REACTION OF A WIDAL REAGENT WITH SERUM SAMPLES FROM SUSPECTED TYPHOID PATIENTS

BY

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DECLARATION

I hereby declare that the work presented in this thesis is original. It was carried out in the laboratories of Microbiology Department, Ahmadu Bello University, Zaria, Nigeria, and composed by me. No part of this research has been submitted for a degree or any other qualification. All sources of information are specifically acknowledged by means of references.

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CERTIFICATION

This thesis entitled "REACTION OF A WIDAL REAGENT IN SUSPECTED TYPHOID AND NON TYPHOID PATIENTS" by Florence Azie MBUH meets the requirements governing the award of the degree of Master of Science in Microbiology of Ahmadu Bello university, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to my mother and father.

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ABSTRACT

Blood samples from 222 patients referred for the Widal test in four hospitals located in Zaria and 52 blood samples from apparently healthy persons (controls) were examined for typhoid fever using the widal test and blood culture methods. Blood smears were also examined for malaria parasites. All serum samples were tested for antibodies to the somatic (0) and flagella (H) antigens of *S. Typhi* by the rapid slide widal screening test and rapid slide titration agglutination test. *S. typhi* isolates were tested for antibiotic susceptibility.

Out of the 222 patient samples *S. typhi O* antibody titres greater than or equal to 1 in 20 were detected in 111 (50%). Antibody titres greater than or equal to 1 in 40 to the H antigen were detected in 190 (86%) patient samples. In the control group, 16 samples (30.1%) had anti O titres of greater or equal to 1 in 20, while anti H titres of greater or equal to 1 in 40 were detected in 40 (77%) samples. Over all a significant anti 0 titre of greater or equal to 1 in 160 was found in 56 patients (25.2%) and in 16 (11.5%) healthy persons. Sixty patients (27%) and 12 healthy persons (23.1%) had malaria parasites. Parasite density (ul) was higher in the patients than in the control group. A correlation analysis showed that there was no correlation between parasite count and Widal titres.

Fourteen patient samples yielded S. typhi on culture while all control samples were culture negative. The difference between the incidence of typhoid fever by Widal (25.2%) and culture (6.3%) was statistically significant ($P<0.01$). Because of the wide distribution of antibodies detected by the widal test, clinicians are advised to be careful in making conclusions based on the Widal test. Laboratory diagnosis of typhoid fever should be based on culture or paired results of Widal tests. Although the level of parasitemia did not influence Widal titres there was wide occurrence of Salmonella antibodies in malaria patients and carriers. All S. typhi isolates were sensitive to chloramphenicol, tetracycline, nitrofurantoin cephalexin and floxacin while 7 (50%) isolates and 4(28.6%) isolates were resistant to ampicillin and co-trimoxazole respectively.

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CHAPTER 1

INTRODUCTION

Typhoid fever caused by infection with *Salmonella typhi* is a widespread and potentially lethal disease that continues to pose a major health problem in many developing countries. It is speculated that there are more typhoid cases in areas of drug resistant malaria (Alaribe, *et.al.*, 1995). This speculation may be a function of the method of diagnosing typhoid fever which allows interference from concurrent malaria infection in such areas.

For many years typhoid fever has caused morbidity and mortality in humans. The disease is endemic or hyper endemic in many economically underdeveloped countries of Asia, Latin America and Africa where there are areas of poor sanitation and living conditions. Although the occurrence of typhoid in countries with good sanitation has shown a steady decline (Hickmann and Farmer, 1978) where most of the cases are presently due to returning travellers from endemic areas (Simpson, *et.al.*, 1993), rates are high and continue to rise in many developing countries, with annual incidence of 16 to 17 million cases and 600,000 deaths (Thong, *et.al.*, 1995). The situation is further compounded by the continuous emergence of drug resistant strains of *S.typhi* (Kohbata, *et.al.*, 1983;

Gulati, *et.al.*, 1991).

In rural tropical countries only rough estimates of the prevalence of typhoid are available (Petit and Wamola, 1994). Edelman and Levine (1986) estimated that 4.38 million cases of typhoid fever occur yearly in Africa alone. In Nigeria typhoid is endemic and has been reported in different parts of the federation (Onile and Odugbemi, 1987). Typhoid is also a common health problem in Zaria (Akoh, 1987) with a prevalence of about 3.1% (Okolocha *et.al.* , 1993).

The clinical signs and symptoms of typhoid fever are protean. The disease is characterized by insidious onset of sustained fever, headache, malaise, anorexia, a relative bradycardia, splenomegaly, rose spots on the trunk, non, productive cough, constipation more commonly than diarrhoea (in adults) and involvement of the lymphoid tissues (Benenson, 1990). The case fatality rate of 10% in typhoid can be reduced to less than 1% with prompt antibiotic therapy which can only be made possible by early diagnosis. Typhoid is transmitted by ingestion of food or water contaminated by faeces or urine of patients or carriers (Lucas and Gilles, 1993) and man is the only Reservoir of infection. The causative agent of typhoid ***S.typhi*** is a Gram-negative, aerobic, non-sporing rod-like organism that does not ferment lactose.

One of the major problems facing the control of typhoid in endemic areas is that of poor diagnosis. The diagnosis of typhoid is based on blood culture (Edelman and Levine, 1986;

Lewis, 1992) or blood culture and antibody estimation (Sivadasan, *et.al.*, 1984). Most rural hospitals in Zaria do not perform blood cultures due probably to the lack of materials, expertise or excess work load upon the few laboratory technicians or simply due to ignorance and negligence. Thus most physicians rely on serology (Widal test) even though the results are often confusing and difficult to interpret (Pang and Ruthucheary, 1983; Ong, *et al.*, 1989; Lewis, 1992; Cheesbrough, 1993). The lack of standard, false positive and negative results, anamnestic responses and cross reaction with micro-organisms unrelated to Salmonella and the effect of previous encounter with ***S.typhi*** or typhoid vaccine make the Widal test unreliable.

Nigeria is in a hollo endemic zone for malaria (Keshinro, 1988; WHO, 1991), and Alaribe, *et.al.*, (1995) suggested that there may be a cross reactivity among Salmonella and malaria parasite antigens, causing false positive widal reaction. In Zaria the incidence of typhoid continues to increase yearly (Okolocha, *et.al.*, 1993). Not surprisingly this is accompanied by the proliferation of laboratories diagnosing typhoid in this environment. Another problem is that in most of the "standard" and "backdoor" laboratories in Zaria typhoid is diagnosed by a simple widal screening test. Also most of the man power in many of the laboratories are not competent and often make random judgements as to whether a Widal test is positive or not. Thus the increasing reports of typhoid fever can only be expected.

Consequently there is increased exposure of individuals to expensive antibiotics which only increase the risk of drug resistance and economic burden on the already needy population. There is the urgent need to examine the actual situation of typhoid fever in Zaria and the impact and efficacy of the widal test in association with malaria parasites in the diagnosis of this disease. This study was therefore designed with the following objectives:

1) To find out the distribution of antibodies to *Salmonella typhi* antigens in hospital patients and healthy individuals using the widal test.

2) To determine the level of *S.typhi* antibodies in malaria patients or carriers of malaria parasites.

3) To investigate the efficacy of a single Widal test compared with blood culture in the diagnosis of typhoid fever.

4) To investigate the antibiotic susceptibility of Salmonella isolates in view of the acclaimed typhoid fever explosion and the high consumption of antibiotics.

CHAPTER II

REVIEW OF LITERATURE

2.1 TYPHOID FEVER

Typhoid fever is a systemic bacterial disease characterized by insidious onset of sustained fever, headache, malaise, anorexia, slow pulse, rose spots on the trunk, non productive cough, constipation more commonly than diarrhoea (in adults), splenomegaly and involvement of the lymphoid tissues (Benenson, 1990). Many mild and atypical infections occur. The acute stages of the disease last from a few days to several weeks with a case fatality rate of 10%.

2.1.1 Etiologic Agents.

Typhoid fever is caused by infection with a motile, gram negative, asporogenous bacillus, ***Salmonella typhi,*** formerly known as ***Eberthella typhosa*** *and* ***Bacillus Typhosus*** (Anderson, *et.al.*, 1969; Ager and Top, 1972). ***S. typhi*** was first isolated in 1880 by Eberth (Brock, 1979). The organism multiplies in milk and other foods that are not too acidic. Presently there are 106 types of ***S. typhi*** that can be distinguished by phage typing which is of value in epidemiologic studies. The organisms may survive for long periods in butter and cheese, water, snow and ice and in frozen soil but it does not multiply in water and can be distinguished from other enteric pathogens

by biochemical tests. The lack of lactose fermenting ability differentiates the genus *Salmonella* from several other genera of *Enterobacteriaceae*. However, there are few reports on some lactose fermenting *S.typhi* (Kohbata, et al., 1983). *S. typhi* produces acid but not gas from glucose and mannitol, may produce acid from dulcitol but it does not grow in citrate medium and does not liquify gelatin. *S.typhi* decarboxylates lysin, produces hydrogen sulphide, but does not decarboxylates ornithine and is generally non lactose fermenting (Collins, *et.al.*, 1995).

In the body the organism can be found in the blood within the first 10 days of infection from which it can be isolated. Later in the infection it can be found in stool and urine. *S. typhi* is a host adapted pathogen with infections limited to man under normal conditions (Simon and Miller, 1966; Anderson, *et.al.*, 1969; Cheesbrough, 1993; Lewis 1992). Therefore man is the only reservoir for *S.typhi*. The organism escapes from the reservoir through faeces and urine of both patients and carriers or vomitus of typhoid patients.

2.1.2 Epidemiology

Typhoid fever is an infectious disease transmitted by water and food which have been contaminated by *S.typhi*. The epidemiology of the disease therefore depends on different factors linked to the mode of transmission of the organism which includes sewage disposal and the quality of water treatment.

Typhoid fever has a world wide occurrence but is particularly endemic in Asia, Africa and Latin America. It is

difficult to determine its exact incidence, which as a rule is considerably underestimated especially so since the disease is not the subject of obligatory report to the World Health Organization (WHO). However, Edelman and Levine, (1986) estimated that about 4.375 million cases of typhoid occur annually in Africa, 7.7 million cases in Asia and 406,000 cases in Latin America.

Typhoid is endemic or hyper endermic in all countries where there are areas with inadequate sewage disposal and unhygienic conditions and these may be found especially in Southern Asia, the middle East, East, West and Central Africa and Central and South America. The disease is endemo-epidemic in the countries of North Africa, certain countries of south-East Asia and some European countries around the mediterranean. It is sporadic in North America and most countries of Europe. In countries where typhoid occurs in the sporadic-endemic form (North America and Europe) cases are largely attributed to carriers working in restaurants or food industries. Recently a majority of typhoid cases reported in the United Kingdom were due to carriers and travellers returning from endemic areas (Simpson *et.al.*, 1993).

While the incidence of typhoid in many developed countries has reduced greatly due to the success of hygiene measures, surveillance and efficiency of treatment with antibiotic, typhoid fever has remained endemic in many developing countries where rates continue to rise and more and more people are

vulnerable to *Salmonella* infection as a result of malnutrition, sickle cell anaemia, Human Immunodeficiency Virus (HIV), malaria and schistosomiasis (Hathout, *et.al.*,1966; Coelum *et.al.*, 1987; Gilks, *et.al.*, 1990). Also the growing number of people with neglected sanitary facilities, lack of portable water and other infections have continued to increase the number of salmonella infection (Ager and Top, 1972; Petit and Wamola, 1994).

Typhoid fever affects individuals of both sexes, and in countries where the disease is endemic young persons are most affected - pre - school and school-age children (Benenson, 1990). Older individuals often seem to have a partial immunity, probably following exposure to frequent, moderate, sub-infectious doses of the typhoid bacillus. Children below the age of 5 years and particularly infants, are rarely affected due to their protected life style. But if they are, the clinical course is mild and difficult to diagnose. Males are slightly more affected than females (Mulligan, 1971; Bodejo and Arigbadu, 1980; Khuri-Bulos, 1981; Akoh, 1987). Okolocha and Kabir, (1994) reported that sex, age and season have no effect on the distribution of typhoid in Zaria, but that personal, environmental and general hygiene could be contributing factors.

In the tropics, carriers among food handlers dealing with high risk foods and beverages such as cold dishes, fresh fruits and cold drinks, especially those that require much handling

during preparation play an important role in maintaining endemic infection (Mandel, 1979; Adesiyun *et.al.*, 1987).

2.1.3 Seasonal variation

Seasonal variation in the incidence of typhoid has been reported by several authors. The disease may have a high incidence in certain months of the year and which may vary according to geographical location. For example, a seasonal variation with two peaks coinciding with the two rainy seasons occurs in Accra, Ghana (Achampong, 1969). In kenya, Huckstep (1962) noted that the disease was more frequent in the hot dry period of January to March. This was attributed to contaminated water supply and poor personal hygiene. In Nigeria the seasonal incidence is not clear but it is likely that, maximum incidence of typhoid occurs during the heavy rainstorms at the beginning and end of the dry season (Mulligan, 1971). However three peaks of typhoid infection were reported in Lagos in the months of April, July and November (Ogunbiyi and Onabowale, 1976). Here most of the patients came from areas of Lagos with over crowding and poor sanitation. In Zaria Akoh (1987) reported a peak incidence of typhoid in October while Okolocha *et.al.*, (1993) reported high prevalence of salmonella infection in Zaria but observed that this distribution of the disease was not affected by season. The isolation of *S.typhi* from patients in Ilorin was shown not to be dependent on season (Onile and

Odugbemi,(1987).

2.1.4 **Mode of transmission**

The spread of *S.typhi* from one host to another can be effected in two ways: Direct and indirect transmission.

Direct transmission occurs by contact with discharges from a sick person or debilitated carrier resulting in direct person to person spread (Hickman and farmer, 1978; Sammalkorpi *et.al.*, 1987).In indirect transmission, the organism spreads by means of a common vehicle. This second mode of transmission is more common and plays a major role in the spread of the disease in countries of high endemicity where sewage disposal system are often inadequate. This thus occurs through ingestion of food and water contaminated by faeces and urine of patients and carriers (Anderson *et.al.*, 1969).Vehicles include shell fish taken from sewage contaminated beds, raw fruits, vegetables fertilized by night soil, milk and milk products contaminated usually by hands of carriers and also missed cases (Benenson, 1990). Flies may infect foods in which the organisms then multiply to achieve an infective dose. This presents a problem in rural areas and places where sewage is disposed of through primitive ways.

2.1.5 *Infection and incubation period*

Infection of humans with *S.typhi* is caused almost exclusively by ingestion of contaminated food, water or milk (Anderson, 1966. Lawson, 1977) and very rarely per return through the use of rectal or enema tubes inadequately

sterilization after use on a typhoid case or carrier (Anderson, *et.al.*, 1969).

Once infection has been established the incubation period may vary depending on the infecting dose (Benenson, 1990), the individual and the actual source of infection (Anderson, *et.al.*, 1969). For this reason different incubation periods have been reported ranging from 3 to 40 days with a mean of 5 to 17 days. According to Anderson, *et.al.*, (1969) water borne infection has long incubation period, milk infection, shorter and food, the shortest. Glynn, *et.al.*, (1995) reported an average incubation period of 5 to 9 days in volunteers challenged with 10^5 to 10^9 organisms and this was the time of the first body temperature of at least 100°F (37.8 °C) from the time of infection.

2.1.6 Pathogenesis,carrier state and complications

The severity of typhoid fever and the rate of spread depends on the size of inoculum swallowed. The disease was more severe in volunteers who swallowed 10^8 to 10^9 organisms than in those who ingested 10^5 organisms. Other factors that may increase host susceptibility include concurrent malaria, schistosome and HIV infections, sickle cell anaemia and malnutrition (Hathout, *et.al.*, 1966; Parry, 1984; Celum *et.al.*; 1987; Christie, 1987; Gilks, *et.al.*, 1990).

The pathogenesis of ***S.typhi*** infection is better understood if we trace the route of the organisms after infection. After

ingestion those bacteria that survive the acid barrier of the stomach are carried to the small intestine where they multiply asymptomatically, penetrate the walls of the small intestine and enter the mesenteric lymph nodes. Here further multiplication takes place and the organism enter the blood stream through the thoracic duct within the first week of infection, and most cases of positive blood culture are obtained within this period. Once in the blood further incubation occurs in distant organs such as the liver, spleen, gall bladder and bone marrow. Multiplication in these sites results in a secondary and more sustained bacteriemia which is an essential pathophysiologic feature of typhoid (Drutz and Graybill, 1987). Invasion of the gall bladder leads to re-entry of the organisms into the gut with contaminated bile. The payer's patches in the terminal ileum are the most affected area in the gut and the pathological changes occurring here take place in stages (Anderson, 1966, Adams, 1983): hyperplasia during the first week, necrosis during the second and ulceration in the third week followed by healing. This sequence of changes is however greatly accelerated in Africans. The liver and the spleen are enlarged, hyperaemic and soft. Both organs may show a collection of *S. typhi* and lymphocytes. From this time too, the presence of antibodies in the patients serum can be demonstrated by the widal test (Lawson, 1977).

The infected gall bladder and kidney may become sites of chronic inflammatory condition, conducive to calculi formation

stimulate the synthesis and release of endogenous pyrogen by the inflammatory cells at sites where *S.typhi* is actively multiplying.

Complications

Lawson (1977) summarized complications resulting from typhoid according to the affected site as follows:

1. Abdominal complications - meteorism, haemorrhage, intestinal perforation and acute cholecystitis which is responsible for chronic stage of faecal carriers.
2. Cardiovascular complication - myocarditis and thrombosis.
3. Respiratory complications - bronchitis and bronchopneumonia.
4. Central nervous system - typhoid meningitis is a rare complication. Others include peripheral neuritis, melancholia and dementia.
5. Local inflammations such as otitis media due to secondary infection, bone abscesses may develop in convalescence or years after the infection has subsided. Spondylitis (or typhoid spine) occurs in adults during convalescence.

The incidence of complications in typhoid varies widely and the responsible factors are not fully understood. Rowland (1961) found a complication rate of 16.3% in Iran and blamed it on the use of chloramphenicol in treatment. Gupta *et.al.*, (1985) reported a complication rate of 48%, while in a review of typhoid in four African countries (Ghana, Kenya, Tanzania

and Zambia), Petit and Wamola (1994) noted that the rate of complications was also high (30%). In these countries they observed a case of myocarditis, relapses and hemorrhage in 6% and 7.6% of cases respectively, and intestinal perforation was four times as often in Ghana. Other complications such as cholecystitis and thrombophlebitis were rare in these countries. A high rate of pneumonia was thought to have resulted from haematogenous or super infection by salmonella.

Intestinal perforation is the most common complication in Africa and accounts for most deaths (Petit and Wamola,1994).

2.1.7 Clinical manifestation

After an incubation period of about 5 to 17 days, typhoid fever starts with a variety of disorders: headache, fatigue, abdominal pains, constipation and vertigo. The patient progressively develops high and persistent fever (39°C to 40°C) A typhoid case may mimic a case of acute bronchitis or full fledged pneumonia (Huckstep, 1962). General symptoms with chills, malaise, headache, fever, cough, body pains vomiting, lose of appetite, sore throat and stiff neck (Ager and Top, 1972; Lawson, 1977, Drutz and Graybill, 1987) may be seen in most cases of acute febrile illness and are therefore not specific.

Specific clinical signs become apparent in the second stage of the disease (Petit and Wamola, 1994). Body temperature rises slowly over a period of days, the increase in temperature

not more than 1 or 2°F per day. Patient's condition becomes worse, both physically and mentally. He is prostrate and may present with severe GIT disturbances accompanied by a moderate splenomegaly. The tongue becomes patched, rose spots appear on the trunk and fade under pressure and bacteriaemia manifests as crops on the sides and back of the chest and upper abdomen. This stage of clinical manifestation coincides with the height of toxaemia and there is the great danger of complications such as perforation and haemorrhage. The body temperature is raised to a peak of 101°F to 105°F (Glynn, *et.al.*, 1995) and maintained for a period of one week or longer with slight remissions not more than a degree or two, after which it falls by lysis if the patient survives. The fall in temperature is observed in the third stage of the disease during which the mental state clears and the patients condition begins to improve and progresses into the convalescence stage. Rarely, some cases initially present as functional psychosis before the manifestations of general signs and symptoms (Baiyewu and Oluboyo, 1991).

Some specific signs are pathognomonic and these include neuropsychiatric symptoms, typhoid face, bradycardia, perforation and rash. These signs show a specific and sometimes unknown relation with geographic areas (Petit and Wamola,1994). Neuropsychiatric or confused state with or without convulsion and psychosis are far more dominating in Africa and Asia, and rash is not commonly found in Africans may be as a result of the dark complexion. In south America and parts of South-East

Asia typhoid fever manifests as a relatively mild illness with low fatality rates and minimal neurologic complications (Thong, *et.al.*, 1996) whereas in Sub-Saharan Africa and Indonesia the disease often present with higher mortality and often accompanied by neurologic signs such as delirium and coma. The various observations are related to differences in health care facilities, host immune responses, genetic factors and perhaps also to differences in strains of ***S. typhi*** circulating in areas of endemicity (Thong, *et.al.*, (1996).

2.1.8 **Immunology**

Typhoid is one of the infectious diseases in which humoral and cell mediated immunity collaborate in host defense.

Evidences of involvement of cell mediated immune responses in typhoid fever (Drutz and Graybill, 1987) are given as follows: i) Occurrence of intracellular parasitism: Demonstrated ability to transfer immunity to normal animals adoptively with lymphocytes of immune animals. ii) Absence of significant participation by neutrophils in the infective process: when killed salmonellae or their extracts are administered to animals in Freud's complete adjuvant, solid immunity is established which is comparable to the immunity achieved by inoculation with attenuated organisms. This is a pattern characteristic of cell mediated immunity. Conversely, administration of killed vaccines will generate high titre antibodies and delay (but does not prevent) infection.
iii) Delayed hypersensitivity skin tests with protein extracts

of Salmonella convert to positive after animals are immunized with salmonellae in Freud's complete adjuvant.

Three major groups of antigens are present in *S.typhi* to which human beings react to produce antibodies (Ager and Top, 1972): the flagella (H) antigen, somatic (O) antigen and Vi (virulence) antigen. Agglutination of serum with H Antigen in high titre gives evidence of either previous typhoid disease, inoculation with typhoid vaccine or period of early convalescence in typhoid fever. Low titres of H antigen may indicate present infection, convalescence from other salmonellae or related organisms (anamnestic reaction), or late convalescence. For the O antigen, high titres against this antigen may mean typhoid infection or infection with some other member of the salmonella group to which *S.typhi* belongs.

The several possibilities are due to the fact that the genus salmonella consists of serologically related organisms with H and O antigens that are not necessarily specific: two or more organisms may have the same O antigen (Christie, 1980; Sleigh and Duguid, 1989). For example *S.typhi* and *S.enteritidis* both have O:9,12 antigens and belong to the D1 group in the Kauffman-White classification (Chessbrough, 1993). Similarly, different members of the genus salmonella may have similar H antigen which are either phase 1 or 2. Furthermore there is the possibility of cross reaction of the salmonella antigens with those of other organisms such as malaria parasites and schistosome (Parry, 1984; Alaribe, *et.al.*,1995).Thus unless a

laboratory is equipped serologically to type O and H antigens properly, a positive Widal test is of little or no value and by itself not necessarily diagnostic. Additional information must come from clinical evidence and the recovery of *S. typhi* by cultural method.

The Vi or virulence antigen is a surface polysaccharide antigen of *S.typhi* and plays an important role in the virulence and pathogenesis of *S.typhi* (Wilson and Miles, 1975; Drutz and Duguid, 1989; Cheesbrough, 1993; Hashimoto, et.al., 1995). In subjects experimentally challenged with *S.typhi*, Vi positive strains were more virulent than Vi negative strains. Following this observation it was suggested that the Vi antigen may play a role in the pathogenesis by shielding *S.typhi* from the action of complement (Edelman and Levine, 1986). Vi positive *S.typhi* are not agglutinated by *S.typhi* O antibodies (Cheesbrough, 1993) but have no effect on H agglutinins. Vi antibodies are present almost exclusively in the blood of chronic typhoid carriers and the O and H antibody titres may be low or nil in such instances. Thus the Vi antibody testing is useful in epidemiologic surveillance (Lucas and Gilles, 1993).

Cellular immunity: Macrophages are important in host defence in typhoid fever. The involvement of cellular immunity was demonstrated when it was observed that peritoneal exudate cells from immunized mice were far more active against *S.enteritidis* than those from unimmunized mice (Drutz and Graybill, 1987). *S.typhi* is easily phagocytized but not killed.

The intracellular multiplication of the organism and its growth in body fluids (Brock, 1979; Keusch and Thea, 1989) is a clear indication of the ability of *S.typhi* to overwhelm the host defense mechanism. In chronic carriers the organism may live only intracellularly where they are protected from the immune mechanism of the host and are less susceptible to drug therapy (Brock, 1979).

One attack of the disease confers lasting immunity with a few exceptions in which another strain of the organism or massive dosage of the same strain may be responsible for the subsequent attack.Artificial immunity is obtained by administration of typhoid vaccine and the immunity is only periodical (Benenson,1990).

2.2 DIAGNOSIS

The diagnosis of typhoid fever can be done based on clinical findings, isolation of ***S.typhi*** by culture, serology, leucocyte count, diazo urine test and molecular methods.

2.2.1. Clinical diagnosis Typhoid fever can be diagnosed upon a close study of clinical signs and symptoms but since early clinical signs are not necessarily specific (Petit and Wamola, 1994), every effort must be made to recover the organism by culture and/or to demonstrate a rising antibody titre to O antigen of ***S.typhi*** in samples from

clinically suspected typhoid patients (Klotz, *et.al.*,1984; Sadallah, *et.al.*,1990).

2.2.2. **Cultural diagnosis**

Since Eberth first isolated ***S.typhi*** in 1880 it has become necessary to demonstrate the presence of the organism in patients samples for definitive diagnosis. According to Lewis (1992) a definitive diagnosis of typhoid fever is based on the isolation of ***S.typhi*** from blood. Blood cultures are positive in 80 to 90% of patients during the first week of infection (Onile and Odungbemi, 1987; Sadallah, *et.al.*, 1995). ***S.typhi*** is easier to isolate from blood than from stool and urine (Onile and Odugbemi, 1987) and bone marrow culture is superior to all specimens in the isolation of the organism (Sadallah, *et.al.*, 1990). ***S.typhi*** can usually be isolated from the faeces of 40%-50% of patients during the second week of infection and from about 80% of patients during the third week of infection, while in urine culture, about 25% of samples are positive in the second week especially those with urinary schistosomiasis (Cheesbrough, 1993). In urine the bacteria are not secreted continuously so that several specimens may be cultured before the organisms are isolated.

Enrichment media for isolation depend on the type of specimen sent for culture. Blood is enriched in thioglycolate broth or tryptic soy diphasic medium at a minimum of 1 in 10 dilution in the broths (Cheesbrough, 1993). Selenite F broth is used for faeces (Ewig, 1982). The use of sodium hydrogen

The isolation of the organism from stool should be accompanied by compatible clinical signs for the diagnosis of typhoid (Klotz, *et.al.*, 1984) because carriers who are not necessarily sick will produce culture positive results. Culture of bone marrow aspirate is most sensitive for the isolation of ***S.typhi*** from patients and is particularly useful for the identification of chronic carriers and patients who take antibiotics before presentation (Basily, *et.al.*, 1980; Sadallah, *et.al.*, 1990). Bone marrow aspiration is however painful and requires technical expertise (Rubin, *et.al.*, 1989). Antigenic analysis and phage typing of ***S.typhi*** are not routine procedures for a clinical laboratory (Cowan, 1981;Tenover,1988) but are necessary for epidemiological investigations (Altwegg *et.al.*, 1989).

2.2.3. **Serodiagnosis**

Demonstration of antibody levels to ***S.typhi*** O, H and Vi antigens in patients's serum is a common method of laboratory diagnosis. Significant antibody titre values vary and each laboratory has to determine its own standard. In endemic areas in developing countries active typhoid is suggested if titres of O or H or both antibodies are greater than 1 in 180 or 200 depending on titres found in local healthy people (Cheesbrough, 1993). Also there is the need to demonstrate a rising titre because titres greater than 200 can occur in non typhoid conditions (Cheesbrough, 1993, Lucas and Gilles, 1993).

After about 10 days of infection the widal test for O and

H antibodies becomes positive and the level of antibodies rises progressively (Lucas and Gilles, 1993) reaching highly significant levels by the third week. A diagnosis of typhoid can be made on the basis of a four-fold rise in antibody titre, or a titre greater than or equal to 1 in 160 to the O antigen of *S.typhi* together with a compatible clinical illness (Klotz, *et.al.*, 1984). However, only a two or three fold rise in titre may occur in patients (Cheesbrough, 1993), and positive result in a single Widal test can not prove the presence of enteric fever nor a negative result its absence (Sleigh and Duguid, 1989).

Limiting factors affecting the diagnostic value of the widal test are well documented (Schroeder, 1968; Wicks, *et.al.*, 1974; Freter, 1980; Abraham *et.al.*, 1981; Pang and Puthucheary, 1983; Raphael and Spencer, 1983; Ong, *et.al.*, 1989, Sleigh and Duguid, 1989, Lewis 1992; Cheesbrough, 1993). These include the stage of illness, normal population titres, effect of previous attack and inoculation with typhoid vaccine, non specific cross reactions, effect of early antibiotic therapy and technical variations. These limitations often present difficulties in interpreting Widal test results thus making the test unreliable. Furthermore a meaningful result always requires the availability of paired sera (Lucas and Gilles, 1993). Therefore Widal titres can only be of diagnostic value when use as adjunct to bacteriological diagnosis. The Widal test alone can be of help only when typhoid is in differential diagnosis but

S.typhi cannot be isolated (Bennett and Hoo, 1959).

2.2.4 Leucocyte count.

In uncomplicated typhoid the blood always shows leucopenia with relative lymphocytosis in early infection (Lucas and Gilles, 1993). A leucocyte count can therefore be valuable in all early cases of suspected typhoid. Leucocyte count is cheap and easy to perform and interpret though it is often neglected.

2.2.5. **Diazo Urine Test**

The diazo urine test is another test often used for screening typhoid fever (Huckstep, 1962, Mulligan, 1971). The test is based on a red coloration given by the froth of urine of typhoid patient when mixed with diazo reagent, the active principle in urine being a product of the putrefaction of protein in the intestine. Diazo urine test is often used in areas where laboratory facilities for culture are minimal (Cheesbrough, 1993; Lucas and Gilles, 1993). However, the limitation in this test procedure is that positive results may occur in patients with pulmonary tuberculosis, measles and typhus. About 5% false positive reaction occurs in non typhoid patients (Cheesbrough, 1993).

2.2.6 Molecular and other Methods of Diagnosis

Molecular techniques used in diagnosing typhoid are not yet fully developed. Nested PCR based ViaB sequence was found to be highly specific in detection of *S.typhi* in clinical specimens including blood (Sharma and Arya, 1995) but the

sensitivity of the method was poor. The polymerase chain reaction (PCR) method based on Hi-d flagellin was reported to detect *S.typhi* from blood but could not be used on stool samples which may contain other serovars of salmonella (Song, *et.al.*, 1993). The nested PCR method can defect even a single cell of *S.typhi* from blood. The combination of PCR primers based on the ViaB region and a nested strategy is a rapid and specific method that could be applied for the defection of *S.typhi* from all clinical specimens including blood (Hashimoto, *et.al.*, 1995).

Immunodiagnostic methods apart from the Widal test have also been developed with the hope to overcome some of the problems encountered with the Widal test. Counterimmuno electrophoresis (Harish and Sambasiva Rao, 1983; Sleigh and Duguid, 1989) gives higher proportion of positive reaction (96%) in typhoid patients and lower proportion of false positive reaction (0%) in uninfected subjects compared to the Widal test which gave 73% and 16% respectively. Specific IgM - adherence test (Ong, et al, 1989) detected 95% of confirmed typhoid cases with no false positive. Monoclonal antibody test (Sadallah, *et al*, 1990) based on flagellin are still under evaluation for their practical use for rapid diagnosis of typhoid. Vi antigen - specific DNA probes (Rubin, *et al*, 1989) detected only 17 of 33 culture confirmed typhoid cases. The probe also hybridized to 4 of 47 patients from whom *S.typhi* was not isolated. Molecular and other methods however promising

will have the limitation of cost, equipment and technical expertise in many laboratories in the developing countries.

2.3 TREATMENT

Chloramphenicol, co-trimoxazole and ampicillin are the standard first line drugs for the treatment of typhoid fever (Soe and Overthurf, 1987; Islam, *et.al.*, 1988; Benenson, 1990).Chloramphenicol is however the most effective antibiotic for treating typhoid (Sleigh and Duguid, 1989; Lucas and Gilles, 1993), although it induces marrow aplasia at high doses administered over a long period so that effective treatment is not without hazards to the patient. Ceftriaxone is a better alternative to chloramphenicol (Islam, *et.al.*, 1988).

Locally, treatment of typhoid fever with chloramphenicol was found to be very effective in Lagos (Ogunbiyi and Onabowale, 1976), in Ilesha (Mulligan, 1971). In Zaria *S.typhi* strains isolated from typhoid patients were sensitive to ampicillin, colistin sulphate and chloramphenicol with low sensitivity to sulphonamides (Akoh, 1987).

The emergence of drugs resistant strains of *S.typhi* to all classical drugs has posed a problem in the management of typhoid fever (Farid, 1992; Yaneza and Kumari, 1993). Gulati *et.al.*, (1991) found 95% of *S.typhi* isolates to be resistant to the first line antibiotics. Ciprofloxacin and intravenous ampicillin are effective and economic drugs for treating

typhoid in developing countries (Finkelstein and Markel, 1990; Gulati, *et.al.*, 1991), though ciprofloxaxin causes acute renal failure as side effect (Simpson, *et.al.*, 1993).

Antibiotic treatment of chronic carriers is not considered effective. High doses and long courses of ampicillin and co-trimoxazole have been used but results are not satisfactory. Kari, *et.al.*, (1987) obtained satisfactory results with ceprofloxacine in adult chronic salmonella carriers. But surgical removal of the focus of infection is the only reliable treatment of chronic carriers (Anderson, *et.al*, 1969; Finkelstein and Markel, 1990).

2.4 PREVENTION AND CONTROL

Typhoid fever is a preventable disease. It can be prevented and controlled in any community in several ways.

2.4.1 Preventive Measures

The public should be educated about the importance of hand washing. Suitable hand washing facilities should be provided especially for food handlers and attendants involved in the care of patients and children (Benenson, 1990).

Human faeces should be disposed of in a sanitary manner and people should endeavour to keep fly-proof latrines. Sufficient toilet paper should be used to minimize finger contamination and hands must be thoroughly washed after using the lavatory. Pit toilets should be located at a distance, and

down stream from the source of drinking water (Lucas and Gilles, 1993)

Scrupulous cleanliness should be maintained in food preparation and handling and attention should be directed to the proper storage of salads and other foods served cold for home or public eating places. Also milk and dairy products should be pasteurized.

Carriers of *S.typhi* should be restricted of occupation unless cured by combined antibiotic therapy (Benenson, 1990) or by surgical excision of focus of infection and proved by 3 consecutive negative cultures obtained from authenticated fecal and urine specimens taken at least one month apart and at least 48hr after antibiotic therapy has stopped.

Typhoid fever immunization: Persons living in highly endemic areas and household members of known carriers should be vaccinated against typhoid. Typhim Vi vaccine administered intramuscularly or by subcutaneous route guarantees protection with a duration of at least three years.

Untreated typhoid carriers should not be allowed to handle food for the consumption of others except members of their immediate families.

2.4.2 Control in Patients, Contacts and immediate Environment.

Typhoid patients should be isolated and released only after 3 consecutive negative fecal and urine cultures.

All cases should be treated with amoxicillin. co-

trimoxazole or chloramphenicol or as indicated by the antibiotic sensitivity test.

The actual or probable source of infection of every case should be determined by search of unreported cases, carriers or contaminated food, water, milk or shellfish. High titres of antibody to the Vi antigen is highly suggestive of typhoid carrier state. Identification of the same phage type in the organisms isolated from patients and a carrier suggests a possible chain of transmission.

2.4.3 **Measures in epidemic situations.**

Intensive search for index case or carrier which is the source of infection and for the vehicle (water or food) by which the infection was transmitted, and suspected food should be excluded.

Pasteurize or boil milk or exclude milk supplies and other foods suspected on epidemiologic evidence until safety is assured.

All water supplies should be chlorinated under competent supervision or the water should not be used. Drinking water should be chlorinated, treated with iodine or boiled before use (Lucas and Gilles, 1993)

CHAPTER 3

MATERIALS AND METHODS

3.1 RETROSPECTIVE STUDY (FIELD SURVEY)

Data on the prevalence of typhoid and malaria fevers were collected from four hospital laboratories in Zaria for the period of January to December 1995. The laboratories were identified by code numbers I, II, III and IV. Information on the method of Widal test for each laboratory was also recorded.

3.2 PROSPECTIVE STUDY

Patients directed to the laboratory for the Widal test by the attending physician at the laboratories mentioned above were sampled in this study. Blood samples were collected from a total of 222 hospital patients comprising 101 females and 121 males with ages between 2 and 64 years. 52 blood samples were also collected from apparently healthy individuals as controls.

3.2.1 Sample Collection

The blood samples were collected by venepuncture. Briefly, an improvised tourniquet was applied firmly on the upper arm of the individual. A prominent vein was located and the area was swabbed clean with cotton wool soaked with ethanol. Then a sterile syringe was used to draw 5 ml of blood. All blood samples were screened for *S. typhi* O and H antibodies, examined for malaria parasites and cultured for the isolation of *S. typhi*

3.2.2 Widal Test

The method described by Raphael and Spencer (1983) was adopted using stained bacterial fibril antigen suspensions of the somatic and flagella antigens of *S.typhi* produced by Cal-Test diagnostic Inc. Chino, CA 91710, USA.

To obtain serum, 3 ml of blood sample were allowed to clot at room temperature. After centrifugation the serum was separated into a micro tube with the aid of a sterile pasteur pipette. The rapid slide screening and slides titration tests were then carried out as follows:

Widal Screening Test.

The rapid slide screening test was carried out by placing 50 uL of serum (using a graduated micropipette) on a clean glass slide. 50 uL of well shaken *S.typhi* O antigen were then added and mixed with a clean plastic applicator stick. This was then rocked gently for one minute and observed for agglutination. The same procedure was also carried out for the H antigen.

Slide Titration Test:

The slide titration method was chosen to reflect the field situation. The rapid slide titration agglutination test was carried out on any sample that showed agglutination in the widal screening test.

A graduated micropipette of 100 uL capacity was used to deliver 80uL, 40 uL, 20 uL, 10 uL and 5 uL of serum onto separate squares made on a large glass slide. 30 uL of well

shaken antigen suspensions was added to the serum and mixed with applicator sticks. Then the slide was rocked back and forth for 3 minutes and observed for agglutination under a table lamp light source. The titre of the serum against the antigen was considered to be shown by the highest dilution of that serum giving a 2 plus agglutination.

3.2.3 **Parasitological Examination**

For the purpose of determining the influence of malaria parasites on the Widal test blood samples of all patients sent for either Widal test or Widal and malaria tests as well as healthy persons were examined for plasmodium parasites. Both thick and thin blood films were prepared from each sample (WHO 1985).

Thin blood films:

A drop of blood was placed on one end of a clean microscope slide and spread out into a thin film one cell layer thick using a spreader. The film was allowed to air dry completely then labelled with an Hb pencil, fixed in methanol for 2 minutes and stained with 10% Giemsa stain for 15 minutes. Stained smears were rinsed in phosphate buffered solution (PBS) and allowed to drain dry.

Thick blood films:

The drop of blood was placed on a clean slide. Using the edge of a spreader the blood was spread out in a circular shape of about 2cm in diameter and allowed to air dry for about 1hr in an insect and dust free environment. Then the film

was dehaemoglobinized by dipping it in PBS for about 30 seconds before staining in the same way as for thin blood films.

Examination of blood films :

Giemsa-stained blood films were examined under the oil immersion (100x) objective of the light microscope. Parasitaemia was evaluated per microlitre of blood using the thick film preparation as described by WHO (1985). Parasite counts were calculated by assuming a leucocyte count of 5400/uL. This value was established for healthy Nigerians as a mean of 175 blood samples (Akinyanju and Grossman, 1973).

Parasite count in relation to leucocyte count was converted to parasite count per uL by the formula:

(No of parasite/No of Leukocytes) x 5400 = parasite per uL

Then a correlation analysis was carried out between parasite count/uL and Widal titre to find out whether parasite counts would influence Widal test.

3.2.4 Bacteriological Blood Culture

Using strict aseptic techniques 5ml of venous blood were collected from each person. 2 ml of the blood were put into a sterile blood culture bottle (McCartney bottle with screw cap having a perforation lined with a rubber bong) containing 18ml of thioglycolate broth (DIFCO, Detroit, Michigan, U.S.A.) as enrichment medium. The broth was gently shaken to mix with the blood and incubated at 37°C for 48 hr, then streaked on MacConkey agar (OXOID Limited, Basingstoke, UK). plate as a differential medium. A further subculture from the

thioglycolate broth of samples that did not show growth on MacConkey agar in the first instance was done on the 7th day and thereafter any sample that did not show growth was considered as negative. All suspect isolates were gram stained and subjected to serotyping and biochemical testing as described below.

Preliminary differentiation of isolates:

Preliminary differentiate was based on the reaction of isolates on triple sugar iron agar (TSI) and urea agar slant. Characteristic non lactose fermenting, gram negative colonies on MacConkey agar plates were inoculated into TSI and urea agar slants and incubated at 37℃ for 24hr. Those which gave typical *S.typhi* reactions of K/A (H_2S) on TSI and were urease negative were stored at 4℃ for further identification.

Serotyping.

This was carried out by the method of Ewig (1982). The isolates were subcultured in nutrient broth and incubated at 37℃ overnight. 5uL of the broth culture was placed on a clean slide. The same amount of Salmonella O polyvalent A to S (Wellcome Laboratories, Daitford, England DAI 5AH) was added and mixed. The slide was tilted back and forth until agglutination was observed in one minute. The purpose of this test was to confirm the isolate as Salmonella or otherwise. Presumptive species identification was based on biochemical reactions due to the lack of specific antisera.

Biochemical characterization

Biochemical characterization of isolates was based on standard techniques (Ewig 1982; Cowan, 1981). All isolates stored at 4℃ after preliminary differentiation were subjected to the following biochemical tests as adjuncts to the serotyping: Indole test, motility test, Lysine iron agar (LIA) test, phenyl alanine decarboxylase test, citrate utilization test, lysine decarboxylase test, methylred and voges-proskauer (MR-VP) tests, gelatin liquefaction test, ornithine decarboxylase test, oxidase test, bile aesculin test, and sugar fermentation tests. A 1% sugar in peptone water was prepared and bromocresol purple indicator was incorporated into all the sugar preparations. The organisms were tested on the following sugars: glucose, maltose, mannitol, xylose, lactose, sucrose, dulcitol rhamnose and salicin.

Antibiotic susceptibility testing:

Antibiotic susceptibility testing was carried out on all *S.typhi* isolates by the disc diffusion method (Stokes and Ridgeway, 1981) on antibiotic medium No.1 (DIFCO) using MASTRINGS antibiotic multodiscs (Mast laboratories limited, Bootle Merseyside, U.K) and BRODISCK antibiotic multodiscs (Bronila Diagnostic Systems, Enugu, Nigeria). All isolates were tested for susceptibility to 13 antibiotics at the following disc contents:

ampicillin (25 ug)	tetracycline (25 ug)
cephalexin (25 ug)	chloramphenicol (25 ug)
cefuroxime (30 ug)	erythromycin (5 ug)

gentamycin (10 ug)	penicillin G (1 unit)
floxacin (10 ug)	streptomycin (10 ug)
nitrofurantoin (200 ug)	cloxacillin (5 ug)
co-trimoxazole (50 ug)	

A colony of the culture was inoculated into sterile tryptic soy broth and incubated at 37°C until the turbidity approximated that of the turbidity standard prepared as described by chessbrough (1993). Briefly 0.5ml of 1.175% W/V barium chloride were added to 99.5ml of 1% sulphuric acid and mixed, then 5 ml of the mixture was put in a bijou bottle and used as the turbidity standard.

DIFCO antibiotic medium No.1 agar plates were inoculated with the organisms in duplicates by placing 3 loopfuls of the broth culture on the centre of each plate and spread uniformly with sterile swabs. The plates were allowed to dry for about 5 minutes after which a sterile forceps was used to pick MASTRINGs antibiotic multodisc and placed on one agar plate and BRODISKS antibiotic multodisc was placed on the other duplicate agar plate. The discs were pressed with the same forceps to ensure complete contact with the agar. The plates were incubated at 37°C for 18 hours. After incubation the zone of complete inhibition was measured to the nearest milimetre and interpreted as sensitive, intern=mediate or resistant compare to the sensitivity of the standard staphylococcus strain obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH) Zaria.

CHAPTER 4

RESULT

4.1: RESULTS OF THE RETROSPECTIVE STUDY

Table I presents the results of a field survey carried out to determine the prevalence of typhoid and malaria fevers from the records of four hospital laboratories in Zaria. The results show that a single widal slide agglutination test was the common method of diagnosing typhoid in these laboratories and that positive titre values varied from one laboratory to another: three laboratories considered either anti *S. typhi* O or H or both anti O and anti H agglutinin titres of greater or equal to 1 in 80 as positive test for typhoid, while one laboratory considered only anti-O agglutinin titre values greater or equal to 1 in 64 as positive for typhoid. Based on these, from a total of 6407 patients examined, between January and December, 1995, 2436 cases tested positive for typhoid , with a prevalence of 38%. Three methods of performing the widal test were also observed (table 1):

Laboratory I, used slide titration using only *S.typhi* O antigen with a positive titre $\geq$ 1 in 64.

Laboratory II and II used rapid slide screening test using both *S. typhi* O and *S. typhi* H antigens.

Laboratory IV used rapid slide screening test using either

Table 1: RECORD OF WIDAL AND MALARIA PARASITE TESTS FROM FOUR LABORATORIES IN ZARIA FOR THE YEAR 1995.

Hospital No.		Widal Test		Malaria parasite test		
	No. tested	No. positive	% Positive	No. tested	No. Positive	% Positive
I	2288	346	15.1	1109	766	69.1
II	1409	541	38.4	46	28	60.1
III	432	241	55.8	42	25	59.5
IV	2278	1308	57.8	1406	720	51.2
Total	6407	2436	38.0	2603	1539	59.1

Table 2: PREVALENCE OF TYPHOID AND MALARIA PARASITES IN PATIENTS AND CONTROLS.

Hospital No. or Control group	No. of Sample per group	Widal positive	Culture Positive	Malaria positive	Negative in all three tests.
I	76	16 (21.1%)	8 (10.5%)	22 (28.9%)	30 (39.5%)
II	64	10 (15.6%)	3 (4.7%)	13 (20.3%)	38 (59.3%)
III	55	20 (33.4%)	0 (0%)	20 (33.4%)	15 (27.3%)
IV	27	10 (37%)	3 (11.1%)	5 (18.5%)	9 (33.3%)
Total (Patients)	222	56 (25.2%)	14 (6.3%)	60 (27.0%)	92 (41.1%)
Control.	52	6 (11.5%)	0 (0%)	12 (23.1%)	34 (65.3%)

KEY: No. = Number
% = percentage

4.2.1 Results of Widal test.

Table 3 presents the distribution of *S.typhi* antibodies in patients and controls. The results show that 111 (50%) and 32(14%) patient had antibody levels less than 1 in 20 to the O and H antigens respectively which could not be detected by the method used. Of the 52 controls, 36(69%) had O antibody titres less than 1 in 20. On the other hand only 12(23%) of the healthy persons had anti H titres less than 1 in 20.

4.2.2 Widal titres and malaria parasites

Table 4 shows the distribution of *S. typhi* antibodies in malaria positive patients. Out of the 60 malaria positive patients, 26 and 4 had *S. typhi* O and *S. typhi* H antibody titres less than 1 in 20 respectively, while 18 had anti O titre of 1 in 160 and 4 had anti O titre of greater or equal to 1 in 320 making a total of 22 patient samples positive for both malaria parasites and the Widal test. Most malaria patients had high levels of H antibodies. Appendix III shows details results of malaria positive samples in which parasite counts ranged from 76 to 99,000 parasites/ul of blood. Table 5 shows the results of Widal test in malaria positive healthy individuals (controls). Most carriers of malaria parasites had low antibody levels to the somatic antigen of *S.typhi* $\leq$ 40. Three malaria carriers actually had anti O titres greater or equal to 1 in 160.Six of the 12 carriers of malaria parasites had anti O titres of greater or equal to 1 in 40 with 3 of them having a titre of greater or equal to 1 in 60.

Table 3. *DISTRIBUTION OF S.typhi O AND H ANTIBODIES IN PATIENTS AND CONTROLS.*

Groupof antigens	Antigen(O orH)	Widal titre values and number of cases				
		<20	40	80	160	320
Patients n=222	O	111(50%)	35(12%)	20(9%)	45(20%)	11(5%)
Patients n=222	H	32(14%)	37(16.8%)	44(20%)	66(29%)	43(19%)
Control n=52	O	36(69%)	8(15%)	2(4%)	5(10%)	1(2%)
Control n=52	H	12(23%)	12(23%)	11(21%)	14(27%)	3(6%)

Key: O = Somatic (O) antigen of *S. typhi*

H = Flagella (H) antigen of *S. typhi*

NO = Number of

n = total number

Table 4. DISTRIBUTION OF S.typhi O AND H ANTIBODIES IN MALARIA PATIENTS.(n=60)

Antibody O titres	<20	40	80	160	>320
No. of persons	27	8	3	18	4
Antibody H titres	<20	40	80	160	>320
No. of persons	4	5	16	19	16

Key: No. = number

Table 6 Shows the results of correlation analysis for Widal titres and parasite counts in malaria positive patients. The results show that there was very little correlation between parasite counts and Widal titre values. The maximum correlation coefficient (r) for O and H antibody titres and parasite count was -0.014 and 0.054 respectively. The negative value for correlation with O antibody titres indicated that many malaria patients had high parasite counts but with low corresponding Widal titre values. Over all there was no correlation between parasite count and antibody levels as the result was not statistically significant ($P<0.1$)

4.2.3 Results of blood culture

A positive culture result refers to the isolation and bacteriological identification of *S.typhi* from sample by standard culture methods. Based on this,14 blood cultures were positive for *S. typhi* giving a percentage isolation of 6.3%. Table 7 gives details of test results of the 14 culture positive patients. One sample designated M26 was positive for the Widal test, culture and malaria parasites. Seven of the 14 culture positive samples were also positive for the Widal test while 5 had 'O' antibody titre of less than or equal to 1 in 20. However, all except one sample (A42) showed antibody levels of greater than or equal to 1 in 40 to the flagella antigen. Also sample number A42 failed to show the presence of antibodies to both the somatic and flagella antigens.

Table 5 : DISTRIBUTION OF S.typhi O AND H ANTIBODIES IN HEALTHY CARRIERS OF MALARIA PARASITES (n=12)

Antigen O titres	<20	40	80	160	>320
No of persons	6	3	0	2	1
Antigen H titres	<20	40	80	160	>320
No of persons	1	2	2	5	2

Key: n = number

Table 6: CORRELATION COEFFICIENTS FOR MALARIA PARASITE COUNTS AND *S.typhi* O AND H ANTIBODY LEVELS IN MALARIA PATIENTS.

Category of patients	Correlation Coefficient (r) for O_{ab} and malaria parasite count	H_{ab} and malaria parasite count
All malaria patient (n=60)	-0.014	0.054
Males (n=30)	0.129	0.033
Females(n=30)	0.021	0.125
Age group 1-10 yrs (n=6)	0.104	0.25
Age group 11-20 yrs (n=16)	0.004	0.048
Age group 21-30yrs (n=29)	0.128	0.021
Age group >30yrs (n=8)	-0.081	0.082

Key: Oab = antibody titres to the somatic (O) antigen of *S.typhi*
Hab = antibody titres to the flagella(H) antigen of *S.typhi*
Mp = Malaria parasite counts.

CULTURE POSITIVE PATIENTS

			Widal titre for		mp
Sample code	Age (years)	Gender	O_{ag}	H_{ag}	Count/ uL
A28	10	M	80	80	0
A42	17	F	<20	<20	0
A59	2	M	160	320	0
G3	17	M	<20	160	0
G11	18	M	40	320	0
G16	26	F	320	320	0
M8	33	M	160	320	0
M12	28	M	160	320	0
M26	23	M	320	160	14580
W8	49	M	160	320	0
W19	6	M	<20	80	0
W20	10	M	<20	160	0
W25	18	F	160	160	0
W31	16	M	<20	320	0

Oag = somatic (O) antigen of *S. typhi*

Hag = flagella (H) antigen of *S. typhi*

M = male

F = female

mp = malaria parasite

The difference in the incidence of typhoid by Widal (25.2%) and culture methods (6.3%) was statistically significant ($P<0.01$). Of all the culture positive patients 11 were males while only 3 were females and ages 10 to 20 years accounted for 50% of the culture positive cases.

Results of Biochemical tests

The result of biochemical tests are shown in appendix 4.

4.2.4 Results of antibiotic susceptibility test

The 14 *S typhi* isolates were tested for susceptibility to 13 antibiotics and the results are presented in table 9. All isolates were sensitive to 6 or more drugs presenting 4 patterns of sensitivity as follows: one isolate was sensitive to 9 drugs, 3 isolates were sensitive to 8 drugs, 8 isolates were sensitive to 7 drugs, and 2 isolates were sensitive to 6 drugs. Isolates with intermediate sensitivity were classified as resistant to the particular drug. Overall, all isolates were sensitive to cephalexin, floxacin, nitrofurantoin, tetracycline and chloramphenicol. Patterns of resistance and susceptibility of isolates are shown in tables 10 and 11.

All isolates were resistant to four or more drugs with a set pattern of resistance to erythromycin, penicillin G, streptomycin and cloxacillin which are drugs that are commonly used on Gram positive bacteria. Eight patterns of resistance to the 13 antibiotics used were observed as follows: one pattern of resistance to 7 drugs, 3 patterns of resistance to

6 drugs, 3 patterns of resistance to 5 drugs and one pattern of resistance to 4 drugs.These resistance patterns are presented in table 11.

Table 8: CONTINGENCY TABLE FOR WIDAL TEST AND BLOOD CULTURE RESULTS

Widal test result	Culture positive	Culture negative	Total
positive	7(TP)	49(FP)	56
negative	7(FN)	159(TN)	166
Total	14	208	222

Key: TP = True positive(positive for widal test and culture).

FN = False negative widal reaction .

FP = False positive widal reaction.

TN = True negatives(negative for widal test and culture).

Calculations:

a) Sensitivity=100xTP/(TP+FN) = 100x7/14=50%

b)Specificity=100xTP/(TN+FP)=100x159/(159+49)=76.4% c)% False negative=100xFN/(FN+TN)= 100X7/166=4.2%

d)%false positive=100XFP/(FP+TP)=100X49/(49+7)=87.5%

Table 9: RESULT OF ANTIBIOTIC SUSCEPTIBILITY TESTING OF *S. typhi* ISOLATES

ISOLATE No	AMP	PG	E	CXM	CX1	CX2	F	FX	S	TE	GEN	C	COT
A28	R	R	R	S	S	R	S	S	R	S	S	S	S
A42	S	R	R	S	S	R	S	S	R	S	I	S	R
A59	S	R	R	S	S	R	S	S	R	S	S	S	R
G3	S	R	R	R	S	R	S	S	R	S	I	S	S
G11	S	R	R	R	S	R	S	S	R	S	R	S	R
G16	S	R	R	R	S	R	S	S	R	S	I	S	I
M8	S	R	R	R	S	R	S	S	R	S	S	S	S
M12	S	R	R	S	S	R	S	S	R	S	S	S	S
M26	R	R	R	R	S	R	S	S	R	S	S	S	S
W8	R	R	R	R	S	R	S	S	R	S	S	S	S
W19	R	R	R	R	S	R	S	S	R	S	S	S	S
W20	R	R	R	R	S	R	S	S	R	S	S	S	S
W25	R	R	R	R	S	R	S	S	R	S	S	S	S
W31	R	R	R	R	S	R	S	S	R	S	S	S	S

S = Sensitive to, R = resistant to,

I = Intermediate AMP=ampicillin

Cx_1= cephalexin S = Streptomycin

PG =Penicillin G Cx_2= cloxacillin

TE= Tetracycline E = Erythromycin

F = nitrofurantoin GEN=gentamycin

CXM=cefuroxime FX = floxacin

C = chloramphenicol COT=co-Trimaxazole

Table 10: RESISTANCE AND SUSCEPTIBILITY PATTERNS OF THE 14 ISOLATES TO 13 DIFFERENT ANTIBIOTICS

Antibiotic	No. of isolates sensitive	No. of isolates resistant *
Ampicillin	7(50%)	7(50%)
Penicillin G	0(0%)	14(100%)
Co - trimoxazole	10(71.4%)	4(28.6%)
erythromycin	0(0%)	14(100%)
Cefuroxime	4(28.6%)	10(71.4%)
Cephalexin	14(100%)	0(0%)
Cloxacillin	0(0%)	14(100%)
Nitrofurantoin	14(100%)	0(0%)
Floxacin	14(100%)	0(0%)
Streptomycin	0(0%)	14(100%)
Tetracycline	14(100%)	0(0%)
Gentamycin	10(71.4%)	4(28.6%)
Chloramphenicol	14(100%)	0(0%)

* All isolates giving intermediate sensitivity as shown in table 9 were considered as resistant to the drug.

Table 11 RESISTANCE PATTERNS OF *S. typhi* ISOLATES

Resistance type	Isolate Number.
1) GEN, PG, E, COT, COX, CX_2 S	G11, G16
2) AMP, PG, E, CXM, CX_2, S	M26, A28, W20, W25 W31, W19
3) GEN, PG, E, CXM, CX_2, S	G3
4) GEN, PG, E, COT, CX_2, S	A42
5) AMP, PG, E, CX S	A28
6) PG, E, COT, CX_2, S	A59
7) PG, E, CXM, CX_2, S	M8
8) PG, E, CX_2, S	M12

GEN = gentamycin
PG = penicillin G
E = Erythromycin
COT = Co-trimoxazole
CXM = Cefuroxime
CX_1 = cloxacillin
CX_2 = cephalexin
S = Streptomycin
AMP = Ampicillin

CHAPTER 5

DISCUSSION

The use of a single Widal test in diagnosing typhoid is highly debatable. From the results of the present investigation the sensitivity of the single slide agglutination Widal test was only 50% and the specificity was 76.4%, and there was also a 4.2% false negatives and 87.5% false positive when the Widal test results were compared with blood culture. Although there is a growing number of literature on the limitations and shortcomings of the Widal test in diagnosing typhoid fever (Pang and Puthucheary, 1983; Ong, *et. al.*, 1989; Lewis, 1992; Cheesbrough, 1993; Petit and Wamola, 1994) it is however recommended that where culture procedures are not available the Widal test if carefully performed can be helpful (Buck, *et. al.*, 1987) especially with the knowledge of the background level of *S. typhi* antibodies in the local healthy population (Cheesbrough, 1993).

The prevalence of typhoid fever in Zaria has reportedly been on the increase ever the years. (Okolocha, *et. al.*, 1993). A study of 1949 blood culture for the isolation of *S. typhi* from patients in Zaria over a 10 year period (Okolocha, *et. al.*, 1993) revealed that the prevalence of typhoid in this environment was 3.1%. In the present study the incidence of typhoid by culture was 6.3% indicating that the number of new

cases is increasing although a small sample size was used. This can be attributed to stagnant or deteriorating sanitary standards and poor living habits in many rural areas(like in suburbs in Zaria where many of the patients come from) which serve as predisposing factors to infection or poor control of patients misdiagnosis and general lack of awareness thus leading to increased spread of the agent in the population. Also in this study males were far more infected by *S.typhi* than females where the sex specific ratio of males to females was 3:1 compared to earlier reports of sex specific ratio of 1.13:1 (Akoh, 1987) and 3.2:3 (Okolocha, *et. al.*, 1993) for male and females respectively. The finding in the present work shows that the males in Zaria are increasingly more exposed to *S. typhi* infection than the females. This may be as a result of their living habits and cultural practices which is further compounded by the present economic crisis in the country. Most reports on typhoid fever show that males are slightly more affected than females and that the highest incidence occurs in people in the late teens and twenties.

In Zaria, Akoh (1987) reported a peak incidence in ages 5 to 19 years involving 54.69% of typhoid patients. In this work also the peak incidence was observed in patients between 10 and 20 years of age who accounted for 50% of the typhoid patients.

Bucks, *et. al.*, (1987) reported that a single pretreatment Widal test detected about 50% of the culture

positive cases which tally with the findings of the present study. From this it is evident that a single Widal test can not be of definitive diagnostic value in deciding a typhoid case, particularly so when in Zaria up to 11.5% of the local healthy persons had antibody titres for the somatic antigen of *S. typhi* of greater or equal to 1 in 160 and a high degree of false positives observed in patients. The presence of high levels of salmonella antibodies in the local healthy population in Zaria and the low sensitivity and specificity of the Widal test is a clear indication that a single widal test will provide negligible diagnostic assistance in the investigation of *S.typhi* infection in this environment.

The most certain method of confirming typhoid fever is by cultural isolation of *S.typhi* from clinical patient samples. However in many developing countries where typhoid is endemic, including Nigeria, this is usually not the general practice. The faster single Widal test is preferred irrespective of the widely documented criticisms. Adherents of Widal test argue that culture procedures are time consuming but in this study all 14 blood culture positive cases were identified within 72 hours and the method provided very reliable information to the physicians who would have had to wait for 7 to 10 days for the demonstration of a four-fold rise in antibody titres or seroconversion before making a definitive diagnosis. Yet there is the fear that many typhoid patients will never be diagnosed by the Widal test because only a 2 or 3-fold rise in titre is

likely to occur in patients (Cheesbrough, 1993).

Complications and seriousness of typhoid may result from delays in diagnosis (Khuri-Bulos, 1981) and misdiagnosis is actually one of the factors contributing to death from typhoid infection among Nigerians (Olubuyide, 1992). Akoh (1987) reported a case fatality rate of 17.2% in typhoid patients in Zaria and according to Benenson (1990) the general case fatality rate of 10% in typhoid can be reduced to less than 1% with prompt antibiotic therapy. This can only be achieved if diagnosis of the disease is made on time. It will therefore be necessary for clinicians to opt for sensitive, reliable and cheap diagnostic techniques such as blood culture for the diagnosis of typhoid. This will also have the advantage of solving the problem of high rate of false positive and the false negative Widal reactions and consequently reducing the risk of exposure of uninfected individuals to anti-typhoid chemotherapy. It will also reduce the rate of emergence of resistant strains of *S.typhi*.

The result of the correlation analysis (table 6) show that malaria parasite density did not affect antibody levels in suspected typhoid patients. Although 34 of the 60 malaria positive patients had detectable levels of O antibodies, parasite counts and antibody levels were shown not to be mutually correlated irrespective of age and sex of the patients. However, from appendix 3 certain individuals showed low parasite counts with correspondingly high antibody titres.

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and other who had high parasite counts showed corresponding low Widal titres while another group with high parasite counts also had high Widal titres. From these observations it seems that the relationship between malaria parasite counts and *S. typhi* antibody titres depend on host immune response.

In a study conducted in Calabar, Alaribe, *et. al.*, (1995) using the same method as in this work, reported that only 12 (15%) of 80 suspected typhoid patients had significant anti 0 titres $\geq$ 160 as against the 22 (36.6%) patients in this study who were positive for both Widal test and malaria parasites. These could be cases of co-infection or antibodies to malaria parasite and other antigens cross react with *S typhi* antigens as earlier reported (Alaribe, *et.al.*, 1995). But from the culture positive results (table 7) it seems that co-infection will occur only in 7.1% of typhoid cases as against the 36.6% shown by the Widal test.

The fact that symptoms of typhoid and malaria fevers may mimic each other in early stages of infection (Petit and Wamola, 1994) poses a major diagnostic problem in areas where both diseases are endemic. For instance the one sample in this study that was positive for culture and malaria parasites (table 7) could be missed if appropriate diagnostic procedures such as blood culture was not employed.

Malaria can be associated with typhoid and could by its immunosuppressive effect induce salmonella infections (Hathout, et al 1966), as well as induce a false positive

Widal reaction (Cheesbrough, 1993; Hyneman and Mekerrow, 1989; Petit and Wamola, 1994). Hence this study also showed that the presence of malaria parasites could result in a high or low antibody level to *S.typhi* antigens in a non specific relationship.

Another important finding in this work was the 23.1% carrier rate for malaria parasites in Zaria. Similar findings have also been reported elsewhere in the country by Somiari and Amanam, (1995) who reported a prevalence of 18.7% in blood donors and 24.1% in 166 controls. Asymptomatic carriers of malaria parasites is a problem in blood banks (Ogunledun *et. al.*, 1990) and could rather aggravate the condition of patients receiving such blood since it is not the usual practice to screen for malaria parasites during blood donation.

On the susceptibility of *S typhi* isolates to antibiotics the finding that all isolates were resistant to erythromycin, streptomycin, cloxacillin and penicillin G does not appear to have any clinical significance since these drugs are not routinely used against gram-negative bacteria. However the observed 50% resistance of isolates to ampicillin and 28.6% resistance to co-trimoxazole which in addition to chloramphenicol are the standard first line drugs for the treatment of typhoid (Soe and Overturf, 1987; Islam, *et. al.*, 1988) is an indication that strains of drug resistant *S.typhi* are common in Zaria. Also of importance was the observation

that resistance to ampicillin and co-trimoxazole was not restricted to isolates from a particular locality implying that the resistant strains are widespread in Zaria. The observed sensitivity of isolates to chloramphenicol, nitrofurantoin, floxacin, cephalexin and tetracycline provide alternative drug list for the management of typhoid fever in Zaria though the use of chloramphenicol is often with reservations because of its suppressive effect on bone marrow proliferation (Anderson, 1966).

From the results of the field survey carried out at the beginning of this study (table I) a single Widal test was the common method of diagnosing typhoid in Zaria. However in the present work the single Widal test and blood culture methods were adopted. In addition instead of using the positive Widal titre of greater or equal to 1 in 80 as was commonly used in the hospitals, a titre of $\geq$ 1 in 160 of O antibodies was used. The advantage of using both methods was to confirm whether the result obtained from a single widal reaction did actually indicate *S.typhi* infection. This proved to be a valuable tool as indicated in the foregone discussion. For there were many patients with a positive Widal test who could have been treated for typhoid when actually they were not having the disease. Also 7 (50%) patients whose blood culture yielded *S.typhi* may have died of typhoid because the single Widal test did not identify them Table 7. From this it would be concluded in agreement with Gaviria-Ruiz and Castro, 1995) that the

cultural isolation of *S.typhi* from blood is one of the best methods of diagnosing typhoid fever.

Judging from the 38% prevalence of typhoid from hospital records in some hospitals around Zaria between January and December, 1995 (table 1) it is possible that many of the patients never really had typhoid due to the 87.% false positive observed in this study using the single Widal test results. There is also the danger that many of the patients who tested negative for typhoid in those hospitals could have had the infection but were never diagnosed since a negative Widal test result does not invariably indicate the absence of typhoid given that the specificity of the test was only 76.4%. Moreover it takes about two weeks for significant amount of antibodies to be produced and the idea of paired sera is completely neglected in this environment.

The lack of interest in following standard laboratory procedures in performing the Widal reaction and the reliance on a single widal test in an environment with a high level of salmonella antibodies in the local healthy population (controls) adds to the abuse of Widal test in Zaria. This reflected in the very high prevalence (38%) of typhoid from hospital records in this community which is detrimental to the already needy population.

CONCLUSION AND RECOMMENDATIONS:

From the results of this work the dependence on single Widal test in the diagnosis of typhoid fever is highly undesirable. Even in the presence of high antibody level, clinician should be careful in reaching conclusion. In the absence of materials for cultural studies, it is strongly recommended that in a malarious region as ours, paired results should be used for a possible rise in antibody level against *S.typhi* in conjunction with compatible clinical signs. The Federal Ministry of Health should clamp down on quack laboratories that are ill-equiped to carry out laboratory diagnosis. Results from such laboratories have given rise to the false epidemic of typhoid fever. In addition it should be made a law that where clinical diagnosis is not sufficient to conclude a case as typhoid, paired sera should always be used and isolation of the offending pathogen as confirmatory.

Regular public lectures and education of the population on the importance of good personal and environmental hygiene and the supply of portable water will greatly reduce the incidence of preventable diseases in zaria, typhoid fever inclusive.

The government should set up a centre for infectious and communicable disease, surveillance, to monitor the epidemiology of significant public health diseases such as typhoid and device and implement effective preventive and

control measures.

Similar studies should be carried out in other parts of the federation in order to set up a standard control scheme for the whole country.

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APPENDIX 1: RESULTS OF WIDAL TEST, BLOOD CULTURE

AND MALARIA PARASITE TEST OF PATIENTS

S/No	Sample code	Age	Gender	O/Ag	H/Ag	mp.count/uL	Blood culture
1	A1	30	F	160	320	9765	N
2	A2	36	M	40	80	0	N
3	A3	16	M	40	40	0	N
4	A4	23	M	160	160	3186	N
5	A5	28	F	<20	40	0	N
6	A6	41	M	80	80	1755	N
7	A7	3	M	<20	40	0	N
8	A8	35	M	40	40	0	N
9	A9	33	M	<20	80	0	N
10	A10	26	F	40	320	11097	N
11	A11	36	M	<20	<20	0	N
12	A12	29	F	<20	80	1323	N
13	A13	40	M	40	40	0	N
14	A14	35	F	80	160	0	N
15	A15	31	M	<20	<20	0	N
16	A16	27	M	<20	<20	0	N
17	A17	23	F	80	320	13824	N
18	A18	35	M	<20	<20	0	N
19	A19	26	F	<20	40	0	N
20	A20	4	M	<20	<20	0	N
21	A21	29	M	<20	20	0	N
22	A22	7	M	<20	<20	0	N
23	A23	26	F	<20	40	0	N
24	A24	27	M	160	80	15780	N
25	A25	29	M	<20	80	0	N
26	A26	28	F	<20	80	0	N
27	A27	17	M	<20	80	0	N
28	A28	10	M	80	80	0	P

S/NO	Sample code	Age	Gender	O/Ag	H/Ag	mp.count/uL	Blood culture
29	A29	22	M	<20	<20	2890	N
30	A30	28	F	80	160	0	N
31	A31	32	M	<20	80	5697	N
32	A32	2	M	<20	160	0	N
33	A33	28	F	<20	160	6912	N
34	A34	22	M	160	320	8559	N
35	A35	23	M	40	40	0	N
36	A36	6	M	40	80	0	N
37	A37	33	M	<20	80	0	N
38	A38	31	F	<20	80	0	N
39	A39	18	M	160	320	0	N
40	A40	4	M	<20	40	0	N
41	A41	5	M	320	320	3195	N
42	A42	17	F	<20	<20	0	P
43	A43	45	F	<20	<20	0	N
44	A44	18	M	<20	40	0	N
45	A45	28	F	<20	40	0	N
46	A46	25	F	<20	80	0	N
47	A47	14	F	80	160	0	N
48	A48	8	F	80	160	0	N
49	A49	5	M	<20	40	0	N
50	A50	16	F	<20	40	0	N
51	A51	11	F	<20	<20	0	N
52	A52	33	F	40	160	48816	N
53	A53	16	M	<20	40	0	N
54	A54	24	M	320	320	0	N
55	A55	24	F	80	160	0	N
56	A56	18	F	80	80	0	N
57	A57	15	F	<20	40	0	N
58	A58	25	M	160	160	0	N
59	A59	2	M	160	320	0	P
60	A60	24	F	<20	80	0	N
61	A61	18	F	<20	<20	0	N
62	A62	26	M	40	160	0	N
63	A63	18	M	160	160	0	N
64	A64	25	F	80	160	0	N
65	G65	31	F	<20	<20	0	N
66	G66	28	M	<20	160	2257	N

67	G3	17	M	<20	160	0	P
68	G4	52	M	<20	<20	0	N
69	G5	56	F	<20	80	3262	N
70	G6	30	F	<20	40	0	N
71	G7	22	F	80	160	0	N
72	G8	25	F	<20	<20	0	N
73	G9	25	F	160	320	0	N
74	G11	18	M	<20	320	0	P
75	G12	56	M	<20	160	0	N
76	G13	31	F	40	80	0	N
77	G14	19	F	40	40	0	N
78	G15	14	M	<20	40	0	N
79	G16	26	F	320	320	0	P
80	G17	22	M	40	80	0	N
81	L1	12	F	<20	160	57547	N
82	L2	11	F	<20	160	20628	N
83	L3	22	M	<20	80	0	N
84	L4	25	M	40	40	0	N
85	L5	18	F	160	160	26021	N
86	L6	17	F	160	160	0	N
87	L7	20	M	<20	80	296	N
88	L8	26	F	40	160	0	N
89	L9	20	F	40	320	0	N
90	L11	24	F	<20	80	0	N
91	L12	23	M	40	80	0	N
92	L13	27	F	160	320	3780	N
93	L14	31	M	<20	40	0	N

S/No	Sample code	Age	Gender	O/Ag	H/Ag	mp.count/uL	Blood culture
94	L15	29	M	160	320	2592	N
95	L16	21	M	<20	160	0	N
96	L17	18	M	<20	160	0	N
97	L18	31	M	160	320	0	N
98	L19	22	F	<20	40	0	N
99	L20	19	F	320	320	0	N
100	L21	23	M	<20	40	3024	N
101	L22	21	F	<20	40	648	N
102	L23	28	M	<20	80	0	N
103	L24	16	M	<20	320	0	N
104	L26	32	F	<20	80	0	N
105	L27	28	M	320	160	0	N
106	L29	18	F	<20	160	0	N
107	L30	24	F	160	80	846	N
108	L32	25	F	<20	40	0	N
109	L33	64	F	<20	<20	0	N
110	L34	50	F	<20	160	0	N
111	L35	21	F	80	160	0	N
112	L36	28	M	40	80	7714	N
113	L38	9	F	40	80	100000	N
114	L39	27	F	320	320	4970	N
115	L40	25	F	80	80	0	N
116	L41	4	F	160	320	91854	N
117	L42	17	F	320	160	6886	N
118	L43	20	M	<20	160	0	N
119	L44	31	F	<20	80	0	N
120	L45	28	F	<20	160	0	N
121	L46	19	F	80	80	21600	N
122	L47	23	M	<20	80	18900	N
123	L48	40	M	<20	160	0	N
124	L49	36	F	160	160	0	N

S/No	Sample code	Age	Gender	O/Ag	H/Ag	mp.count/uL	Blood culture
125	L50	38	F	160	160	0	N
126	L51	25	F	160	320	0	N
127	L52	16	M	160	160	0	N
128	L53	22	F	<20	320	1322	N
129	L54	29	F	160	160	0	N
130	L56	19	F	160	320	4940	N
131	L57	16	F	160	320	2700	N
132	L58	25	M	<20	160	0	N
133	L59	10	M	160	160	1800	N
134	L60	31	F	40	160	0	N
135	L61	26	F	160	320	0	N
136	M3	25	M	40	20	0	N
137	M4	48	M	<20	20	0	N
138	M5	24	M	160	320	0	N
139	M6	27	M	160	320	0	N
140	M8	33	M	160	320	0	P
141	M9	40	M	80	80	0	N
142	M10	30	M	80	160	0	N
143	M12	28	M	160	320	0	P
144	M22	27	F	40	160	0	N
145	M25	31	M	<20	160	10800	N
146	M26	23	M	320	160	14580	P
147	M32	25	F	<20	160	0	N
148	M34	19	F	<20	40	0	N
149	M37	29	F	320	320	0	N
150	M41	33	M	40	40	0	N
151	M44	25	F	40	160	0	N
152	M61	28	M	160	160	0	N
153	M62	21	F	<20	80	569	N
154	M63	38	M	<20	40	0	N
155	M64	8	F	160	320	0	N

S/No	Sample code	Age	Gender	O/Aq	O/Aq	mp.count/uL	blood culture
156	M65	38	F	<20	<20	0	N
157	M66	16	M	<20	40	1789	N
158	V1	9	M	160	160	9307	N
159	V2	25	F	160	320	0	N
160	V4	37	M	<20	80	0	N
161	V5	27	F	<20	<20	0	N
162	V6	32	M	<20	40	0	N
163	W1	29	F	40	40	0	N
164	W2	31	M	<20	40	5859	N
165	W3	30	M	160	320	12150	N
166	W4	26	M	<20	<20	0	N
167	W5	20	F	40	80	7110	N
168	W6	19	F	160	160	0	N
169	W7	21	F	<20	40	0	N
170	W8	49	M	160	320	0	P
171	W9	26	M	40	80	0	N
172	W10	24	F	80	160	0	N
173	W11	16	M	80	160	0	N
174	W14	25	M	160	320	684	N
175	W15	31	F	<20	<20	0	N
176	W16	25	M	<20	<20	0	N
177	W17	22	F	<20	40	0	N
178	W18	13	F	<20	20	5211	N
179	W19	6	M	<20	80	0	P
180	W20	10	M	<20	160	0	P
181	W21	26	F	<20	<20	0	N
182	W22	8	M	<20	<20	0	N
183	W23	20	M	<20	<20	0	N
184	W24	32	M	<20	<20	0	N
185	W25	18	F	160	160	0	P
186	W26	24	M	160	320	21600	N

S/No	Sample code	Age	Gender	O/Ag	H/Ag	mp.count/uL	blood culture
187	W27	6	M	<20	<20	0	N
188	W28	28	M	40	160	0	N
189	W29	9	F	80	320	0	N
190	W30	23	F	<20	160	1577	N
191	W31	16	M	<20	320	0	N
192	W32	54	F	<20	160	0	P
193	W33	25	F	<20	160	0	N
194	W34	28	M	<20	160	0	N
195	W35	32	M	160	320	0	N
196	W36	18	M	<20	160	3248	N
197	W37	25	M	160	160	1512	N
198	W38	33	F	160	320	0	N
199	W39	29	M	<20	320	76	N
200	W40	25	M	<20	160	297	N
201	W41	23	M	320	320	0	N
202	W42	21	F	<20	160	26065	N
203	W43	10	M	<20	<20	41200	N
204	W44	6	M	<20	80	108	N
205	W45	29	M	40	40	0	N
206	W46	28	M	160	160	0	N
207	W47	23	M	<20	80	210	N
208	W48	33	M	40	160	0	N
209	W49	18	F	<20	40	513	N
210	W50	27	M	<20	<20	0	N
211	W51	24	M	<20	80	0	N
212	W52	22	M	40	160	459	N
213	W53	10	M	40	320	0	N
214	W54	18	F	160	<20	292	N
215	W55	18	M	160	160	0	N
216	W56	22	M	320	320	0	N
217	W57	20	F	40	80	0	N
218	W58	3	M	40	80	0	N
219	W59	31	F	40	40	9000	N
220	W60	25	M	80	160	0	N
221	W61	16	M	40	80	788	N
222	W62	13	M	<20	40	0	N

KEY: F = Female

M = Male

O/ag = Somatic (O) antigen (ag) of *S.typhi*

H/ag = Flagella (H) antigen of *S.typhi*

MP = Malaria parasite.

P = Positive

N = Negative

* = Hospitalised patients (A41 and L38)

APPENDIX 2: **WIDAL AND MALARIA TEST RESULTS OF HEALTHY CONTROLS.**

S/No	Sample code	Gender	Age	O/Ag titres	H/Ag titres	mp. count/uL	Blood culture
1	M4	M	24	20	20	0	N
2	M7	M	29	20	160	0	N
3	M11	M	42	20	20	0	N
4	M13	F	28	40	80	0	N
5	M14	M	18	40	20	0	N
6	M16	M	27	80	80	0	N
7	M17	F	35	320	320	3780	N
8	M18	M	25	20	20	0	N
9	M19	M	27	20	20	0	N
10	M20	M	25	160	160	0	N
11	M21	M	31	160	160	2052	N
12	M23	M	23	20	80	0	N
13	M24	M	35	20	320	702	N
14	M27	M	26	20	80	0	N
15	M28	M	29	20	160	518	N
16	M29	M	31	20	160	0	N
17	M30	M	27	20	40	0	N
18	M31	M	24	20	80	0	N
19	M33	M	27	20	80	0	N
20	M35	M	18	20	40	0	N
21	M36	M	20	20	20	0	N
22	M38	M	25	20	20	261	N
23	M39	M	26	20	20	0	N
24	M40	M	19	20	40	0	N
25	M42	F	29	160	320	0	N
26	M43	F	18	20	40	0	N

APPENDIX 2: Continued

Widal and malaria results of healthy controls.

S/No	Sample code	Age	Gender	O/Ag titres	H/Ag titres	mp. count /uL	Blood culture
27	M45	26	M	20	20	0	N
28	M47	21	F	20	40	0	N
29	M49	40	M	20	20	0	N
30	M50	17	M	20	80	538	N
31	M51	24	F	20	80	0	N
32	M15	22	M	40	20	0	N
33	M18	34	M	20	80	0	N
34	M22	12	M	20	40	0	N
35	M48	25	F	40	40	0	N
36	M54	4	M	20	20	0	N
37	M55	29	M	20	160	0	N
38	M60	25	F	40	80	732	N
39	M67	28	F	160	160	0	N
40	L10	24	M	20	160	0	N
41	L22	23	F	20	40	648	N
42	L25	25	M	40	160	2700	N
43	L28	18	M	20	160	0	N
44	L31	23	F	40	160	0	N
45	L35	21	F	80	80	0	N
46	L37	18	M	20	40	778	N
47	L55	23	F	160	160	956	N
48	V3	25	F	20	80	0	N
49	G10	19	F	40	160	1836	N
50	W12	26	M	20	40	0	N
51	W13	30	M	20	40	0	N
52	W63	47	M	20	40	0	N

KEY: F = Female

M = Male

O/ag = Somatic (O) antigen (ag) of *S.typhi*

H/ag = Flagella (H) antigen of *S.typhi*

MP = Malaria parasite.

P = Positive

N = Negative

APPENDIX:3 **WIDAL AND BLOOD CULTURE RESULTS OF MALARIA PATIENTS.**

S/No	Sample code	Age (years)	Gender	O/Ag	H/Ag	mp. count /uL	Blood culture
1	A1	30	F	160	320	9765	N
2	A4	23	M	160	160	3186	N
3	A6	41	M	80	80	1755	N
4	A10	26	F	40	320	11097	N
5	A12	29	F	<20	80	1323	N
6	A17	23	F	80	320	13824	N
7	A24	27	M	160	80	15870	N
8	A29	22	M	<20	<20	2890	N
8	A31	32	M	<20	80	5697	N
10	A33	28	F	<20	160	6912	N
11	A34	22	M	160	320	8559	N
12	A41	5	M	320	320	3915	N
13	A52	33	F	40	160	48816	N
14	G2	28	M	<20	160	2257	N
15	G5	56	F	<20	80	3262	N
16	L1	12	F	<20	160	57547	N
17	L2	11	F	<20	160	20628	N
18	L5	18	F	160	160	26021	N
19	L7	20	M	<20	80	296	N
20	L13	27	F	160	320	3780	N
21	L15	29	M	160	320	2592	N
22	L21	23	M	<20	40	3024	N
23	L22	21	F	<20	40	648	N
24	L30	24	F	160	80	846	N
25	L36	28	M	40	80	7714	N
26	L38	9	F	40	80	99000	N
27	L39	27	F	320	320	4970	N
28	L41	4	F	160	320	91854	N
29	L42	17	F	320	160	6886	N
30	L46	19	F	80	80	21600	N

APPENDIX:3 Continued

WIDAL AND BLOOD CULTURE RESULTS OF MALARIA PATIENTS.

S/No	Sample code	Age (years)	Gender	O/Ag titres	H/Ag titres	mp. count /uL	Blood culture
31	L47	23	M	<20	80	18900	N
32	L53	22	F	<20	320	1322	N
33	L56	19	F	160	320	4940	N
34	L57	16	F	160	320	2700	N
35	L59	10	M	160	160	1800	N
36	M25	31	M	<20	160	10800	N
37	M26	23	M	320	160	14580	P
38	M62	21	F	<20	80	569	N
39	M66	16	M	<20	40	1789	N
40	V1	9	M	160	160	9307	N
41	W2	31	M	<20	40	5859	N
42	W3	30	M	160	320	12150	N
43	W5	20	F	40	80	7110	N
44	W14	25	M	160	320	684	N
45	W18	13	F	<20	<20	5211	N
46	W26	24	M	160	320	21600	N
47	W30	23	F	<20	160	1577	N
48	W36	18	M	<20	160	3348	N
49	W37	25	M	160	160	1512	N
50	W39	29	M	<20	320	76	N
51	W40	35	M	<20	160	297	N
52	W42	21	F	<20	160	26065	N
53	W43	10	M	<20	<20	41200	N
54	W44	6	M	<20	80	108	N
55	W49	18	F	<20	40	513	N
56	W52	22	M	40	160	459	N
57	W54	18	F	160	<20	292	N
58	W59	31	F	40	40	9000	N
59	W61	16	M	40	80	788	N

APPENDIX 4

BIOCHEMICAL REACTIONS OF ISOLATES

Sample code	Lactose	TSI	LIA	L.D	H_2S	P.A	Urease	Indole	Motility	Oxidase	B.E
A28	-	K/A*	K/K*	+	+	-	-	-	+	-	-
A42	-	"	"	+	+	-	-	-	+	-	-
A59	-	"	"	+	+	-	-	-	+	-	-
G3	-	"	"	+	+	-	-	-	+	-	-
G11	-	"	"	+	+	-	-	-	+	-	-
G16	-	"	"	+	+	-	-	-	+	-	-
M8	-	"	"	+	+	-	-	-	+	-	-
M12	-	"	"	+	+	-	-	-	+	-	-
M26	-	"	"	+	+	-	-	-	+	-	-
W8	-	"	"	+	+	-	-	-	+	-	-
W19	-	"	"	+	+	-	-	-	+	-	-
W20	-	"	"	+	+	-	-	-	+	-	-
W25	-	"	"	+	+	-	-	-	+	-	-
W28	-	K/A*	K/A*	-	-	-	+	+	+	-	-
W31	-	"	"	+	+	-	-	-	+	-	-
W60	-	K/A*	K/A*	-	-	+	+	+	+	-	-

Biochemical reactions of isolates

Sample code	Gl	G.F.G	Mn	Dc	Rn	Xl	Slc	Ct	M.R	V.P.	Glt	Ot
A28	+	-	+	-	+	+	-	-	+	-	-	-
A42	+	-	+	-	+	+	-	-	+	-	-	-
A59	+	-	+	-	+	+	-	-	+	-	-	-
G3	+	-	+	-	+	+	-	-	+	-	-	-
G11	+	-	+	-	+	+	-	-	+	-	-	-
G16	+	-	+	-	+	+	-	-	+	-	-	-
M8	+	-	+	-	+	+	-	-	+	-	-	-
M12	+	-	+	-	+	+	-	-	+	-	-	-
M26	+	-	+	-	+	+	-	-	+	-	-	-
W8	+	-	+	-	+	+	-	-	+	-	-	-
W19	+	-	+	-	+	+	-	-	+	-	-	-
W20	+	-	+	-	+	+	-	-	+	-	-	-
W25	+	-	+	-	+	+	-	-	+	-	-	-
W28	+	+		-	-	+	-	-	+	-	-	-
W31	+	-	+	-	+	+	-	-	+	-	-	-
W60	+	+		-	+	-	-	-	+	-	-	-

KEY: Lysine decarboxylase L.D + positive
Hydrogen Sulphite H_2S - negative
Phenyl alamine P.A * Gas
Bile esculine B.E K/A" K/A(H_2S)
Glucose Gl.
Gas from Glucose G.F.G
Manitol Mn.
Dulcitol Dct.
Rhamnore Rn.
Xylose Xl.
Salicin Slc.
Citrate Ct.
Methyl red M.R.
VOGES PROSKAUER V.P.
Gelatin Glt.
Ornithine Ot.