positive results showed partial or complete agglutinations with variable degree of clearing of the supernatant fluid. The highest dilution of the sera in which agglutinations occurred was taken as titres for the positive reactions. When there was no agglutination the result was recorded as negative or <1:20 titre.

3.4.2 Bacterial Isolation

(A) Processing of Blood Samples

Clot culture technique was used as against standard cultural techniques. The clots were lysed by mincing with sterile scissors to free any salmonella organisms trapped in the clot. About 5 ml of the prepared bile salt broth were pipetted into each of the containers. These were incubated overnight at 37°C for 18 to 24 hours. After this subcultures were made from each of the containers unto freshly prepared and dried SSA (Biotec) and incubated at 37°C.

(B) Processing of Stool Samples

The stool samples were first inoculated into the enrichment medium (Selenite-F broth) and after incubation for 24 hours at 37°C each was sub-

cultured into Salmonella-Shigella agar. The SSA plates were incubated overnight at 37°C and examined for growth.

(C) Processing of Water Samples

About 100mls of each water sample were filtered through a membrane which retained the bacteria on its surface. The membrane was removed aseptically and placed on the Salmonella-Shigella agar media. This was incubated at 37°C for 24 hours. After incubation the colony growth of each water sample was counted and recorded.

3.4.3 Characterization of Isolates.

(A) Sugar fermentation tests

Nutrient broth cultures were prepared. Bijou bottles containing the basal medium and appropriate prepared carbohydrate (mannitol, maltose, dulcitol, sucrose and glucose) were inoculated with drop of the nutrient broth suspension of the test isolate and were loosely capped and incubated at 35°C overnight. Each was observed for change in colour from amber to red and for gas production (in the medium filled inverted Durham tube).

(B) Urease Test

The test organisms were inoculated heavily on the entire slope surface of the urea agar slants prepared in capped tubes. The tubes were placed in racks and incubated at 37°C up to 48 hours. Tubes were thereafter examined for change of colour from plain to pink.

(C) Hydrogen Sulphide production

Test organisms were inoculated into the triple sugar iron agar slants contained in test tubes. These were incubated at 35°- 37°C for up to 48 hours.

After incubation the TSI agar media were checked for blackening and change in colour from amber to red at the bottom (butt) of the tube.

(D) Serological Identification (Serotyping).

Suspected colonies were picked and sub-cultured onto moist nutrient agar slopes in MacCartney bottles. These were incubated for minimum of 4 hours.

One to two loopfulls of the agar cultures were mixed with normal saline on clean microscope slides to form a paste. A drop each of the O and H polyvalent sera were added and further mixed with the organisms on the slide. Positive results were indicated by visible agglutination within 30 seconds. Slide tests were repeated for the positive cultures using single factor sera.

3.4.4 Haematological Assays.

(A) Measurement of haemoglobin (Haemiglobincynade technique)

Each sample was dispensed in 0.02 ml volumes into 4 ml Drabskin's diluting fluid. The tubes were stoppered, the content mixed and left at room temperature protected from sunlight, for 4 to 5 minutes. A yellow green filter (Liford 605) was placed in colorimeter and wavelength set at 540 nm. The colorimeter was set to zero using the Drabskin's fluid and the absorbances of the samples were read. The haemoglobin values were read using calibration table.

(B) White cell count

Approximately 0.38 ml of the white cell diluting fluid (weak acid and gentian violet) was dispensed into small tubes. Exactly 0.02 ml of well mixed EDTA anticoagulated venous blood samples were added to the tubes. The solutions were remixed using bulb Pasteur pipettes. The grids of the already assembled counting chamber were charged with cell suspension and the chamber was left undisturbed for 2 minutes for the cells to settle. The cells were microscopically examined with $\times 10$ objective lens and those contained in four large corner squares were counted and the counts per litre of blood estimated

(C) Blood film Preparation and Microscopic Examination for Malaria Parasites

Thin blood films were made and allowed to dry and fixed with absolute methanol for 2 minutes. The fixed blood films were covered with undiluted Leishman's stain and stood for further 2 minutes. The stain was double diluted with buffered saline water (pH 6.8). This was mixed with the plastic bulb pipette and the stain was allowed to stay for another 8 minutes. The stain was washed off with tap water. The slides were placed on the draining rack and allowed to dry and the films were examined using x 10 objective lens.

3.4.5 Antibiotic Sensitivity Testing

(A) Standardization of Bacterial Inoculum (McFarland Nephelometry)

One percent (v/v) solution of sulphuric acid (BDH) and 1% barium chloride (w/v) were each prepared separately in distilled water; then 0.6 ml of the 1% BaCl₂ and 99.4ml of the 1% H₂SO₄ solutions were mixed. The resultant turbid suspension of BaSO₄ was used for standardizing the inocula of the *Salmonella* isolates and the control strains tested for antibiotic susceptibility.

(B) Modified Agar Disc Antibiotic Sensitivity Testing

Sensitivity testing was carried out according to the National Committee on Clinical Laboratory Standards (2006). An 18 h culture of the test strain in nutrient broth was diluted to 0.5 McFarland standard (= 1×10^6 cfu/ml) and

0.2ml dropped on sterile Muller Hinton agar. Inoculum was spread evenly over the agar surface, allowed to dry and selected antibiotic discs aseptically placed and gently pressed on to the agar. Incubation was at 37°C for 24hour. Inhibition zone diameters (IZD) of the active antibiotics were measured and an interpretative chart was used to interpret the results.

(C) Statistical Analysis

Data were collected, computed and analysed statistically using SPSS, version 15.0. Chi-square formulas were used for two by two contingency tables while the students T-test were used for frequency calculation. Measurement of association between ranks of variables was by correlation test of Kendall rank correlation coefficient. The corelation tests were also carried out to determine the Pearson coefficient (r) value. The values of 0.05 and 0.001 properbility were determined at the 95% (5%) and 99.9% (0.1%) confidence limits, respectively.

CHAPTER FOUR

RESULTS

4.1 Distribution of *Salmonella enterica* variants of O and H antibody.

Distribution of *Salmonella enterica* variants among symptomatic and asymptomatic individuals in the sample population was studied using serological method (Widal test) and culture of stool and blood of the individuals. The panel of antigen could discriminate against O and H antigens of *S. paratyphi A*, *paratyphi B*, *paratyphi C* and *S. typhi D*.

Among the 557 samples from symptomatic cases screened, distribution of sera positive for O and H antibodies at titre of $\geq 1:160$ was as follows; 56 (10.1%) for *S. paratyphi A*, 35(6.3%) for *S. paratyphi B*, 30 (5.4%) for *S. paratyphi C* and 118 (21.2%) for *S. typhi D*. Similarly, among the 208 asymptomatic individuals screened, 7(3.4%) had O and or H antibodies against *S. paratyphi A*, 6 (2.9%) for *S. paratyphi B*, 3 (1.4%) for *S. paratyphi C* and 16(7.7%) for *S. typhi D* (Table 3). The proportions in the total sample population (symptomatic and asymptomatic) that tested positive (titre of $\geq 1:160$) for the O antibody singly for each serovar were *S. paratyphi A* (3.7%), *S. paratyphi B* (7.7%), *S. paratyphi C* (4.3%) and *S. typhi D* (17.5%); and for H antibody ($\geq 1:160$), *S. paratyphi A* (1.9%), *S. Paratyphi B* (1.6%), *S. paratyphi C* (1.6%) and *S. typhi D* (7.2%.); (Figure 2).

Looking at the frequency distribution of titres of H and O antibodies against *S. paratyphi* A (Figure 3) majority of the samples had no detectable antibodies (titre $\leq 1:20$); but at 1:20, 1.0% of the individuals were positive for H antibody and 1.6% for O; at 1:40 it was 2.2% (H) and 2.4% (O); at 1:80, 2.5% (H) and 3.1% (O); at 1:160, 1.6% (H) and 3.3% (O), while at 1:320 they were 0.3% (H) and 0.4% (O). Similar distribution for *S. paratyphi* B showed greater proportion of individuals to have O antibody titre at dilution of 1:160 (7.6%). The distribution of subjects positive at other dilutions was 1:20 (1.8%), 1:40 (2.5%), 1:80 (5.9%) and 1:320 (1.0%); (Figure 4). For *S. paratyphi* C the greater proportion of individuals (2.9% and 2.4%) tested positive for respective O and H titres of 1:80 titres; whereas the least occurring titre for both antibodies was 1:320 (Figure 5).

Frequency distribution of antibody titres against *S. typhi* D showed some individuals (0.3%) exhibiting O antibody at titre of 1:640; a greater proportion (13.3%) of the subjects had O antibody titre of 1:160. For H antibody the highest frequency (7.8%) was at 1:80 titres and the least (2.1%) was at 1:320 (Figure 6).

Table 3 Frequency of Occurrence of *Salmonella enterica* O and H antibody variants in the samples of symptomatic and asymptomatic subjects (Titre \geq 1:160)

Subjects.	Total number of samples	Paratyphi A (%)	Paratyphi B (%)	Paratyphi C (%)	Typhi D (%)
Symptomatic	557	56(10.1)	35(6.3)	30(5.4)	118(21.2)
Asymptomatic	208	7(3.4)	6(2.9)	3(1.4)	16(7.7)
Total	765	63	41	33	134

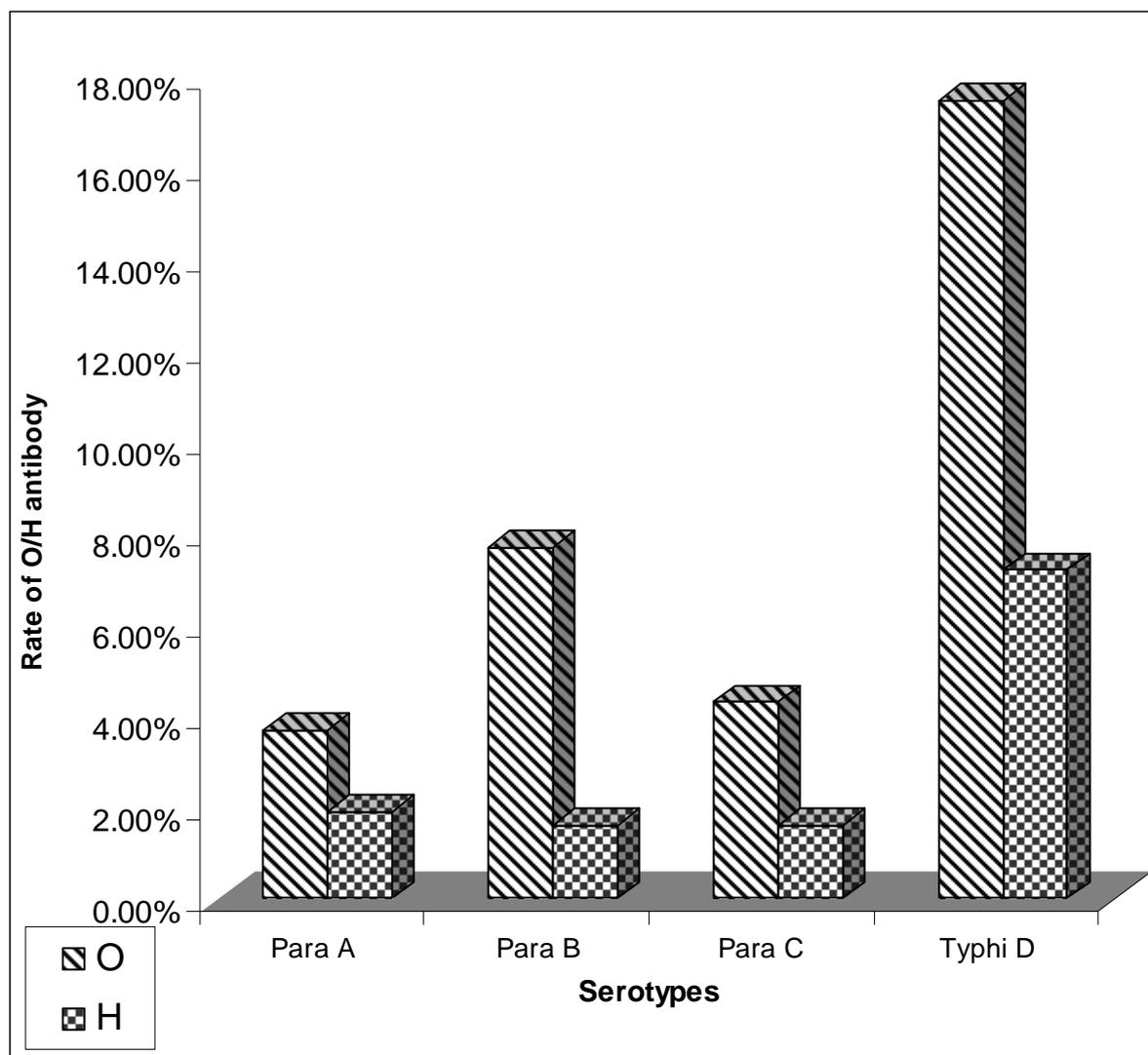


Figure 2: Distribution of *Salmonella enterica* serotypes of O and H Antibody in Subjects (Titre \geq 1/160).

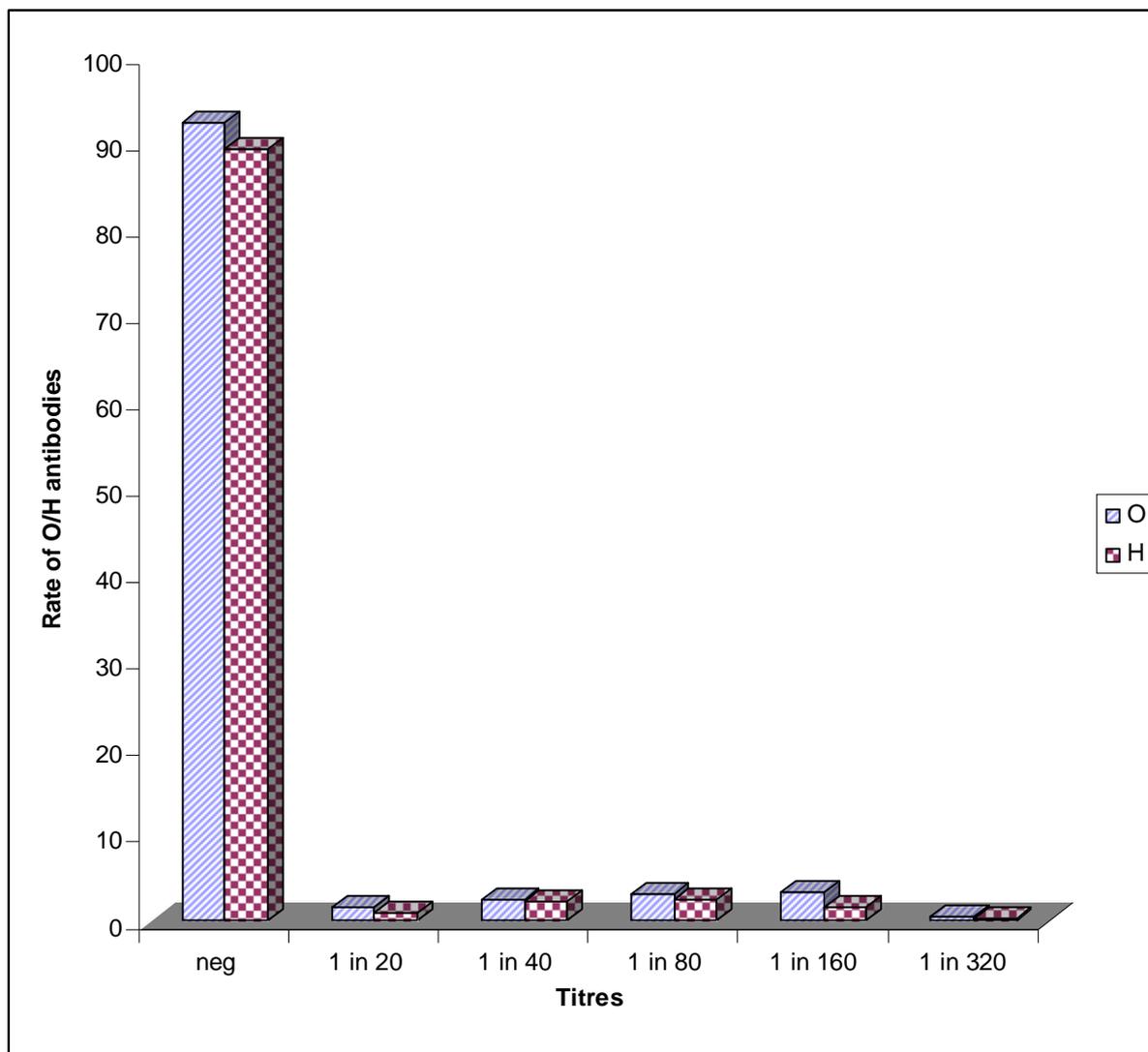


Figure 3: Distribution of *Salmonella* O and H Antibody of *S. paratyphi* A Variant in the Blood Samples of Subjects by Titre Dilutions of Widal Screening Test.

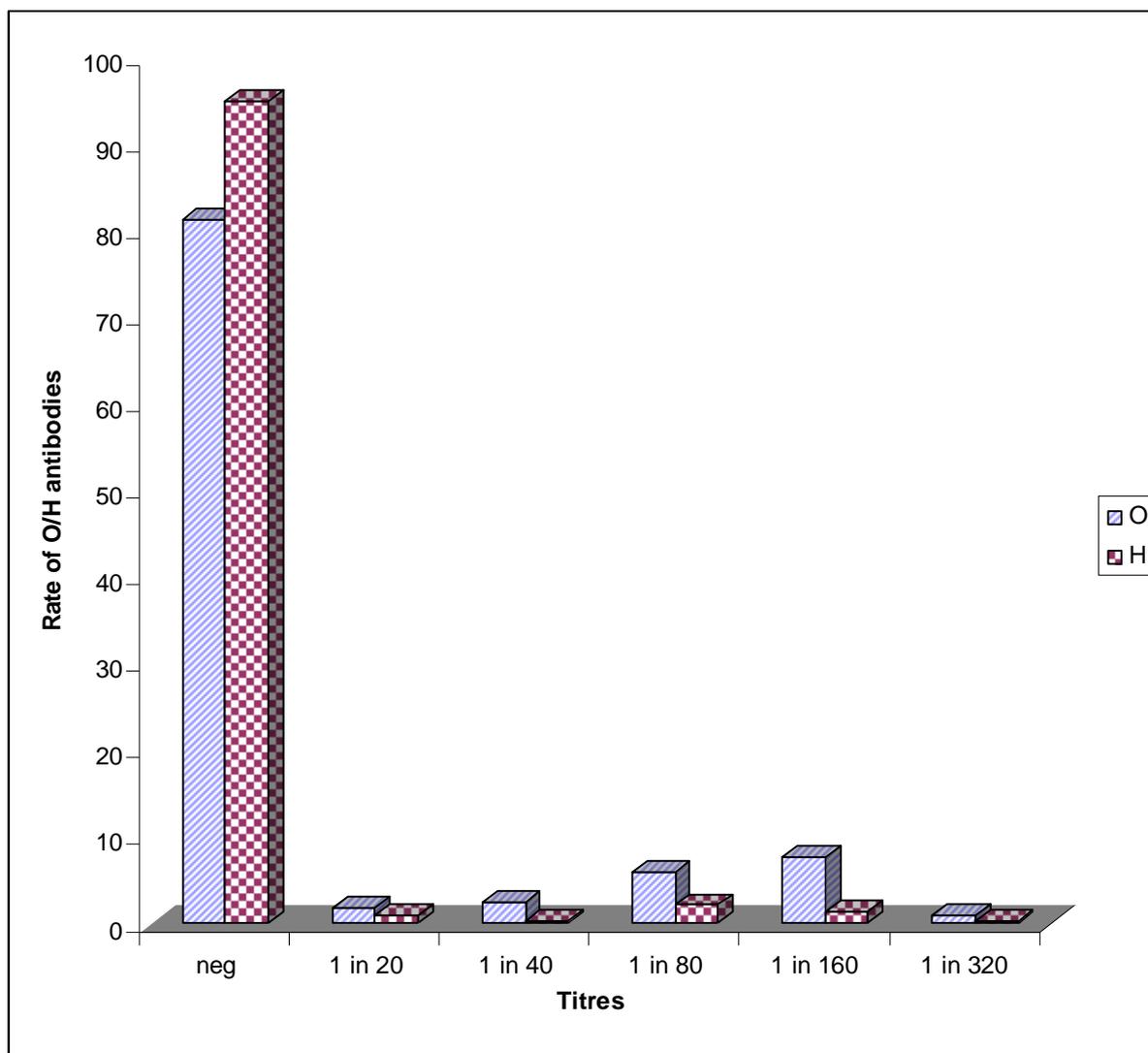


Figure 4: Distribution of *Salmonella* O and H antibody of *S. paratyphi B* Variant in the Blood Samples of Subjects by Titre Dilutions of Widal screening test.

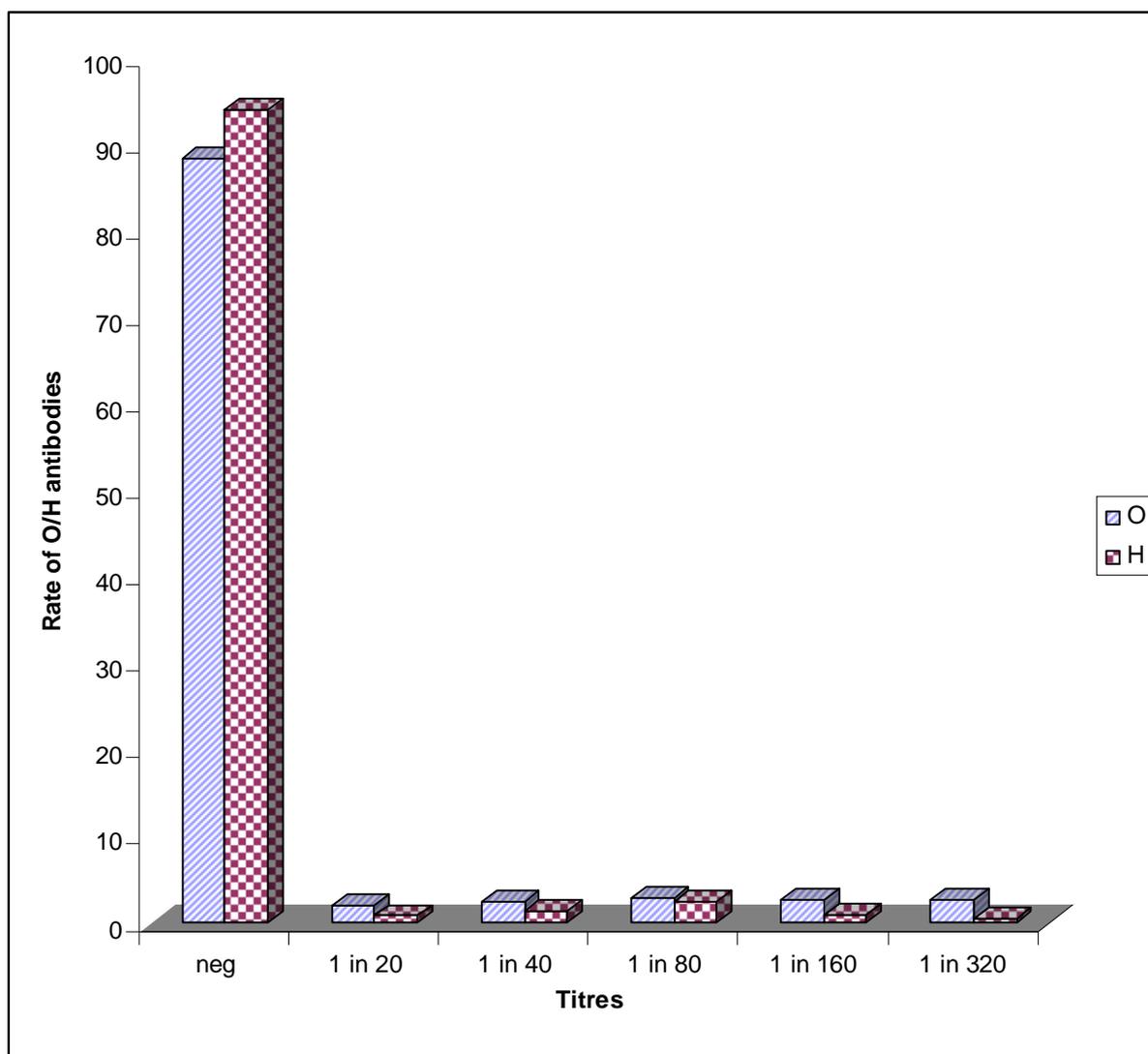


Figure 5: Distribution of O and H Antibody of *S. paratyphi* C Variant in the Blood Samples of Subjects by Titre Dilutions of Widal screening test.

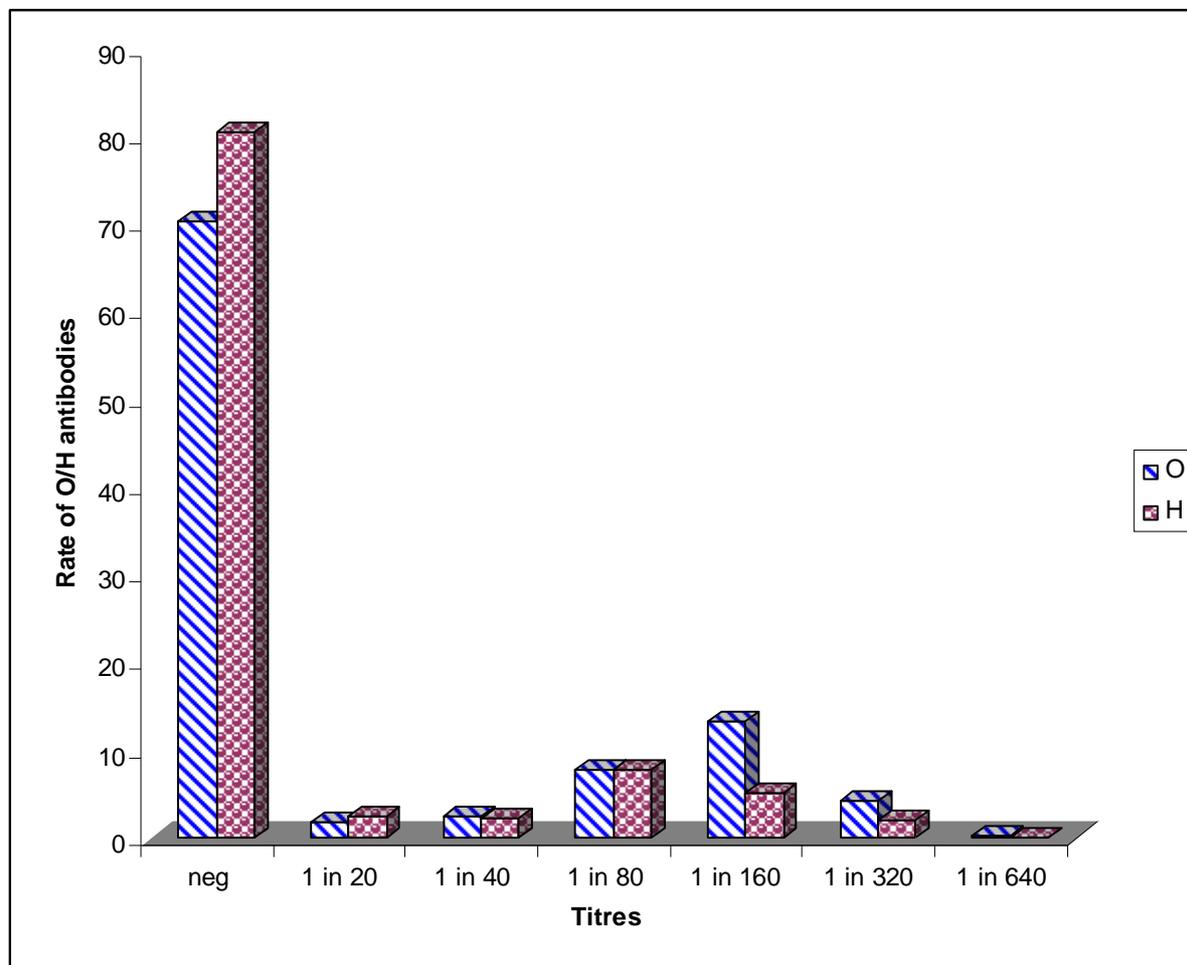


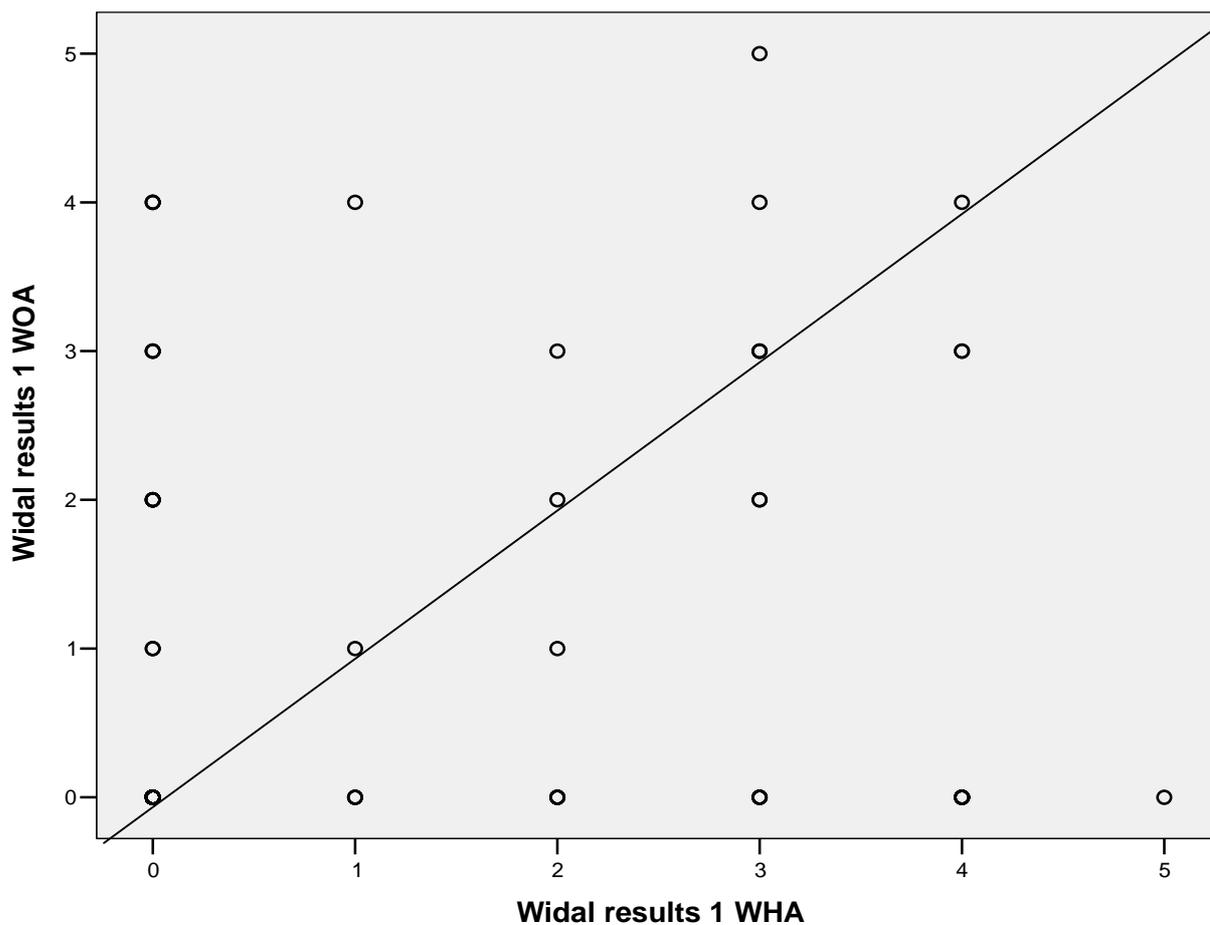
Figure 6: Distribution of O and H Antibody of *S. typhi* D Variant in the Blood Samples of Subjects by Titre Dilutions of Widal screening test.

4.2: Correlations of the O and H Antibodies

A Pearson coefficient, r , of 0.370 at a probability level of 0.001 shows a negative correlation between the O and H antibody titres of *S. paratyphi A* (Table 4); similar correlation is shown in the scatter plot (Figure 7). Similar data were obtained for the other variants as shown in Tables 5, 6, 7 and Figures 8; 9, 10, respectively. The r value for *S. paratyphi B*, *S. paratyphi C* and *D* O and H antibody were 0.304 and 0.317, respectively. There was a slight correlation between the O and H antibodies of *S. typhi D*.

TABLE 4 Statistical Values for Widal test Correlations of O and H Antibodies of *Salmonella paratyphi* A Variant

	Widal results WOA	Widal results WHA
Widal results WOA		
Pearson Correlation	1	.370(**)
Sig. (2-tailed)		.001
N	364	364
Widal results WHA		
Pearson Correlation	.370(**)	1
Sig. (2-tailed)		.001
N	364	364



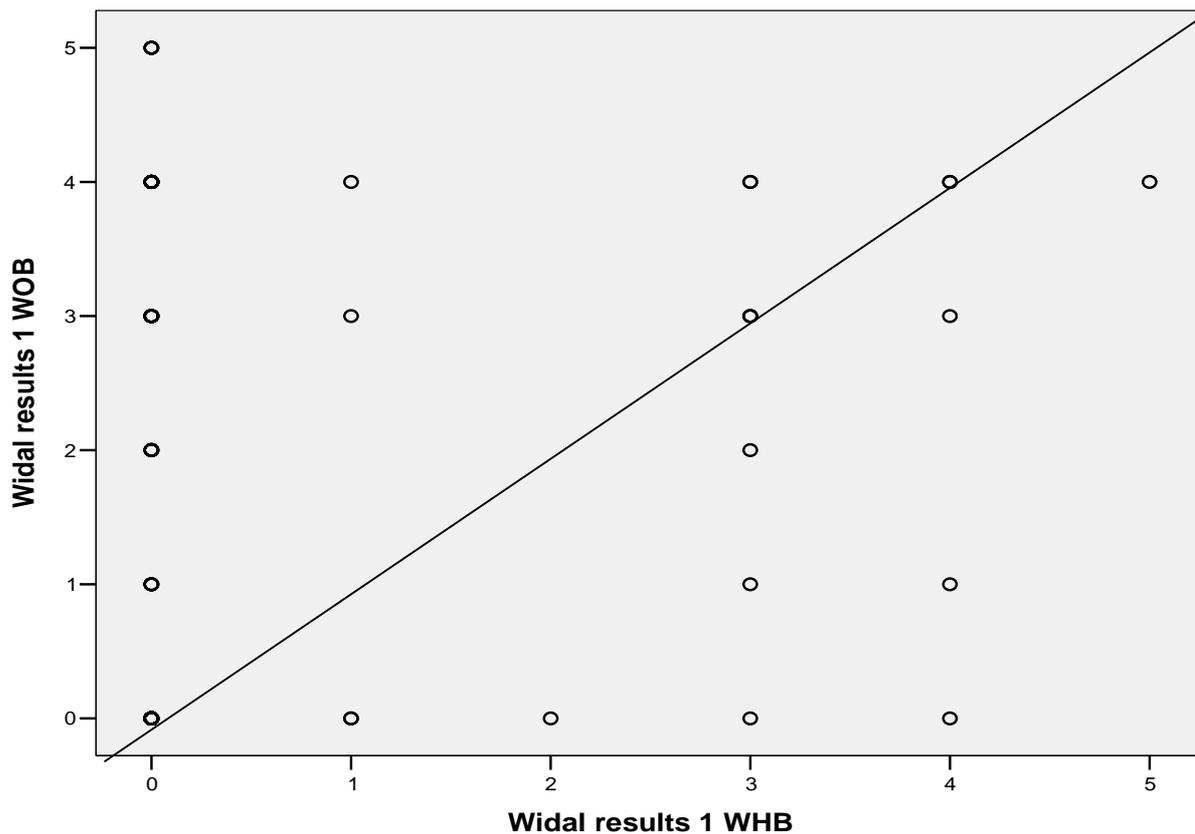
$$Y = \frac{1}{2}(n_1(n_1-1) + n_2(n_2-1)), \quad X = \frac{1}{2}(n_1(n_1-1) \div n_2(n_2-1))$$

$$r = \frac{S/V}{(1/2 N(N-1) - X) \sqrt{(1/2 n(n-1) - Y)}}$$

Figure 7: Scatter plot showing the statistical co-efficient of O and H antigens of *Salmonella paratyphi A* variants

TABLE 5 Statistical Values for Widal Test Correlations of O and H Antibodies of *Salmonella paratyphi B*

	Widal results WOB	Widal results WHB
Widal results WOB		
Pearson Correlation	1	.304(**)
Sig. (2-tailed)		.001
N	364	364
Widal results WHB		
Pearson Correlation	.304(**)	1
Sig. (2-tailed)		.001
N	364	364



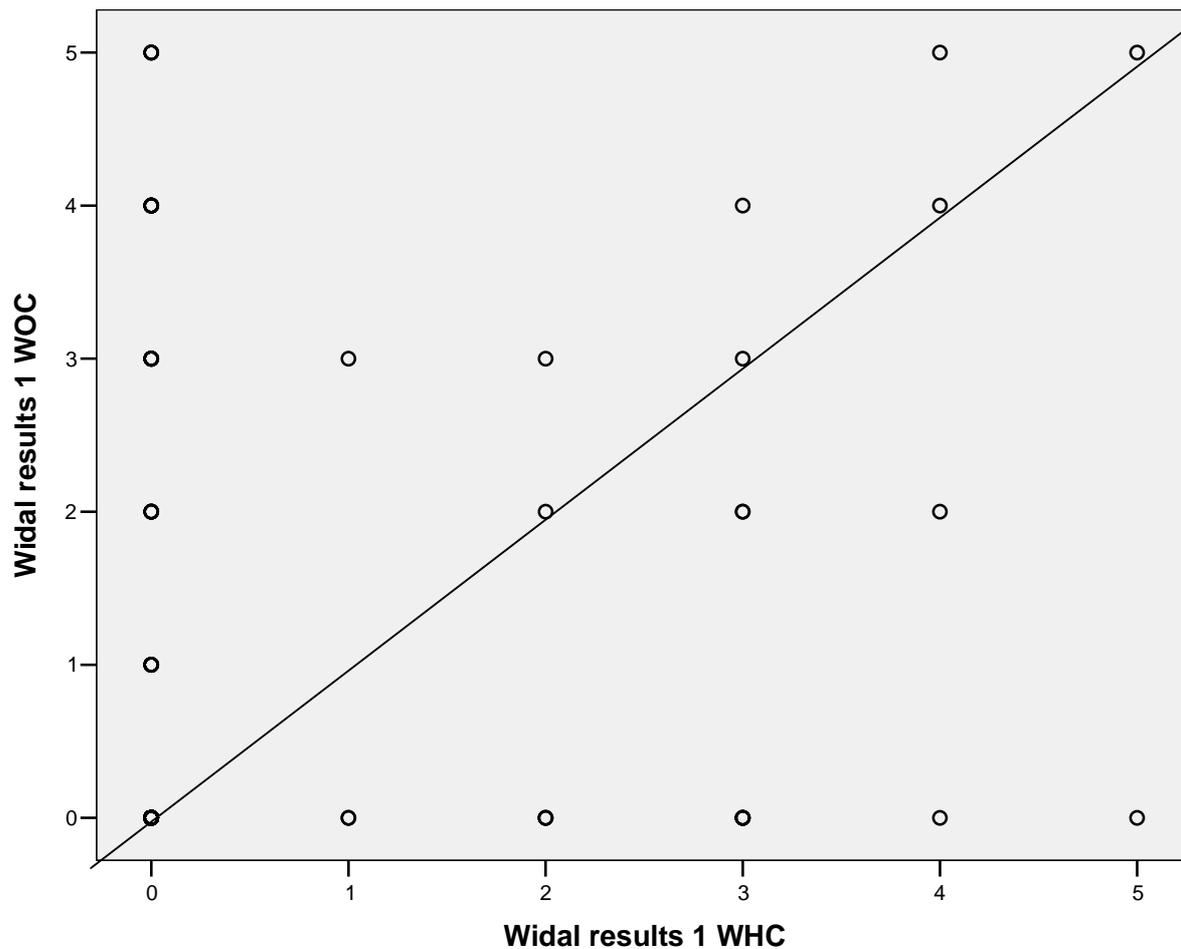
$$\bar{Y} = \frac{1}{2}(n_1(n_1-1) + n_2(n_2-1)), \quad \bar{X} = \frac{1}{2}(n_1(n_1-1) + n_2(n_2-1))$$

$$r = \frac{S}{\sqrt{V}} \left(\frac{1}{2} N(N-1) - X \right) \sqrt{\frac{1}{2} n(n-1) - Y}$$

Figure 8: Scatter Plot Showing the Correlation Coefficient of O and H Antibodies of *Salmonella paratyphi* B Variants.

TABLE 6 Statistical Values for Widal Test Correlations of O and H Antibodies of *Salmonella paratyphi C* Variant

	Widal results WOC	Widal results WHC
Widal results WOC		
Pearson Correlation	1	.317(**)
Sig. (2-tailed)		.001
N	364	364
Widal results WHC		
Pearson Correlation	.317(**)	1
Sig. (2-tailed)		.001
N	364	364



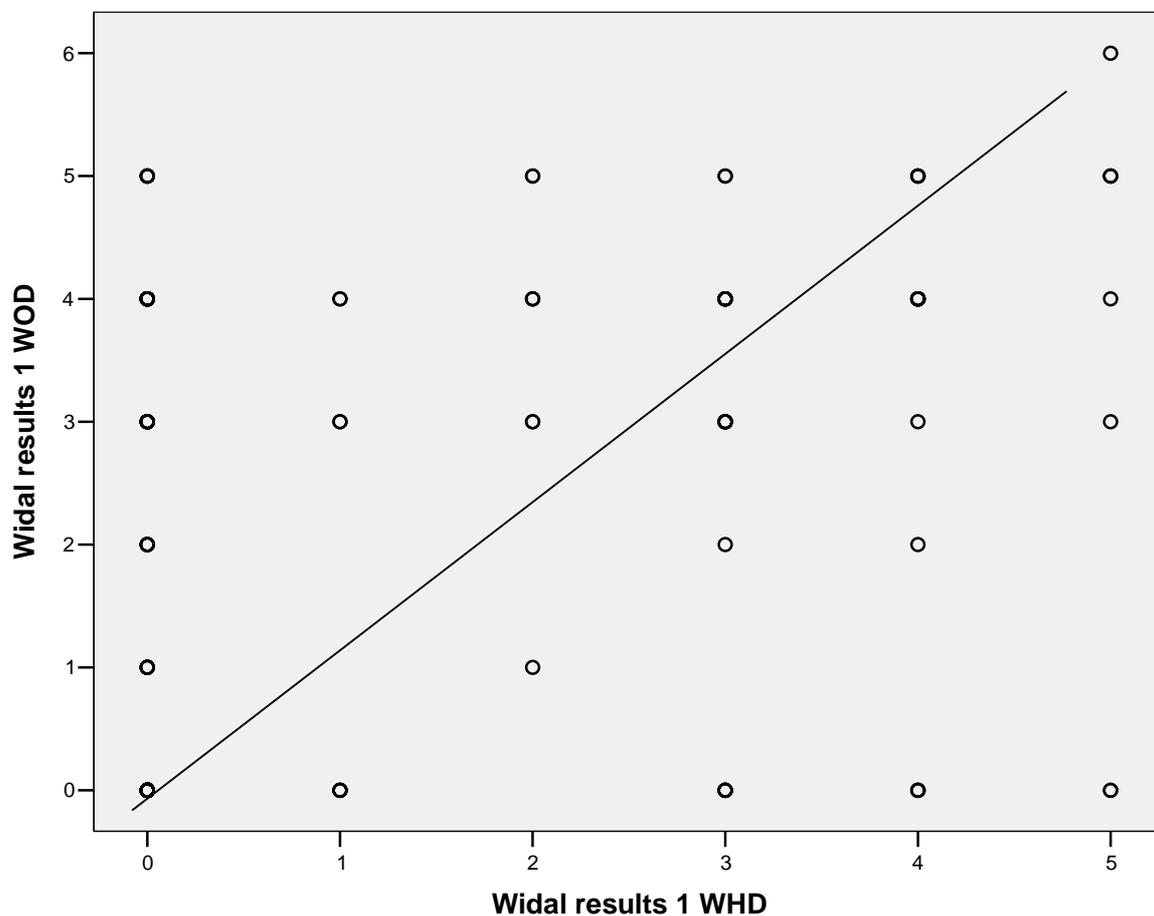
$$Y = \frac{1}{2}(n_1(n_1-1) + n_2(n_2-1)), \quad X = \frac{1}{2}(n_1(n_1-1) \div n_2(n_2-1))$$

$$r = \frac{S}{V} \sqrt{\frac{1}{2} N(N-1) - X} \sqrt{\frac{1}{2} n(n-1) - Y}$$

Figure 9: Scatter Plot Showing the Correlation co-efficient of O and H Antibodies of *Salmonella paratyphi* C variant

TABLE 7 Statistical Values for Widal Test Correlations of O and H Antibodies of *Salmonella typhi* D Variant

	Widal results WOD	Widal results WHD
Widal results WOD		
Pearson Correlation	1	.530(**)
Sig. (2-tailed)		.001
N	364	364
Widal results WHD		
Pearson Correlation	.530(**)	1
Sig. (2-tailed)		.001
N	364	364



$$Y = \frac{1}{2}(n_1(n_1-1) + n_2(n_2-1)), \quad X = \frac{1}{2}(n_1(n_1-1) \div n_2(n_2-1))$$

$$r = \frac{S/V}{(1/2 N(N-1) - X) \sqrt{(1/2 n(n-1) - Y)}}$$

Figure 10: Scatter Plot Showing the Correlation Coefficient of O and H Antibodies of *Salmonella typhi* D Variants

4.3: Comparative Diagnoses of Typhoid Fever using Widal test, Blood and Stool Culture Methods

Out of the 765 subjects evaluated for enteric fever illness, 164(21.5) were positive by stool culture singly, 125(16.3) by blood culture singly and 75(9.8) by both stool and blood cultures (Figure 11). Table 8 shows the isolation rate of the salmonella organism from the blood and stool samples. Age group 31-40 years had the highest rate of isolation from stool (35.4%); age group 21-30 years had the highest rate from blood (25.2%) while the highest rate of isolation from both stool and blood was recorded among the age group of 41-45 years (20.2%).

A total of 107 (19.7%) *S. enterica* isolates were from the blood of symptomatic individuals and 18 (9.8%) from the asymptomatic; but from stool cultures, 90 (16.2%) were isolated from the symptomatic and 74 (35.6%) from the asymptomatic (Table 9). Among symptomatic subjects blood isolates, 72(21.8%) were from females and 35(15.4%) from males; and from stool samples, 58(17.6%) were from female and 32(14.1%) from males. The isolate from both blood and stool of female was 34(10.3%) and that of male was 31(13.7%) (Table10). There was significant difference between the isolation rate from blood of symptomatic and asymptomatic males (0.001) similar statistical value was obtained in comparing number of isolates from both their blood and stools. There was also a significant statistical difference between the values obtained from symptomatic and asymptomatic female blood samples

($p=0.005$). The total incidence rate of typhoid fever in males and females was 32.8% and 42.8% respectively (Table 11).

The rates of recovery of *S. paratyphi* A, B, C and *typhi* D from stool and blood cultures of symptomatic individuals were matched with respective antibody titres. For *S. paratyphi* A the titre with highest isolation rate was at 1:160 for stool (37.5%), blood and stool combined (39.1%) at 1:80, (Table 12). For *S. paratyphi* B the titres with peak isolation rates were at 1:160 (46.7%) by stool and culture (Table13). The symptomatic subjects stool samples singly yielded *Salmonella paratyphi* C isolates with corresponding titres as follows: 1/20 (1.3%), 1/40 (10%), 1/80 (4.3%), 1/160 (27.8%) and 1/320 (16.7%). The titre with peak isolation rates for *paratyphi* C from blood sample singly was 1:320 (41.7%); from stool sample singly it was 1:160 (27.8%) while that from both blood and stool was 1:80 (39.1%); (Table14). In the case of *S. typhi* D the peak isolation rate was from blood (34.6%) at titre of 1:320 (Table 15). Blood sample yielded 7.1%, 10.5%, 7.4% and 17.8% and 34.6% at titre of 1/20- 1/320.

Table 16 displays the rate of false positive and false positives, sensitivity, specificity rates and the positive and negative predictive values of Widal screening method. The false negative was 19.7 % while false positive result was 8.4 %. Sensitivity rate of Widal test was 88.4% and specificity rate was 72.4% Positive predictive value (PPV) and negative predictive values (NPV) were 74.8% and 52.7%, respectively.

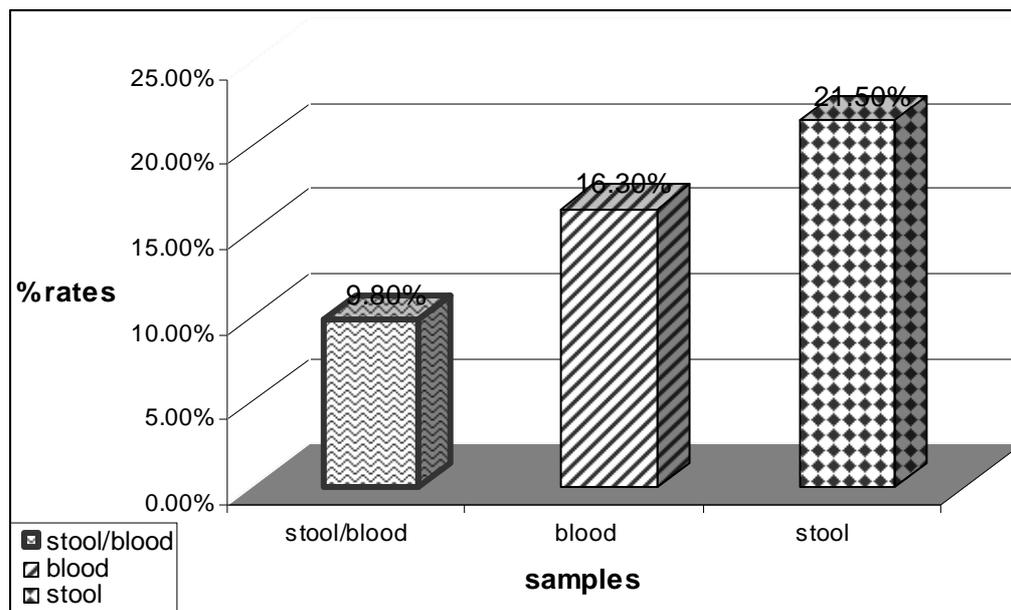


Figure 11: Distribution of *Salmonella enterica* in Stool, Blood, and Blood/stool of Subjects.

TABLE 8 Isolation of *Salmonella enterica* from samples of subjects of various age groups using stool and blood cultures

Age groups	Total no. screened	Stool culture Positives (%)	Blood culture Positives (%)	stool/blood culture Positives (%)	Total Positives (%)
0-11 mths	4	0(0.0)	0(0.0)	(0. 0)	0
1-10 yrs	39	3(7.7)	7 (17.9)	2 (5.1)	12
11-20 yrs	147	24 (16.3)	35 (23.8)	13 (8.8)	72
21-30 yrs	131	25(19.1)	33 (25.2)	15 (11.5)	73
31-40 yrs	175	62 (35.4)	21 (12.0)	17 (9.7)	100
41-50 yrs	104	27(25.9)	17 (16.3)	21 (20.2)	65
51-60	83	16 (19.2)	12 (14.5)	6 (7.4)	34
61-70	60	7(11.7)	0 (0.0)	1 (1.7)	8
71-80	22	1 (4.5)	(0.0)	(0 .0)	1
Total		164	125	75	364
Std. Dev.		0.475	0.449	1.032	

TABLE 9 Distribution of *Salmonella enterica* in Blood Samples of the Symptomatic and Asymptomatic Subjects.

Subjects.	Nos.Screened.	Positive in stool (%)	Positive in blood (%)	Positive in stool/blood (%)
Symptomatic	557(72.8)	90(16.2)	107(19.2)	65(11.7)
Asymptomatic	208(27.2)	74(35.6)	18(8.7)	10(4.8)
Total value	765(100.0)	164(21.4)	125(16.3)	75(9.8)
P value		0.035	0.001	0.001

Table 10 Distribution of *Salmonella enterica* in Samples of Symptomatic and Asymptomatic Subjects by Gender

Subjects	Negative (%)	Stool pos (%)	Bld pos (%)	Stl/bld pos (%)	Total
Male (symp)	129(56.8)	32(14.1)	35(15.4)	31(13.7)	227(64.1)
Male (asymp)	58(45.7)	52(41.0)	12(9.4)	5(3.9)	127(35.9)
Total	187(52.8)	84(23.2)	47(13.3)	36(10.2)	354(100.0)
P value		0.082	0.001*	0.001*	
Female (symp)	166(50.3)	58(17.6)	72(21.8)	34(10.3)	330(80.3)
Female (asymp)	48(59.3)	22(27.2)	6(7.4)	5(6.2)	81(19.7)
Total	214(52.0)	80(19.5)	78(19.0)	39(9.5)	411(100.0)
P value		0.436	0.005*	0.044*	

Table 11 Distribution of *Salmonella enterica* by Sex in Sample Population

Subjects	Total	Nos. Positives	% Positive
Male	354	132	37.3
Female	411	160	38.9
Total	765	292	38.2

P = 0.103

Table 12: Evaluation of Symptomatic Subjects for *Salmonella enterica* Serotype *paratyphi* A Infection Using Stool and Blood Cultures

Antibody Titre.	Nos. positive at titre	Nos (%). Positive by stool culture	Nos. (%) positive by blood culture	Nos. (%) positive by stl and bld cultures
1/20	15(2.6)	0(0.0)	0(0.0)	6(2.0)
1/40	16(2.9)	1(6.3)	1(6.3)	2(12.5)
1/80	22(3.9)	2(9.1)	7(31.8)	13(39.1)
1/160	48(8.6)	18(37.5)	4(8.3)	10(20.8)
1/320	8(1.4)	1(12.5)	0(0.0)	2(25.0)
1/640	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total	109	22 (20.2)	12 (11.0)	33 (30.3)
Correlation		0.234	0.040	0.249
P-value		0.001	0.566	0.001

**Table 13: Evaluation of Symptomatic Subjects for *Salmonella enterica*
Serotype *Paratyphi B* Infection Using Stool and Blood Cultures**

Antibody Titre.	Nos. positive at titre	Nos (%). Positive by stool culture	Nos. (%) positive by blood culture	Nos. (%) positive by stl and bld cultures
1/20	8(1.4)	1(0.0)	0(0.0)	0(0.0)
1/40	17(3.1)	1(5.9)	4(23.5)	4(23.5)
1/80	20(3.6)	1(5.0)	5(25.0)	2(10.0)
1/160	15(2.7)	3(20.0)	1(6.7)	7(46.7)
1/320	2(0.4)	0(0.0)	1(50.0)	1(50)
1/640	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total	80	6 (7.5)	11 (13.8)	14 (17.5)
Correlation		0.-037	0.110	0.125
P-value		0.763	0.078	0.027

Table 14 Evaluation of Symptomatic Subjects for *Salmonella enterica* Serotype *Paratyphi C* Infection Using Stool and Blood Cultures.

Antibody Titre.	Nos. positive at titre	Nos (%) Positive by stool culture	Nos. (%) positive by blood culture	Nos. (%) positive by stl and bld cultures
1/20	14(2.5)	2(1.3)	1(7.1)	4(2.6)
1/40	17(3.1)	0(10)	1(5.7)	6(35.5)
1/80	23(4.1)	1(4.3)	2(8.7)	9(39.1)
1/160	18(3.2)	5(27.8)	6(33.3)	4(22.2)
1/320	12(2.0)	2(16.7)	5(41.7)	1(8.3)
1/640	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total	84	10 (11.9)	15 (17.9)	24 (28.6)
Correlation (r)		0.060	0.116	0.210
P-value		0.242	0.001	0.001

**Table 15 Evaluation of Symptomatic Subjects for *Salmonella enterica*
Serotype *Typhi D* Infection Using Stool and Blood.**

Antibody Titre.	Nos. positive at titre	Nos (%). Positive by stool culture	Nos. (%) positive by blood culture	Nos. (%) positive by stl and bld cultures
1/20	14(2.5)	0(0)	1(7.1)	2(14.3)
1/40	19(3.4)	2(5.3)	2(10.5)	2(10.5)
1/80	54(9.6)	3(7.4)	4(7.4)	6(11.1)
1/160	90(16.2)	9(23.3)	16(17.8)	2(2.2)
1/320	26(4.7)	6(15.4)	9(34.6)	2(7.7)
1/640	2(0.4)	0(0.0)	0(0.0)	0(0.0)
1/1280	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total	205	20(9.8)	32 (15.7)	14 (6.8)
Correlation		0.256	0.309	0.235
P-value		0.001	0.001	0.001

TABLE 16 Sensitivity and specificity rates of the Widal,

Total	True pos. (%).	False pos. (%).	True neg. (%).	False neg. (%).	PPV.	NPV.	Sens. Rate	Spec. Rate
765	190 (24.8)	64 (8.4)	168 (22.0)	151 (19.7)	74.8	52.7	88.4	72.4

$$\text{Sensitivity rate} = \frac{\text{true positive}}{\text{true pos.} + \text{false neg.}} \times 100$$

$$\text{Specificity rate} = \frac{\text{true negative}}{\text{true neg.} + \text{false pos.}} \times 100$$

$$\text{Positive predictive value} = \frac{\text{true positive}}{\text{True pos.} + \text{false pos.}} \times 100$$

$$\text{Negative predictive value} = \frac{\text{true negative}}{\text{True neg.} + \text{false neg.}} \times 100$$

4.4: Distribution of *Salmonella enterica* infections in the various Localities.

Table 17 shows the total incidence rate of typhoid fever in the three years. The distribution in 2006, 2007 and 2008 were 35.9%, 34.0% and 40.8%, respectively. Subjects in Peri-urban area had the highest incidence of infection in 2006 and 2007(39.7% and 40.9%).

There was an increasing incidence rate of total typhoid illness in 2008(40.8%). During 2006 and 2007, peri-urban population had the highest incidence of typhoid (39.7% and 40.9%, respectively), but in 2008 Enugu North recorded the highest incidence of 44.8%. The overall incidence was highest among the peri-urban population (40.7%). There was, however, a progressive increase in the incidence of typhoid within Enugu East, Enugu North and Peri-urban populations during the three years of study

**TABLE 17 Annual Distribution of Salmonella enterica Cases in Subjects
Population by Localities**

Localities.	2006				2007		2008	
	Total tested.	Total pos (%).	Nos tested (%).	Nos pos (%).	Nos. tested (%).	Nos. pos (%).	Nos. tested (%).	Nos pos (%)
Enugu West	105	37(35.2)	26	8(30.7)	33	10(30.3)	46	19(41.3)
Enugu East	130	50(38.5)	43	16(37.2)	37	14(37.8)	50	20(40.0)
Enugu North	133	48(36.1)	54	16(29.6)	50	19(38.0)	29	13(44.8)
Enugu South	203	78(38.4)	65	25(38.5)	68	26(38.2)	70	27(38.6)
Peri-urban	194	79(40.7)	63	25(39.7)	66	27(40.9)	65	27(41.5)
Total	765	292(28.2)	251	90(35.9)	254	96(34.0)	260	106(40.8)

4.5 Comparative Distribution of *Salmonella enterica* and Plasmodium species

Blood smears of subjects with confounding typhoid/malaria symptoms were stained for malaria parasite microscopy and other pathologic features. Typical morphological appearances of plasmodium species indicated cases of malaria diseases. Findings of monocytes and some neutrophils having brown-black pigments also aided the diagnosis. Neutrophils containing darkly staining coarse granules and some vacuoles were considered as toxic granulation. These were also considered as abnormality involving left shift of the neutropils commonly seen in acute bacterial infections and inflammatory conditions like typhoid fever. Subjects within the age range of 21-30 years were mostly infected with *Salmonella typhi/paratyphi* while children within 0-11 years were mostly positive for malaria parasites (Table 18). In 11.4% of symptomatic cases there was concomitant typhoid and malaria -11.7% male and 11.1% female. The male and female subjects had 11.7% and 11.1% typhoid/malaria co-existent, respectively. Singly, 27.4% males were diagnosed with typhoid and 10.1% with malaria; similarly 28.2% females were sick with typhoid and 14.2% with malaria. Altogether, there were 237 (47.4%) confirmed cases of typhoid and 118 (23.6%) cases of malaria (Table 19).

Figure 12 shows that the highest percentage (38.0%) of *S. enterica* strains was isolated from the stool samples of the patients concomitantly affected by typhoid and malaria. A rate of 29.0% typhoid fever co-infection was obtained using Widal test (titres \geq 1:160), 11.0% with blood culture singly and 22.0% with dual culture positive

blood and stool samples. Enugu North had the highest rate of typhoid infection (56.1%) while Enugu west recorded the highest rate of malaria infection (34.1%). (See table 21). Figure 13 shows that the highest rate of typhoid infection (40.0%) was recorded among students while petty traders had the highest rate of malaria infection (51.4%). Table 21 shows the distribution of clinical symptoms. Headache with fever was the most prevalent confounding symptoms; High proportions of patients with these symptoms tested positive for both malaria (21.5%) by microscopy and typhoid fever by Widal test (33.2%) and culture method (32.1%)

Table 18 Age Distribution of Diagnosed Typhoid and Malaria Cases

Age range	Total no. tested (%)	Malaria Pos. (%)	Typhoid pos (%)	Mal\typhoid pos (%)
0-11 mths	4	2(50.0)	0(0.0)	0(0.0)
1-10 yrs	24	2(8.3)	8(33.3)	1(4.2)
11-20 yrs	93	11(11.8)	36(38.7)	12(12.9)
21-30 yrs	113	10(8.8)	50(44.2)	11(9.7)
31-40 yrs	111	24(21.6)	27(24.3)	18(16.2)
41-50 yrs	83	9(10.8)	34(40.9)	10(12.0)
51-60 yrs	50	2(4.0)	20(40.0)	5(10.0)
61-70 yrs	22	1(4.5)	5(22.7)	0(0.0)
Total	500(100)	61(12.2)	180(36.2)	57(11.4)

Table 19 Sex Distribution of Typhoid Fever and Malaria Cases

Sex	Total samples	Total typhoid (%)	Total Malaria (%)	Typhoid/malaria (%)	Typhoid Singly (%)	Malaria Singly (%)
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Male	248	97(39.1)	54(21.7)	29(11.7)	68(27.4)	25(10.1)
Female	252	140(55.5)	64(25.4)	28(11.1)	112(28.2)	36(14.2)
Total	500	237(47.4)	118(23.6)	57(11.4)	180(36.0)	61(12.2)

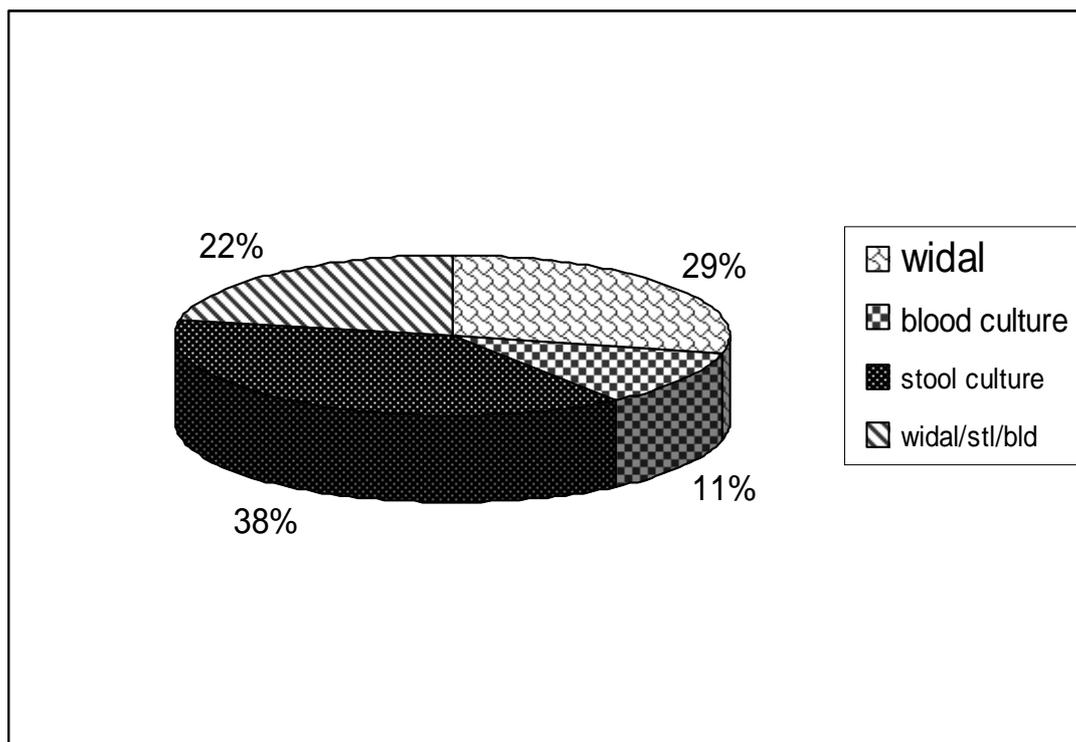


Figure 12: Evaluation of Subjects for *Salmonella typhi/paratyphi* with Malaria Parasites Co-existent Using Widal, Stool and Blood Cultures

Localities	No. of subjects (%)	Typhoid pos (%)	P value	Malaria pos (%)	P value
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Enugu south	142(28.4)	61(43.0)	0.138	33(23.2)	0.688
Enugu North	50(10.0)	29(58.0)	0.338	10(20.0)	0.656
Enugu East	80(16.0)	44(55.0)	0.433	15(18.8)	0.451
Enugu west	65(13.0)	16(24.6)	0.143	22(33.9)	0.398
Peri-urban	163(32.6)	88(54.0)	0.464	30(18.4)	0.113
Total	500(100)	238(47.6)		110(22.0)	

TABLE 20: Distribution of Typhoid and Malaria Cases according to Localities

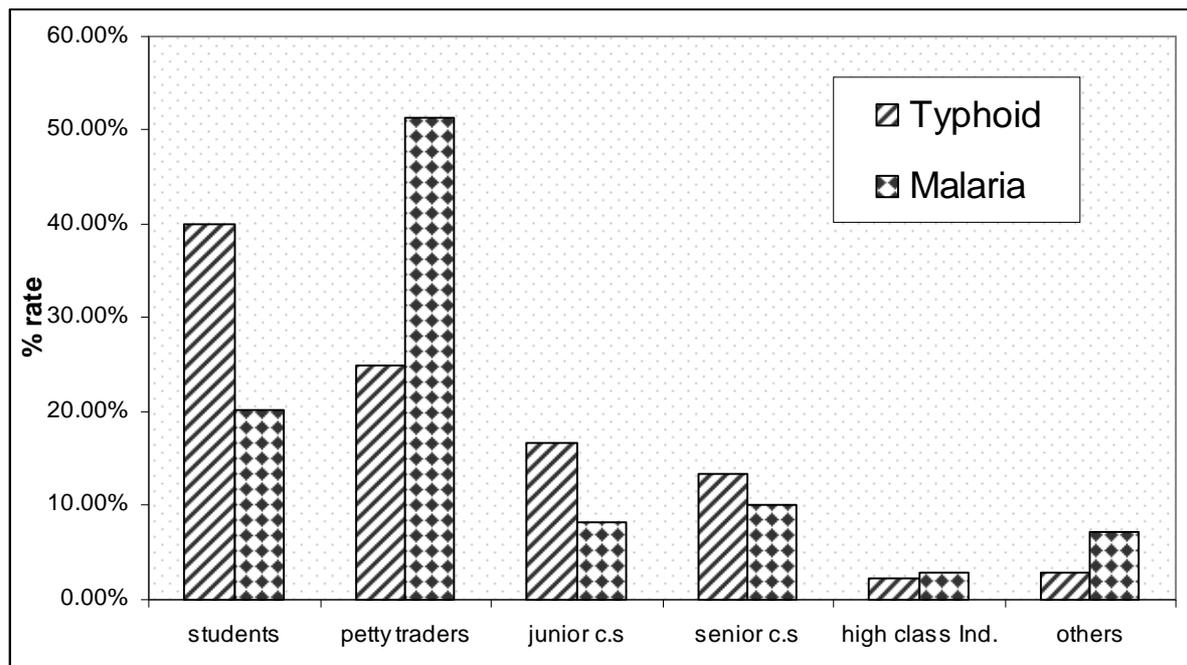


Figure 13: Distribution Rate of Typhoid and Malaria Infections by Occupations of Individuals.

Table 21 Distribution of Patients with Symptoms confounding Diagnosis of Typhoid and Malaria

Symptoms	Malaria parasites. pos. (%)	widal screening. pos. (%)	blood culture. pos (%)	microscopy/widal culture neg.(%)	Total no tested (%)
Headache					
Only	9 (8.0)	4 (3.5)	1 (8.9)	99 (87.6)	113 (22.6)
Fever only	4 (23.5)	1 (5.9)	1 (5.9)	11 (64.7)	17 (3.4)
Headache/ Fever					
	57 (21.5)	88 (33.2)	85 (32.1)	35 (13.2)	256 (53.0)
Headache/fever / abdominal upsets					
	9 (16.7)	2 (3.7)	2 (3.7)	41 (75.9)	54 (10.8)
Other related Symptoms					
	30 (58.9)	7 (13.7)	7 (13.7)	7 (13.7)	51 (10.2)
Total number of samples tested					
	109 (21.8)	102 (20.4)	96 (19.2)	193 (38.6)	500 (100)

4.6 Distribution of *Salmonella enterica* in Carriers/ Household Contacts and Water Samples

Out of the 1060 subjects in the whole population tested for carrier status (480 males and 580 females), 49.0% males and 44.7% females' yielded *S. enterica* (Table 22). Table 23 shows the number and rate of subjects infected by carrier contact within household. The total number of infected subjects within the 15 household was 22 (36.7%) among whom 10 (16.7%) were confirmed carriers. Amongst the 60 subjects living within the 15 household, 23 (38.3%) were exposed. The total number/percentage infected by carrier contact was 9 (39.1%).

The total number of water samples analysed was 1000, consisting of 200 each of 5 different water sources. *Salmonella* species were mostly prevalent in well water (30.0%). The rate of isolation of *Salmonella enterica* from water of other sources was as follows: bottled water (3.5%), sachet water (6.0%), pipe-borne water (8.5%), and tank water (15.0%). See table 24. Figure 14 shows the relative risk and the attack rate resulting from often drinking water from a particular source. Consumption of well water often, gave rise to highest attack rate (60.0%) of typhoid fever infection. The relative risk (RR) was 2.7. There was no risk of infection presented in consumption of only boiled water. The attack (incidence) rate of often consuming water from other sources was as follows: Filtered (18.8%), stream (22.2%), tank (26.6%), pipe-borne (37.1%), sachet (38.7%).

Table 22 Prevalence of Carriers by Gender using Stool Cultures

Gender	Total nos. tested	Nos. positive	Percentage
Males	480	235	(49.0)
Females	580	259	(44.7)
Total	1060	494	(46.6)

Table 23 Carrier Contact Case Rate of Subjects

Households infected	Subjects within households.	Infected subjects (%).	Carriers (%).	Exposed subjects (%)	Subjects infected by contact (%)
1	5	1(20.0)	1(20.0)	4(80.0)	1(25.0)
2	5	0(0.0)	1(20.0)	4(80.0)	0(0.0)
3	4	1(25.0)	0(0.0)	0(0.0)	0(0.0)
4	4	1(25.0)	0(0.0)	0(0.0)	0(0.0)
5	4	1(25.0)	0(0.0)	0(0.0)	0(0.0)
6	6	0(0.0)	3(50.0)	3(50.0)	0(0.0)
7	4	1(25.0)	2(50.0)	2(50.0)	1(50.0)
8	5	3(60.0)	1(20.0)	4(80.0)	3(75.0)
9	4	3(75.0)	1(25.0)	3(75.0)	3(100.0)
10	4	2(50.0)	0(0.0)	0(0.0)	0(0.0)
11	2	2(100.0)	0(0.0)	0(0.0)	0(0.0)
12	3	2(66.7)	0(0.0)	0(0.0)	0(0.0)
13	4	1(25.0)	1(25.0)	3(75.0)	1(33.3)
14	3	2(66.7)	0(0.0)	0(0.0)	0(0.0)
15	3	2(66.7)	0(0.0)	0(0.0)	0(0.0)
Total	60	22(36.7)	10(16.7)	23(38.3)	9(39.1)

Key:

No. of exposed subjects = No. of infected subtracted from number living within the household

No. infected by contact = No. infected who have been in contact with (exposed to) carriers.

Rate (%) infected by contact is calculated per number of exposed.

Rate of infected, carriers and exposed are each calculated per number living within household

Table 24 Distribution of *Salmonella enterica* in water samples

Water sources	Total sample analysed	<i>Salmonella enterica</i> (%)
Bottled water	200	7(3.5)
Sachet water	200	12(6.0)
Pipe-borne water	200	17(8.5)
Tank water	200	30(15.0)
Well water	200	60(30.0)
Total	1000	126(12.6)

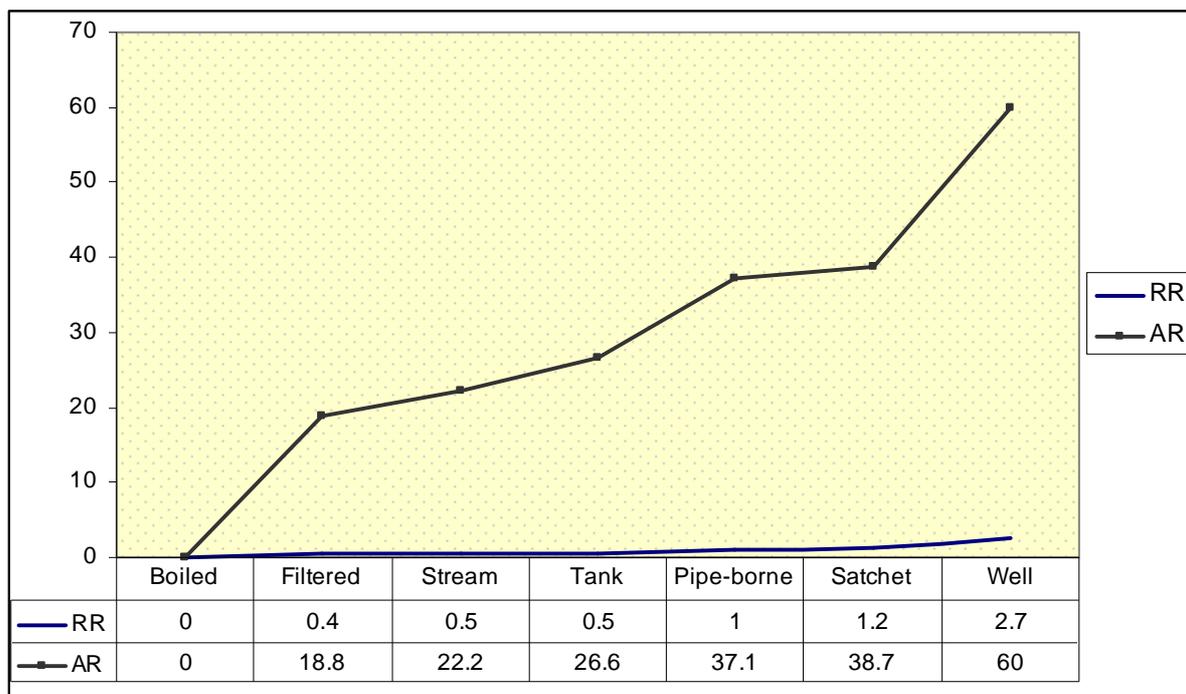


Figure 14: Relative risks and Infection Rates for Domestic Water Consumption

4.7 Follow up/Cohort Study Report

The surveillance study was carried out on two male subjects of age 40 and 46. Both were petty traders at Kenyatta market situated at Uwani urban, close to Achara layout and Maryland urban and sub-urban, respectively, within Enugu South locality. They often ate food from the same source from the commercial food vendor. Widal test analysis of the two subjects showed positive with typhi O antigen up to 1:320 titres. Organisms were also isolated from the blood and stool samples during first cultures. The isolates were sensitive to ciprofloxacin and perflacin antibiotics. The clinician administered them with ciprofloxacin. After treatment the forty year old (subject A) had good prognosis and recovered completely after about a month. He was re-tested after 6 months. Result showed falling titre of 1:80 and negative stool and blood culture. There has been no relapse of re-infection after a year. The 46 year old (subject B) had a relapse. Second test result still gave high titre (1:320) in Widal test result after proper treatment, he was treated again and re-tested, and blood and stool cultures were negative with falling Widal titre. During the year 2007 the test was repeated 4 times, he continually tested positive with either one or all test methods. The whole of his house hold members, who were apparently healthy as of that time, had their stool cultured to check for carrier status. The commercial food vendor where he regularly ate food from the market square was also tested.

Result revealed that two members of the family, the wife and the senior daughter, excreted the organism in the stool. Repeated culture showed the same result. They admitted having the infection sometime in the past. The commercial food vendor in the market also had positive stool culture results. Only the wife accepted medication and tested negative after a year. The daughter and the cook both refused medication. Subject was advised to stop eating food from the commercial source. However, subject B continued to have relapsis of infection after medication. He had another 3 episodes within one year period.

Cohort/surveillance study of subjects fed from contaminated water source was also conducted. The subjects consisted of 7 residents from peri-urban area who used the same water source from busted/perforated pipes for over a period of one year from 2006 to 2007. Subjects' age ranged 2 to > 60 years. All subjects, except the >60 years age woman who had recently visited and spent only 3 weeks then, were either positive with high titre (>1/160) or blood isolates of *Salmonella enterica serotype typhi*. Strains were highly sensitive to ciprofloxacin and perflacin. All subjects, except the 3 and 6 years old boy, were administered ciprofloxacin. The 3 and 6 years old were treated with perflacin. Treatment with the antibiotics yielded good prognosis except in one subject who substituted the ciprofloxacin with ampicillin. He later repeated the test and was administered the right antibiotic dosage. Subjects were advised to stop drinking from the contaminated source and none complained ill again.

Table 25: Cohort Study Result of Subjects ‘A’ with Relapsing Typhoid Fever Symptoms

Samples collected.	Date of analysis.	Widal test.	Blood culture.	Stool culture
1 st	13 th July, 2006	positive	negative	positive
2 nd	5 th August, 2006	negative	negative	negative
3 rd	20 th July, 2007	negative	negative	negative

Table 26 Cohort Study Result of Subjects ‘B’ with Relapsing Typhoid Fever Symptoms

Samples collected.	Date of analysis.	Widal test.	Blood culture.	Stool culture
1 st	15 th Feb., 2006	Positive	positive	positive
2 nd	10 th Feb., 2007	Positive	negative	negative
3 rd	26 th Feb., 2007	Positive	positive	negative
4 th	27 th June., 2007	Positive	negative	negative
5 th	24 th July., 2007	Positive	positive	negative
6 th	28 th Dec., 2007	Negative	negative	positive

Table 27. Cohort/surveillance Study of Subjects Fed from Contaminated Water Source

Age of subjects.	No. of subjects tested.	Nos. positive (%)	Nos. negative (%)
0-10 years	3	3 (100)	0 (0.0)
11-20 years	2	2 (100)	0 (0.0)
21-30 years	0	0(0.0)	0(0.0)
31-40 years	0	0(0.0)	0(0.0)
41-50 years	1	1(100)	0(0.0)
51-60 years	0	0(0.0)	0(0.0)
61-70 years	1	0(0.0)	1(100)
Total	7	6(85.7)	1(14.3)

4.8 Antibiotic Sensitivity

All the isolates were tested against the selected antibiotic and the sensitivity rates/pattern shown in Figure 15. Ciprofloxacin showed activity against the highest proportion of the *Salmonella enterica* isolates (95.7%). Distribution of activity of other antibiotics against the isolates was as follows; Ofloxacin (92.0%), Perflacin (75.3%), Gentamicin (47.8%), Chloramphenicol (40.7%), Ceftriaxone (36.0%), Amoxicillin (30.0%) and Cefuroxin (30.0%). The isolates were not susceptible to Ampicillin and Nalidixic acid.

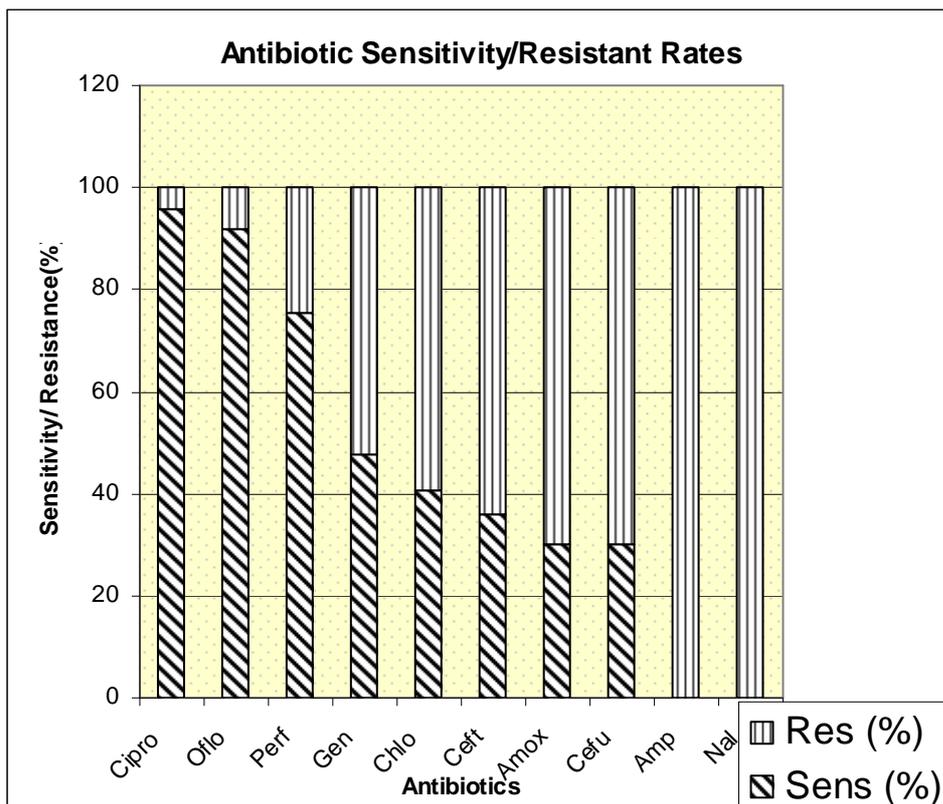


Figure 15: Antibiotic Sensitivity Pattern of the *Salmonella enterica* Isolates

CHAPTER FIVE

DISCUSSION

5.1. Serological Evidence of Typhoid and Paratyphoid in the Sample Population

Serologically (titres $\geq 1:160$) typhoid cases singly, was more prevalent than each paratyphoid case; this is consistent with the observations made elsewhere in Asia and other parts of Africa (Hirose *et al.*, 2001; Smith *et al.*, 2004; Siddiqui, 2006). Dimotrov *et al.* (2007) recorded a prevalence rate of 82.4% O antibody of *S. typhi* D variant at 1:160 titres as against 11.8% for *S. paratyphi* A. The H antibodies of A, B and C variants appear to be more prevalent when taken into consideration with that of O antibody. It would be noted that the anti O agglutinis disappears quickly after recovery but the anti H persists longer in the blood stream after recovery and are still detectable after several years (Le Minor, 2006). This implies that most of the *S. paratyphi* A and B antibodies being detected may not reflect current infection unlike the predominant *S. typhi* D infections shown by antibodies against O antigen. In a study in Nepal *S. typhi* D and *S. paratyphi* A variants were reported to be more common causes of typhoid fever illnesses whereas *S. paratyphi* B infections were rare (Maskey *et al.*, 2008; Porkharel *et al.*, 2006; Bharat *et al.*, 2009). In this study higher titres of the somatic O antibodies against each variant were detected compared with antibodies against the flagellar H antigen, which indicates that the infections

were current. The four-fold rise in the O antibody coupled with the clinical symptoms of the patient, even in convalescent period without clinical symptoms was strongly indicative of infection (Olopoenia and King, 2000).

5.2. Cultural Evidence for infection by Salmonella variants in the Sample Population

Blood and stool samples for culture were selected based on finding the clinical symptoms for enteric fever in patients. However, *Salmonella typhi* D and *paratyphi* (A, B or C) variants were isolated from apparently healthy individuals. The isolation was presumably made at the incubation period. The highly sensitive method of diagnosis (blood clot culture, bile salt enrichment broth and highly selective Salmonella-Shigella agar), employed in this study was a major aid to recovery and isolation of the organism in this early stage of infection. Blood clot culture is highly sensitive when proper isolation method is used. This confirms the ability of this method to accurately detect those infected in the population irrespective of whether they are symptomatic or not. At Zaria, Nigeria, Florence *et al.*, (2004) obtained 22 positive from Widal test and only 1 isolate from blood culture using thioglycolate broths and Macconkey agar. Similarly, at Ebonyi State, Nigeria, using same isolation techniques, Nwuzo *et al.* (2009) 2(0.8%) isolate was obtained by blood culture against 53(21.2%) obtained by widal. This inconsistent figures and low numbers of isolates obtained with blood culture may have been as a result of the inappropriate

technique used. Blood culture for Salmonella is most appropriately carried out using clot culture technique and SSA selective media (Wain *et al.*, 2001). Previous antibiotic intake can, however, reduce the chances of isolation of organism from blood culture. Pearson and Guerrant (2000) however, confirmed this by noting that bacteria could be isolated from blood cultures in 73 to 97% of cases before antibiotic use. The organism is obtained from blood during the first week of the illness (Bhutta, 2006).

Isolation of the Salmonella variants from blood samples of a significantly higher proportion of symptomatic individuals than asymptomatic ones. This is not surprising since the symptomatic cases are in the acute state of infection and additionally. Symptoms of the disease remain positive predictors for choosing which samples to culture in event of scarce resources or large number of samples requiring evaluation. This does not underate the public health importance of isolation of the organism from apparently healthy individuals in an epidemiological survey setting. Most asymptomatic individuals showed no detectable anti-O and/or H antibodies or the antibodies were detected at very low titres for this category of cases and could only be confirmed by isolation of the Salmonella organism from the stool or blood. Colle *et al.* (1996) had reported that about 70% adults formed antibody within the first week of infection. Le Minor (2006) further specifically stated that anti-O agglutinins were detected after eight day of the disease while the anti-H agglutinins showed up after 10 days. It is not very clear why individuals harboring the organism

could not exhibit detectable antibodies or what factors suppressed expression of antibodies when they were provoked. Typically, antibodies to O and H antigens start appearing during the end of the first week and peaks at the end of the third week (Levine *et al.*, 1987). As explained earlier these could infections at the incubation stage which may later manifest the symptoms with rising antibodies. The significance of these findings lies in the chances of missing these cases when Widal test method is employed exclusively for diagnosis of enteric fevers.

The prevalence of salmonella isolates from stools of both symptomatic and asymptomatic subjects may suggest carrier status. Those positive with stool culture alone were likely to be chronic carriers. Such carriers may or may not have had history of typhoid fever. Parry (2008) reported that up to 25% of established chronic carrier cases have no history of clinical enteric fever. Many people become carriers as a result of inadequate medication following diagnosis and self-medication, a practice which is prevalent in Nigeria. There was, however, significant difference in the rate of isolation from stool samples from the symptomatic and asymptomatic individuals. This implies that stool culture may also be of value for the diagnosis of acute infection; though not very specific.

5.3 Determination of baseline titre for the Widal (Serological) Test

Baseline value of Widal test result is determined by comparison of efficiency of isolation methods at various antibody titre levels. Significant

numbers of *Salmonella* organisms were isolated from the blood and blood /stool samples of symptomatic subjects with antibody titre of $\geq 1/160$, confirming that they were undergoing active clinical infections. Organisms were also isolated from the blood and blood/stool of patients who had no detectable or very low ($1/20$) *S. typhi D* antibodies (Table 15). This goes to confirm that one third of typhoid patients mount no detectable antibody response or no demonstrable rise in titre (Parry, 2008). Up to 50% of the patients' blood samples singly; and blood and stool yielded *Salmonella* isolate at $1/320$ antibody titre (by Widal test) for *S. paratyphi B* variant. This titre is, therefore, regarded as the base-line titre for the study population. Using the blood culture singly and stool/blood culture the highest rate of isolation of the *Salmonella* organism were obtained at the titre of $1/80$ for *S. paratyphi A* and *C* variants. The rate of isolation of *Salmonella typhi D* from blood culture was highest (34.6%) among individuals with antibody titres of $\geq 1/320$. These can also be regarded as the base-line titres of these variants for the study population. Endemicity may affect the baseline titre for that population. In such population the baseline titre may be high.

The positive predictive values (PPV) and negative predictive values (NPV) show the ability of the test method to accurately predict the number of positives and negatives in the study population. The 74.8% PPV and 52.7% NPV obtained by Widal test in this study was the resultant effect of the false positive and the false positive result values obtained, respectively; being that the low rate of false positive result gives rise to high percentage rate of PPV. Also

the NPV is higher when the number/rate of the false negative result is less and vice versa (Table 16). The 88.4% sensitivity rate of Widal technique obtained in this study is consistent with the value of 72.5% obtained by Roxas and Mendoza in 1989, but contrasts with that of Ayse *et al.* (2002) which showed a sensitivity and specificity of 52% and sensitivity of 88%.

5.4. Effect of Age and Sex on Infection/Clinical Manifestation of Typhoid

No age group was observed in this study to be particularly at risk of infection although younger children were less affected. Using the stool isolation method, the infection was found to be more prevalent among subjects of age range 31-40 years; but with blood culture it was observed within age range of 21-30 years. This indicates that older adolescents/young adults were the most vulnerable age group. Indiscriminate eating habit indulged by individuals at this age may serve as risk. Ameh and Okpara (2004) in their study reported that the young adults of 11-20 years and adolescents of 21-30 years age grades were the most vulnerable members of the community with prevalence rates of 36.5% and 31.6%, respectively. Usually, the age range considered to be at greatest risk was 5 to 25 years (Saha *et al.*, 2001). The enteric fever infection was, however, seen to decrease with age, probably because of immunity derived from prolonged infections (Idoko *et al.*, 1998). Arti *et al* (1997) also recorded a mean age of 20.1 years. In other regions where typhoid is considered endemic, including India and Pakistan, incidence of the disease has been reported to be low in the

first few years of life, peaking in the school age group (children and young adults; then falling in the middle age and older adult groups (Graham, 2002; Brooks *et al.*, 2005). This study recorded higher rate of typhoid fever infection in females than males, however, no sex (male or female) was observed to be particularly at risk of typhoid fever. As shown in table 12 there was no significant difference ($P=0.103$) between male and female in the rate of infections by *Salmonella enterica typhi/paratyphi*. This explains the fact that the typhoid disease affects both sexes equally (Osuafor and Mgbor, 1990). However, in the study conducted at Sokoto, Ameh and Opara (2004) reported that more males (313 or 58.9%) than females (218 or 41.1%) were infected with the enteric fever illness. Arti, (1997) also recorded higher incidence in males while Smith *et al* (2004) recorded higher incidence in females. Table 18 showed that the total incidence rate of typhoid fever recorded between 2006 and 2008 had a rising trend 35.9% to 40.8%. In a study conducted at Sokoto state of Nigeria at College of Health Sciences, Usman Dan Fodio University by Ameh and Okpara (2004), a similar trend of incidence rate was obtained; 9.2% in 1985, 12.1% in 1986 and 38.8% in 1989.

5.5 Malaria and Typhoid; Confounding Diagnoses

The 11.0% typhoid/malaria co-existence obtained in this study, using Widal test and blood cultures, correlated with such ones published in Nigeria and some African countries. The 10.1% prevalence rate of co-existence was

obtained by Mbuh *et al.*, (2003) in Zaira using Widal test, while 18.0% was obtained using only bacterial culture. Most recently, Akinyemi *et al.*, (2007) obtained a co-existence rate of 14.9%. The 70.0% and 26.6% rates of typhoid/malaria fever concomitant infections obtained by Onuigbo (1990) and Ohanu *et al.*, (2003) in Enugu, however, contrasted with the figure in this study. Most studies carried out to check typhoid/malaria co-existence had used indices of high titre of Widal tests only (Onuigbo, 1990; Samal and Sahu, 1991; Jhaveri *et al.*, 1995; Ibadin and Ogbimi, 2004).

Evaluation of the 500 patients with confounding symptoms for malaria and typhoid diseases showed that 29.0% of the patients with concomitant infection had typhoid detected by Widal screening method only. This was higher than that detected by blood culture method which was 11.0% (Fig 12). In contrast Florence *et al.* (2004) obtained 36.7% co-infection rate with Widal test and 1.6% rate with blood culture at Zaria, Nigeria. This low rate of isolation from blood may have been hampered by the inappropriate culture media and techniques used. However the blood culture isolation method is quite reliable and actually confirms the presence of typhoid disease. In yet another study in Lagos, Nigeria, Olopaenia *et al* (1996) noted that the presence of Widal agglutinin under conditions of positive malaria and negative culture would suggest that malaria parasite may have some undefined antigenic determinants similar to *S. typhi* which can induce antibody production and could explain the febrile condition seen in some of the patients. In Benin City, Nigeria Widal

agglutination reaction at $> 1/80$ was seen to be significantly higher in patients with malaria than in controls without malaria. The outcome of the Widal reaction for patients with a clinical suspicion of typhoid and malaria may depend on individual immune response, which becomes stimulated in fibrile conditions associated with malaria fever. Other similar studies within Nigeria and other African countries with disparities in Widal test and cultures results may serve as pointers to the above opinions (Ameh *et al.*, 1999).

Malaria had a rising incidence in the very early age of 0-11 months (9.2%) and thereafter a sharp fall at the age of 1-10 years (3.7%); children in the endemic regions usually acquire semi-immunity against *P. falciparum* early in life (Riley *et al.*, 1994). Considering the various P-values shown in Table 20, at the 95% confidence limit, the rate of typhoid and malaria infections were not significantly high in the urban and peri-urban populations. Figure 13 shows that enteric fever was more prevalent among the students population (40.2%). This may underscore the fact that this group may be more at risk because of indiscriminate eating and drinking habits. They did not prepare their own food but often ate food prepared outside the homes.

Clinical signs and symptoms of malaria do overlap. In this study, those with fever only and those with headache, continuous fever and stomach pains were mainly positive for malaria parasites while those with headaches and fever combined were mainly positive for enteric fever bacilli (33.2%). Persistent headaches were more consistent with concomitant or dual infections being

positive by both microscopy and Widal test (Table 21). In Pakistan, however, it was observed that subjects with dual infection had significantly higher rates of nausea, vomiting, abdominal pain and diarrhea all presenting as features of enteric fever (Khan *et al.*, 2005). Furthermore, it was noted that unlike the intermittent fever pattern generally seen with malaria patients with co-infection tended to exhibit a continuous fever more typical of enteric fever. Crump *et al* (2003) recorded 23.0% fever in their study. Secmeer *et al* (1995) and Anad (1993) recorded 40% and 25% respectively on the varied reports of headache symptoms on patients studied.

5.6 Transmission of Infection from Asymptomatic Carriers to Contacts

Some cases of typhoid infection were obviously transmitted through carrier contact as recorded in this study. Cohort studies of this research show that subject 'A' was most likely to have been infected by carrier contact. Subject 'B' infection was also likely to be a case of carrier contact either through food eaten out of home or at home disseminated by carrier family member (household carrier contact). About 1-5% of people who are infected with *S. typhi* become asymptomatic chronic carriers (Levine, 1999; American Public Health Association (APHA), 2000). About 10% of untreated typhoid fever patients excrete the bacteria for 3 months after onset of symptoms (APHA).

5.7 Relationship between Source of Water in the Project area and incidence of Salmonella infection/Distribution of Typhoid

Drinking of infected water was mostly found to be a risk factor in acquisition of Salmonella infection in this study (Table 24). *Salmonella enterica* was isolated at high rate from the well water, tank and pipe-borne water. Well water may have been contaminated because of high bacterial load that may have ensued from some septic tanks sipping into the source (Bhutta, 2006). Occasionally wastes and heaps of refuse or sewage drain into broken water pipes or surface water system that supply the public (Ameh and Okpara, 2004). At Sokoto Ameh and Okpara (2004) also discovered that drinking water was the most likely source of typhoid irrespective of the source (stream, pipe-borne, well and commercial water). The high rate of isolation from tank water shows that such water may have been collected from surface water, streams or even from untreated bore-hole water sources. Pipe-borne water was easily infected too. The water pipes serving as common domestic water source was found to be contaminated through the negative pressure inside the pipes created by intermittent water supply. The relative risk of consumption of well water, sachet and pipe-borne water was also found to be high giving rise to high rate of attack of typhoid fever (Figure 13). The infection rate was directionally proportional to the risk of consumption. The result of the cohort/surveillance study of subjects fed from contaminated water source over a period of time gave a significantly

high rate ($p < 0.05$) of typhoid fever disease among the consumers (table 27). The continuance exposure to contaminated water source may be consistent to incessant and relapsing typhoid fever attacks in exposed individuals.

5.8 Susceptibility of Isolates to Antibiotics Tested

Result of antibiotic sensitivity (Figure 15) showed that the fluoroquinolone (ciprofloxacin) was most sensitive both in the acute and carrier state. In the surveillance study conducted during the course of this research, subjects who had been through many episodes of typhoid fever and those who were confirmed as carriers when administered with ciprofloxacin were reported to have good prognosis. When carriers were administered with other antibiotics that produced sensitivity zones invitro, there was no effective elimination of the organism invivo as evidenced by relapsing typhoid infections. Bhutta, (2006) reported that standard treatment with chloramphenicol or amoxicillin is associated with a relapse rate of 5-15% or 4-8% respectively, whereas the newer quinolones and third generation cephalosporins are associated with higher cure rates. Recent studies have shown a rise of the MDR organisms (Walia *et al.*, 2005), also isolates high-level resistance to fluoroquinolones have become common in Asia (Dutta *et al.*, 2001). The flouroquinolones-oflaxacin and ciprofloxacin, the third generation cephalosporins: - ceftriazone and cefxime and azithhromycin were found to be the drug of choice for MDR typhoid fever (Renuka *et al.*, 2004).

CONCLUSION

There is need to improve personal hygiene using public awareness and educational campaigns with trained personnel. Supply of portable drinking water and, targeted vaccination, and rational use of antibiotics can help prevent and control the dissemination of enteric fever. Regular screening and licensing of public food and water vendors may help control the dissemination of the infectious organisms.

Widal test is an easy and inexpensive test which can be of diagnostic value in situations where blood culture are not obtainable and other automated and sophisticated test are not available. However, the results have to be interpreted with caution considering the base-line titre for each locality; also as negative results do not exclude typhoid fever. It is imperative that definitive diagnosis be made to confirm presence of enteric and malaria fever with confounding clinical symptoms by the demonstration of plasmodium species and the *Salomonella enterica enterica typhi/paratyphi* serotypes in the patients' blood, using appropriate culture techniques. However, it is important to note that the most widely used approach to malaria diagnosis, being clinical diagnosis, is unreliable because the symptoms of malaria are not specific. Reliable facility should be made available in rural areas. In areas where the required facilities are available blood culture should be used for diagnosis of the typhoid fever as a confirmatory instead of repeating the Widal test for a rising titre, being that such

time lapse may lead to bad prognosis due to complications. It is better to obtain confirmatory result within three days using isolation method rather than wait for two or three fold rise in titre after 7 days. Stool culture is not a reliable tool for diagnosis of current or clinical infection. The result of stool culture for the diagnosis of typhoid fever should, therefore, be interpreted with caution, especially in the endemic area.

Contrary to popular belief that routine blood culture for isolation of enteric fever bacilli is cumbersome and expensive, this analysis successfully employs the simpler and inexpensive method with high rate of sensitivity and specificity to obtain a more reliable result. It is hereby suggested that physicians should routinely request for blood cultures for diagnosis of enteric fever which may be of more advantage especially in cases of confounding clinical symptoms. Furthermore, prompt and definitive diagnoses of enteric fever may help in intervention and reduction of morbidity and mortality. There is an urgent need to develop a method that may allow the rapid and specific diagnosis of common febrile illnesses such as malaria and typhoid fever.

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APPENDIX

RAW DATA TABLE**Results of Some Patients with Confounding Features**

S/N	Area	Sex	Age	Samples	Antigen O Result				Antigen H Result				Stl. culture result	Bld culture result	MP	Isolates
					A	B	C	D	A	B	C	D				
1	E. South	F	42	Bld/Stl	-	-	-	160	-	-	-	-	-	+	+	Typhi D
2	P-urban	F	48	:: ::	160	80	80	80	80	160	-	80	+	+	-	Typhi D
3	E. South	F	40	:: ::	-	80	-	80	-	-	-	-	+	-	-	Typhi D
4	E. South	F	45	:: ::	-	-	-	-	-	-	-	-	-	-	+	-
5	E.North	M	16	:: ::	-	-	-	160	-	-	-	-	-	-	+	-
6	E.North	F	23	:: ::	40	80	40	320	-	-	-	80	+	+	-	Typhi D
7	E.North	F	35	:: ::	-	-	-	320	-	-	-	80	-	-	-	Typhi D
8	P-urban	F	38	:: ::	40	-	-	-	-	-	-	-	-	-	++	-
9	E.North	F	52	:: ::	-	-	-	160	-	-	-	40	-	+	-	Typhi D
10	P-urban	F	19	Blood	-	-	-	-	-	-	-	-	-	+	+	Typhi D
11	“ “	F	31	::	-	-	-	-	-	-	-	-	-	-	++	-
12	E. South	F	21	::	-	20	40	40	160	160	160	160	+	+	-	Typhi D
13	“ “	M	14	::	-	-	-	-	320	-	-	-	-	+	-	Para. A
14	“ “	F	46	::	-	-	-	-	-	-	-	-	-	-	-	-
15	E North	F	45	Bld/ Stl	-	40	40	160	80	80	80	320	+	+	+	Typhi D
16	P-urban	F	31	:: ::	160	-	-	320	20	20	20	320	+	-	-	Typhi D
17	E.South	F	38	:: ::	-	-	-	-	-	-	-	-	+	-	-	Para. A
18	“ “	F	36	Blood	20	20	20	160	-	-	-	-	-	-	++	-
19	“ “	F	18	::	-	-	-	-	-	-	-	-	-	-	+	-
20	“ “	M	48	::	-	-	-	160	-	-	-	-	+	+	-	Typhi D
21	“ “	M	41	::	-	-	160	-	80	-	-	160	+	+	-	Para. C
22	E. East	F	23	Bld/stl	-	-	-	-	-	-	-	-	+	-	+	Typhi D
23	E. East	F	18	:: ::	-	-	-	-	-	-	-	-	+	-	++	Typhi D
24	“ “	F	19	:: ::	-	-	-	320	-	-	-	40	+	-	-	Typhi D
25	“ “	F	38	:: ::	-	-	-	320	-	-	-	40	-	-	+	-
26	E. South	M	58	:: ::	-	160	-	160	-	-	-	40	+	+	-	Typhi D
27	E.East	F	35	:: ::	-	-	-	160	-	-	-	80	+	+	++	-
28	E.. North	F	26	:: ::	-	-	-	-	-	-	-	-	-	-	++	-
29	E. North	F	26	:: ::	20	40	40	80	20	-	-	-	-	+	-	Typhi D
30	E. South	F	36	:: ::	-	-	-	-	-	-	-	80	-	-	-	-
31	E. South	F	35	:: ::	-	-	-	-	-	-	-	-	+	-	-	Para .A
32	E. North	F	26	:: ::	-	-	-	80	-	-	-	-	-	-	++	-
33	E. South	F	5	:: ::	-	-	-	-	-	-	-	-	-	-	++	-
34	E. South	F	42	:: ::	-	-	-	80	40	-	-	-	-	-	-	-
35	E. South	F	<2	:: ::	-	-	-	-	-	-	-	-	-	-	++	-
36	E. North	F	26	:: ::	80	40	40	160	40	40	40	40	+	+	-	Typhi D
37	E. South	F	20	:: ::	-	-	-	40	-	-	-	-	-	-	-	-
38	E. South	F	18	:: ::	-	-	-	-	-	-	-	-	-	-	++	-
39	E. South	F	47	:: ::	-	160	-	160	-	20	-	80	-	+	++	Para. B

40	E. South	F	37	::	::	-	160	-	160	-	-	-	80	+	+	-	Typhi D
41	E. North	F	45	::	::	-	-	-	-	-	-	-	-	-	+	++	-
42	E. South	M	23	::	::	-	40	-	-	-	-	-	-	-	+	-	Para. B
43	E. South	F	56	::	::	-	40	-	-	-	-	-	-	-	-	+	-
44	E. North	M	12	::	::	-	-	-	-	-	-	-	-	-	-	+	-
45	E. North	M	12	::	::	-	160	160	160	-	160	-	-	+	-	++	Para. B
46	Periurban	M	23	::	::	-	-	-	-	-	-	-	-	-	-	-	-
47	Periurban	F	27	::	::	-	-	-	-	-	-	-	-	-	-	-	-
48	E. South	M	15	::	::	-	-	-	-	-	-	-	-	-	-	+	-
49	E. North	M	30	::	::	-	-	-	-	-	-	-	-	+	-	-	Para .A
50	E. South	M	17	::	::	-	-	-	-	-	-	-	-	-	-	-	-

Key: Widal Antigen O and H results: Negative = -, 1/20 titre = 20, 1/40 titre =

40, 1/80 titre = 80, 1/160 titre =160, 1/320 titre =320, 1/640 titre = 640. Malaria

parasite results: Negative= -, positive = +, strongly positive = ++.

PREPARATION OF MEDIA

(I) Selenite-F Broth

Add 19 grams of part A to 1 litre of distilled water to which 4 grams of Part B has been added. Warm to dissolve, mix well and filter out into containers, sterilize in autoclave for 10 minutes.

Part A: peptone (5.0 grams), Lactose (4.0 grams), Sodium phosphate (10.0 grams).

Part B: Sodium biselenite (4 grams).

(II) Bile Salt Broth

<u>Formulation</u>	<u>Quantity</u>
Nutrient broth	1 litre
Sodium taurocholate	5 gram

Preparation

Adjust the pH of the broth to 7.6 and dissolve the bile salt (sodium taurocholate) in it. Autoclave at 108°C for 15 minutes.

(III) Peptone Water Sugars

Prepare solution A (peptone water) and distribute in 100ml aliquots.

Add 1 gram of appropriate sugar (glucose, sucrose maltose and galactose).

Add 1ml of solution B (Andrades indicator) to each of the sugars.

Mix and distribute to bijou bottles wit Durham tubes.

Sterilize at 115°C for 10 minutes or steam for 3 days at 100°C for one hour interval.

Solution A: Peptone (10grams), Sodium chloride (5grams), Distilled water (1000ml).

Solution B (Andrades indicator): Acid fuchin (0.5gram), Sodium hydroxide (16ml), Distilled water (100ml).

(IV) Nutrient Broth (Lab. M).

<u>Formulation</u>	<u>Grams per Litre</u>
Lab-lemco powder	1.0
Yeast Extract	2.0
Peptone	5.0
Sodium Chloride	5.0

Preparation

Dissolve 8 grams of powder in 1 litre of distilled water. Warm slightly, if necessary. Sterilize at 121°C for 15 minutes.

(V) Salmonella Shigella Agar (Biotec)

<u>Formulation</u>	<u>Gram per Litre</u>
Lab-Lemco	5.0
Peptone	5.0
Lactose	10.0
Bile salt	5.5
Sodium citrate	10.0
Sodium thiosulphate	8.5
Ferric citrate	1.0
Brilliant green	0.00033
Neutral red	0.025
Agar	12.0

Preparation

Suspend 57 g of the ingredient in 1 litre of distilled water. Bring to boil with frequent agitation to dissolve the agar. Allow to sterilize by boiling without autoclaving. Cool and pour in Petri dishes.

(VI) MacConkey Agar (Biotec)

<u>Formulation</u>	<u>Quantity</u>
Peptone	20 g
Sodium taurocholate	5.0 g
Neutral red	2.0%
Lactose	10.0%

Preparation

Add 49 gram in 1 litre of distilled water and autoclave at 121°C for 15 minutes.

Allow to cool to about 70°C. Distribute into Petri dishes

(VI1) Muller Hinton Agar (Oxoid)

<u>Formulation</u>	<u>Gram per litre</u>
Beef infusion from casein hydrolysate	17.5
Stach	17.5 g
Agar	10.0 g

Preparation

Suspend 35 grams in 1 litre of distilled water. Bring to boil to dissolve the medium completely. Filter if necessary and adjust pH to 7.4. Sterilize by autoclaving at 121°C for 15 minutes. Dispense into Petri dishes.

(VIII) Urea Agar (Lab M)

<u>Formulation</u>	<u>Gram per litre</u>
Peptone	1.0
Dextrose	1.0
Sodium chloride	5.0
Disodium phosphate	1.2
Potassium dihydrogen phosphate	0.8
Phenol red	0.012

Preparation

Suspend 2.4 grams in 95 ml of distilled water. Bring to boil to dissolve completely. Sterilize by autoclaving at 115oc for 20 minute. Cool to 50oc and aseptically introduce 5 ml of sterile 40 % urea solution. Mix well, distribute to 10 ml amounts into sterile containers and allow to set in the slope position.

(IX) Hydrogen Sulphide Agar (Lab M)

<u>Formulation</u>	<u>Gram per litre</u>
Lab lemco	3.0
Yeast extract	3.0
Peptone	20.0
Sodium chloride	5.0
Lactose	10.0
Surose	10.0
Dextrose	1.0
Ferric citrate	0.3
Sodium thiosulphate	0.3
Phenol red	0.012

PREPARATION OF REAGENTS**(I) Drabkin Solution**

<u>Formulation</u>	<u>Quantity</u>
Potassium ferricyanide	440mg
Potassium cyanide	100mg
Anhydrous potassium dihydrogen phosphate	280mg
Distilled water	2litres

Preparation

Dissolve all the chemicals in the water, accordingly and lock away in a cupboard ready for use.

(II) White Cell Diluting Fluid

<u>Formulation</u>	<u>Miliimitre</u>
N/10 hydrochloric acid	4.3
1% gentian violet	10
Distilled water	500

Preparation.

Half fill a 500ml volumetric flask or measuring cylinder with distilled water.

Add 4.3 ml of concentrated hydrochloric acid. Add 1% gentian violet

Make up to the 500 ml mark with distilled water and mix.

(III) Leishman's Stain

<u>Formulation</u>	<u>Quantity</u>
Leishman powder	1.5 gram
Methyl alcohol	1 litre

Preparation

Rinse out a 1 litre bottle with methanol (methyl alcohol) and place in it a few glass beads.

Add the staining powder and methanol.

Mix at interval over a period of one day, until stain is completely dissolved.

Filter for use the following day.

