PERFORMANCE OF WIDAL TEST AND STOOL CULTURE AS DIAGNOSTIC METHODS FOR *SALMONELLA TYPHI* INFECTION IN CHUKA GENERAL HOSPITAL, THARAKA NITHI COUNTY

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DECLARATION

This thesis is my original work and has not been presented for the award of a degree in any other university.

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DEDICATION

This thesis is dedicated to my parents Mr and Mrs Gerald Gitonga for their moral support during the struggle to attain my academic success, to my husband Mr John Gitonga for his encouragement and inspiration during the writing and to my children Lenza Kathomi, Evans Mwenda and Ann Nkatha for their patience during my busy schedule.
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ACRONYMS AND ABBREVIATIONS

DC       Dendritic cells
DCA      Deoxycholate citrate agar
EMB      EosineMethlyene Blue
FAE      Follicle associated epithelium
GALT     Gut associated lymphoid tissue
GIT      Gastro intestinal tract
SS       *Salmonella* - *Shigella* agar
*S. typhi*  *Salmonella typhi*
MLN      Mesentric lymph nodes
NPV      Negative predictive value
PP       Peyer’s patches
PPV      Positive predictive value
TMP-SMZ  Trimethoprim-sulfamethoxazole
TSI      Tripple Sugar Iron agar
WBC      White blood cells
XLD      Xylose Lysine Deoxycholate agar
Typhoid fever is one of the infectious human diseases. Outbreaks of typhoid fever caused by *Salmonella typhi* remains a serious health problem worldwide. There are a number of tests available presently, from molecular to immunological and biochemical to microbiological. However, misdiagnosis is usually experienced since most health care facilities use only Widal test without confirmation of results with a second test method. This study aimed at evaluating the performance of Widal test and stool culture in the laboratory diagnosis of typhoid fever using blood culture as gold standard. Presenting patients aged between 5 to 82 years with symptoms clinically suspected to be of typhoid fever visiting Chuka General Hospital for a period of eighteen months were recruited for the study. Informed consent of volunteers and guardians were obtained. Serum samples from a total of 126 patients were subjected to Widal agglutination tests. Blood and stool samples from the same individuals were analyzed for typhoid fever infection using blood and stool cultures respectively. Serotyping was performed using agglutination with *Salmonella* O, H and Vi antisera. In Widal agglutination test, titre values from 1:160 and above were regarded as significant and therefore positive for the *Salmonella* antigen. Isolation of *Salmonella typhi* from stool and blood culture indicated an infection. Raw data were entered into Microsoft excel and analyzed using statistical package for social sciences (SPSS). Analysis involved computation of descriptive statistics such as frequencies, means and standard deviations. Comparison of categorical data was made using Chi square or fisher’s exact test, as appropriate. The confidence intervals for sensitivity and specificity were computed using the Wilson’s score method. A P value ≤ 0.05 was considered statistically significant. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for Widal test and stool culture were calculated. The total number of patients positive for typhoid fever based on Widal test were 70 (55.6%). The prevalence of typhoid fever was 42.9% and 15.1% based on the findings from stool and blood culture respectively. Widal test recorded 73.7% sensitivity, 47.7% specificity, 20% positive predictive value and 91.1% negative predictive value. Stool culture showed 84.2% sensitivity, 64.5% specificity, 29.6% positive predictive value and 95.8% negative predictive value. These results demonstrate that Widal test is not very reliable for diagnosis of typhoid fever since false positive and false negative results are common. The low PPV means that Widal test could only be useful for excluding the disease from the population hence health care personnel should not totally depend on this test alone for diagnosis of enteric fever but should use other diagnostic methods to differentiate *Salmonella* infection from other infection. There is therefore, an urgent need to develop a rapid, highly sensitive and cheap diagnostic tool for diagnosis of typhoid fever.
CHAPTER ONE: INTRODUCTION

1.1 Background information

The bacterium, *Salmonella typhi*, is known to cause typhoid fever, a life threatening illness (Crump *et al.*, 2004). Annually, typhoid fever is known to record cases estimated to be between 13-16 million, which result in 216,000 deaths. The most affected are children and young adults between the ages of 5 and 19 year. As of 2010, these cases caused about 190,000 deaths, a rise from 137,000 recorded in 1990. In Kenya, typhoid incidence rate of 39/100,000 have been reported but these figures may be underestimates because facilities capable of performing the blood culture tests essential for diagnosis are absent from many regions (Kariuki *et al.*, 2008). A study carried out by Breiman *et al.* (2008) found out that typhoid incidence rate is higher in urban slums than in rural areas. The rates of typhoid fever among children aged 2 to 4 years were 15 times higher in Kibera (urban slum) than in Lwak (rural setting) for the same age group. This can be linked to overcrowding and suboptimal access to safe water, sanitation and proper hygiene. In Kenya, multidrug *Salmonella typhi* isolates from adults and school age children associated with sporadic outbreaks in resource poor settings especially in slum areas have been reported.

Although oral contact with an infected person may result to the spread of the bacteria, in most cases it is spread through contaminated food or water. Contaminated water and poor sanitation are the most common causes of typhoid fever in developing nations (Hook, 2005). Though one can be treated with antibiotics, a few who recover are known to still harbor the bacteria for years in their gall bladder or intestinal tracts. These chronic
carriers as they are referred to may infect others through shedding the bacteria in their feces though they might not have signs or symptoms (Chart et al., 2000). Diagnostic methods currently used in health facilities in Kenya include Widal test, stool and blood cultures (Bhutta, 2006). Cases of typhoid fever can be confirmed using the current gold method of isolating the bacteria, Salmonella typhi, from blood (House et al., 2001). The problem however, is the fact that in developing nations, the equipments, supplies and trained laboratory personnel required especially in primary health-care can rarely be found. Due to this, the most commonly used diagnostic methods in most rural health care facilities in Kenya include Widal test, stool and blood culture since these methods are cheaper and do not require complicated technical expertise. However, most health-care facilities use only one of these methods without confirmation of results with a second test method. This at times leads to unnecessary and inappropriate antimicrobial treatment of patients without the typhoid fever due to misdiagnosis. The objective of the present study was to evaluate Widal test and stool culture performance as diagnostic methods for Salmonella typhi infections with a view to review the testing algorithms for the benefit of the patient.

1.2 Problem statement

Typhoid fever is a life threatening illness. It is important to diagnose and treat it early since serious complications that may include severe intestinal bleeding or perforations can arise within a week. Currently, there is emergence and recurrence of disease due to floods, poor sanitation and emergence of strains that are resistant to antibiotics (Francis et al., 2008). There has been cases of misdiagnosis due to the local health facilities adopting
Widal test diagnostic method as it is quicker, simpler and less costly. There has been no use of other methods to confirm *S. typhi* infection. Therefore, there was a need to evaluate the performance of Widal test and stool culture diagnostic methods to guide on the need to review the testing algorithms to benefit the patient.

1.3 Justification of the study

Outbreaks of *S. typhi* infection could be due to misdiagnosis, therefore, a need to determine the best and less costly method of diagnosis. Widal test and stool culture are cheap and do not require complicated technical expertise hence are applicable in rural setting. Use of insensitive diagnostic methods may lead to wrong medication, prolonged pathogenesis and bacterial resistance to antibiotics, which can be fatal and hence the need for re-evaluation of diagnostic methods in order to advice the medical practitioners on the most sensitive method of diagnosing *Salmonella typhi* infections.

1.4 Research questions

i. What is the sensitivity and specificity of Widal test in detecting *S. typhi* infection in patients attending Chuka General Hospital?

ii. What is the sensitivity and specificity of stool culture in detecting *S. typhi* infection in patients attending Chuka General Hospital?

iii. What is the positive and negative predictive values of Widal test and stool culture in detecting *S. typhi* infection in patients attending Chuka General Hospital?
1.5 Hypothesis

The sensitivity, specificity, positive predictive value and negative predictive values of Widal test and stool culture in detecting *S. typhi* infection are the same.

1.6 Objectives of the study

1.6.1 General objective

To determine the sensitivity, specificity, positive predictive value and negative predictive values of Widal test and stool culture as diagnostic methods for *S. typhi* infections in patients attending Chuka General Hospital in Tharaka Nithi County, Kenya.

1.6.2 Specific objectives

i. To determine the sensitivity and specificity of Widal test in detecting *S. typhi* infection in patients attending Chuka General Hospital.

ii. To determine the sensitivity and specificity of stool culture in detecting *S. typhi* infection in patients attending Chuka General Hospital.

iii. To determine the positive and negative predictive values of Widal test and stool culture in detecting *S. typhi* infection in patients attending Chuka General Hospital.
1.7 Significance of the study

The evaluation of performance of Widal test and stool culture in clinical diagnosis of *S. typhi* infection determines the most sensitive and effective diagnostic method. This provides information to policy makers to guide the health workers on the most appropriate method for diagnosing typhoid fever. This will help reduce misdiagnosis, ensure specific drug for the disease and reduce bacterial resistance to antibiotics.
CHAPTER TWO: LITERATURE REVIEW

2.1 Typhoid fever

The bacterium, *Salmonella typhi* (*Salmonella* enteric serotype *typhi*), causes typhoid fever. *Salmonella typhi* is an obligate, motile, gram-negative, rod-shaped enteric bacillus and belongs to enterobacteriaceae family. The intestines of humans are known to be its principal habitat. The major route of transmissions of enteric fever (typhoid fever) is either through faeco-oral route or urine-oral route (Mtove et al., 2010).

2.1.1 Pathology

When the bacteria is ingested through food or water that is contaminated (containing at least $10^4$ bacteria), it finds its way into the small intestine after 1-2 weeks period of incubation which varies. After they attach themselves to the intestinal epithelium where they penetrate lamina propria and sub mucosa, *S. typhi* are engulfed by monocytes (Gopalakrishnara et al., 2002). Bacteria have host defense evasion mechanism that enable them to resist intracellular killing and continue to multiply. They move to the blood stream through the thoracic duct and as a result, there is primary bacteremia. The liver, lymph node, spleen, gall bladder and the bone marrow seed the bacteria during this transient bacteremia. The onset of clinical disease is marked by this. After being shed from the gall bladder along the bile juice, they reach the small intestines again (Figure 2.1). Here, bacteria results in inflammation and ulceration after they infect the peyer’s patches and lymphoid follicles of ileum (Bhutta, 2006).
Figure 2.1 Course of *Salmonella typhi* infection (Adopted from Bhutta, 2006).
2.2 Epidemiology of typhoid fever

Typhoid fever is prevalent in areas that are characterized by overcrowding and at the same time have poor access to proper sanitation (Charles et al., 2012). Estimates by the WHO depict South East Africa, South Central Asia and Southern Africa as having high incidences of Salmonella typhi infection i.e. more than 100 cases per 100,000 persons per year (Crump et al., 2004) (Figure 2.2). Those with medium incidences include the Caribbean, Latin America among some other regions of Asia as well as Africa (10-100 cases per 100,000 persons per year) (Bhutta, 2006). The factual incidences of typhoid fever are however, difficult to estimate especially in developing countries due to what Hook (2005) terms as lack of rapid inexpensive diagnostic tools, poor disease reporting systems, laboratory testing infrequency and the often common confusion between the clinical presentation of the disease and other common febrile illness.

In developed countries for example UK, typhoid fever incidences are low as a result of proper sanitation, safe drinking water and also hygiene education. The rare cases reported are mostly from travellers returning from endemic areas or contact with carriers (Francis et al., 2008). California and Nerada state in India were in 2010 hit by an outbreak of typhoid fever. United States reports approximately 200-300 cases of S. typhi annually (WHO, 2006). This is especially among travellers that is about 80% of the total cases, who travel to countries which have been hit by the typhoid fever endemic, particularly the South Central Asian countries (Hohman, 2011). Tajikistan was also hit by a large typhoid outbreak between 1996 and 1998 which affected more than 24,000 people with cases of fatality rate of around 1%. 
Figure 2.2. Global incidence of typhoid fever (Adopted from Crump et al., 2004)
Central and southern Africa states such as Uganda, DRC, Zambia and Zimbabwe have had an inrush of typhoid fever outbreaks form early November 2011. According to WHO, approximately 21.6 M cases of typhoid in school and pre-school children and 200,000 deaths of the same has been reported. All these cases are attributed to problems with getting safe water and accessing proper sanitation. For example, in Kikwit DRC, typhoid is termed as endemic due to contamination of local water source that caused a spike of cases to thousands resulting in life threatening intestinal perforation, severe cases of peritonitis and death (Olopoenia and King, 2004).

In Kenya, urban slums have a higher rate of typhoid fever compared to rural areas (Hohman, 2011). This can be linked to overcrowding with poor access to safe drinking water and also proper sanitation facilities. A study carried out by Kariuki (2008) that compared the incidence of typhoid fever between Kibera (urban slum) and Lwak (rural setting) found out that the rates of typhoid fever among children aged 2-4 years were 15 times higher in Kibera than in Lwak for the same age group. The rates of typhoid fever in Kibera were comparable to those documented in endemic regions for example South East Asia. According to this study, 75% of S. typhi strains from these children were resistant to commonly used antibiotics and this posed challenges to the treatment and management of typhoid fever. The urban population grows quickly due to migration and new births causing strain on available resources and a variety of public health challenges (Lin et al., 2010). Typhoid fever still remains a serious public health problem in Kenya. According to Kariuki et al. (2004), the fever is still a major cause of mobility and mortality. However, the accurate data on true prevalence of typhoid fever is hard to get because
many hospitals do not have the facilities for blood culture which is regarded as the gold standard to diagnose typhoid fever (Gilman et al., 2011).

The fact that there is an emergence of *S. typhi* that resist most drugs, complicates the treatment and management of typhoid fever (Dutta *et al.*, 2006). Multi-drug typhoid which resists all three of the first line antibiotics; nalidixic acid, chloramphenicol and ciprofloxacin has been linked to more serious illness, a higher rate of complication and loss of life, especially among children aged below the age of 5 years (Mweu and English, 2008). India, Pakistan, Bangladesh, Vietnam and Africa have experienced such outbreaks which have these strains (Gopalakrishnan *et al.*, 2002) (Figure 2.3).
Figure 2.3. Global distribution of antimicrobial resistance in *S. typhi* (1990-2004) (Adopted from Crump *et al.*, 2004).
2.3 Clinical presentation of typhoid fever

The course of typhoid fever occurs in phases which last approximately one week (Hook, 2005). In the course of these phases, patients appear worn out and wasted. The patient has high fever, malaise, leucopenia, headache, bradycardia (faget sign), abdominal pain, cough, bloody nose, eosinopenia and relative lymphocytosis (Mweu, 2008). Rose spots are visible on the lower chest and also on the abdomen. The lung bases have rhonchi. The abdomen is stretched or becomes swollen and hurts at the right lower quadrant, where borborygmi can be heard. The patient trots frequently and suffers from hepatosplenomegaly (Prajapati et al., 2008). Many other complications may occur during the third week of infection e.g. congested peyer’s bleeding that results in intestinal hemorrhage, intestinal perforation, encephalitis, symptoms of neuropsychiatry, metastatic abscesses, cholestasis, endocarditis and osteitis (Hook, 2005). Dehydration ensures and the patient is delirious (typhoid state). Platelet level goes down (Francis et al., 2008).

2.4 Transmission and risk factors

The natural hosts of Salmonella enteric serovar typhi are the humans (Parry et al., 2009). This bacteria can survive for some period in ground or sea water and for a long period in contaminated food (Lunguya, 2012). Organisms of between $10^3$-$10^6$ bacteria can cause an infection (Prajapati, 2008). Typhoid fever is spread through consumption of contaminated water and food with the bacteria (Shrivastava et al., 2011). The other risk factors associated with transmission of typhoid fever include; having oral contact with someone who is infected (or a carrier) or eating contaminated foods for example flavored ice drinks and ice cream from street vendors and eating food that is raw or vegetables grown using human faeces as fertilizer.
(Parry et al., 2002). A study from a slum in Bangladesh found out that raw papaya provides optimum temperatures for survival of *S. typhi* since it has a neutral PH (Lin et al., 2010). Areas that are overcrowded especially the urban slums are highly associated with typhoid fever (Mweu and English, 2008). In this state, the sanitation facilitates are strained and people are not able to access clean water thereby promoting person to person transmission resulting to endemicity. Proper personal hygiene for example washing of fruits and vegetables and use of latrine for defecation are protective measures against typhoid fever. Antimicrobial exposure can lead to extended changes in gastro intestinal flora and reduced barrier to bacterial colonization increasing susceptibility to *Salmonella* (Okonko et al., 2010).

There is an association between serum anti-*Helicobacter pylori* IgG antibodies and infection with typhoid fever (Chart et al., 2000). *Helicobacter pylori* lowers gastric acidity thereby increasing the risk of infection with typhoid fever. Host genetic factors are also important in determining the susceptibility or resistance to infection with typhoid fever. Possession of nucleotide polymorphism in specific HLA alleles and the TNF-alpha promoter are associated with a lower risk (House et al., 2001). HLA-DRB provides protection against complicated typhoid fever. The wide variation of incidence of typhoid fever in many countries with similar standards of public health and hygiene could be due to differences in genetic makeup of individuals in these countries (Breiman et al., 2012). Indian sub-continent is considered as a hot spot of disease activity therefore travellers returning from it are likely to carry with them resistant strains of *Salmonella typhi.*
2.5 Immune response to *Salmonella*

A number of mechanisms of innate and acquired immunity are available that respond to different stages of *S. typhi* infection and those mechanisms differ in their importance during distinct infection stages. Phagocytes such as macrophages and neutrophilic granulocytes play important roles in controlling *Salmonella* infection (Berkley *et al.*, 2005). Macrophage phagocytose *S. typhi* and this process is enhanced by receptor mediated uptake after opsonization of *Salmonella* with antibodies or complement. Cytokines such as IFN-γ and TNF-α are involved in activation of macrophage to kill *S. typhi*. These cytokines are involved in induction of bacteriocidal mechanism in macrophages. Such mechanisms include not only production of nitrogen intermediates and reactive oxygen intermediates but also better handling of bacteria containing phagosomes, and the bacteria is rendered accessible to lytic effector molecules from the lysosomes (Jesudason *et al.*, 2002).

Macrophage express a functional Nramp 1 molecule, a decisive factor for the potential of macrophage to kill *S. typhi*. Massive inflammatory responses are induced by wall components of *Salmonella* such as LPS and certain lipoproteins during the initial stages of infection. As a result, inflammatory cytokines e.g. TNF-α, IL-1, IL-6, IL-12 and IL-18 and a variety of chemokines that recruit cells of the immune system to those sites are produced (Lin *et al.*, 2010). Natural killer cells produce IFN-γ at early stages. Other cells that produce IFN-γ include macrophages, B cells and specialized cell population such as Natural killer T cells that are able to recognize conserved structural patterns of bacteria (Dutta *et al.*, 2006). Interleukin-18 and IL-12 produced by macrophages and dendritic
cells play an important role in inducing the expression of IFN-\(\gamma\). Interferon-\(\gamma\) then enhances the expressions of IL-12 through a feedback loop (Hook, 2005). Inteleukin-12 induces polarization of T-helper cells towards the TH1 pole. The major source of IFN-\(\gamma\) are most likely \textit{Salmonella}-specific TH1 cells during the secondary response and especially in later stages of infection.

\subsection*{2.5.1 Innate immune response}

A number of cells of innate immunity are involved in rapidly recognizing the microbes that are invading the body and then eliminates them through phagocytosis-mediated killing and the induction of inflammation (Jesudason \textit{et al.}, 2002). Acute inflammation is characterized by rapid influx and activation of immune cells, increased blood flow and release of pro-inflammatory cytokines (Barrett \textit{et al.}, 2002). This results in redness of the affected area, heat, swelling and serves to promote pathogen clearance, prevent the spread of infection and promotes tissue repair.

A chemical and physical barrier is provided by the intestinal epithelial cells which are involved in uptake of nutrients. This layer blocks the entry of pathogens and commensals and also ensures that the commensal-derived antigens are not encountered by the innate immune system (Tanyigna \textit{et al.}, 2011). Non-hematopoietic cells e.g. goblet cells secrete mucus while paneth cells secrete antimicrobial peptides and both mucus and anti-microbial peptides help maintain the integrity of epithelial layer. Lamina propria which is found underlying epithelial layer has a highly organized lymphoid tissue called gut
associated lymphoid tissue (GALT). Aggregated and isolated lymphoid follicles form the GALT. T cells and B cells are the lymphocytes that populate the GALT and also phagocytic cells including dendritic cells, neutrophils and macrophages (Gizachew and Andalem, 2011). These cells induce inflammatory responses to antigen and bacteria that breach the GIT barrier, scavenge dead cells and foreign debris and protect the mucosa against harmful pathogens.

Immune surveillance of the intestinal lumen is carried out by aggregated lymphoid follicles called peyer’s patches. Peyer’s patches facilitate the induction of defense against pathogens or immune tolerance as a result of the complex interplay between immune cells located in the lymphoid follicles and the follicles-associated epithelium (FAE) (Crump et al., 2004). M-cells present in follicle associated epithelium transport luminal antigens and bacteria to the basolateral side by transcytosis. At their basal surface, cell membrane of M-cells is extensively folded around underlying lymphocytes and antigen presenting cells that activate or inhibit the immune response leading to either tolerance or systemic immune response (Lunguya et al., 2012). The dendritic cells (DCs) sample antigens from commensal and pathogenic GIT bacteria. Antigen presenting cells in peyer’s patches activate T cells and memory cells. They then travel to the mesentric lymph nodes (MLN) where they amplify the immune response (Mussa, 2011).
2.5.2 Acquired immune response

The innate immune mechanism fails to achieve sterile elimination of bacteria. Once the bacteria penetrates into deeper tissues, it expresses diverse virulence factors that counteract the bactericidal host mechanisms (WHO, 2001). For effective eradication of bacteria, specific lymphocytes are generated which also provide increased protection against subsequent encounter with the pathogen (Bhutta and Mansurali, 1999).

2.5.2.1 Role of T cells during the immune response

Large amount of IFN-γ are produced by Salmonella specific memory T-cells, a characteristic of TH1 response, providing increased protection against secondary infection (Shrivastava et al., 2011). Clearance of bacteria in addition to IFN-γ is preceded by activation markers on both CD4 and CD8 T cells (Gilman et al., 2011).

2.5.2.2 Role of antibodies in immunity against Salmonella

Protein antigens and lipopolysaccharides which are non-protein antigens induce production of antibodies which participate in protection against Salmonella (Pang et al., 2010). Antibodies such as IgM and IgA in the intestinal lumen can block penetration of Salmonella infection. Antibodies can speed up bacterial engulfment via FC receptor (FCR) mediated phagocytosis in peyer’s patches, mesentric lymph nodes, spleen and liver. Bactericidal activities of macrophages are activated by fragment crystallizable receptor-mediated uptake thereby increasing their bactericidal activities. The classical pathway of complement is activated by antibodies (Olsen et al., 2004). Although
Salmonella seems to be insensitive to complement lysis, complement-receptors facilitate uptake by phagocytosis and clearing of bacteria from serum through phagocytosis. Surface structures on Salmonella are blocked by antibodies and toxic components such as LPS are neutralized.

**2.6 Salmonella survival mechanisms**

Salmonella has defense mechanism that enables it to sense host defense responses (Ley et al., 2010). The bacteria then respond by upregulating its corresponding virulence mechanisms for example the PhoP-PhoQ, two component signal transduction system which Salmonella shares with many other bacteria and can sense the presence of cationic antimicrobial peptides as well as divalent cations and low pH (Crump et al., 2008). When PhoQ is activated, a transcription program is induced. This results in increased resistance against antimicrobial peptides that are achieved by modification of lipid A cation by acylation (Pag P) or addition of aminoarabinose moiety (PMR) system (Parry et al., 2002).

**2.7 Prevention of typhoid fever**

**2.7.1 Safe water and food**

Eating food or drinking water contaminated with *S. typhi* are the major modes of transmission of typhoid fever. Transmission can hence be prevented by having access to safe water and proper food handling practices. Public awareness should be raised on modes of transmission through health education and this may induce behavior change
Water may be disinfected by bringing it to a rolling boil. Chemical treatments such as the use of chlorine dioxide are very effective at inactivating parasites. Portable, battery operated devices utilizing UV light can also be used to purify water (Pang et al., 2010). Wells in rural areas must be screened for pathogens and treated if necessary. Basic hygiene measures must be reinforced during food processing and handling. Hands must be washed with soap before preparing or eating food and raw food must be avoided especially during epidemics. Travellers should be advised to choose recently prepared food that is thoroughly cooked and served hot.

2.7.2 Proper sanitation

Typhoid fever is transmitted through faeco-oral route, therefore, proper sanitation is paramount to preventing its spread (Parry et al., 2002). Due to this, facilities for disposing human waste must be provided. Sewage must be collected and treated especially during the rainy season. In endemic areas, the use of human waste as fertilizer must be avoided (Mweu and English, 2008).

2.7.3 Health education

Awareness must be raised through public education. Preventive measures must be emphasized. Those measures include personal hygiene, setting up and maintenance of facilities for disposing waste, isolation measures for typhoid fever patients and the importance of disinfection (Mtose et al., 2010). Therefore, community must be involved in order to instill behavior change with regard to hygiene.
2.7.4 Typhoid fever vaccines

A number of vaccines are available that offer protection against typhoid fever. Heat-phenol inactivated whole-cell vaccine is administered subcutaneously and it has a protective efficiency rate of 51-67% though its reactogenicity is relatively high (9-34%) (Parry et al., 2009). This vaccine has been associated with severe reactions such as hypertension, chest pain, fever and shock. For this reason, its use has been discontinued in the USA. Acetone-inactivated parenteral vaccine was used by US armed forces. Another vaccine called Vi polysaccharide vaccine comprise of purified Vi capsular polysaccharide from the Ty21 S. typhi strain (Gilman et al., 2011). It can either be administered subcutaneously or intramuscularly thereby eliciting a T-cell independent IgG response. However, it does not offer a patent protection, but it is safe, efficient and it provides a programmatic impact. This vaccine can be co-administered with other vaccines e.g. yellow fever, viral hepatitis A for international travellers or with vaccines used in childhood immunization (WHO, 2006).

The live oral Ty21a vaccine is an attenuated Ty2 strain with the Vi gene chemically mutated (House et al., 2001). There are two formulations of this vaccine: enteric coated capsule for travellers to endemic areas and a liquid suspension for young children in developing countries. This vaccine requires multiple doses taken every day and this could be a reason why it is used by travellers to endemic areas and not for controlling endemic cases in developing countries (Table 2.1). It can be co-administered with vaccines against
polio, cholera, yellow fever etc (Ley et al., 2010). Development of newer vaccines that could confer high levels of protective immunity in all age groups is paramount (WHO, 2006). A prototype Vi conjugate vaccine, using a recombinant exotoxin A of *Pseudomonas aeruginosa* (Vi-rEPA) as a carrier protein to increase immunogenicity has been shown to confer 89% efficiency over 46 months of follow up (Cunha, 2004).
Table 2.1 Comparison of the parental Vi polysaccharides vaccine and the live oral Ty21a vaccine (Adopted from Chart et al., 2000)

<table>
<thead>
<tr>
<th></th>
<th>Vi polysaccharides vaccine</th>
<th>Ty21a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Licensed group</td>
<td>Aged 22 yrs.</td>
<td>Aged 25 yrs.</td>
</tr>
<tr>
<td>Schedule</td>
<td>Administered as one dose to achieve immunity after 7 days</td>
<td>Administered in three doses everyday. Seven days after the third dose is when immunity is achieved.</td>
</tr>
<tr>
<td>Revaccination</td>
<td>Every 3 years</td>
<td>After primary immunization, 1 booster dose 3-7 years for people in endemic area. One booster dose for travelers after 1-7 years of primary immunization.</td>
</tr>
<tr>
<td>Contraindication</td>
<td>Reports of severe systemic or local reactions to any component of the vaccine. Not recommended for patients having fever.</td>
<td>Not recommended for patient having gastrointestinal illness or patients who are immunocompromised. Antibacterial and antimalarial drugs may destroy live bacterial vaccine. Little is known if it may have negative effects on the foetus when given to pregnant women.</td>
</tr>
<tr>
<td>Efficacy</td>
<td>64-72%, last 3 years</td>
<td>53-78%, last 3-7 years</td>
</tr>
<tr>
<td>Adverse reaction</td>
<td>Minimal (local-side effect)</td>
<td>Well tolerated</td>
</tr>
<tr>
<td>Storage</td>
<td>Recommended at 2-8°C, 6 months at 37°C, 2 years at 22°C</td>
<td>Recommended at 2-8°C, 14 days at 25°C</td>
</tr>
</tbody>
</table>
2.8 Diagnosis of typhoid fever

Various methods including Widal test, stool and blood culture are used to diagnose *S. typhi* infections (Table 2.2).

**Table 2.2: Laboratory diagnostic methods (Adopted from Parry et al., 2002)**

<table>
<thead>
<tr>
<th>Diagnostic test</th>
<th>Sensitivity range (%)</th>
<th>Specificity range (%)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbiological tests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood culture</td>
<td>40-80</td>
<td>NA</td>
<td>Is mostly regarded as the gold standard. It has low sensitivity in endemic areas where the rate of antibiotic use is high. It is difficult to estimate its true specificity.</td>
</tr>
<tr>
<td>Bone marrow cultures</td>
<td>55-67</td>
<td>30</td>
<td>Registers a higher sensitivity but has a limited clinical value due to its invasiveness, especially in ambulatory management</td>
</tr>
<tr>
<td>Urine culture</td>
<td>0-58</td>
<td>NA</td>
<td>Sensitivity varies</td>
</tr>
<tr>
<td>Stool culture</td>
<td>30</td>
<td>NA</td>
<td>In developing countries sensitivity is low therefore, it is not used routinely for follow up</td>
</tr>
<tr>
<td>Molecular diagnostics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymerase chain reaction</td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
<td>Promising, but has lower specificity. Sensitivity is similar to blood cultures according to earlier reports</td>
</tr>
<tr>
<td>Diagnostic test</td>
<td>Sensitivity range (%)</td>
<td>Specificity range (%)</td>
<td>Remarks</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------------------</td>
<td>-----------------------</td>
<td>--------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Nested polymerase chain reaction</td>
<td>100</td>
<td>100</td>
<td>Promising and may replace blood culture as the new “gold standard”</td>
</tr>
<tr>
<td><strong>Serological diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Widal test (tube dilution and slide agglutination)</td>
<td>47-77</td>
<td>50-92</td>
<td>Classic and inexpensive. It does well when screening large volumes though mixed results have been reported in endemic areas. There is need for standardization and quality assurance of reagents</td>
</tr>
<tr>
<td>Typhidot</td>
<td>66-88</td>
<td>75-91</td>
<td>Lower sensitivity than Typhidot-M</td>
</tr>
<tr>
<td>Typhidot-M</td>
<td>73-95</td>
<td>68-95</td>
<td>Sensitivity and specificity are higher than classic Typhidot in some series. Performance is better in hospital than in community setting.</td>
</tr>
<tr>
<td>Tubex</td>
<td>65-88</td>
<td>63-89</td>
<td>Initial results showed that it is promising but its effectiveness in large trials in community settings need to be evaluated</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine antigen detection</td>
<td>65-95</td>
<td>NA</td>
<td>Preliminary data only</td>
</tr>
</tbody>
</table>
2.8.1 Widal agglutination test

Widal agglutination test is a serologic reaction between an antigen and an antibody that results in agglutination of cell suspension (William et al., 2001). A killed suspension of \textit{S. typhi} is used to detect typhoid fever in serum of patients presenting with febrile illness. \textit{Salmonella typhi} bacteria is treated to retain only ‘O’ (somatic) or ‘H’ (flagella) antigens and these antigens are used to detect specific antibodies in serum of patients suspected to be suffering from typhoid fever (Youssef et al., 2010). Agglutination is a positive test and suggests that the patients serum has the antibodies to ‘O’ and ‘H’ antigens hence shows infections with typhoid fever. Lack of agglutination is a negative test that implies that the patient is not suffering from typhoid fever. The initial serologic response in acute typhoid fever is represented by appearance of IgM somatic O antibody, while the IgG flagella H antibody develops more slowly but persists for longer (Parry et al., 2001).

The agglutination techniques that are commonly used include the slide test and the tube test. Widal slide test is a rapid diagnostic test and is used as a screening procedure. The tube test is a macroscopic test and serves to confirm the result of the slide test (Bhutta, 2006). The tube test clarifies erratic or equivocal agglutination reaction of more rapid slide test (Parry et al., 1999). A mixture of antigen and antibody is incubated at 37\(^\circ\)C for 24 hours in a water bath. Agglutination is visualized in form of pellets, clumped together at the bottom of the test-tube. Results are scored from 0 to 4\(^+\) positive agglutination.
Since agglutination involves formation of antigen antibody complexes, cross reaction occurs when antibody produced by non-typhoid antigens reacts with typhoid-specific antigens. Diseases that are caused by non-Salmonella organisms for example Malaria, dengue fever, military tuberculosis, endocarditis, chronic liver disease, brucellosis etcetera exhibit this cross reactivity in typhoid endemic region and those cross reactions may produce false positive results leading to misdiagnosis of typhoid fever (Olopoenia and King, 2004). Lack of standardization of antigens also compromises this technique (Gilman et al., 2011).

2.8.2 Culture methods

2.8.2.1 Stool culture method

Stool culture is a laboratory test used to determine the etiology of infective bacterial diarrhea. It refers to the inoculation of selective agar plates with feaces and incubation for 1-2 days to detect the presence of pathogenic bacteria within the bowel flora of enterobacteriaceae (Crump et al., 2004). Enterobacteriaceae is a large family of Gram negative bacteria that includes many harmless symbionts and many of the more familiar pathogens such as Salmonella, Yersinia pestis, Escheria coli, Klebsiella and Shigella (Renuka et al., 2005). Other disease causing bacteria in the family include Enterobacter, Proteus Serratia and Citrobacter.

Isolation of enteric bacteria involves separating the pathogens from non-pathogens using both selective and differential media such as macCkonkey agar, Deoxycholate Citrate
Agar (DCA) or selenite F. Most non-pathogens have an enzyme which hydrolysis the sugar, breaking it into acids that change the pH of the media (Lin et al., 2010). Also incorporated in the media is a colourless indicator, neutral red which turns red in colour when it comes into contact with acids, therefore, all the lactose fermenting colonies appear reddish in colour. Non-lactose fermenters for example Salmonella, Shigella and Proteus appear colourless. Diagnostic identification of Salmonella typhi can be attained by growth on MacConkey agar and Eosin Methylene Blue (EMB) agar and the bacteria is strictly non-lactose fermenting. It also produces no gas when grown in Tripple Sugar Iron (TSI) agar which is used to differentiate it from other enterobacterioceae (Pang et al., 2010).

2.8.2.2 Blood culture method

Blood culture is a microbiological culture of blood (Gilman et al., 2011). It is employed to detect infections that are spreading through the blood stream (such as bacteremia and septicemia amongst others). Blood cultures are inoculated in suitable differential and selective media and incubated at 37°C for 3 days (Altman and Bland, 2011). Results are reported as positive (cultures with bacteria present thus indicating the patient is bacteremic) or negative blood culture. If the culture is positive, biochemical tests are carried out to confirm the presence of bacteria which are as follows; Gram reaction, lactose fermentation, motility test, citrate utilization test and glucose fermentation test (Chessbrough, 2000).
In mild typhoid fever, the number of *S. typhi* bacteria that grows in culture may be as low as one colony-forming unit per milliliter of blood (Lin et al., 2010). The blood of adults usually registers lower levels of bacteria than that of children, possibly because of developed immune system in adults that fights off the bacteria. Recovery of the organism from the blood cultures depends on a number of factors that may include the volume of blood cultured, the ratio of the volume of blood to the volume of culture broth in which it is inoculated (the ratio should be at least 1:8) and inclusion of anticomplementary substances in the medium (Parry et al., 2002).

### 2.8.2.3 Blood culture to PCR as gold standard for diagnosis of typhoid fever

The gold standard for typhoid fever diagnosis is currently the blood culture and when evaluating diagnostic tests for typhoid fever, blood culture is universally used as the reference standard (Gizachew, 2011). Where culture is conducted, bone marrow culture is not common due to invasiveness and technical difficulty of the procedure (Gilman et al., 2011). The potential of using molecular detection of *S. typhi* from blood has been demonstrated by a number of studies. However, the true utility of PCR as a typhoid fever diagnostic tool is still being debated. A study carried out by Abera et al. (2010) found out that 22 (32%) PCR negative samples were blood culture positive. Duthie and French (1990) also demonstrated that blood samples that were culture positive could be PCR negative, where 58% of culture-positive samples were negative using detection by real-time PCR. In the context of diagnostic evaluation, polymerase chain reaction is useful as a complementary form of diagnosis and provides the advantage of being able to detect non-viable cells for example in patients who have recently commenced antibiotics. For
routine diagnostic use, PCR is not currently applicable. The major limiting factor in routine use of PCR test in busy clinical laboratories are lack of technical expertise, methodologies are time consuming and expensive equipment are required such as PCR machines. Until large population based studies are conducted to establish the true diagnostic value of this test, blood culture remains the gold standard in typhoid fever diagnosis (Hook, 2005). The most effective diagnostic method in suspected typhoid fever is the isolation of causative organism and blood has been the main sample for culture for S. typhi since 1900 (Farooqui et al., 1991).

A patient with Salmonella bacteremia has small number of Salmonella bacteria circulating in the blood. One study by Pang et al. (2010) in 15 patients with typhoid fever found 0.5-22 bacteria per milliliter of blood, and another from 81 patients with typhoid fever showed a median of 0.3 bacteria per millilitre of blood (Duthie and French, 1990). Since the ratio of bacterial to human DNA is very low, PCR template in clinical preparation is dominated by mammalian DNA resulting in reduced sensitivity due to false negative results. In practice, samples with low bacterial numbers cause problems for PCR- based pathogen detection in blood because of large excess of human DNA.

2.8.2.4 Colony characteristics in culture media

Isolation of S. typhi involves culturing blood or stool samples suspected of having S. typhi on media suitable for its growth. Some media are selective while others are differential. The bacteria possess certain biochemical characteristics that affect the media,
and include lactose fermentation and hydrogen sulphide production among others (Onyokewere, 2007). Identification of the bacteria is through observation of appearance of the colony or the surrounding media. Such media include blood agar on which bacteria are distinguished by the type of haemolysis produced. *Salmonella typhi* on blood agar produce non hemolytic smooth white colonies (Altman and Bland, 2011).

MacConkey agar is a media that is both selective and differential. It contains bile salts and crystal violet which selects for gram negative enterics and differentiate lactose fermenters from non-lactose fermenters. *Salmonellae* on this media produce lactose non-fermenting colonies (Bhanu *et al.*, 2011). On *Salmonella-Shigella* agar, desoxycholate agar and bismuth sulfite agar, *Salmonellae* produce lactose non-fermenting colonies with black centres whereas in xylose-lysine-desoxycholate agar, *Salmonellae* produce transparent red colonies with black centres. *Salmonella typhi* produces no gas when grown in triple sugar iron agar which is used to differentiate it from other enterobacterioceae (Bhutta, 2006).

### 2.9 Treatment of typhoid fever

Where resistance is uncommon, the treatment of choice is fluoroquinolones such as ciprofloxacin. Otherwise, a third generation cephalosporin such as ceftriaxone or cefotaximine is the first line drug (Table 2.3).
Table 2.3. Treatment of uncomplicated typhoid fever (Adopted from Bhatta, 2006)

<table>
<thead>
<tr>
<th>Optimal therapy</th>
<th>Antibiotic</th>
<th>Daily dose mg/kg</th>
<th>Days</th>
<th>Alternative effective drugs</th>
<th>Daily dose mg/kg</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>Fluoroquinolone e.g. ofloxacin or ciprofloxacin</td>
<td>15</td>
<td>5-7</td>
<td>Chloramphenicol</td>
<td>50-75</td>
<td>14-21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15-20</td>
<td>7-14</td>
<td>Amoxicillin</td>
<td>75-100</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TMP-SMX</td>
<td>8-40</td>
<td>14</td>
</tr>
<tr>
<td>Fully sensitive</td>
<td></td>
<td></td>
<td></td>
<td>Azithromycin</td>
<td>8-10</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cefixime</td>
<td>15-20</td>
<td>7-14</td>
</tr>
<tr>
<td>Multidrug resistant</td>
<td>Fluoroquinolone or cefixime</td>
<td>15</td>
<td>5-7</td>
<td>Cefixime</td>
<td>8-10</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15-20</td>
<td>7-14</td>
<td></td>
<td>15-20</td>
<td>7-14</td>
</tr>
<tr>
<td>Quinolone resistance</td>
<td>Azithromycin or ceftriaxone</td>
<td>8-10</td>
<td>7</td>
<td></td>
<td>8-10</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75</td>
<td>10-14</td>
<td></td>
<td>15-20</td>
<td>7-14</td>
</tr>
</tbody>
</table>

2.9.1 General management

Typhoid fever requires appropriate supportive measures in its management. Such measures may include intravenous or oral hydration, blood transfusion, use of antipyretics and suitable nutrition (House et al., 2001). Reliable care and oral antibiotics can be used to manage more than 90% of patients. Such cases should be monitored closely for failure to respond to therapy or any complication. However, patients vomiting
persistently and those with severe diarrhea or distended abdomen may require parenteral antibiotic therapy and hospitalization (Shrivastava et al., 2011).

2.9.2 Treatment for complicated typhoid fever

In developing countries, the first line antibiotics to be selected should meet certain criteria that is they should be readily available, efficient and cheap (Onyokewere, 2007).

In adults, treatment of typhoid fever is optimal when fluoroquinolones are used (Table 2.4).

**Table 2.4 Treatment of severe typhoid fever (Adopted from Bhutta, 2006)**

<table>
<thead>
<tr>
<th>Susceptible</th>
<th>Antibiotic</th>
<th>Daily dose mg/kg</th>
<th>Days</th>
<th>Antibiotic</th>
<th>Daily dose mg/kg</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully sensitive</td>
<td>Fluoroquinolone e.g. ofloxacin</td>
<td>15</td>
<td>10-14</td>
<td>Chloramphenicol Amoxicillin TMP-SMX</td>
<td>100 100</td>
<td>8-40 14</td>
</tr>
<tr>
<td>Multidrug resistant</td>
<td>Fluoroquinolone</td>
<td>15</td>
<td>10-14</td>
<td>Ceftriaxone or cefotaxime</td>
<td>60 80</td>
<td>10-14</td>
</tr>
<tr>
<td>Quinolone resistant</td>
<td>Ceftriaxone or cefotaxime</td>
<td>60 80</td>
<td>10-14</td>
<td>Fluoroquinolone</td>
<td>20</td>
<td>7.14</td>
</tr>
</tbody>
</table>
Flouroquinolones are well tolerated, relatively effective and cheaper than the first line drugs that were formerly used like amoxicillin, chloramphenical and trimethoprim-sufamethoxazole (Kariuki, 2008). They excellently penetrate tissues, kill *S. typhi* in monocytes/macrophages during its stationary intracellular stage and in gall bladder they achieve higher active drug levels than other drugs. A rapid therapeutic response is produced ie symptoms and fever are cleared in 3-5 days, and the rates of post- treatment carriage are very low (Cunha, 2004).

In children, ciprofloxacin is not recommended for use for concerns that it may cause articular damage, though in endemic areas it has been used to treat typhoid fever and no case of complication has been reported (Francis *et al.*, 2008). Ceftriaxone/cefotaxime or ampicillin (where intravenous RX is required) are suitable alternatives for children (Berkley *et al.*, 2005).

Ceftriaxone is recommended for use in pregnant women (Nsutebu *et al.*, 2002). Ideally, the alternative therapy should have as many advantages in common with the treatment of choice. Ceftriaxone is administered intravenously and can be used as short term treatment. It is comparable to ciprofloxacin in clearance of the organism from the gut and as a short term treatment (Charles *et al.*, 2012). Co-trimoxazole (bactrim) and ampicillin/amoxicillin have a disadvantage in that a long period of time is required during treatment (at least 14 days) and patients may not comply. In addition, these drugs are not as effective in clearing gut organism which is a significant disadvantage in an outbreak situation (Francis *et al.*, 2008).
2.10 Resistance of S. typhi to antibiotics

Even after new antibacterial drugs have emerged, typhoid fever still remains a major health problem. The bacteria S. typhi has not only become resistant to antibiotics like cotrimoxazole, ampicillin and chloramphenical, but also to drugs that were initially effective such as ciprofloxacin (Bhutta, 2006). The management and treatment of typhoid fever has been complicated by the emergence of multidrug resistant strains to antibiotics that were commonly used thereby posing great challenges to the management of the disease. When such strains infect children, they cause typhoid fever that is more severe, take long to be cured and results in high mortality rate (WHO, 2006). However, there are no pathognomonic features to distinguish such infection with fully sensitive S. typhi at presentation. Since the emergence of multidrug resistance typhoid in the 1970s and 1980s, the broad spectrum fluoroquinolones especially ciprofloxacin and ofloxacin, have been the treatment of choice for suspected typhoid fever especially in South Asia where the disease is endemic (Cheesbrough, 2000). However, this indiscriminate use has led to reports of S. typhi strains that are resistant to fluoroquinolone. The use of appropriate antibiotic is of paramount importance in the successful treatment of enteric fever with minimal complications (Onyokewere, 2007).

The therapeutic options for treatment include fluoroquinolones such as ciprofloxacin or ofloxacin; and expanded spectrum cephalosporins such as ceftriaxone or cefotaxime (Nsutebu et al., 2002). In both developing and developed countries, treatment of typhoid fever is by ciprofloxacin. Indiscriminate usage of ciprofloxacin should be avoided if its efficacy is to be maintained (Beyene et al., 2008). Cromosomaly encoded resistant to
ciproxacin has been observed in a small number of strains isolated in UK from patients returning from Indian subcontinent and patients with this strain did not respond to therapy by this antibiotic. It is unfortunate that MDR \textit{S. typhi} to ciproxacin has emerged (Crump \textit{et al.}, 2004).

\textbf{2.10.1 Possible misdiagnosis- induce drug resistance}

Based on the above literature, it is clear that misdiagnosis leads to incorrect treatment, delayed treatment or no treatment at all. Patient’s condition can be made much worse and this can lead to tens of thousands of deaths. Bacteria mutate into a tougher strain that can no longer be killed by drugs (Parry \textit{et al.}, 2002). Indiscriminate use of antibiotics may cause fungal overgrowth because their natural competitors commonly called normal flora have been killed. Administration of wrong drugs exposes a patient to multiple drugs that can interact negatively (Okonko \textit{et al.}, 2010). Antibiotics and antiviral medications compromise body physiology in predictable ways. High number of allergic and adverse reactions to medications arise directly as a result of toxic shock from medicines. The overuse and misuse of drugs is making bacteria mutate at an alarming rate. Misdiagnosis is increasing drug resistance and allowing other illnesses to go untreated. It is also increasing poverty because of loss of productivity from prolonged illness and money spent on incorrect treatment (Renuka \textit{et al.}, 2005). The success of treatment of \textit{S. typhi} is therefore dependent on proper diagnosis of the disease so that correct and proper medication can be administered. Gaps exist which need to be addressed especially by use of the most appropriate diagnostic tool hence the importance to evaluate the performance of Widal test and stool culture.
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

The study was carried out at Chuka General Hospital, Maara District, Tharaka Nithi County. Chuka General Hospital is located within Latitude 0° 19’ 59” S and Longitude 37° 38’ 45” E and is at altitude 1445 m above sea level. It lies along Meru – Nairobi Highway, 50 and 40 kilometers from Meru and Embu towns respectively. It’s a referral hospital and attends to patients of Tharaka – Nithi County and neighbouring Meru and Embu Counties. Chuka General Hospital is rural based and a majority of people are not accessible to piped treated water. The major sources of water for this population are rivers and boreholes. These waters are prone to contamination from runoff water that may be harboring pathogens posing a health threat to the people.

3.2 Study population

The subjects of the study were patients clinically diagnosed as typhoid fever who presented with fever of 38°C and above that had lasted for at least three days, had bowel disturbances, headache, malaise, anorexia et cetera who were seeking medical attention at the study site. Controls for the study were patients with other laboratory-confirmed illness such as malaria, amoebic dysentery, diabetes, AIDS, cholera, parasitic worm infestation, infectious hepatitis etc. Individuals selected for the study were identified by special code numbers.
3.3 Research design

This study utilized a descriptive cross sectional design. The study involved obtaining blood and stool samples from patients suspected of having typhoid fever. Blood samples were centrifuged to obtain serum that was subjected to Widal test. Blood was inoculated into suitable blood agar for growth of *S. typhi* bacteria. Stool samples were inoculated into selenite F. broth suitable for cultivation of *S. typhi*.

3.3.1 Inclusion criteria

The inclusion criteria for enrolment in the current study included patients who presented with fever at Chuka General Hospital, aged five years or more and willing to participate.

3.3.2 Exclusion criteria

The exclusion criteria for the study subjects included patients who did not present with fever at Chuka General Hospital, aged less than 5 years and declined to participate in the study.

3.4 Sample size calculation

Sample size determination was calculated according to the method described by Daniel (1999).

\[ N = \frac{z^2 \cdot p(1-p)}{d^2} \]
Where \( n \) = sample size, \( Z \) = \( Z \) statistic for level of significance (1.96), \( P \) = expected prevalence or proportion and \( d \) = precision (\( d = \alpha (0.05) \)). Prevalence for typhoid fever in Tharaka Nithi County has been indicated to be 9\% of all patients attending hospitals (Kariuki et al., 2004).

\[
N = 1.96^2 \times 0.09(1 - 0.09)/0.05^2 = 3.8416 \times 0.09 \times 0.91/0.0025
\]

\[
= 0.31462704/0.0025 = 125.850
\]

The calculated sample size of 125.850 was rounded to 126 samples. One hundred and twenty six samples of serum for Widal test, blood, and stool for culture were analyzed.

### 3.5 Specimen collection

Specimens were collected from patients \( \geq 5 \) yrs old with symptoms of typhoid fever who presented themselves at the study site. They were asked to give verbal consent and answer a brief questionnaire about clinical signs and symptoms, antimicrobial treatment, history of typhoid fever and vaccination.

#### 3.5.1 Blood collection

Samples of 5 ml of blood were obtained from the study population upon routine venipuncture for blood culture and Widal test. Blood samples were allowed to clot and the clot was removed by centrifuging at 1,000-2,000 xg for 10 minutes in a refrigerated
centrifuge and the supernatant obtained was serum. Serum samples were stored at -20°C until analyzed.

3.5.2 Stool collection

Freshly passed feces were collected in a sterile wide mouthed container. Each sample container was labelled with the patient code number, date and time.

3.6 Laboratory analysis

3.6.1 Widal test

Two types of Widal tests are available; standard tube test and slide test.

3.6.1.1 Standard tube test method

This was done according to the procedure described by Crump et al. (2004). Two sets of test tubes were labelled 1-8 for O and H antibody detection. Tube 1 of the two sets was pipetted 1.9 ml of isotonic saline. The remaining tubes (2-8) were put 1.0 millilitre of isotonic saline. To the tube number one in each row, 0.1ml of the serum sample to be tested was added and mixed well. One millilitre of the diluted serum was transferred from the tube number one to the tube number two and this serial dilution was continued till tube number seven of each set. Tube number eight in the two sets served as a saline control. The dilution of the serum sample achieved in each set was as follows;

<table>
<thead>
<tr>
<th>Tube no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8 (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilutions</td>
<td>1:20</td>
<td>1:40</td>
<td>1:80</td>
<td>1:160</td>
<td>1:320</td>
<td>1:640</td>
<td>1:1280</td>
<td></td>
</tr>
</tbody>
</table>
To all tubes (1-8) of each set, one drop of the respective Widal test antigen suspension (O, H) from the reagent vials was added and mixed well. The tubes were covered and incubated at 37°C overnight (approximately 18hrs). The titre of the patient’s serum using Widal test antigen suspension is the highest dilution of the serum sample that gives a visible agglutination. The sample which showed a titre of 1:160 or more was considered as clinically significant.

3.6.1.2 Slide test procedure

Slide test was done according to the procedure described by Olopoenia (2004). One drop of positive control was placed on one reaction circle on the slide. One drop of isotonic saline was pipetted on the next reaction circle (negative control) before addition of one drop of patient’s serum to be tested into the remaining two reaction circles. One drop of Widal test antigen suspension ‘H’ and ‘O’ was added to the first two reaction circles (positive control and negative control). One drop each of ‘O’ and ‘H’ antigens was added to the reaction circle with the patient’s serum. Contents of each circle were mixed uniformly over the entire circle with separate mixing sticks. The slide was rocked gently back and forth and agglutination was observed macroscopically within one minute. Agglutination was a positive test result and if the positive reaction was observed with the test sample, it indicated presence of clinically significant levels of the corresponding antibody in the patient’s serum.

3.6.2 Stool culture

Feces were inoculated onto Deoxycholate Citrate agar (DCA) and highly selective media such as Wilson and Blair agar. The plates were incubated at 37°C overnight. Subculture
was made from enrichment broth onto Tripple Sugar Iron agar (TSI) for subsequent overnight incubation. Isolation of *S. typhi* in stool culture indicated an infection. If there was no growth, the culture was considered negative.

### 3.6.3 Blood culture

Collected samples were cultured in suitable brain heart infusion broth culture media and blood culture bottles were incubated at 37°C. Cultures were observed everyday for turbidity for at most three days. If there was growth, subculture was done the next day on blood agar. After obtaining good growth, it was plated on selective media, Wilson and blair bismuth sulphite agar. Isolates were Gram-stained and were then identified by standard biochemical methods described by Francis *et al.* (2008). Serotyping was performed using agglutination with Salmonella O, H and Vi antisera. If there was no growth after 3 days, the culture was considered negative.

### 3.7 Data management and statistical analysis

Raw data were entered into Microsoft Excel. Statistical analysis were conducted using IBM SPSS Statistics 21.0 (IBM Corp., Armonk, NY). Analysis involved computation of descriptive statistics; frequencies, means and standard deviations. Statistical analysis outputs were presented in form of tables, charts and graphs. Comparisons of categorical data were made using the Chi square test while Fisher’s exact test was for small values that were less than five. The confidence intervals for sensitivity and specificity were computed using the Wilson's score method. A P value ≤ 0.05 was considered statistically significant.
3.8 Ethical approval

Permit to conduct the study was obtained from the National Commission for Science and Technology (NCST) and Maara District Hospital Ethical Committee. Approval was also done by the Kenyatta University Ethical Review Committee and the School of Graduate studies.
CHAPTER FOUR: RESULTS

4.1 Demographic characteristics of the study participants

A total of 126 patients presenting with febricity at Chuka General Hospital for a period of eighteen months were enrolled in the current study. Majority of the study participants were female (89; 70.6%) while a minority were male (37; 29.4%). The age of the sampled patients ranged from 5 to 82 years. Their median (interquartile range) age was 12.0 (9.0 to 30.3) years. Analyses of the highest level of education attained by the study participants revealed that 53.2%, 15.1% and 31.7% had achieved Primary, Secondary and post-Secondary educational qualifications respectively. The main sources of water for the respondent’s households were borehole (23.0%), piped (39.7%) and river (37.3%) (Table 4.1).
Table 4.1: Demographic characteristics of the sampled patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number (n=126)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>89</td>
<td>70.6%</td>
</tr>
<tr>
<td>Male</td>
<td>37</td>
<td>29.4%</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 8</td>
<td>32</td>
<td>25.4%</td>
</tr>
<tr>
<td>9-35</td>
<td>68</td>
<td>54.0%</td>
</tr>
<tr>
<td>&gt; 35</td>
<td>26</td>
<td>20.6%</td>
</tr>
<tr>
<td><strong>Level of education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>67</td>
<td>53.2%</td>
</tr>
<tr>
<td>Secondary</td>
<td>19</td>
<td>15.1%</td>
</tr>
<tr>
<td>Post-secondary</td>
<td>40</td>
<td>31.7%</td>
</tr>
<tr>
<td><strong>Source of water</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piped</td>
<td>50</td>
<td>39.7%</td>
</tr>
<tr>
<td>Borehole</td>
<td>29</td>
<td>23.0%</td>
</tr>
<tr>
<td>River</td>
<td>47</td>
<td>37.3%</td>
</tr>
<tr>
<td><strong>Disposal of fecal matter</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pit latrine</td>
<td>82</td>
<td>65.1%</td>
</tr>
<tr>
<td>Flush toilets</td>
<td>29</td>
<td>23.0%</td>
</tr>
<tr>
<td>Bush</td>
<td>15</td>
<td>11.9%</td>
</tr>
<tr>
<td><strong>Awareness on mode of transmission</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aware</td>
<td>90</td>
<td>71.4%</td>
</tr>
<tr>
<td>Not aware</td>
<td>36</td>
<td>28.6%</td>
</tr>
</tbody>
</table>
Most of the patients (54.0%) were in the age bracket of nine to thirty five years. Thirty two (25.4%) patients were aged eight years or less while 26 (20.6%) were more than 35 years old.

4.2: Factors that may affect results of typhoid fever diagnostic tests

Factors that affect results of Widal test and stool culture were considered and included history of vaccination, typhoid fever and antimicrobial treatment. Only one (0.8%) patient reported to have been vaccinated against S. typhi. Additionally, 33 (26.2%) patients reported to have suffered from typhoid fever at least once in the period preceding the current survey. Twenty (15.9%) patients had used antibiotics prior to seeking health services from the hospital (Table 4.2).

Table 4.2: Factors that affect results of Widal test and stool culture

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number (n=126)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>No</td>
<td>125</td>
<td>99.2</td>
</tr>
<tr>
<td>History of typhoid fever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>33</td>
<td>26.2</td>
</tr>
<tr>
<td>No</td>
<td>93</td>
<td>73.8</td>
</tr>
<tr>
<td>Antimicrobial treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>20</td>
<td>15.9</td>
</tr>
<tr>
<td>No</td>
<td>106</td>
<td>84.1</td>
</tr>
</tbody>
</table>
4.3: Diagnostic tests

Out of the 126 suspected cases, seventy (55.6%) were positive for typhoid fever based on Widal test with an antibody titer of 1:160 for O and/or H antigens being taken as cut off values indicative of a recent typhoid infection (positive titers). Further, the prevalence of typhoid fever was 42.9% and 15.1% based on the findings from stool and blood culture respectively (Table 4.3).

Table 4.3: Results from diagnostic tests under study

<table>
<thead>
<tr>
<th>Diagnostic Test</th>
<th>Positive</th>
<th></th>
<th>Negative</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Widal</td>
<td>70</td>
<td>55.6</td>
<td>56</td>
<td>44.4</td>
</tr>
<tr>
<td>Stool Culture</td>
<td>54</td>
<td>42.9</td>
<td>72</td>
<td>57.1</td>
</tr>
<tr>
<td>Blood Culture</td>
<td>19</td>
<td>15.1</td>
<td>107</td>
<td>84.9</td>
</tr>
</tbody>
</table>
4.4: Percentage prevalence of typhoid fever based on age and gender

Out of the 32 patients aged 8 years and below, 17 (53.1%) tested positive for typhoid fever using the Widal agglutination test whereas 15 (46.9%) did not show agglutination with antisera ‘O’ or ‘H’ antibodies. Of the 68 patients aged between 9-35 years that were tested for *S. typhi* antibodies, 39(57.4%) showed positive results but 29 (42.6%) showed negative results. Females showed more positive results (56.6%) to Widal test than males (54.0%). The highest isolation rates of *S. typhi* bacteria using the stool culture was 46.9% among children aged 8 years and below. Out of 50 male samples that were cultured, 17 (34.0%) showed positive culture. Thirty seven (48.7%) out of 76 female stool cultures were positive for *S. typhi* (Table 4.4).

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of patient</th>
<th>Widal test results</th>
<th>Stool culture results</th>
<th>Blood culture results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;=8</td>
<td>32</td>
<td>17(53.1%)</td>
<td>15(46.9%)</td>
</tr>
<tr>
<td></td>
<td>9-35</td>
<td>68</td>
<td>39(57.4%)</td>
<td>29(42.6%)</td>
</tr>
<tr>
<td></td>
<td>&gt;35</td>
<td>26</td>
<td>14(53.8%)</td>
<td>12(46.2%)</td>
</tr>
<tr>
<td>overall</td>
<td>126</td>
<td>70(55.6%)</td>
<td>56(44.4%)</td>
<td>54(42.9%)</td>
</tr>
</tbody>
</table>
### Table 4.4: Analysis of patient Widal test result, stool culture results, and blood culture results

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of patient</th>
<th>Widal test result</th>
<th>Stool culture results</th>
<th>Blood culture results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>50</td>
<td>27(54.0%)</td>
<td>17(34.0%)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>76</td>
<td>43(56.6%)</td>
<td>56(44.4%)</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>126</td>
<td>70(55.6%)</td>
<td>56(44.4%)</td>
</tr>
</tbody>
</table>

### 4.5: Performance of diagnostic tests

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were computed for the various diagnostic tests. In the present study, blood culture-confirmed typhoid fever cases were regarded as the "true positives" and all other febrile patients with blood culture negative for *S. typhi* as the "true negatives" (Table 4.5).

Table 4.5: Analysis of the performance of Widal test and stool culture

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No(n=19)</td>
<td>% (95%CI)</td>
<td>No(n=107)</td>
<td>% (95%CI)</td>
</tr>
<tr>
<td>Widal</td>
<td>14</td>
<td>73.7(51.2-88.2)</td>
<td>51</td>
<td>47.7(38.5-57.0)</td>
</tr>
<tr>
<td>Stool</td>
<td>16</td>
<td>84.2(62.4-94.5)</td>
<td>69</td>
<td>64.5(55.1-72.9)</td>
</tr>
</tbody>
</table>
4.6: Prevalence of typhoid fever and associated factors

Out of the 126 blood specimens cultured, nineteen were found positive for *Salmonella typhi*. Thus, the overall prevalence of typhoid fever in the study population was 15.1% (95% CI: 8.8%-21.3%).

Factors associated with having typhoid fever were evaluated using chi square test or Fishers’ exact test (FET) where the number of participants in a category was small (<5). A higher proportion of females were positive for *Salmonella typhi* as compared to males (respectively, 18.4% versus of 10.0%). Nonetheless, the difference in the proportions was not statistically significant ($X^2 = 1.670$, degrees of freedom (df) = 1, $p = 0.217$). Fewer cases of typhoid fever (10.9%) were reported amongst patients aged twelve years or less when evaluated against their counterparts who were above twelve years of age (19.4%). Again, the association did not attain statistical significance ($X^2 = 1.742$, df = 1, $p = 0.187$). Having been vaccinated against *S. typhi*, having a history of *S. typhi* infection or having taken antimicrobials before seeking treatment at the hospital were not associated with infection with *S. typhi* (Table 4.6).
Table 4.6: Factors associated with infection with *S. typhi*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total</th>
<th>Negative (n=107)</th>
<th>Positive (n=19)</th>
<th>OR* (95%CI)</th>
<th>$X^2$, df= , p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>50</td>
<td>45 (90.0%)</td>
<td>5 (10.0%)</td>
<td>0.492 (0.165-1.465)</td>
<td>1.670, 1, 0.217</td>
</tr>
<tr>
<td>Female</td>
<td>76</td>
<td>62 (81.6%)</td>
<td>14 (18.4%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤12</td>
<td>64</td>
<td>57 (89.1%)</td>
<td>7 (10.9%)</td>
<td>0.512 (0.187-1.400)</td>
<td>1.742, 1, 0.187</td>
</tr>
<tr>
<td>&gt;12</td>
<td>62</td>
<td>50 (80.6%)</td>
<td>12 (19.4%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td><strong>History of typhoid fever</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>33</td>
<td>29 (87.9%)</td>
<td>4 (12.1%)</td>
<td>0.717 (0.220-2.340)</td>
<td>0.306, 1, 0.779</td>
</tr>
<tr>
<td>No</td>
<td>93</td>
<td>78 (83.9%)</td>
<td>15 (16.1%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td><strong>Vaccination</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>1 (100.0%)</td>
<td>0 (0.0%)</td>
<td>1.179 (1.095-1.270)</td>
<td>0.179, 1, 0.999</td>
</tr>
<tr>
<td>No</td>
<td>125</td>
<td>106 (84.8%)</td>
<td>19 (15.2%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td><strong>Prior antimicrobial treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>20</td>
<td>16 (80.0%)</td>
<td>4 (20.0%)</td>
<td>1.517 (0.446-5.159)</td>
<td>0.450, 1, 0.502</td>
</tr>
<tr>
<td>No</td>
<td>106</td>
<td>91 (85.8%)</td>
<td>15 (14.2%)</td>
<td>Reference</td>
<td></td>
</tr>
</tbody>
</table>

Generally, an upward trend in prevalence of typhoid fever was observed. The group of participants aged between nine and 35 years had the highest prevalence followed by those aged more than 35 years. The lowest prevalence of typhoid fever was reported in participants of eight years of age or less. Nevertheless, the variations in prevalence by age groups were not statistically significant ($X^2 = 1.537$, df = 1, p = 0.215 and $X^2 = 0.072$, df = 1, p = 0.999) as shown in Table 4.7.
Table 4.7: Other factors associated with infection with *S. typhi*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total</th>
<th>Typhoid fever status</th>
<th>OR* (95% CI)</th>
<th>X², df, p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative (n=107)</td>
<td>Positive (n=19)</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-35</td>
<td>68</td>
<td>55 (80.9%)</td>
<td>13 (19.1%)</td>
<td>2.039 (0.625-6.656)</td>
</tr>
<tr>
<td>&gt;35</td>
<td>26</td>
<td>23 (88.5%)</td>
<td>3 (11.5%)</td>
<td>1.231 (0.271-5.596)</td>
</tr>
<tr>
<td>≤8</td>
<td>32</td>
<td>29 (90.6%)</td>
<td>3 (9.4%)</td>
<td>Ref</td>
</tr>
<tr>
<td><strong>Level of education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>19</td>
<td>15 (78.9%)</td>
<td>4 (21.1%)</td>
<td>2.015 (0.659-6.163)</td>
</tr>
<tr>
<td>Post-Secondary</td>
<td>40</td>
<td>32 (80.0%)</td>
<td>8 (20.0%)</td>
<td>1.914 (0.751-4.88)</td>
</tr>
<tr>
<td>Primary</td>
<td>67</td>
<td>60 (89.6%)</td>
<td>7 (10.4%)</td>
<td>Ref</td>
</tr>
<tr>
<td><strong>Main source of water</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borehole</td>
<td>29</td>
<td>26 (89.7%)</td>
<td>3 (10.3%)</td>
<td>1.293 (0.311-5.378)</td>
</tr>
<tr>
<td>River</td>
<td>47</td>
<td>35 (74.5%)</td>
<td>12 (25.5%)</td>
<td>3.191 (1.107-9.205)</td>
</tr>
<tr>
<td>Piped</td>
<td>50</td>
<td>46 (92.0%)</td>
<td>4 (8.0%)</td>
<td>Ref</td>
</tr>
<tr>
<td><strong>Disposal of faecal matter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pit latrine</td>
<td>82</td>
<td>69 (84.1%)</td>
<td>13 (15.9%)</td>
<td>2.299 (0.552-9.579)</td>
</tr>
<tr>
<td>Bush</td>
<td>15</td>
<td>11 (73.3%)</td>
<td>4 (26.7%)</td>
<td>3.867 (0.797-18.750)</td>
</tr>
<tr>
<td>Flush toilet</td>
<td>29</td>
<td>27 (93.1%)</td>
<td>2 (6.9%)</td>
<td>Ref</td>
</tr>
<tr>
<td><strong>Awareness on modes of transmission</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>36</td>
<td>30 (83.3%)</td>
<td>6 (16.7%)</td>
<td>1.185 (0.412-3.403)</td>
</tr>
<tr>
<td>Yes</td>
<td>90</td>
<td>77 (85.6%)</td>
<td>13 (14.4%)</td>
<td>Ref</td>
</tr>
</tbody>
</table>

The main source of water for the household a participant hailed from was associated with typhoid fever with those who relied on water from the river being three times more likely to be infected as opposed to those who used piped water (OR = 3.191 (95%CI: 1.107-9.205); p = 0.028). The level of education of the study participants was not associated
with typhoid fever ($\chi^2 = 1.492$, df = 1, $p = 0.215$ and $\chi^2 = 1.896$, df = 1, $p = 0.169$). Similarly, being awareness of the modes of transmission showed no statistically significant relationship with the disease ($X^2 = 0.099$, df = 1, $p = 0.753$). Although the prevalence of typhoid fever was higher in those who practiced open defecation (bush) when evaluated with those who used toilets and/or latrines, the association did not attain statistical significance (26.7% versus 13.5% respectively, $p = 0.241$).
CHAPTER FIVE: DISCUSSION

5.1 Prevalence of typhoid fever

Chuka General Hospital attends to patients of Tharaka Nithi County and the neighbouring Embu and Meru counties hence a larger catchment area. The findings in the present study suggested high rates of bacteremia (15.1%) in typhoid fever patients. These results concur with the study carried out in Kibera by Breiman et al. (2012), where results showed a high prevalence of typhoid fever (24.7%) due to overcrowding with poor access to clean water and sanitation. Water from rivers and borehole was not safe because feecal matter may gain access into the water through runoff water or through seepage from sewers and pit latrines. This could have been a major contributing factor to the high bacteremia cases considering that a number of patients reported to have used bushes to dispose off their faecal waste and urine. Wild animals and even domestic animals may drink from the same rivers. Their feaces and urine contaminate the river water. River water is further contaminated by people who may bath in them (Mweu and English, 2008).

In the present study, the finding that people of all levels of education were susceptible to infection by typhoid fever suggest that due to public health education and awareness on the modes of transmission, people are able to practice healthier lifestyles for example hand washing with soap, proper sewage disposal et cetera. Awareness campaigns during outbreaks also encourage people with symptoms to seek immediate healthcare. The use of modern communication channels like mobile phones and social media makes these campaigns effective. Earlier report by WHO (2006) suggested that provision of piped water systems
with water treatment facilities as well as construction of systems for sewage disposal and latrines greatly reduces infection by typhoid fever.

The findings in the present study suggested that people of all ages are susceptible to infection by \textit{S. typhi}. These results concur with the study carried out by Parry \textit{et al.} (1999) where results showed that both children and adults can get typhoid fever through ingestion of contaminated food and water. Children at early ages are playful, very active curious et cetera and such behaviors expose them to risks of contracting typhoid fever. Travelers to high risk destinations for example South East Asia have a high risk of contracting typhoid fever (Lin \textit{et al.}, 2010). People living in overcrowded areas with poor access to safe drinking water and proper sanitation facilities are prone to infection by typhoid fever.

The results of the present study found out that both gender were susceptible to infection by typhoid fever. These findings concur with the study carried out by Youssef \textit{et al.} (2010) which showed that the attack rates by \textit{S. typhi} are similar for both men and women. This possibly could be because both men and women are involved in outdoor activities exposing them to high risk of infection. In addition, most of the health care facilities lack the equipment and technical expertise to properly diagnose typhoid fever (Pang \textit{et al.}, 2010). Most of the cases go untreated. Bacteria persist for long in the body resulting to a high population of carriers. Other patients do not follow doctor’s prescription and instead they take the antibiotics and when the symptoms are gone they stop taking them. These patients therefore, continue to shed the bacteria thereby infecting more population. Due to indiscriminate use of antibiotics, \textit{S. typhi} bacteria strain that is resistant to commonly used antibiotics has emerged.
Since multidrug resistant strains to antibiotics like ampicillin, gentamycin, cloxicillin, augmentin, ceftazidimine have emerged, when these antibiotics are used for treatment, the bacteria is not eliminated and therefore it is excreted for months or years thereby infecting more population (Parry et al., 2009).

The finding that immunization is insufficient to offer full protection in the present study support an earlier report by WHO (2006) that vaccination does not offer potent protection in case of ingestion of a large dose of infectious organism. Vaccination is not routinely recommended except for travelers or laboratory workers who are likely to be exposed to the bacteria. All the same, travelers should be advised to exercise care in selecting food and drinks. The community should be educated about personal hygiene and the need for proper sewage disposal.

5.2 Widal test

Results in the present study found out that Widal test has low sensitivity and specificity. These findings concur with the study carried out by Parry et al. (2009) which found out that Widal test had registered high cases of false positives. The case of false positive in the present study are possibly because the ‘O’ and ‘H’ agglutination usually appear around 8-12 days of infection, hence the probability of them not detected in case of early diagnosis. Immunosuppressed patients lack antibody responses. Infection of a site such as the synovial cavity which is not part of the reticuloendothelial system or early treatment
with ampicillinor chloramphenical can have a profound inhibitory effect on agglutinin production (Mweu and English, 2008).

In the present study, a single sample test was used, as is the case in hospitals since outpatients rarely return for medical follow up. This could be a drawback to interpreting the results because of high background rates of circulating antibodies to *S. typhi*. Due to polyvalent nature of Widal antigens, there is a high possibility of cross-reactivity with bacterial and non-bacterial infections. Since some diseases such as Malaria, ulcerative colitis, non-typhoidal *Salmonellae*, rheumatoid arthritis and nephrotic syndrome may show similar symptoms and produce high ‘O’ antibody test, they should always be evaluated as differential diagnosis (Bhutta, 2006).

The base titre value of 1:160 may change in the population so the titre values may not have had much significance. The accurate antibody levels of individuals (steady-state baseline titre) are difficult to establish. The patients who had already been treated with antibiotics showed false positive results because even though, the bacteria was dead, there were still circulating antibodies in serum, which agglutinated with ‘O’ and ‘H’ antigens.

The case of many false positive in Widal test in the present study could be because individuals who had *S. typhi* infection in the past may have developed *Salmonella typhi* antibodies during
an unrelated or closely related infection (anamnestic response) thereby giving false positive results. Patients who had received vaccines against *Salmonella* gave false positive reactions since antibodies to ‘O’ and ‘H’ antigens were detectable in serum. Other causes of drawback are the procedural aspects when conducting the tests and variability in the reagents as well, since varying antigens in the different test kits available perform differently. Positive Widal agglutination tests have also been reported in association with dysgammaglobulinaemia of chronic active hepatitis and other autoimmune diseases (Pang *et al.*, 2010). In addition, the infecting strains of *S. typhi* are poorly immunogenic. There is therefore, big conflicting evidence as to the relative importance of somatic and flagella agglutinin tests for the diagnosis of typhoid fever.

### 5.3 Stool culture

The findings in the present study indicated that stool culture has a high sensitivity and specificity. This finding support an earlier study by Hohman (2011) that established that stool culture picked highest typhoid fever cases. *Salmonella typhi* is an enteric microorganism and its principle habitant is in the intestine of humans, so it is easily detectable in stool even in cases of early ingestion of bacteria (Prajapati *et al.*, 2008). Stool culture is an important adjunct for diagnosis; it may be positive even when blood culture is negative and it is also important for the monitoring of carriage of *S. typhi* after apparent clinical cure, which is a risk factor for people involved. Improvement of stool culture sensitivity can be done by culturing stool in triplicate. Additionally, increasing the quantity of stool used for culture has been shown to increase the sensitivity with culturing
two grams of stool rather than the standard one gram increasing isolation by 10% (Bhanu et al. 2011).

5.4 Blood culture

Blood culture is the standard diagnostic method for Salmonella typhi infection. Blood is normally a sterile environment, so detection of bacteria in the blood is an indication of systemic infection. Culture of blood is the most sensitive method for detection of bacteremia (Lin et al., 2010). However, even though the blood culture has the highest sensitivity in detecting S. typhi infection, it is not widely applicable in most of the rural health care facilities because these health centres lack trained laboratory personnel, scientific know how and technical expertise necessary to perform a blood culture. Due to lack of financial resources, setting up and maintaining a microbiology laboratory with a blood culture facility is a big challenge. The inability to maintain stable and reliable electricity supply in rural health facilities poses a great challenge to performing a blood culture. Access to blood cultures is limited to the well-equipped private hospitals located in urban centres. The inability to perform effective diagnosis has led to poor health outcomes, indiscriminate antibiotic use and increasing drug resistant. It has also made targeted cost effective vaccination strategies impossible to implement. Some of the limitations of blood culture include venipuncture which is associated with pain, therefore creating fear to patients especially children. The size of veins vary in different individuals, therefore locating one is sometimes difficult. Risk factors associated with giving a blood sample include blood accumulating under the skin (hematoma), excessive bleeding especially those that are haemophilic, then fainting and infections.
5.5 Factors predictive of typhoid fever

Unspecified host or bacterial factors, as well as prior antibiotic use, have been given as possible explanations for this observation and may explain why some blood cultures from Widal positive cases never yielded bacterial growths (Schroeder, 1968).

Unlike in the present study where age was not significantly associated with *S. typhi* infection, a study by Lunguya *et al.* (2012) found that older age and long duration of fever were predictive of typhoid fever. The study, however, had a different focus as compared to the present study; it focused on invasive *Salmonellosis* among hospitalized children between 2 months to 14 years of age.

In the present study, males and females were equally susceptible to infection with *S. typhi*. This finding contrast that of Prajapati *et al.* (2008) whereby higher proportions of infection were observed in males than females in Nepal. The difference could be attributed to the fact that, the study population in the Nepal were children. Outdoor activities are more pronounced in males than the females in most of rural communities, exposing them to high risk of infection.
CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i) This study established that Widal test is not very reliable for the diagnosis of typhoid fever since false positive and false negative results are common. The present study found that in this population a Widal titer of 1:160 had a sensitivity of 73.7% and NPV of 91.1%. On the other hand, the test has a specificity of 47.7% and a PPV of 20.0%. The low PPV value meant that Widal test could only be useful for excluding the disease from the population.

ii) Stool culture had a sensitivity of 84.2% and a specificity of 64.5%, PPV of 29.6%, NPV of 95.8%. Looking at these results, stool culture performed better than Widal test since there are low false negative rates.

iii) Comparing the two diagnostic tests for detection of S. typhi infection; Widal test and stool culture, stool culture performed better by detecting 16 out of 19 cases. Widal test had the lowest sensitivity resulting in high rates of false-positive result. Out of 19 positive cases detectable by blood culture, Widal test could only detect 14 cases correctly.
6.2 Recommendations

i) Widal test is most widely used test for diagnosis of typhoid fever in most of the health care facilities. Due to its low sensitivity, there is a need to develop a rapid, highly sensitive and cheap diagnostic tool for diagnosis of typhoid fever. Health care personnel should not totally depend on Widal test alone for the diagnosis of typhoid fever but should use other diagnostic methods to differentiate *Salmonella* infection from other infection. This would reduce misdiagnosis, ensure proper antimicrobial therapy, and would also minimize the emergence of MDR strains. The use of rapid screening of *S. typhi* antigen directly from stool of a patient suspected of having typhoid fever would be more accurate.

ii) It is clear that typhoid fever is still a serious health problem, therefore, it is recommended that the public should be made aware of the modes of transmission, and the need for proper hygiene and sanitation should be emphasized.

iii) Since both gender are susceptible to infection by typhoid fever, there is need to target both males and females by public health authorities in control of typhoid fever.

iv) A national wide study should be carried out to investigate the true prevalence of typhoid fever in Kenya. There is also the need to carry out a longitudinal study on the temporal variations/seasonality of *S. typhi* transmissions in this area. Also a study assessing performance of the different approaches in diagnosis of typhoid
fever in light of the duration of fever. The performance of various cut-offs of an anti-TH and -TO titers for the diagnosis of typhoid fever should be evaluated in order to come up with the optimum cut-off values for performing Widal test in this population.
REFERENCES


APPENDICES

Appendix I. Map of study area
Appendix II: GRAM’S STAIN

Equipment

Bunsen burner, alcohol cleaned microscope slide, distilled water, inoculating loop, bibulous paper, and microscope.

Reagents

Crystal violet, Gram’s iodine solution, acetone/ethanol, 0.1% basic fushsin solution.

Procedure

1. Using an inoculating loop, transfer a drop of suspended culture on a microscope slide. Spread the culture using the inoculating loop to end up with a very thin film.
2. Allow the smear to air dry.
3. After air drying the culture heat fix it by passing it over a Bunsen burner flame while moving it in a circular motion, 2-3 times with the smear side up. Heat fixing kills the bacteria in the smear and helps to adhere the smear on the glass slide and allows the sample to move readily to take up the stains.
4. Add crystal violet stain over the fixed culture, let it stand for 10-60 seconds. Pour off the stain and gently rinse the excess stain with a stream of water.
5. Gently flood the smear with Gram’s iodine and let it stand for 1 minute. Pour off the iodine solution and rinse the smear with running water. The smear will appear as a purple circle on the slide.
6. Add a few drops of 95% ethyl alcohol or acetone to dicolourize for about 5-10 seconds then rinse with water.
7. Counter stain with basic fushsin solution for 40-60 seconds. Tilt the slide slightly and gently rinse with tap water or distilled water from the wash bottle.
8. Blot dry the slide with bibulous paper to remove the excess water.
9. View the smear using a light microscope under oil immersion.
Appendix III: Preparation of Media

a). Blood agar

Components

Pancreatic digest of casein

Papain digest of soy meal

Sodium chloride

Agar

Distilled water

Procedure for preparation of blood agar

1. Combine the ingredients and adjust the pH to 7.3. Sterilize by autoclaving at 121°C for 15 mins.

2. Transfer the prepared blood agar base to a 50°C water bath.

3. When the agar base to is cooled to 50°C, add sterile blood agar aseptically and mix well taking care not to form air bubbles. You must have warmed the blood agar to room temperature at the time of dispensing to molten agar base.

4. Dispense 15 ML amounts to sterile petridish aseptically. Store the plates at 2-8 °C preferably in sealed plastic bags to prevent loss of moisture.
b). Selenite F. broth

An enrichment medium for the primary isolation of Salmonella species.

Formulation

<table>
<thead>
<tr>
<th>Material</th>
<th>Concentration in medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone mixture</td>
<td>5.0 g/l</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.0 g/l</td>
</tr>
<tr>
<td>Sodium phosphate</td>
<td>10.0 g/l</td>
</tr>
<tr>
<td>Sodium hydrogen Selenite</td>
<td>4.0 g/l</td>
</tr>
</tbody>
</table>

Procedure

1. Dissolve 4g of sodium biselenite LPO 121 in 1 litre of distilled water and then add 19g of Selenite broth base.

2. Heat gently to dissolve

3. Dispense into suitable containers (bottles or tubes). A depth of atleast 5 cm is recommended as Salmonella survive better at low oxygen tensions.

4. Sterilize by boiling in hot water bath for 10 mins.

5. Inoculate with faeces directly.

c) Tripplesugar iron agar (TSI)

This media is used for identification of Gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production.

Procedure

1) Suspend 65.0g of TSI in 1000ml distilled water.
2) Heat to boiling to dissolve the medium completely.
3) Mix well and distribute to test tubes.
4) Sterilize by autoclaving at 101 bs pressure (115 °C) for 15 minutes.
5) Allow the medium to set in sloped form with about 1 inch long.
APPENDIX IV: REQUEST LETTER TO PERFORM RESEARCH

CIRIAKA M. GITONGA
CHOGORIA GIRLS
P.O. BOX 76
CHOGORIA
30-1-2014

THE DIRECTOR OF HEALTH
THARAKA NITHI COUNTY
P.O BOX 8
CHUKA

RE: REQUEST TO PERFORM RESEARCH IN YOUR HOSPITAL

I am a student of Kenyatta University pursuing a master's degree in Immunology. My research topic is 'comparative sensitivity of Widal test, stool and blood culture as diagnostic methods for Salmonella typhi infections'. I am requesting to be granted an opportunity to do my research at your hospital.

Yours faithfully

CIRIAKA MUTHONI GITONGA
MOBILE N0: 0701174645
APPENDIX V: UNIVERSITY REQUEST LETTER

KENYATTA UNIVERSITY
Department of Zoological Sciences
P.O. Box 43844, Nairobi, Kenya
Tel: 810901/811278, Ext. 57305/307
Website: http://www.ku.ac.ke
E-mail: zoologicalku@yahoo.com

28th November, 2013

Medical Superintendent
P.O Box 8-60400
CHUKA

RE: GITONGA CIRIAKA MUTHONI
REG. NO. 156/CE/2600/2011

This is to confirm that the above named is a student in this department pursuing Masters degree in Immunology.

Ms Gitonga has completed her course work for the first year of MSc. The second year is usually a research project that culminates in thesis writing. She is requesting to be granted opportunity to do her research in your institution.

Any assistance accorded to her will be highly appreciated.

Yours sincerely,

[Signature]

Dr. Gichuru UI
Chairman, Dept. Of Zoological Sciences

[Stamp: KENYATTA UNIVERSITY]

[Stamp: 28 NOV 2013]
APPENDIX VI: CONSENT FORM

KENYATTA UNIVERSITY
P.O BOX 43844
NAIROBI
KENYA
TEL:810901-19

CONSENT FORM

I..............................................................................................................................have
consented to be involved in research study of typhoid.

I AM AWARE OF THE FOLLOWING CONTRACTUAL TERMS

1. That the information shared will be treated confidentially.
2. That the services are absolutely free of charge.
3. That the samples to be involved in the tests are blood and stool.

OTHER DETAILS

Signature of the client........................................Date...........................................

Signature of the researcher ..................................Date .........................................