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BY

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A COMPARATIVE STUDY OF TYPHIDOT AND WIDAL TESTS FOR THE DETECTION AND DIAGNOSIS OF TYPHOID FEVER IN PATIENTS.

A Thesis Submitted to the Department of Clinical Microbiology, Kwame Nkrumah University of Science and Technology in Partial Fulfillment of the Requirement for the Degree of MASTER OF PHILOSOPHY (Clinical Microbiology)

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DECLARATION

I declare that thesis is an original result resulting from my personal effort. With the exception of other literally works of scholars duly been acknowledged, this thesis is the result of my own study done at, Department of Clinical Microbiology KNUST under the supervision of Prof. E.H Frimpong. This research has neither in part nor in whole been submitted anywhere for another Master's Degree or of a kind.

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DEDICATION

This work is dedicated to my husband Mr. Appiah Ntim James and my kids Darius Kerris Appiah Ntim and Zynnell Nasia Nkrumah Ntim.

ACKNOWLEDGEMENT

I personally wish to express my profound appreciation to the various persons who have contributed in diverse ways to make this study a success. I am personally indebted to God, for his faithfulness and grace given me to complete this study. I personally express my gratitude to my supervisor Prof E.H Frimpong for the demonstration of patience in correcting my mistakes and offering invaluable advice. I remain grateful to all the lecturers and the entire staff of Clinical Microbiology Department, KNUST. The staff of Bekwai Government Hospital deserve commendation for their support during the data gathering phase of the study.

ABSTRACT

Typhoid fever continues to be a public health issue in most developing countries. In Ghana, Typhoid Fever ranks among the first twenty causes of outpatient illness. Among the numerous challenges in addressing the disease burden of Typhoid is the quest to secure a more reliable and standardized laboratory diagnoses of Salmonella infections to be able to win the surveillance battle on Typhoid fever. The purpose of this study was to compare the specificity and sensitivity of Typhidot and Widal serological tests for the detection of typhoid fever in the Bekwai Municipality. A prospective hospital based longitudinal study was conducted by gathering samples of 292 potential patients who showed features of Typhoid infection. Adopting a purposive sampling technique, patients who met the inclusion criteria were conveniently sampled and enrolled. Blood culture was used as the standard protocol after which sensitivity, specificity and positive predictive values were compared between Typhidot and Widal serological test kits. Data was analyzed using SPSS version 20 to process after which results were presented descriptively. Chi square test of association was performed for categorical variables .The study on 292 individuals recorded sensitivity of widal (95%) and typhidot (85.8%) with the specificity of widal (54.0%) and Typhidot (90.1%). This observation indicates Widal test is much more sensitive than the typhidot in the study area. The study established that both tests could be equally, sensitive and specific in diagnosing typhoid since they both had an equal accuracy (98.0) making them suitable for rapid diagnosis. The study found high detection of S. typhi by blood culture diagnosis, with S. paratyphi and S. typhimurun occurring least. This study has implication for further study to be carried out for confirmatory purposes as well as to determine prevalence of infection and antigenic variants that were not captured in the present study.

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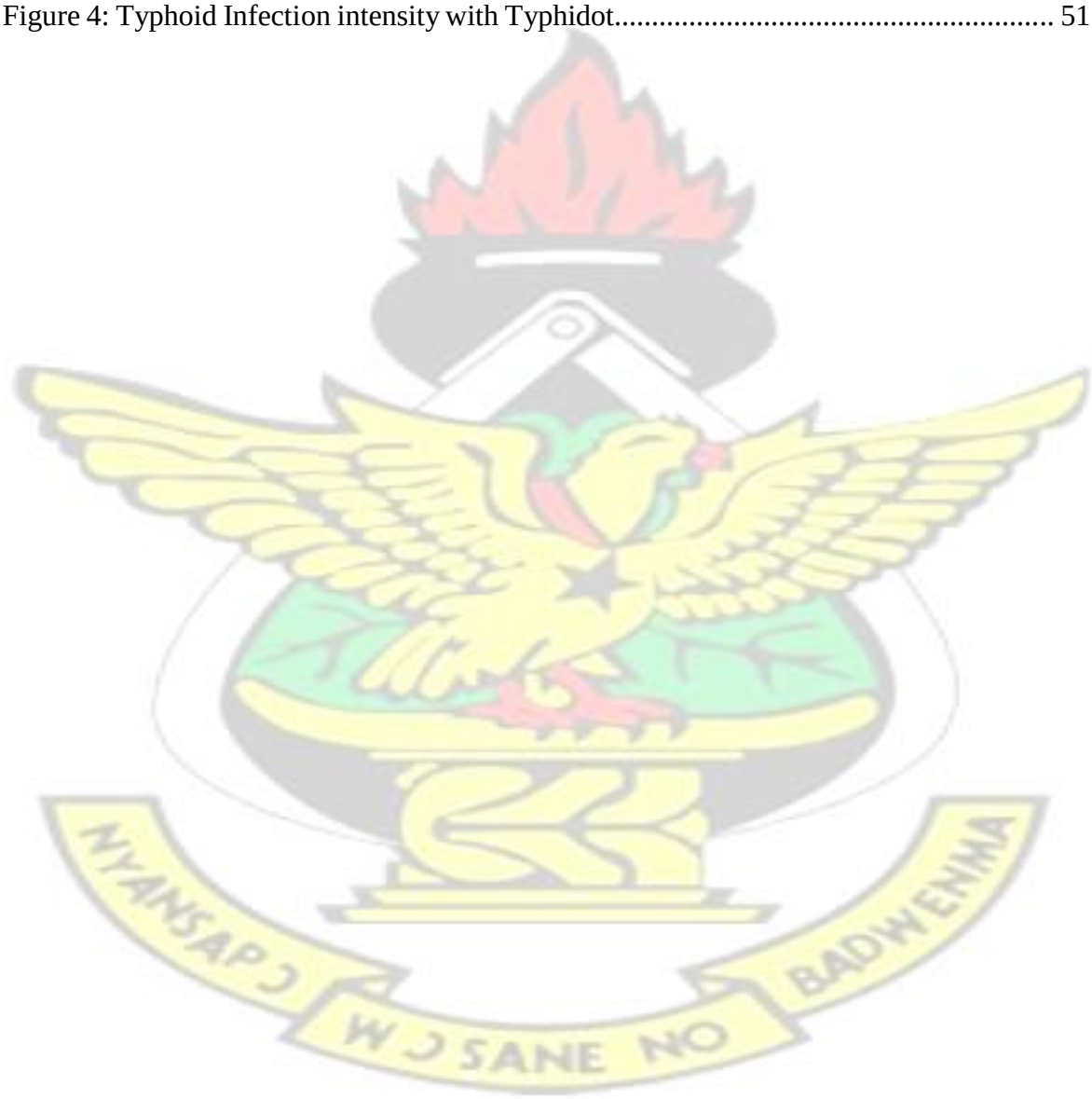

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background to the study

One of the principal diseases causing morbidity and mortality in the tropical regions is typhoid fever. Typhoid fever is a serious acute febrile syndrome that is very common in tropical countries. (Nguri, 2011). The mode of transmission of Typhoid fever is through food ingestion. It could also be as a result of contamination of food with infected persons feces which has the presence of Salmonella enteric serovar Typhi (Magnum & Magnum, 2014). There is the movement of the bacteria resulting from perforation into the intestinal.

This results in macrophages phagocytizing it. The family of the Genus Salmonella is the Entero bacteria facultative Gram negative bacilli. It is identified by a biochemical test and antigen structure. Patients infected with Typhoid fever demonstrate the features of high fever, myaliga, headache, stomach pain, sore throat, and diarrheoa. In some instances the term "enteric fever" collective represent all typhoid and paratyphoid cases

(Kanungu, Dutta & Sur, 2008)

In most cases, when Typhoid fever is not attended to early, it may end in the death of the individual. Heyman reports as cited from the Center for Disease Control and Prevention that, globally there e over 17 million cases of Typhoid which results in more than 600,000 deaths yearly. In the developing world, Nguri (2011) reports that, is threat affecting about 12.5 million persons annually. Notwithstanding, in most situations, the effect of the incidence and the actual health effect of Typhoid remains unexplored within the Sub

Saharan region. Marks et al (2010) report that key among the barriers to knowing the effect and incidence of Typhoid within the Sub-Saharan Africa is the issue of lack of diagnostic laboratories. As a result, there are wrong diagnosis as fatal Typhoid Fever is often interpreted as malaria (Evans et al, 2004; Reddy,2010).

1.2 Statement of Research Problem

In Ghana, Typhoid Fever ranks among the first twenty causes of outpatient illness. This according to Sorry (2009) as cited by Marks (2010) accounts for 0.92% of hospital admissions. In the literature on Typhoid Fever, little has been documented in Ghana. A study conducted by Marks (2010) in Ghana at the Agogo Presbyterian Hospital in the Ashanti Region of Ghana using 1,456 children provides further insights into researching into the diagnosis of Typhoid Fever. In this study, using Blood culture method, it was established that Children<2 years of age had the highest proportion of positive blood cultures (164/1,456). Typhoid fever was low among children <2 years of age (7/1018, 0.7%).Though the blood culture method was able to record high incidence figure, the authors admit the low sensitivity of standard microbiologic methods which was given as being up to 50%.As a result the results were prone to under diagnosing moderate bacteremia in Salmonella infections (Gilman et al., 1975; Wain et al., 2001).

There is the need to obtain a more reliable and standardized laboratory diagnoses of Salmonella infections to be able to win the surveillance battle on Typhoid Fever. The diagnosis of Typhoid fever on clinical grounds is difficult sometimes. Blood culture and Widal test are the commonest forms of laboratory test for the detection of typhoid fever in

almost all clinical settings (Hasan et al., 2013). In developing countries with Ghana inclusive, diagnosis is mostly done by Widal test. It is a serological test that has moderate sensitivity and specificity (Marks, et al, 2010).This is due to the lack of laboratory capacity for culture experiments. More to this is the associated cost in conducting these experiments. Another rapid serological test has been introduced for an early detection and diagnosis of tyhoid fever .The study by Olsen et al (2004) report high sensitivity and good specificity for Typhidot compared to Widal test making its application in developing countries very timely. More to its advantage in the same finding was that, it was known to simple to use by clinicians, very reliable when compared to Widal test. It additionally provides quick results within 1 hour when it is compared to 48 hours for blood culture and 18 hours for Widal test.

However, the usefulness of it has not been explored much as to how sensitive and specific it is when compared to Widal test. In Asia, Sherwal et al (2004) has documented that among the relatively small number of studies that have been done in south India and the rest of Asia, was not encouraging. Notwithstanding, Olsen et al (2004) and Andualem et al (2014) posit that there is a continuing debate about Widal test utility in the early detection and diagnosis of the disease. In view of this a serodiagnostic test kit with a higher specificity and sensitivity than Widal test was introduced. In light of this the research questions that arise are

1. Is Typhidot test simpler and quicker than Widal test?
2. Is the Typhidot more sensitive and reliable than Widal test for the dection and diagnosis of Enteric fever?

1.3 Research Objective

Research Aim or main Objective

This study mainly aims at comparing the results of Widal test and Typhidot in the early diagnosis and management of enteric fever cases.

1.3.1 Specific Objectives

The specific objective of this study is ;

To compare the specificity and sensitivity of Typhidot and Widal serological tests to help in the detection and diagnosis of typhoid fever in the Bekwai Municipality.

1.4 Research Hypothesis

The following hypothesis test will be conducted

HO: There is no significant difference in the sensitivity and specificity of widal and Typhidot serological test in the diagnosis of febrile fever.

H1: There is a statistically significant difference in the specificity and sensitivity of Widal serological and Typhidot test.

1.5 Scope of the Study

The study was limited to both its geographical scope and conceptual scope. The geographical scope of the study was the Bekwai Municipal. The research setting was the Bekwai Municipality. The study setting was chosen because of the incidence of enteric fever in the Municipal. A prospective cross sectional design will be used. Conceptually, the study examined, Typhoid acquisition, types and nature of diagnosis, the risk factors to acquisition and patients levels of knowledge on typhoid acquisition and treatment

alternatives.

1.6 Significance and justification of the study.

The comparative study of the testing methods for the detection and diagnosis of Typhoid is justified on the counts of the growing Typhoid fever cases in the Ghana. The difficulty in early diagnosis of Typhoid is crucial for saving precious lives. A more accurate, affordable and reliable means of diagnosing typhoid early is urgently. This has necessitated the comparative study of the test options so as to reach a decisive point on the test options. The study will provide useful insight into Typhoid test options, their specificity and sensitivity which will be useful to hospital laboratory scientist for improved health care delivery. The findings of this study will add to the body of knowledge on Typhoid test globally and contribute significantly to the limited empirical scholarly works on Typhoid and Typhoid test in Ghana in particular. The usefulness of the study findings to policy makers in public health education on environmental health and sanitation is very timely.

1.7 Organization of the study

The entire research work will be presented in five chapters. The first chapter ;chapter one will include the background of the study, the problem statement, leading research questions, study objectives, research hypothesis, justification, contextual and geographical scope and the organization of the study. The chapter two will concern itself with a review of scholarly works on Typhoid diagnosis, Widal and Typhidot test. A critical review of the risk factors in acquiring Typhoid will be part of the chapter two. The methodology that will detail the processes and procedures in conducting the study will occupy the chapter three. The results and discussion will be presented in the chapter four of the study. Summary of key findings,

conclusions and recommendations for their policy implication will constitute the entire chapter five.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

This part of the study concerns itself with the review of the extant literature on the research topic. It is the part of the study that critically situates the research in context of other scholars' positions on typhoid test, test procedures, varying levels of specificity and sensitivity and the extent of population knowledge on febrile Typhoid

2.2 DEVELOPMENT OF TYPHOID FEVER

Globally, enteric fever is a public health concern (Nagashetty, et al 2010). According to

Kanungo et al, (2008) two broad categories of Salmonella infection in humans can be identified. These include Salmonella enterica with low virulence which causes food poisoning and second Salmonella enterica typhi (S. typhi) with high virulence. These ones account for the serovars known as Samonella Paratyphi A, B and C, which cause Paratyphoid and typhoid. Humana are the only host of Salmonella Paratyphi A, B and C group of pathogen (Bhan, Bahl & Bhatnagar, 2005Typhoid effect on man traces its roots to the around the year 323 during the death of Alexander the Great where his death is linked with Typhoid infection (Berth et al, 1990) The disease is linked with symptoms such sharp abdominal pain, chills and sharply rising fever- The history of the Salmonella typhi bacteria is traceable to Karl Joseph Eberth finding in 1880 when a during the identification of a bacterium located in the spleen and mesenteric lymph nodes of a patient dying of typhoid fever. From its Greek origin, Typhos means smoke and as such as it was believed that typhus fever was gotten from smoke .Typhoid suggest typhus like and thus the emergence of the name Typhoid. Historically, it is believed that May Mallon is first patient from the United States who was noted to be a healthy carrier of enteric fever. Public health authorities told. After her quarantine period, Mary died of pneumonia with an autopsy reporting demonstrating the presence of live typhoid bacteria in her gallbladder. (Sultana, 2012)

2.3 TYPHOID AETIOLOGY

The causative of Typhoid is Salmonella typhi, which is a Gram-negative strand of bacteria. Salmonella typhi belong to the family Enterobacteriaciae. According to Grossman et al (1995), there is a positive prediction of bacterium serologically, for detection the antigens O9 and O12 proteins which are lipoglycans, flagella antigen H, and polysaccharide capsular antigen (Connertonet al., 2000). Characteristically, cells of Salmonella typhi are

identified by their rod shape. A wide variety of populations are infected by Salmonella strains. These include reptiles, birds and mammals. The variations in infection which generate antigenic variations is as result of difference in this equally affecting the molecules produced by the pathogen (Fierer &Guiney, 2001). Typhoid fever is highly adapted, human-specific pathogen and have developed mechanism for growth and still remain clued to the host so that it can subsequently survive and transmit further). There is a multiplication of Salmonella typhi in the mononuclear phagocytic cells. This take place before it enters the bloodstream. When food is ingested, the pylorus serves as medium for the typhoid bacteria and arrives at the small intestine. There is a fast penetration of the epithelium through enterocytes to arrive in the propria. After this process, the bacteria rapidly elicit an influx of macrophages ingesting the bacillus which are generally not killed (WHO, 2003). It is believed that the bacteria of typhoid end in the person's bloodstream through the lymph drainage. It occurs through the mesenteric nodes,
thoracic duct and then through to the general circulation (WHO, 2003).

In the monoclonal phagocytic lymphoid follicles cells, there is the multiplication and survival of Salmonella organisms in the spleen and liver (House et al., 2001). The critical point is often determined by the number (quantum) of bacteria, the degree molecules produced by the pathogen, and the host response .There is the release of bacteria from this intracellular habitat (comfortable zone) into the bloodstream. There is mostly an incubation period of 7 to 14 days for the bacteria. The liver, spleen, bone marrow, gallbladder, and are the commonest locations for secondary infection for Peyer's patches of the terminal ileum (Butler et al., 1978). Bile excreted organism either destroy the intestinal wall or are rather excreted into the faeces. The presence of bacteria counts in a patient with history of acute

typhoid fever infection show a concentration of 1 bacterium per milliliter of blood and about 10 bacteria per milliliter of bone marrow (Wain et al., 1998).

2.3.1 CHARACTERISATION AND SYMPTOMSOF TYPHOID FEVER

The bacterium Salmonella causes Typhoid fever. Typhoid fever is a global in scope. It is transmitted through the ingestions of food or water that has come into contact with feces from an infected person. Bacteria spread from the host intestine through the bloodstream to the intestinal lymph nodes, liver, and spleen via the blood and multiplies after ingestion. The gall bladder may be infected by the Samonella through the hepatic duct. This infection may spread to other parts of the body through the bloodstream. Nguri (2011) cites from Poweish and others in their study that included in the early symptoms of Typhoid infection are fever, malaise and abdominal pain with a progression in the fever to as high as over 103 degrees Fahrenheit showing prominence in diarrhea. There are complications of typhoid infection which includes intestinal hemorrhage (severe GI bleeding), perforation of the intestines, and peritonitis kidney failure. According to Ivanoff et al (1994), Typhoid fever threatens life systematically so an extent that 16 million new cases are estimated globally. In Asia over 13 million cases are recorded annually with more than six deaths are reported globally.

In the opinion of Anggraini et al (2004) despite the disease occurrence in all ages, it is very high among children. Jerrold and Turner (2010) in their study add that the elderly are one group of persons among whom Typhoid infection is very high in the rest of the world aside Africa, the peak incidence are recorded during summer and fall. The presumption has been that Typhoid infection is high in regions of the world with difficulty in having access to safe drinking water and proper sanitation. Clinically, enteric fever ranges from mild illness

with low-grade fever, with features of malaise, and slight dry cough to a severe clinical condition with some abdominal discomfort and several complications. The symptoms include; constipation (adults), presence of diarrhoea (children), headache condition, malaise and anorexia and infarction of spleen (Mehta et al., 2007).In the early stages of the illness bronchitic cough is common. In the period of infection, close to 25% of patients demonstrate rose spots on their chest region, abdomen and also at back. There are additional conditions of stool blood. The WHO (2003) report of Intestinal perforation among about 3% of hospitalized cases increasing abdominal discomfort.

2.3.2 MODE OF TRANSMISSION OF TYPHOID

Humans are known to be reservoirs and natural host of bacteria. When contaminated food and water with faeces is ingested, typhoid infection can be transmitted. One other risk factor for typhoid transmission is ice cream. Others include, Shellfish from contaminated water, and raw vegetables and fruit that has receive fertilization from water sewage (Edelman & Levine Myron, 1986). Mostly, there is higher incidence when water supplies to very large populations get contaminated with faeces of animals or humans. In most of the situation, the transmission of Samonella typhi from waterborne occurs through small inocula. On the other hand, food borne transmission also occurs with large inocula with high attack rates within short periods. There is a great influence of the inoculum size and medium through which the organisms are ingested on the attack rate and the incubation period (WHO, 2003).

2.3.3 PATHOGENESES OF TYPHOID

The production of the disease relies on a myriad of factors. These factors may include; organisms swallowed, ii) state of gastric acidity and iii) possession of Vi antigen by the organisms (Jenkins& Vinetz 2009). In the case of the clinical syndrome of fever, the disease production is through the release of pro inflammatory cytokines (IL-6, IL-1ß, and TNF-Ü) from infected cells. It is not only the virulence of the organisms infected but also the other factors and immunity may also play key in predisposing a person to bacterial infection (Bhutta, 2008). Both Salmonella typhi and Salmonella paratyphi A and B possess high invasive character. They pass through the pylorus to the intestines of humans quickly to reach the mononuclear phagocyte system, where, within 8 to 14 days of incubation, they affect the systemic illness. This, results in ingestion by humans of the causative organisms in contaminated the foods or water. The degree and type of medium in which it is ingested greatly influence the attack rate for the typhoid fever (Khan et al., 2008). Earlier studies conducted by Hornick et al (1970) found out that samonella enterica serotype infected dose in volunteers range between 1000 and million organisms. Further studies have shown that Vi-positive strains are more infectious than Vi-negative serovar typhi strain of Samonella enteric. In most cases, to reach the small intestine, The Samonella enterica serotype typhi survives the gastric acid barrier. This explains why the pH of gastric acid is a necessary defence mechanism. The adherence of the bacteria to mucosal cells of the small intestine would be penetrated by the mucosa (Parry et al., 2002). The M cells, specialized epithelial cells overlying Peyer's patches, most likely form the site of the internalization of Salmonella enterica serotype typhi and its transport to the underlying lymphoid tissue. When penetration has been completed, the bacterium invading translocate to the intestinal lymphoid follicles and the draining mesenteric lymph nodes, and some pass on to the

reticuloendothelial cells of the liver and spleen (House et al., 2001). Butler et al (1993) in their study reported that even notwithstanding the admission that salmonella. enterica serotype typhi gives out a potent endotoxin binder and the death rate from treated typhoid fever for patients at this stage is less than 1 per cent. Later studies conducted by Butler et al have revealed the levels of circulating pro inflammatory and anti-inflammatory cytokines have increased in patients with typhoid. This has lessened the ability of whole blood to produce inflammatory cytokines in patients with severe disease (Bhutta et al., 1997).

There is a strong association of Typhoid disease with low socioeconomic status and poor hygiene. It has been argued that humans serve as the only natural hosts or reservoir (nidus) of Typhoid infection. The year 2000 recorded an approximated 21.5 million infection of Typhoid out of which, 200,000 die annually across the world (Bhutta, 2006). According to some authorities, it is one of the serious infectious diseases that threaten the global public. Major concerns around it have been the quick and widespread emergence of antibiotic resistance patterns (Akinyemi et al., 2005). Sultana (2012) as cited from Brooks et al (2005) opines that during 2000-2001 among children in an urban slum in Bangladesh, the incidence of typhoid fever was established at 390 cases per 100,000 populations annually. Out of this, the incidence among >5 years was 210 per 100,000 per year and among children <5 years the rate was 1870 per 100,000 per year.

2.4 DIAGNOSIS OF TYPHOID

Clinically the diagnosis of Typhoid is difficult due to the non-availability of specific symptoms or signs of the condition. Some of the features of Typhoid in endemic regions are fever reason that lasts over a week (Parry et al., 2002). Blood, urine, stool and bone marrow

are the samples used in the detection of salmonella organism. Historically, blood culture and Widal test are the diagnostic procedure in detecting Typhoid fever.

Widal tests stand out to be the commonest test in typhoid detection. However the study of Hoa et al (1998) suggest that Culture isolation of the S. typhi remains the most effective diagnostic procedure when typhoid fever is suspected in patients. Though Widal test is very common it becomes very controversial, as how sensitive, specific, with its predictive values most used test differ considerably among areas (geographic). Antibodies to the O and H antigens of Samonella enterica typhi are detected by (Clegg et al., 1994) agglutination through the quantitative method and there are other salmonella serotypes that share cross-reacting epitopes of antigens with Samonella enterica serotype typhi

Enterobacteriaceae. This is however the evidence that Widal's test is much more useful when used with locally determined cut off points (Parry et al., 1999)

Blood cultures serves as the bench mark diagnostic method. The process involves taking a large pool blood which is later cultured 10 ml (adults), blood cultures are positive for patients with typhoid infection. Sensitivity of bone marrow culture is very high as its ability to predict positive results ranges from about 80 to 95 per cent of sick individuals with typhoid and even patients who have been taking antibiotics for several days, regardless of the duration of illness. Bone marrow has now become bench mark diagnosis for bacteriological confirmation of enteric fever. The advantage of the Widal is that it has the utility to be useful in areas where financial constraints make it more difficult to secure diagnostic methods. This necessitates for the urgency of using a quicker and reliable diagnostic test for typhoid fever. The continuing progress in the IDL Tubex® test, can reportedly detect IgM O9 antibodies from patients within a minute. Another rapid serological test, Typhidot®, takes three hours

to perform. It is specific for the detection of IgM and IgG antibodies against Salmonella typhi antigens (Jackson et al., 1995). TyphidotM® test can detect specifically IgM antibodies only. The dipstick , operate by the principle of binding Salmonella typhi-specific IgM antibodies in samples to Samonella typhi lipoglcan antigens, and the staining of bound antibodies by an anti-human IgM antibody conjugated to colloidal dye particles.

There are many efforts that has been applied in developing methods that detect Salmonella typhi antigens in body fluids, blood, urine, thereby providing a rapid diagnostic test for typhoid fever. The diagnosis was performed using immunoassay. The basis of immunoassay of serovars of Salmonella typhi the for detection of the O an Vi antigens found blood or urine using methods like co-agglutination, ELISA, or counter current immune electrophoresis. Polymerase chain reaction (Levy et al., 2008) and DNA search work to apply Salmonella typhi genes and hybridize them with known specific gene probes

2.4.1 TYPES AND METHODS FOR THE EARLY DETECTION OF TYPHOID FEVER

Laboratory detection and diagnosis of typhoid fever is based on isolation and identification of Salmonella typhi from a suitable clinical specimen such as blood, stool, urine, bone marrow, and duodenal aspirates through culture. Serological markers are used to detect S. typhi-specific antibodies while the S typhi-specific antigens are detected by immunological test and identification of nucleic acid by Polymerase chain reaction (Pearson & Guerrant 1995). S. typhi can maximally be isolated from the blood during the first week of disease; faeces in the second and the ensuing weeks and urine in the third and fourth weeks (Old, 2006). The various culture methods available are: Blood culture, faeces culture, clot cultures, bone marrow culture, urine culture, bile culture and duodenal aspirate culture. According to

Wilke (2002), the definition for diagnosis of typhoid fever requires the isolation of Salmonella enterica subspecies enterica serovars Typhi (Salmonella Typhi) from the patient. Specimens such as blood culture and stool, rose spots, urine, blood mononuclear cell platelet fraction and bone marrow has been found useful for diagnosis. Priority has been given to the development of an inexpensive and rapid diagnostic test for typhoid fever that is both sensitive and specific. There has been the development of new serologic tests of Typhoid fever for detecting detect IgM or IgG antibodies to various purified antigens of S. Typhi as TUBEX test.(6) Studies have shown that TUBEX test has marked variation in its results.(7,8

2.4.1.1 WIDAL TEST

The Widal test was named after a French physician and bacteriologist Georges Fernand Isidore Widal. There was the development of Widal in 1896, to diagnose typhoid fever. This was on the basis that antibodies in the blood of an infected individual cause the bacteria to come together into clumps (the Widal reaction). The Widal agglutination test was introduced as a serologic technique to aid in diagnosis of typhoid fever. (Encyclopaedia Britannica, 2011). The test identifies the presence of agglutinin (antibody) in the serum of an infected individual, against the H (flagellar) and O (somatic) antigens of Salmonella typhi. The O antigen of S. typhi and is shared by S. paratyphiA, S. paratyphi B (Rodrigues 2003). IgM is the predominant antibodies against the O antigen with high early titers (appear on day 6-8) during infection and disappear early (Rodrigues 2003). The H antigens are known to be flagella antigens of S. typhi, paratyphiA and paratyphiB. Antibodies against H antigen have both IgG and IgM, rise late (on days 10-12) during illness and last for longer (Olopoenia & King 2000; Rodrigues, 2003). Serological diagnosis is evident on the rising of antibody titers in paired samples at an interval of 1014 days (Parry

et al. 1999). In typhoid fever, however, a four- fold rises after 2 weeks in not always noticeable, even blood culture confirmed cases. This situation develops resulting from obtaining an acute phase sample at a latter data in the history of the disease, because of high levels of background antibodies in an endemic region. Another reason is also due to the fact that in some people the antibody response is blunted by the initial administration of an antibiotic (Schroeder 1968).

2.4.1.2. IMMUNOCHROMATOGRAPHIC METHOD

Specificity and sensitivity of ICT has been observed to be very high according to studies done in many countries (Pastoor et al. 2008; Anusha, Ganesh & Lalitha 2011). One evaluation done on ICT (Typhidot) in India gave100% sensitivity and 80% specificity as compared to the bench mark technique(blood culture)

2.4.1.3 HAEMAGGLUTINATION (HA) TESTS

Many works have been done to evaluate the usefulness of haemagglutination tests in different countries. The results of one study in India showed that using anti-lipoglycans of the haemagglutination test; sensitivity was 60% with specificity of 98.2%. The study found 66.7% and 96.7% for its positive and negative predictive value respectively. Haemagglutination inhibition test that had Salmonella antigens as target was found useful in detecting of S. typhiin culture early (Shukla, Patel & Chitinnis, 1997). Reverse Passive Haemagglutination Test (RPHA) in a different study was designed in detecting S. typhi antigen recorded sensitivity of 70% and specificity of 92% for diagnosing acute typhoid fever (Kalhan et al. 1998).

2.4.1.4 COUNTERCURRENT IMMUNOELECTOPHORESIS (CIE)

This method uses electrophoresis and the visualization of the precipitin band of antigen-antibody complexes formed to detect the presence of the Salmonella typhi. There is some similarity in sensitivity to that of Widal test with a quicker procedure when the test is labeled, but bands are often problematic to see. The cost is advancely equated to Widal, and some research findings suggest that countercurrent immunoelectrophoresis has a short sensitivity for Vi antigens. A section of serovars of Salmonella typhi antigens (somatic (O), flagellar (H) and capsular polysaccharide (Vi) are recommended for the detection and diagnosis of typhoid fever (Sharma et al. 1979).

2.4.1.5 ANTIBODY DETECTION: DOT ENZYME IMMUNOASSAY (EIA) TEST

Dot enzyme immunoassay helps to detect IgG and IgM antibodies against the outer membrane proteins. This is different from the somatic (O), flagella (H) or capsular (Vi) antigen of Salmonella typhis that is mostly commercially available similar to Typhidot. It is available in two properties commercially. These Typhidot M® detects only IgM antibodies of Salmonella typhi. some findings have suggested that it has higher specificity (Choo et al., 1999). The second Typhidot® test detects specific IgM and IgG antibodies of Salmonella typhi. It has history of full scale multinational clinical evaluation of its diagnostic value (Ismail, Kader & Ong 1991). In highly endemic areas, the detection of specific IgG increases with high rates of Salmonella typhi transmission. The IgG antibodies can persist for over two years after enteric fever Infection (Choo et al. 1997; Bhutta & Mansurali 1999). The detection of specific IgG antibodies cannot distinguish between serious and recuperating cases (Choo et al., 1999). There is false positive outcome linked to previous infection. There could also be IgG positivity also occurs in the event of current re-infection. In cases of re-

infection there are secondary immune responses with a significant boosting of IgG over IgM, such that the later cannot be detected and its effect is covered. To be able to solve this problem requires enabling the detection of IgM in an uncoverd form (Bhutta, 1996).

2.4.1.6 ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

Herath's work (2003), indicated that the usefulness of the enzyme linked immunosorbent assay (ELISA) for the detection and diagnosis of typhoid fever has been determined by various investigators using serum and urine (Beasley, et al 1981; Nardiello et al, 1984; Appassakij et al, 1987). Early studies done by Barrett et al (1982); Banchuin et al (1987) and lately by Mekara et al (1990); even though they established that, ELISA using these biological fluids has superior sensitivity and specificity to Widal test, but the invasiveness and the challenge is how to maintain the samples until tested. This called for the development of an ELISA that could detect detect anti-S typhii lipopolysaccharide (LPS) IgA antibodies in a single salivary.

2.4.1.7 IgM dipstick test

Past records is evident that rapid dipstick assay detects S. typhi-specific IgM antibodies in serum and whole blood samples were previously reported and the sensitivity and specificity was evaluated (Gasemet al. 2002; Hatta et al., 2002; House et al., 2005). Dipstick assays stands to be useful for the serological diagnosis of culture-negative patients with clinical signs and symptoms consistent with typhoid fever. Obtaining reports on same day of test performance gives dipstick kit and advantage allowing prompt treatment. In test performance, only a small volume of serum is needed and that no special laboratory equipment is needed to perform the assay. The stability of the reagents of the dipstick and

the simplicity of the assay allows its use in places that lack laboratory facilities (Hatta et al., 2002).

2.4.1.8 ANTIGEN detection test

There is a high demand for Simple diagnostic tools for typhoid fever test. For a test to be considered ideal, it should be reliable, simple, and affordable for the countries where the need is the greatest. Many of the affected countries are poor, and some places do not have electricity. Detection of antigens, rather than antibodies could provide such a test (Wain & Hosoglu, 2008).

2.4.1.9 PROTEIN AND VI ANTIGENS

Vi antigen sensitivity was discovered to be superior than somatic and flagellar antigen, was been reported as ranging from 50-100% in different studies (Fadeel et al. 2004; Kalhan et al. 1999; Raoet al. 1999), but enzyme immunoassay, counter immune electrophoresis and co-agglutination tests can detect serum or urinary somatic/flagella/Vi antigens of Samonella typhi have been evaluated (Fadeel et al., 2004; Kalhan et al. 1999). Also, specificity estimates have been reported to vary from 25% -90%. The suboptimal and variable sensitivity and specificity estimates, inability to detect S. paratyphi infection and Vi antigen negative strains of salmonella. typhi are serious limitations of the Vi antigen detection tests. A monoclonal antibody specific for group D Salmonellae antigen 9 was used in an indirect enzyme-linked immunosorbent assay (ELISA) for detecting the antigen in urine specimens collected from patients with clinical typhoid fever in Jakarta, Indonesia. The ELISA had a sensitivity of 95% in identifying patients in whom Salmonella typhi was isolated from blood cultures, 73% in patients in whom Salmonella typhi was isolated from stool specimens, and

40% in patients in whom the organism was isolated from bone marrow cultures, but specificity varies from 25-90% (Chaicumpa et al., 1992).

2.4.2 Molecular methods

Molecular method of diagnosis of typhoid fever has evolved with time to cover the limitations of cultures and serologic test. Use of polymerase chain reaction has been explored by many authors for the detection of specific DNA sequence of the organism present in the clinical specimens. The PCR for the detection of existence of typhoid fever was first evaluated in 1993, successfully when there was the amplification of the flagellingene of Salmonella typhi in all cases of culture proven typhoid fever and from none of the healthy controls. In Song and colleagues study, two pairs of primers were used in the evaluation; amplification of the flagellingene of S. typhi confirmed the presence of the organism in the patient's blood (Song et al. 1993). In addition nested PCR has been shown promising results. These results show that, the nested PCR has good potential to be a rapid tool for the definitive, differential diagnosis of typhoid and is superior to conventional methods. In 2005, Massi conducted a study to establish the nested PCR for DNA detection of S.typhi in the urine of patients with suspected typhoid fever. This research used 107 urine samples from patients suspected with typhoid fever which were examined with nested PCR using two primer pairs with the final amplification result of 343 base pair (bp). The study reported that 64 (59%) urine samples were positive with Samonella typhi DNA. This research concluded that nested PCR specifically from urine specimen can be used as an alternative method in diagnosis and management of typhoid fever (Massi et al., 2005). Similarly, Kumar et al used blood samples from 40 clinically suspected cases of typhoid fever, and found 20 of 20 culture positive and 12 of 20 culture negative cases to be positive

by PCR in Delhi, India (Kumar et al. 2002). Using single primer in South Sulawesi, Indonesia, 46 of 73 (63.0%) blood samples collected from patients with clinically suspected typhoid fever were positive by PCR compared to 13.7% positive by blood culture (Massi et al. 2003). In Varnassi, India, nested PCR was again better 53 of 57 (73.0%) were positive than blood culture 17 of 53 (32%) were positive on specimens from 63 clinical typhoid fever cases (Prakashet al., 2005).A study in Indonesia, investigated 131 patients with a clinical diagnosis of typhoid fever and diagnosed the cases through blood culture and PCR from blood (84.5%) and urine samples (69.3%). Sensitivity of PCR diagnosis was found to be higher than that of blood culture (Hatta & Smith 2007). Another study from Nepal was conducted on specimens from 71 children with suspected typhoid fever reports 82.7% positivity for PCR from blood and urine, showing similar results for each specimen and PCR results were much higher than that of blood culture (26.9%). Also in Pakistan, 55 cases of suspected typhoid fever and a control group of 20 healthy persons were diagnosed by PCR from blood samples and blood culture. The PCR and blood culture gave 58.2% and 14.5%. Positivity, respectively showing significantly better results by PCR. Again in Pakistan, a multiplex PCR targeting five different genes for differential diagnosis of typhoid pathogens has been developed for use directly on clinical blood samples. Of 42 multiplex PCR-positive blood samples, 35 were positive for Salmonella typhi and two for S. Paratyphi A and interestingly remaining 5 patients were found to have mixed infection (Ali et al., 2009). Moreover, some patients with culture negative results for typhoid fever were PCR positive suggesting that PCR diagnosis of typhoid fever may have superior sensitivity than cultures.

2.5 COMPARATISM OF SENSITIVITY AND SPECIFICITY OF TIPHYDOT AND WIDAL TEST

2.51 WIDAL TEST

Widal test measures the agglutinating antibody levels against O and H antigens. The levels are measured by serial dilutions of serum in large test tubes. Usually, O antibodies appear on days 6-8 and H antibodies on days 10-12 after the onset of the disease. The test is usually performed on the serum (at first contact with the patient). The test has only moderate sensitivity and specificity (Clegg et al., 1994). It can be negative in up to 30% of culture-proven cases of typhoid fever. This may be because of prior antibiotic therapy that has blunted the antibody response. On the other hand, Salmonella typhi shares O and H antigens with other Salmonella serotypes and has cross-reacting antigenic determinant with other Enterobacteria, and this can lead to false-positive results. Such results may also occur in other clinical conditions, e.g. malaria, bacteraemia caused by other organisms, and cirrhosis (WHO, 2003).

2.5.2 TYPHIDOT ® TEST

This particular test makes use of a 50- kD plasma membrane antigen to detect specific IgM and IgG antibodies specific to Salmonella typhi (Ismail et al., 1991). It has undergone full-scale multinational clinical evaluation of its diagnostic value (Jackson et al., 1995). This dot EIA test offers simplicity, speed, specificity (75%), economy, early diagnosis, sensitivity (95%) and high negative and positive predictive values. The detection of IgM reveals acute typhoid in the early phase of infection, while the detection of both IgG and IgM suggests acute typhoid in the middle phase of infection. In endemic areas where the rate of typhoid transmission is high the detection of specific IgG increases. Since IgG can persist for more

than two years after typhoid infection (Choo., 1999), the detection of specific IgG cannot help to differentiate between acute and chronic cases. Furthermore, false-positive results attributable to previous infection may occur. On the other hand, IgG positivity may also occur in the event of current re-infection.

Widal test have been used for centuries in the developing countries for diagnosing typhoid fever but it has a low sensitivity, specificity and positive predictive value, which changes which is dependent on ones area of settlement. However widal test particularly when used along with conventional widal test has a greater sensitivity (Paiet al., 2003). Typhidot is a new, inexpensive, and reliable serological diagnostic test recently available commercially and studied in many endemic areas with reports of higher sensitivity and specificity. Sherwal and colleagues in (2004) did a comparative study of typhidot and Widal test in patients of typhoid fever. The study included 80 patients of acute febrile illness. The patients for the study were separated into 2 groups. Group I included 56 patients with clinical diagnosis of typhoid fever and group II comprised of 24 patients of suspected typhoid fever with alternative diagnosis. From the 56 patients clinically diagnosed of typhoid fever, 32(57%) were positive to Widal test and 44(79%) were positive to Typhidot. Of the 24 patients, 4(17%) were positive to Widal and (12.5%) were positive to Typhidot. In their studies on Typhidot test is useful in patients of typhoid fever, they also observed that it has a sensitivity of 92% and specificity of 87.5%, which was higher than that of Widal test, and comparable to the studies done elsewhere in India and its environs. A similar study carried out in the southern part of India reported that Typhidot have a sensitivity of 100% and a specificity of 80% and was recommended for its utility in conjunction with Widal test for an early diagnosis of typhoid fever (Jesudasson et al.,

2002).

In another study, group of known patients with typhoid in Pakistan were sampled, Typhidot test had a comparable sensitivity of 94% and specificity of 77%, while Widal test had sensitivity and specificity of 63% and 83% only8. The effectiveness of Typhidot test in early diagnosis of typhoid fever patients was also studied in two different studies in Malaysia. Its sensitivity and specificity was reported as 90.3% and 91.9% respectively in the first study, and was significantly higher, while the second study also showed a sensitivity and specificity of 98% and 76.6% respectively (Chooet al., 1999; Gopalakrishan et al 2002). Both Malaysian studies showed typhidot as a better test in contrast to widal test for rapid diagnosis of typhoid fever as well as for its simplicity of ease in use. Results of all the studies done to evaluate typhidot test in developing countries have consistently shown similar and comparable results. This is presented below.

2.5.3 COMPARATIVE ASSESSMENT OF TYPHIDOT TEST IN DIFFERENT STUDIES

Out of 109 patients, the sensitivity of typhidot test was reported to be 95% with 75% specificity (Choo, 1991). Again among 149 patients studied, the sensitivity of typhidot test was 90% with 91% specificity (Choo, 1994). Butta et al (1999) also evaluated that, there is 94% sensitivity and 89% specificity in using the typhidot test. Furthermore, Jesudasson et al (2002) also established that the typhidot test has a sensitivity of 100% with 80% specificity in 150 patients studied. Among 144 patients studied, there was 98% sensitivity with 76.6% specificity (Gopalakrishan et al., 2002). Shawal et al (2008) has also reported that, typhidot test has 80% sensitivity with 92% specificity when 80 patients were studied. Olopoenia & King (2000) states the Widal Test being the most widely used serological assay come along with some disadvantages in endemic areas. Previous exposure to S typhi or antigenically

related Gram negative bacilli and vaccination against typhoid can result in raised titres in the absence of a current infection (Schroeder, 1968;

Thevanesam, 1992). In contrast, a poor antibody response to either the ―O‖ or ―H‖ antigen (or both) can occur in some patients (Butta & Mansurali, 1999; Jesudasson, Esther and Mathai, 2002; Pai, Koppikar, and Deshpande, 2003). Mandell et.al, (2000) argue that the Widal test results often leads to confusion and, on occasions, to misdiagnosis of other febrile illnesses as typhoid fever. To them, this calls for simple, rapid, and sufficiently sensitive technique that is capable of detecting most patients with typhoid, which at the same time is specific enough to avoid misdiagnosis of other febrile illnesses.

2.6 LEVEL OF PATIENT KNOWLEDGE ON CAUSES AND TREATMENT OF TYPHOID FEVER

The socio-demographic characteristics of suspected cases of typhoid fever in a current study have shown that the majority of the respondents were pre-school children 53 (35.3%) and 26 (17.3%) were illiterate, belonged to lower class 121 (80.7%), used open latrine 79 (52.7%) and drinking from tube well 112 (74.7%). Related findings also have been reported by Sur and others showing that the illiteracy rates were highest in the cases of typhoid fever. From the study, it was observed that unhygienic latrines were the main sources of spreading typhoid diseases and the sanitation condition of low income areas was remarkably poor (Sur et al. 2007).

Major contributing factors for typhoid fever transmission were attributed to lack of safe drinking water and unhygienic sanitation. Leakage and cross-contamination of water and

sewage pipelines was observed in slummy areas in Bangladesh. More so residents cannot afford the sanitary latrines in Bangladesh due to poverty which further carries the risk of exposure to Salmonella typhi. A significant relationship has been observed between water bodies and the incidences of typhoid. The finding reveals that, people living closer to water bodies may have elevated risk of infection. Though there is no earlier report, however, case-control studies in India (Sur et al, 2007) and Vietnam (Tran et al., 2005) revealed that people closer to water bodies, and who use surface water for drinking tend to have more typhoid risk. Such results were evident in a diarrhoea incidence report (Emch, 2000). The areas supporting this hypothesis of inverse relationship between typhoid occurrence and distance to water bodies might explained by the fact that there is a higher faecal contamination load in rivers (Horman et al.,2004). As a result, contamination of these water bodies may have substantial impact on the disease dynamics in the communities. As

Samonella Typhi bacteria can survive in water for days (Cho & Kim, 1999) contaminated surface water; such as sewage, freshwater and groundwater would act as etiological agents of typhoid (Thong et al., 1996).

2.6.1 RISK FACTORS INFLUENCING TYPHOID FEVER PREVALENCE

WATER CONTAMINATION

Samonella bacterium thrives well inside humans. The blood stream and the digestive tract serve as a medium for bacterial growth and multiplication. Transmission is through contaminated faeces in water or food (Ray, 2002). Faecal pathogens are frequently transferred to the water borne sewage system, through flush toilets and pit latrines subsequently contaminating surface and ground water. In regions with poor sanitation, the bacteria often spread after water supplies are contaminated by humans waste (WHO,

2000). Water has been a principal carrier of typhoid and becomes extremely dangerous when it serves as a vehicular carrier for the transmission of the disease. Human activities, animals and bird extract have been the primary source of water contamination. Untreated sewage is dangerous to public health as it contributes to environmental water, land and air pollution. Discharging untreated water waste into water bodies has negative effects on human, animal and plant life. It has been observed that too many pollutants reduce the self-purification capacity of water, especially at the point of mixing and, they promote excessive growth of aquatic plants.

2.6.2 LACK OF HYGIENE

Transmission OF Typhi is as a result of a person getting in contact with contaminated water and food through food handlers, sewage. Majority of typhoid case has been attributed to faecal contamination of water supplies or street foods. Therefore, province or regions with poor hygienic practices and poor sanitation records higher incidence of typhoid cases. Typhoid fever can also be spread through irrigation of crops using sewage contaminated with S. typhi (Donald, 2004). The majority of urban populations are tenants in informal settlements where basic services such as water and sanitation are inadequate. Therefore people living in such areas and regions are at a higher risk of typhoid infection.

2.6.3 SANITATION PRACTICES

Sanitation refers to the safe collection, storage and disposal of various wastes resulting from human activities. These include solid wastes, refuse and liquid wastes effluent from sewage works, kitchen sink and even hazardous waste from industries. It also refers

to the general maintenance of the human environment in a safe condition free from pollution. Poor sanitation practices are a cause of bacterial, viral, protozoa and helmintic infections.

2.6.3 HEALTH CARRIERS OF TYPHOID DISEASE

Because humans are the natural host for the bacteria, it is humans who can be carriers. It has been observed that a few groups of people recover from typhoid but continue to carrier the bacteria and these are known as health carriers. Both ill persons and carriers shed Salmonella typhi in stool (Connerton et al, 2000). Failure of typhoid fever carriers to wash their hands thoroughly with soap and clean water after defecation poses a risk of bacterial transfer.

2.6.4 TREATMENT OF TYPHOID FEVER

Oral or intravenous hydration is seen as supportive measure for the management of typhoid fever; include the use of antipyretics, appropriate nutrition and blood transfusions if indicated. More than 90% of patients can be managed at home with oral antibiotics, reliable care and close medical follow-ups for complications or failure to respond to therapy or treatment For the detained (hospitalized patients) ; effective antibiotics, good nursing care, adequate nutrition, careful attention to fluid and electrolyte balance, and prompt recognition and treatment of complications are necessary to avert death. Work done by Chinh and others cited by Connerton et al (2000) revealed that fluoroquinolones have been proven evident to be the most effective drugs for the treatment of typhoid fever. In randomized, controlled trials involving patients infected by quinolone-susceptible samonella enteric serotype typhi, these drugs have proved safe in all age groups and are rapidly effective even with short courses of treatment (three to seven days). In Gotuzzo &

Carrillo's work on quinolones on typhoid fever, the average fever clearance time is less than four days, and the cure rates exceed 96 per cent. Their research showed that, less than 2 per

cent of treated patients have persistent faecal carriage or relapse. The published data also suggested that, the fluoroquinolones are more rapidly effective and are associated with lower rates of stool carriage than the traditional first-line drugs (chloramphenicol and trimethoprim–sulfamethoxazole). The challenge concerning the use of fluoroquinolone drugs for the treatment of typhoid fever has been the potential for toxic effects in children, the cost, and the potential emergence of resistance. Schaad and colleagues (1995) in a preclinical testing found that, the fluoroquinolones damages the articular cartilage of young beagles. There is now a considerable body of reassuring evidence from the long-term use of fluoroquinolones in children with cystic fibrosis and from the short-term use of fluoroquinolones to treat typhoid fever and of fluoroquinolones or nalidixic acid to treat bacillary dysentery in children. Long-term follow up report was evident that, there was no evidence of bone or joint toxicity, tendon rupture, or impairment of growth. The production of generic fluoroquinolones in Asia has reduced the price considerably. However, the emergence of quinolone resistance in areas where these drugs are inexpensive and readily available is likely to be the greatest limitation on their use.

Fortunately, full fluoroquinolone resistance is still rare.

In areas where quinolone-resistant strains are uncommon, the fluoroquinolones becomes the next treatment of choice for all age groups (Parry et al., 2002). Short courses of treatment (three to five days) are particularly useful to contain epidemics. Among patients with quinolone-resistant Salmonella enterica serotype typhi infection, the rate of treatment failure is higher for those treated for less than seven days than for those treated for a longer period (Wain et al., 1997). Fluoroquinolones should be used at the maximal possible dose for a minimum of 10 to 14 days, and the patients should be carefully followed to determine

whether they are excreting S. enterica serotype typhi in their faeces. Unfortunately, quinolone-resistant strains are often also multidrug resistant, and therefore the choice of drugs is limited to azithromycin or the cephalosporins, which are expensive. The third-generation cephalosporins (ceftriaxone, cefixime, cefotaxime, and cefoperazone) and azithromycin are also effective drugs for typhoid. In randomized, controlled trials of third-generation cephalosporins, principally ceftriaxone and cefixime, the fever-clearance times averaged one week and the rates of treatment failure were 5 to 10 percent. The relapse rates were 3 to 6 percent, and the fecal-carriage rates were less than 3 percent. Cure rates of 95 percent were achieved with five to seven days of treatment with azithromycin (Butler, 1999). Fever resolved in four to six days, and the rates of relapse and convalescent fecal carriage were less than 3 percent. Aztreonam and imipenem are potential third-line drugs (Bhutta, 1997).

2.6.5 PREVENTION OF TYPHOID FEVER

One gets infected with typhoid fever by drinking water or eating food contaminated with S. typhi. For this reason, prevention is based on ensuring access to safe water and by promoting safe food handling practices. Health education is paramount to raise public awareness and induce behaviour change (WHO, 2003).

2.6.5.1 SAFE DRINKING WATER

Typhoid fever is a waterborne disease, and the main preventive measure is to ensure access to safe water. The water needs to be of good quality and must be sufficient to supply all the community with enough drinking water .And also for all other domestic purposes such as cooking and washing.

Control measures during outbreaks:

- In urban areas the treatment of water supply systems must be strengthened from catchment to consumer. Safe drinking water should be made available to the population through a piped system or from tanker trucks.
- In rural areas wells must be checked for pathogens and treated if necessary.
- At home particular attention must be paid to the disinfection and the storage of the water to make the source save. Drinking-water can be made safe by boiling it for one minute or by adding a chlorine-releasing chemical. Narrow-mouthed pots with covers for storing water are helpful in reducing secondary transmission of typhoid fever. Chlorine is ineffective when water is stored in metallic containers.
- In some situations: such as poor rural areas in developing countries or refugee camps, fuel for boiling water and storage containers may have to be supplied.

2.6.5.2 SANITATION

Good sanitation contributes to reducing the risk of transmission of all diarrhea pathogens including Salmonella. typhi.

- Appropriate facilities for human waste disposal must be available for all the community. In an emergency, pit latrines can be quickly built.
- Collection and treatment of sewage, especially during the rainy season, must be implemented
- In areas where typhoid fever is known to be present, the use of human excreta as fertilizers must be discouraged.

2.6.5.3 FOOD SAFETY

One of the major vehicles for typhoid fever transmission is contaminated food. Appropriate food handling and processing is paramount and the following basic hygiene measures must be implemented or reinforced during epidemics:

- washing hands with soap before preparing or eating food; • avoiding raw food, shellfish, ice;
- Eating only cooked and still hot food or re-heating it.

During outbreaks, food safety inspections must be reinforced in restaurants and for street food vendor's activities. Typhoid can be transmitted by chronic carriers who do not apply satisfactory food-related hygiene practices. These carriers should be excluded from any activities involving food preparation and serving. They should not resume their duties until they have had three negative stool cultures at least one month apart.

2.6.5.4 HEALTH EDUCATION

Public awareness of infection and a step in the right direction towards prevention is through health education. Health education messages should reach every community and translations to local languages should be employed either by media, schools, committees, religious groups etc

Maintenance of the needed infrastructures and building of new ones with regard to hygiene is the cornerstone of behavioral change for community involvement.

In health facilities, all staff must be repeatedly educated about the need for:

- Excellent personal hygiene at work.
- Isolation measures for the patient.
- Disinfection measure.

2.6.5.5. VACCINATION

The Centre for Disease Control and Prevention, immunization has become one of the important public health advances in the 20th century. Vaccination is an easy and highly effective way to keep travelers healthy (Sturchler & Steffen, 2001). Studies have the evidence that, despite effective treatment of typhoid fever, the increasing report of MDRST make it necessary for vaccine to be used as a public health tool in developing countries. Control and clearance of a vaccine strain rely on the phagocyte oxidative burst, reactive nitrogen intermediates, inflammatory cytokines, CD4 (+) TCR- alpha beta T cells and are controlled by genes including NRAMPI and MHC class 11. Vaccine-induced resistance to re-infection requires the presence of TH1-type immunological memory and antiSalmonella antibodies. The interaction between T and B cells is essential for the development of resistance following vaccination (Mastroeni & Menager, 2003). Levine & Noriega's (1993) work showed that considerable effort and progress have been made in the last decade to develop vaccines against the enteric infections which is of greatest public health importance. Parenteral VI polysaccharide and oral Ty2la are the two vaccines against typhoid fever that have been licensed in many countries. A new typhoid vaccine has also been reported to be composed of VI capsular polysaccharide (Plotkin & Bouveret 1995). The Vi polysaccharide is a well standardized antigen that is effective in a single parenteral dose. It has been observed to be safer than whole cell vaccine and may be used in children of two years of age or older. Much effort in recent years has led to the development of attenuated Salmonella enterica Serover typhi strains as candidate typhoid fever vaccines. Clinical trials has proven that the vaccine tolerable and immunogenic. For example, the attenuated S.enterica var. typhi strains CVD 908-htr A (aroCaroDhtrA), Ty 800 (pho Ppho Q) and chi 4073 (cyacrpcdt) are all

promising candidate typhoid vaccine (Garmory et al., 2002). Ty2la live vaccines given by oral route has been exclusively tested in several studies in developing countries. The liquid formulation was more potent, providing more than 60% of protection after 7 years of follow up. The Vi polysaccharide vaccine has been put to trial and provided more than 65% protection; after 3 years of follow up the Vi antibody was still at a high level. These two vaccines have become proven candidates for use in public health control programs. Aromatic dependent Salmonella live vaccine has been also reported (Stocker, 2000). Killed whole cell bacterial vaccines of typhoid generally show a high degree of stability of potency. Corbal (1996) reported that, Ty21a vaccine is susceptible to ultraviolet irradiation and has low thermal stability. Specific antibody secreting cells (ASC) appear in the blood as a response to oral vaccination in humans. Information obtained from animal experiments has shown that, these cells are believed to be migrating to the mucosa lining. A series of studies aimed at a detailed characterization of the ASC response to a prototype oral vaccine Salmonella typhi Ty21a with respect to its kinetic, IgG class distribution, antigen specificity, influence of the administrative route, nature of the antigen, and the corresponding antibody responses in serum (Khan et al., 2008). Live Salmonella vaccine has been reported as a route towards oral immunization. Vaccines are composed of viral or bacterial strains, which are deprived of their pathogenicity but can still replicate in the organism.

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 Introduction

The chapter three of the study gives a description of the methods that was employed in conducting this study. It details the specific research methods and techniques used for the study. It also looks at the appropriate data collection procedures and the tools used for the data analysis. In this part of the study, the study setting, study design, population and parameters of interest, sampling and sampling technique, experimental design and the data analysis are presented.

3.2 Study Area and setting

The study, was conducted within the Bekwai Municipal Assembly with the study site being the Bekwai Government Hospital .The Bekwai Municipal is one of the twenty-seven (27) administrative District in the Ashanti Region of Ghana, established under legislative instrument (L.I. 1906, 2007). Bekwai is the capital of the Bekwai Municipal. The 2010 population and housing census (GSS, 2010) puts the population of the district at 118,024with a growth rate of 3.1%. The Municipality was projected to have in 2013 a population of 129,000 with a density of 204 persons per square kilometer. Females constitute the dominant sex in the district constituting 52.9% while the males make up 47.1. Health accessibility is more skewed towards urban and semi-urban parts towns within the Municipality. There are a few community and mission clinics situated in the rural areas of the Municipality. The Municipal Government Hospital where the present study was conducted is located at Bekwai and attends to all referred cases from other parts of the Municipality. There are public-private partnership built clinics and hospitals within the communities in the Municipality to providing Mission Clinics.

3.3 Research design

The prospective hospital-based longitudinal design with analytical dimensions was adopted for this study. The study approach was quantitative. The design is appropriate for this study because the study is it is over a time span, descriptive and experimental in nature with analytical dimensions. The study sought to both describe the state of affairs as exist in relation to incidence of typhoid and to compare the sensitivity and specificity levels using Typhidot and the Widal Test for its early diagnosis.

3.4 Study Population

The study population included all patients above 24 Months (2years) attending the Bekwai Government Hospital from the period Jan 2015 to June 2015.

3.4.1 Target Population/Cases/Unit of Analysis

The target population for the study constituted febrile patients who were suspected of typhoid fever attending medical examination at the outpatient departments (OPD) of Bekwai Government Hospital. The basis for this suspicion was on the features that were presented as symptoms of typhoid fever by the medical officer who diagnosed the patient at the various OPD.s.

3.5 Sampling and Sampling technique.

The study employed both probability and non-probability sampling techniques. The purposive sampling techniques were adopted in sampling research participants for the study. The population of interest comprised patients who accessed health care at the Bekwai

government hospital with symptoms of fever. The patients were screened by physicians, for the clinical symptom of typhoid fever often identified as fever of 2 or more days before admission with other clinical symptoms of typhoid fever in the absence of any other known febrile illnesses. The patients after purposely identified as having symptom of Typhoid were recruited randomly. The first ten eligible patients were recruited daily. The laboratory request slip with a request test was placed at the sample collection area together with patient details and entered into a sample collection book. This was done after the patients had been recruited. A laboratory number was assigned to each sample collection. Patients were made to sit and wait for some few minutes. Babies, Children were helped by their mothers, guardians or caretakers after the procedure has duly been explained to them. The methylated spirit was applied to the middle finger of adult and the heel of babies to disinfect them. Using a sterile lancet, the cleaned area, thus the middle finger or heel was pricked deep to allow free flow of blood and disposed into a safety puncture/sharps container. A maximum volume of 1.5ml blood was adequate and collected into the sterile container. The blood collected was mixed by turning up and down about six times to prevent the blood from clotting. After this, the pricked part of the patient was cleaned with a new swab and plastered when it becomes necessary. The Blood samples and request forms were sent to t the laboratory as soon as possible for processing.

3.5 1. Inclusion Criteria

The following feature or conditions were considered as the eligibility criteria for inclusion to become a study participant

- Children attending the Bekwai Government Hospital, for the first time who have fever or any history indicative of malaria and had not taken any antimalaria drugs

within fourteen days of reporting to the hospital whose parents or care givers agrees to consent by writing or orally.

- A patient with history of fever, characteristics for typhoid fever for 3 days or more, irrespective of antibiotic treatment.
- Any patient with clinical presentation suggestive of typhoid fever like abdominal discomfort with diarrhea, soft enlarged spleen, coated tongue, toxic look and relative bradycardia headache, anorexia, nausea and vomiting,
- Individual Patients of both male and female sexes representing all category of ages.

3.5.2 Exclusion criteria

- Children who were attending the Bekwai Government hospital for reviews
- Persons who had taken any antimalaria drugs within fourteen days prior to the day of reporting to the hospital
- Any symptom of fever with an obvious focus for other infection such as urinary tract infection, otitis media etc.
- Patient with known History of immunization with typhoid vaccines

3.5.3 Sample Size determination

The sample size was determined based on Fisher (1998). The population of Bekwai Municipal is 118,024. The proportion of the population possessing the outcome attributed to the outcome since the population was large, the prevalence typhoid was unknown and , was as 20% (0.2), the Confidence interval was taken as ±1.96 at 95% Confidence level.

The Sample size was being calculated as; n

= Z2 (P) (q) D /d2.

n= desired sample size, valid only when the population is more than10, 000. z= the standard normal deviation 1.96, Confidence interval (usually taken 95% p= Relates to proportion of the target population estimated to have the particular characteristics under the study. Prevalence of the disease / problem in community (incidence of typhoid fever is taken as 25 percent based on Afoakwa et al.(2011) whose study had the incidence rate of Typhoid in Sunyani and Kumasi Metropolis to be 24.8%.. D= design effect usually 1 where there are no replications or comparisons

p=0.25

q= 1.0-p, 1-0.25= 0.75 d= degree of

accuracy desired at 0.05 n= (1.96) 2

(0.25)(0.75)(1)

(0.05)2 n

= 288.12

The 288.1 was adjusted by 1.2 design effect to account for the errors in the study design.

The sample size became 292.

3.6 Data Collection Techniques and Tool

Each of the study participants was given a set of questionnaire. All relevant information including history of presenting complains and laboratory findings of every case were systematically recorded in a pre-designed data sheet. The patients either approved of their decision to participate through signed informed consent or oral consent. In the case of children above 2 years, data was obtained if the potential parent, guardian or care giver shows willingness and understanding of the study and is willing to enroll. The interview was done in Twi which is the local language in the district.

3.7 Plans for Data Handling

Data collected on participants was be given maximum confidentiality. They were kept in envelopes and separated from each other with labels. These envelopes were kept in a safe under lock and key and only the researcher had access to the data. Names of patients did not form part of data that collected as identification numbers were assigned to the folders with the results.

3.8 Data Analysis plan

Data input was performed by the researcher. This was done on a weekly basis. A codebook for each variable was prepared beforehand. The data was analyzed using SPSS version 20.Sensitivities, specificities; positive predictive, negative predictive values were determined for the various tests and compared with one another. The statistical tests used were mainly sensitivity, specificity, positive predictive value, negative predictive value and accuracy. These were calculated by using the following formulas: sensitivity is a/(a + c), specificity is d/(d + b), positive predictive value a/(a+b), negative predictive value d/(c+ d), accuracy a+d/(a+b+c + d), where a is positive Typhidot and Widal test, b is negative Typhidot , but positive Widal test, c is positive Typhidot , but negative Widal test, and d is negative Typhidot, and negative Widal test. In addition, chi-square and Pearson correlation will be performed. Differences were considered significant at P value <0.05.

3.9 Ethical Consideration

This study was reviewed and received ethical approval the Committee on Human Research Publication and Ethics, Kwame Nkrumah University of Science and Technology College of

Health Sciences-School of Medical Sciences. All data gathered from the records were strictly used solely for academic purpose.

3.11 Limitations of Study

The study is likely to be limited by caretakers of children giving consent. However, the researcher will encourage them to grant consent by assuring them of the highest level of confidentiality

3.10 EXPERIMENTAL DESIGN

Typhidot test is a kit that detects IgM and IgG antibodies against the outer membrane protein (OMP) of the Salmonella typhi. The typhidot test becomes positive within 2-3 days of infection and separately identifies IgM and IgG antibodies. The test is based on the presence of specific IgM and IgG antibodies to bind to a specific 50-KD outer membrane protein (OMP) antigens, which is impregnated on nitrocellulose strips. The reaction tray was divided into 2 columns marked as G and M. 250µl of sample diluents will be dispensed in each well and 2.5µl of test /control was added and then incubated for 20 minutes. The strips were washed with washing buffer thrice, 250µl of anti- human IgG and IgM will be dispensed in each well and incubated for another 15 minutes. These were washed again, dispensed with 250µl of color development solution, and incubated for another 15 minutes and results interpreted. A positive IgM was interpreted clinically as acute typhoid illness, while IgM and IgG positive was taken as acute typhoid illness in middle stage of infection and IgG positive was interpreted as chronic carrier or previous infection or re-infection. After the Typhidot Test has been performed the Widal test was performed after which the sensitivity and specificity were calculated

CHAPTER FOUR

RESULTS

4.1 Introduction

This chapter presents the study findings in relation to the objectives of the study. The results are presented according to the key specific objectives of the study.

4.2 Blood culture test results

A total of 292 individuals were sampled for the presence of typhoid using the Typhidot, Widal and Culture techniques. The results indicate that there was 41.10% (120) tested positive for Blood culture. As presented in Fig 1, 172(59%) tested negative with Blood culture test.

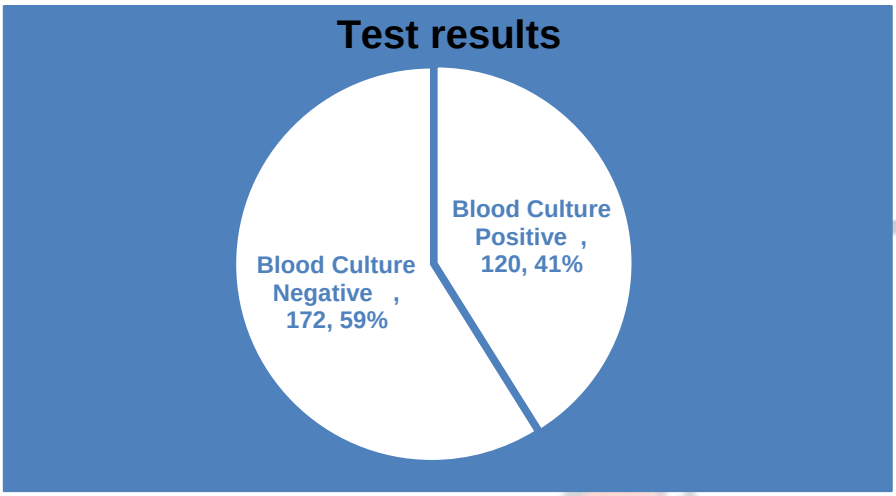

Fig 1: Blood Culture results in all sampled cases.

4.3 Specificity for Typhidot and Widal

The results on the negative predictive values of the study findings are presented in Table 2. Out of the total 172(100%) negative prediction of all the 292 suspected cases of Typhoid, the predictive value of Widal was lower recording 54.0% as compared to Typhidot, 156 (90.7%). The results is presented in Table 2.

Table 2: Specificity for Typhidot and Widal

Test Parameter	Population	Negative (%)
Typhidot	156	90.7
Widal	93	54.0
Blood culture	172	100

Source: Authors construct, 2016

4.3 Positive prediction of typhoid with Typhidot and Widal test

Of the 120(100%) individuals that tested positive for blood culturing, 86.0% (103) and 95.0% (114) of individuals recorded positive for Typhidot and Widal tests, respectively (Table 2). Typhidot test recorded a relatively lower detection rate compared to the Widal

test. Widal however recorded a higher negative predictive value of 54.0% than Typhidot which had only 46% negative prediction outcome.

Table 3: Prediction of typhoid with Typhidot and Widal test in Blood cultured cases.

PARAMETER	WIDAL TEST	TYPHIDOT TEST (%)
Positive predictive value	86	95
Negative predictive value	54	46

Source: Authors construct 2016

4.4 Comparative Typhidot and Widal detection rates for typhoid

A representative 98 (81.7%), of the 120 individuals positive for culture technique recorded an accuracy rate for both Typhidot and Widal tests. Of the same 120 samples, 16(13.3%) recorded negative for Typhidot and positive for Widal, 5(4.1%) recorded positive for Typhidot but negative for Widal test and 1(0.8%) showing negative for both Typhidot and Widal tests (Table 4 and Fig 2).

Table 4: Variation of detection between Typhidot and Widal tests

	Population affected	Population affected (%)
Thyphidot/Widal	98	81.67
Thyphidot-/Widal+	16	13.33
Thyphidot+/Widal-	5	4.17
Thyphidot/Widal	1	0.83

Source: Authors construct 2016

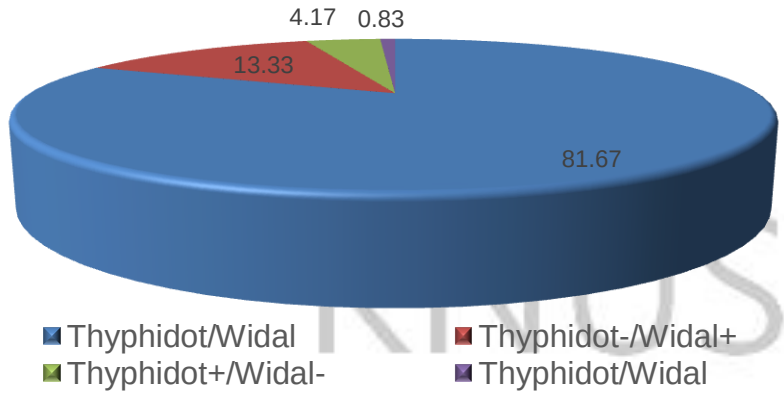

Figure 2: Comparison of Typhidot and Widal infection

4.5 Specificity, sensitivity, Accuracy and predictive value of Typhidot and Widal Test

Sensitivity, specificity, Accuracy as well as prediction rates for both Widal and Typhidot tests were determined for the study population. Sensitivity of Widal was recorded at 95% with 54.0% specificity .Typhidot test recorded a 86.0% sensitivity, 90.1% specificity, a high positive predictive value of 95% and a lower negative predictive value of 46% .

There was however no statistical significance between the various parameters for each tests ($p > 0.05$).

Table 5: Typhidot and Widal test parameters compared

Parameter	Widal test%	Typhidot test %
Sensitivity	95.0	86.0
Specificity	54.0	90.7
Positive predictive value	86.0	95.0
Negative predictive value	54.0	46.0
Accuracy	98.01	98.01

Source: Authors construct, 2016

4.6 Sensitivity test for Widal and Typhidot tests cross tabulated with age.

The age range 21-31 recorded the highest positive prediction of typhoid for both Typhidot and Widal test. These were 32.5% (37) and 31.0% (32) for Widal and Typhidot sensitive cases respectively. The age cohort 11-20 followed with being sensitive for both Widal and Typhidot test.

Table 6: Sensitivity of Typhidot and Widal with Age

	Positive	
Age(yrs.)	Widal	Typhidot
1 – 10	11(9.7%)	10(9.7%)
11-20	28(24.6%)	24(23.3%)
21-30	37(32.5%)	32(31.0%)
31—40	11(9.6%)	10(9.7%)
41-50	12(10.5%)	12(11.7%)
51-60	9(7.9%)	9(8.7%)
61-70	4(3.4%)	4(3.9%)
71-80	1(0.9)	1(0.9%)
81-90	1(0.9)	1(0.9%)
Total	114(100)	103(100)

Source: Authors construct 2016

4.6.1Comparison of test sensitivity with age

Blood culture analysis for typhoid infection recorded the highest sensitivity with age, followed by Typhidot and Widal test. There was however no significant difference between sensitivity of Typhidot (86.0%), Widal (95.0%) and Blood culture test with age.

Table 7: Comparison of sensitivity of the various tests with age

ANOVA						
		Sum of Squares	Df	Mean Square	F	Sig.
Widal_test	Between Groups	2.310	48	.048	.662	.934
	Within Groups	5.157	71	.073		
	Total	7.467	119			
Typhidot_test	Between Groups	4.685	48	.098	.699	.905
	Within Groups	9.907	71	.140		
	Total	14.592	119			
Blood_culture _test	Between Groups	.000	48	.000	.	.
	Within Groups	.000	71	.000		
	Total	.000	119			

Source: Authors construct 2016

4.7 Microbial prevalence and level of infection

Blood culture analysis of samples recorded 99.33% of S. typhi and 0.83% prevalence of S. paratyhi and S. Typhimurun respectively. S. typhi was observed as the most prevailing microorganism in the study population.

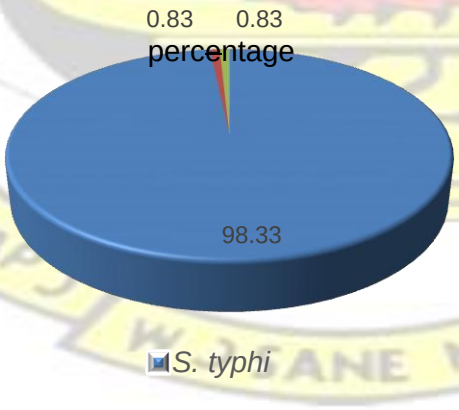

Figure 3: Microbial prevalence by blood culture diagnosis

4.8 Level of infection with Typhidot test

Typhidot test recorded 17% of infected typhoid individuals out of the 120 positive blood culture samples, had no infection. A representative 44% showed middle level infection and majority of the remaining 56% showed acute infection whereas a little less of this recorded chronic infection level.

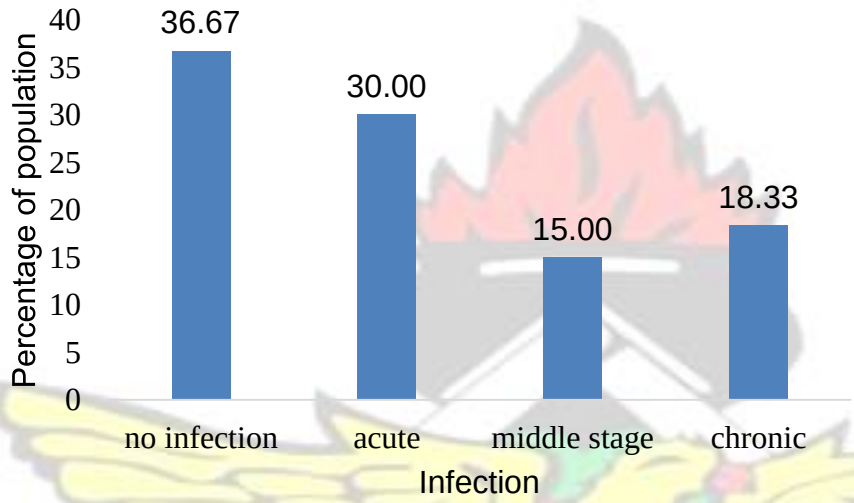

Figure 4: Typhoid Infection intensity with Typhidot

4.9 Correlation of test

The study showed a positive correlation between Typhidot and Widal test. However, the correlation was weak (0.013) and statistically insignificant (p= 0.89).

Table 5: Pearson Correlation analysis of the various tests

		Widal_test	Typhidot_test	Blood_culture_test
Widal_test	Pearson Correlation	1	.013	.a

	Sig. (2tailed)		.890	.
	N	120	120	120
Typhidot_test	Pearson Correlation	.013	1	.a
	Sig. (2tailed)	.890		.
	N	120	120	120
Blood_culture_test	Pearson Correlation	.a	.a	.a
	Sig. (2tailed)	.	.	
	N	120	120	120

a. Cannot be computed because at least one of the variables is constant.

CHAPTER FIVE

DISCUSSION

The study on 292 individuals, showed the presence of typhoid within the sample population using the Blood culture, Typhidot and Widal diagnosing techniques. Blood culture test detected the 41.10% (120) typhoid infection. Detection of typhoid was highest in the blood culture technique, which does not appear a surprise, as it has been identified as one the most effective typhoid diagnosis technique.

There was a high detection of Salmonella typhi by blood culture diagnosis, with Salmonella paratyphi and Salmonella typhimurun occurring least, in this study. This observation indicates that S. typhi is selected against other typhoid causal variant organisms. Of the samples positive for blood culture, 86% (103) and 95.% (114) were positive for Typhidot and widal tests respectively. There was however no significant difference between the observations for the two tests (Table 6).This observation indicates widal test is much more sensitive than the Typhidot test. This observation is contrary to previous studies by Choo et al. (1999) and Gopalakrishan et al. (2002) in Malaysia which predicted 90.3% and 98.0% sensitivity for Typhidot. Contradictions in sensitivity of Typhidot and widal tests in this study in comparison to previous study could be attributed to host-parasite variation due to differences in the geographical location. Previous studies in Asia with different climatic conditions could have favoured the expression of genetic variants of Salmonella (Bhan, Bahl & Bhatnagar, 2005) easily detected by antibodies employed in the test. This could have accounted for the low sensitivity of the two test in this current study as compared to higher sensitivity recorded in previous studies. High levels of probable background antibodies in regions where study was conducted could also have accounted for high sensitivity (Schroeder, 1968).

Specificity is indicative of the test's ability to correctly predict false negatives where they occur. Typhidot in this study recorded a high specificity to typhoid, relative to Widal test. However, there was no statistical significance in this observation between these two tests. The rate of positive prediction in typhidot was observed to be relatively higher (95%) than in Widal (86%), whereas negative prediction of typhoid was vice versa for the two tests. This indicates that Typhidot is a good test for the diagnosis of infection in endemic regions

as it has the potential to detect most true positive cases of typhoid infection as compared to Widal test. The study observed that both tests had equal accuracy (98.1%). Irrespective of the differences in sensitivity and specificity of the Widal and typhidot tests, the study observed an equal accuracy in their prediction of typhoid. There was a positive correlation (0.13) between Widal and typhidot indicating their ability to predict and efficiently detect typhoid concurrently. The correlation was however weak.

The current study indicated three microorganisms (S. typhi, S. paratyphi and S. thyphmurun) responsible for typhoid prevalence within the population. It also showed to some extent that there is not much of a difference in the sensitivity and specificity of the Typhidot and Widal techniques for the diagnosis of typhoid infection.

The study found high positive prediction among children using all the test kits. It was established that almost a third, 32.2% (39/114) of all positive Widal test were children. Similarly, close to a third 33% (34/104) of Typhidot positive predictive values were recorded among children. This finding corroborates earlier studies by Anggraini et al (2004); Jerrold &Turner (2010) whose works established high prevalence of typhoid among child cohorts, a finding additionally confirmed by the results of Sultana (2012) and Brooks et al (2005). More to the high prevalence among children, the prevalence of typhoid was confirmed among the productive population (21-50) in the study area. In Widal positive test, 52.6% (60/114) of all positive predictive cases were recorded among populations within the 21-50 years range.

In contrast to studies by Jerold & Turner (2010), whose finding report on high typhoid prevalence in children and the elderly, in the present study, high detection rates of typhoid was characteristic of children and the productive population. Akinyemi et al (2005)

study confirmed the findings in the present study. This finding appears to be consistent with other studies within the Sub-Saharan setting. Proffering explanation for the unique detection of typhoid in children and the productive cohort has not been consistent however; it could be attributed to the heavily youthful population in the Sub Saharan region which places majority of the population in the productive youthful region and child dependency cohorts.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The study identified S. typhi, S. paratyphi and S. typhimurun as the causal organisms for typhoid in the study population. The study revealed a high sensitivity of Widal test relative to Typhidot test. However, specificity for Widal test was relatively lower than Typhidot. This finding is contrary to most previous studies conducted in Asia (Choo et al., 1999; and Gopalakrishan et al., 2002) that recorded a higher sensitivity (above 90%) and a lower specificity for Typhidot in comparison to the Widal test. Since there was no significance between the observations in the two tests, the differences could be attributed to chance. It can be deduced from the study that both tests could be equally, sensitive and specific in diagnosing typhoid and since they both have an equal accuracy (98.0) either is suitable for rapid diagnosis. In remote areas where there is the absence of proper laboratory equipment to carry out rapid test especially within the Ghanaian situation, Typhidot comes as the best alternative for early diagnosis of fever. Though in this study, sensitivity for Typhoid was relatively lower as compared to Widal, Typhidot comes as easy to use, cheaper and very

affordable. Additionally, it has the potential to be used in rural settings hence its often application. It possesses the ability to detect high true negatives making the specificity higher than Widal in the present study.

6.2 RECOMMENDATION

From the current study it is recommended that:

i. Residual antibody should be done to help in detection of the true titre.
ii. Further study should be carried out around the study area to determine other variant that were not carried out in the present study.

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APPENDIX 1

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
DEPARTMENT OF CLINICAL MICROBIOLOGY SCHOOL OF MEDICAL SCIENCES

DATA COLLECTION INSTRUMENT

Topic: Comparative study of Typhidot and Widal test in the early diagnosis of Typhoid.

Study site: __ Bekwai __

Data entry start time: __ __:__ __ AM / PM (circle one)

Data entry outcome code: __ __ 01 completed
02 partially completed
04 partially refused

Section A: Demographic Characteristics A1.

1 Sex

Male……. 1

Female……2

A2. Age

A3. Have you shown any symptoms indicative of Typhoid Fever infection in the two weeks

Period before coming to the hospital?

Yes 1

No 2

Don't know 3

Section B: Test Mediums

A.4 IGM predictive value for Typhoid test

Yes 1

No ……..2

A5. IgG predictive value for Typhoid test

Yes 1

No ……..2

A.6 S. typhi agglutinins (antibodies) distribution in the serum of infected persons

Agglutinin O [ ]

Agglutinin H [ ]

A.7 Type of microbial prevalent in serum of infected persons

S. paratyhi [ ] ……..1

S. thyphumurun [ ] ……..2

Section C: Clinical and medical History

A.8 History of antimalarial drug within the last fourteen days prior to day of reporting to the hospital

Yes 1

No…….2

A.9 Any known history of immunization with typhoid vaccines

Yes 1

No…….2

A.10 Any known or medically diagnosed symptom of fever with an obvious focus for other infection such as urinary tract infection, otitismedia?

Yes 1

No…….2