ISOLATION AND CHARACTERIZATION OF BACTERIA PATHOGENS IN BLOOD AND STOOL SAMPLES AMONG PATIENTS PRESENTING WITH TYPHOID FEVER SYMPTOMS IN ALUPE, BUSIA COUNTY

MUREITHI MARYANNE WANJIRU (BSC. Biochemistry)

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A thesis submitted in partial fulfillment of the requirement for the award of the degree of Master of Science (Medical Biochemistry) in the School of Pure and Applied Sciences of Kenyatta University.

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Mureithi Maryanne Wanjiru

Signature………………………. Date……………………………

We as supervisors confirm that the work reported in this thesis was carried out by the candidate.

Supervisors:
Dr. Marion Burugu
Department of Biochemistry and Biotechnology
Kenyatta University
P.O.Box 43844-00100
Nairobi, Kenya

Signature……………………….. Date……………………………..

Dr. Fathiya Mbarak Khamis
Department of Biochemistry and Biotechnology
Kenyatta University
P.O.Box 43844-00100
Nairobi, Kenya

Signature……………………….. Date……………………………..

Dr. Matilu Mwau
Kenya Medical Research Institute,
Centre for Infectious and Parasitic Diseases Control Research (CIPDCR)
P.O.BOX 3,
BUSIA- KENYA

Signature……………………….. Date……………………………..
DEDICATION

To one, Winfred Mureithi.
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TABLE OF CONTENTS

DECLARATION ................................................................. II
DEDICATION ................................................................... III
ACKNOWLEDGEMENTS .................................................. IV
LIST OF TABLES ............................................................. VII
LIST OF FIGURES ........................................................... VIII
LIST OF ABBREVIATIONS ............................................... IX
ABSTRACT ...................................................................... X

CHAPTER ONE .................................................................... 1
1.0 INTRODUCTION .......................................................... 1
1.1 Background information ............................................. 1
1.2 Problem statement and justification ......................... 4
1.3 Research questions .................................................. 5
1.4 Research hypotheses ............................................... 6
1.5 Objectives .............................................................. 6
1.5.1 General objective ............................................... 6
1.5.2 Specific objectives .............................................. 6

CHAPTER TWO .................................................................. 7
2.0 LITERATURE REVIEW ............................................... 7
2.1 Common bacteria pathogens associated with poor hygiene 7
2.1.1 Klebsiella pneumonia .......................................... 7
2.1.2 Staphylococcus aureus ....................................... 9
2.1.3 Escherichia coli ................................................ 9
2.1.4 Salmonella enterica serovar Typhi ..................... 10
2.2 Typhoid fever Epidemiology ................................ 11
2.3 Transmission and risk factors of typhoid fever .......... 12
2.4 Clinical features of diseases caused by bacteria normally isolated alongside S. Typhi ......................................................... 13
2.5 Laboratory diagnosis of typhoid fever ..................... 14
2.6 Treatment and control of diseases associated with poor hygiene .................................................. 18
2.6.1 Drug resistance ................................................ 19

CHAPTER THREE ............................................................ 21
3.0 MATERIALS AND METHODS .................................... 21
3.1 Ethical approval ........................................................ 21
3.2 Study sites ............................................................. 21
3.3 Inclusion criteria ...................................................... 23
3.4 Exclusion criteria .................................................... 23
3.5 Sample size determination .................................... 24
3.6 Sampling procedures .............................................. 24
3.7 Laboratory analysis ................................................ 26
3.7.1 Widal test ....................................................... 26
3.7.2 Culture of samples ............................................ 26
3.7.2.1 Preparation of culture media ......................... 26
3.7.2.2 Blood Cultures ............................................ 26
3.7.2.2.1 Sub-cultures onto agars ............................ 27
3.7.2.2.2 Stool cultures ........................................ 27
3.7.3 Identification of Salmonella and other bacterial pathogens .................................................. 27
3.7.3.1 Catalase test ............................................... 28
3.7.3.2 Coagulase test ............................................ 28
3.7.3.3 Oxidase test ............................................... 28
3.7.3.4 Biochemical tests .......................................................... 29
3.7.4 Antimicrobial susceptibility testing .................................. 30
3.8 Data analysis ........................................................................ 31
CHAPTER FOUR ........................................................................ 32
4.0 RESULTS .............................................................................. 32
4.1 Patients demographics ...................................................... 32
4.2 Most common symptoms presented .................................... 34
4.3 Typhoid fever diagnosis .................................................... 35
4.4 Bacterial pathogens isolated .............................................. 36
4.5 Antibiotic susceptibility patterns ........................................ 37
4.5.1 Overall antibiotic profile of the isolates ......................... 38
4.5.2 Antibiotic susceptibility profiles of the individual species .... 39
4.6 Distribution of enteropathogens ........................................ 43
CHAPTER FIVE ........................................................................ 44
5.0 DISCUSSION ...................................................................... 44
5.1 CONCLUSION .................................................................... 52
5.3 RECOMMENDATIONS ......................................................... 52
REFERENCES ............................................................................ 54
APPENDIX I ............................................................................. 61
APPENDIX IIA .......................................................................... 62
KIAMBATISHO IIA ................................................................. 63
APPENDIX IIB .......................................................................... 64
KIAMBATISHO IIB ................................................................. 65
APPENDIX III .......................................................................... 66
APPENDIX IV .......................................................................... 67
APPENDIX VA .......................................................................... 69
APPENDIX VB .......................................................................... 70
APPENDIX VI .......................................................................... 71
LIST OF TABLES

Table 1. Patient’s age statistics ................................................................. 32
Table 2. Geographical location of study participants ................................. 34
Table 3. Clinical presentation by study participants ................................. 35
Table 4. The zone size interpretive chart .................................................. 37
Table 5. Distribution of enteropathogens .................................................. 43
LIST OF FIGURES

Figure 1. Map of Kenya and Busia County (flickr © Albert Kenyani Inima). 22
Figure 2. Age distribution .......................................................... 33
Figure 3. Distribution of occupation of the participants enrolled into the study .......................................................... 34
Figure 4. *Staphylococcus aureus* isolates on an agar plate .................. 38
Figure 5. Antibiotic susceptibility test .................................................. 38
Figure 6. Susceptibility profile for *Staphylococcus aureus* isolated in blood . 40
Figure 7. Susceptibility profile for *Proteus mirabilis* isolated in blood ....... 40
Figure 8. Susceptibility profile for *Escherichia coli* isolated in blood ........ 41
Figure 9. Susceptibility profile for *Escherichia coli* isolated in stool .......... 42
Figure 10. Susceptibility profile for *Shigella dysentriae* isolated in stool...... 42
LIST OF ABBREVIATIONS

ADH  Alupe district hospital
AMC  Amoxicillin-clavulanic acid
ATCC American type culture collection
Ax   Ampicillin-cloxacillin
C    Chloramphenicol
CAZ  Ceftazidine
CIP  Ciprofloxacin
CIPDCR Centre for infectious and parasitic disease control
COT  Cotrimoxazole
CXM  Cefuroxime
EDTA Ethylenediaminetetraacetic acid
EPI  Expanded program on immunization
GEN  Gentamicin
KEMRI Kenya Medical Research Institute
KMLTTB Kenya Medical Laboratory Technicians and Technologists board
MIL  Motility indole lysine medium
NCCLS National committee for clinical laboratory standards (now CLSI (Clinical and laboratory standards institute))
Te   Tetracyline
TSI  Triple sugar iron
WHO World Health Organization
Typhoid fever is a waterborne and foodborne disease caused by *Salmonella enterica* serotype Typhi (*S.* Typhi). Studies have established that some patients presenting with typhoid-like symptoms are usually inflicted by other bacteria pathogens, which may or may not be transmitted by fecal oral route. It is common belief that typhoid fever cases are high in Alupe and continue to cause significant morbidity among the people of Alupe, Busia County. The study focused on isolation and characterization of the bacteria pathogens in blood and stool among patients presenting with typhoid fever symptoms at two health facilities located in Alupe, Busia County. A total of one hundred and fifty patients were recruited and thereafter their blood and stool samples collected. Subsequently, laboratory analysis at KEMRI-CIPDCR was done to isolate and characterize bacteria pathogens as well as their antibiotic susceptibility profiles done. One hundred and forty nine blood cultures and 140 stool cultures were performed. Bacterial pathogens were detected in 11 positive cultures with both gram-positive (27.3%) and gram-negative bacteria (72.7%) obtained. Bacteria isolated from blood cultures included *Staphylococcus aureus* (50%), *Proteus mirabilis* (16.67%) and *Escherichia coli* (33.33%). Those from stool cultures included *Escherichia coli* (80%) and *Shigella dysentriae* (20%). Using Widal test, 73 (50%) samples tested positive for typhoid but there was no *S.* Typhi isolated from both blood and stool cultures. All the isolates showed moderate to high resistance to ampicillin-cloxacillin, tetracycline and cefuroxime. The high susceptibility of the isolates to gentamicin, chloramphenicol and ciprofloxacin is a welcome relief to increasing widespread multi-drug resistance. In Alupe, a malaria endemic region, *S.* Typhi is not the main cause of invasive bacteremia in patients presenting with signs and symptoms of typhoid fever. There is need for improved diagnostic methods; patients presenting with typhoid fever symptoms should also be screened for other bacteria pathogens in addition to *Salmonella* to enhance proper treatment and overall control of multi-drug resistance due to misdiagnosis and improper prescription of antibiotics.
CHAPTER ONE: INTRODUCTION

1.1 Background information

Typhoid fever is a common febrile illness caused by *Salmonella enterica* serotype Typhi. The clinical presentation of the disease is non-specific as the symptoms displayed overlap with those present during infections with other types of bacteria, viral and parasitic pathogens. *S. Typhi* is commonly associated with poor hygiene and sanitation conditions as it is mainly transmitted via the fecal oral route. Therefore, many bacteria pathogens associated with poor hygiene and sanitary conditions are common in patients presenting with typhoid fever-like symptoms.

Many studies done to either establish the prevalence or incidences of *S. Typhi* have reported other bacterial pathogens other than *S. Typhi* isolated from the blood and stool of febrile patients presenting with typhoid fever symptoms. Bacterial pathogens that are commonly isolated alongside *S. Typhi* in patients suspected of typhoid fever include *Staphylococcus* species, *Escherichia coli*, *Shigella* species, *Klebsiella pneumonia*, *Enterobacter*, *Citrobacter* and *Streptococcus* species (Kariuki et al., 2004; Vollard et al., 2005; Adabara et al., 2012).

In a study done in southern coastal Pakistan to establish the incidence of typhoid bacteremia, *Streptococcus pneumonia*, *Acinetobacter* spp., *Pseudomonas* spp., *E. coli, Campylobacter jejuni*, *Hemophilus influenza* type b,
**Kingella** spp., β-hemolytic *Group B Streptococcus* alongside *S*. *Typhi*, *S*. Paratyphi A and *S*. Paratyphi B were isolated from the blood of febrile patients (Owais *et al*., 2010). Similarly, in a study carried out to determine the cause of non-malaria febrile illness in Papua, Indonesia, *S*. *Typhi*, *E*. *coli*, *Streptococcus pneumonia*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Klebsiella pneumonia* were obtained from the positive blood cultures (Punjabi *et al*., 2012).

In Jakarta, India *S*. *Typhi*, *S*. Paratyphi, *S*. *aureus*, *K*. *pneumonia* and *Streptococcus* species were bacteria pathogens obtained from blood of patients in a study done to identify the presentation of typhoid fever and paratyphoid fever cases (Vollard *et al*., 2005). In Kenya and Nigeria, bacteria isolated in blood and stool of suspected typhoid fever patients included *Staphylococcus aureus*, *Klebsiella pneumonia*, *Streptococcus* species and non-typhoidal *Salmonella* species (Kariuki *et al*., 2004; Smith *et al*., 2011). Furthermore, cases of co-infections with more than one bacterium have been reported in India, where *S*. *Typhi* was isolated in a preschool child with pneumonia (Brooks *et al*., 2005).

The diagnosis of typhoid fever on clinical grounds is difficult as the presenting symptoms are diverse and similar to those observed with other common febrile illnesses (House *et al*., 2001). The most common symptoms singled out as the main presentation of typhoid fever include diarrhea, vomiting, abdominal pain, headache, splenomegally, anorexia and hepatomegally (Mweu and English, 2008).
The recommended methods used for diagnosis of typhoid fever include culture of blood, bone marrow aspirates, stools or urine. These methods are costly and most health amenities lack facilities to perform these tests. This leads to the wide use of serological methods and clinical presentation of the patient for the diagnosis of typhoid fever; methods which are also inefficient and insufficient since in most instances they are non-specific. Ultimately, this has resulted to rising cases of misdiagnosis which has had serious implications both at individual and society level.

At the individual level, the insufficient or inappropriate treatment leads to unnecessary exposure of patient to antibiotics in the case of a viral cause of febrile illness (Vollard et al., 2005). This inturn has led to widespread emergence of multidrug resistance in individuals to the commonly used antibiotics for typhoid fever. At the community level, lack of correct diagnosis has made it difficult to monitor the transmission chain of typhoid fever, to determine the carrier patients and local transmission routes which are considered essential in containing diseases in case of an outbreak (Vollard et al., 2005).

Research findings in the past have indicated that typhoid outbreaks are increasingly becoming a major health problem in several parts of Kenya (Kariuki et al., 2004). Particularly, sporadic outbreaks have led to high typhoid fever incidence rates reported in densely populated urban slums in Kenya with over 100,000 cases per year (Breiman et al., 2012). This has led to calls for mass vaccination as well as the common use of the Widal test by the clinicians.
to screen adults and young children, in whom they perceive the disease takes a toll on, presenting with febrile illness in health facilities. The use of the Widal test and the clinical presentation for diagnosis of typhoid fever instead of the gold standard methods that include blood and bone marrow cultures is of great public concern as the methods lack both standardization and specificity (Smith et al., 2011). Furthermore, the use of the Widal test was banned in Kenya but it is continually being used in most health centres due to lack of resources for other diagnostic procedures such as the blood culture (KMLTTB, 2012).

It is common belief that typhoid fever cases are high in Alupe and continue to cause significant morbidity among the people of Alupe, Busia County. There was need to confirm experimentally if typhoid fever is indeed common or the high cases reported are due to misdiagnosis since the clinical presentation of typhoid fever is non-specific and could resemble those of other clinical bacterial pathogens. The study focused on isolation and characterization of the bacteria pathogens in blood and stool among patients presenting with typhoid fever symptoms at two health facilities located in Alupe, Busia County.

1.2 Problem statement and justification

Bacteria pathogens remain a serious public health problem to a wider population of humans in developing countries. Most of these pathogens are associated with poor sanitation and hygiene. The diseases have important socio-economic impacts in the affected communities because in most instances
several months are required for a patient to recover and resume duty as well as the costs incurred during treatment.

The clinical presentation of most of these bacteria pathogens is similar to that of typhoid fever, and as such, clinically many patients are treated against typhoid fever and this has resulted into multidrug resistance amongst the populations. This study was initiated with an aim of shedding light into the true picture of the causative agents in patients, in Alupe, Busia County, that present with typhoid fever like symptoms. The findings of this study will go a long way in informing the policy makers on the need to emphasize the use of the recommended gold standard methods for the correct and specific diagnosis of diseases. This will also help them to strategize on ensuring public health facilities are provided with the appropriate equipment for various diagnostic tests and that gold standard methods (culture tests) are provided at subsidized prices that are affordable to all.

1.3 Research questions

i) What are the most prevalent bacteria pathogens in patients presenting with typhoid fever symptoms with typhoid fever symptoms in Alupe, western Kenya?

ii) Are the bacteria pathogens isolated from patients presenting with typhoid fever symptoms in Alupe, Western Kenya resistant to any of the antimicrobials used?
iii) What are the distribution patterns of enteropathogens amongst the study participants?

1.4 Research hypotheses

i) S. Typhi is the major cause of febrile illness in Alupe, western Kenya and it shows no resistance to the antimicrobial drugs in common use.

ii) There is no significant difference in the distribution of enteropathogens amongst the study participants.

1.5 Objectives

1.5.1 General objective

To isolate and characterize bacteria pathogens in blood and stool samples among patients presenting with typhoid fever symptoms in Alupe district hospital and KEMRI-CIPDCR clinic, western Kenya.

1.5.2 Specific objectives

i) To isolate and characterize the bacteria pathogens in blood and stool samples of patients using biochemical and serological methods.

ii) To determine the susceptibility of the isolates to various antimicrobial agents.

iii) To determine the distribution of enteropathogens amongst the population.
CHAPTER TWO: LITERATURE REVIEW

2.1 Common bacteria pathogens associated with poor hygiene

Infections associated with poor hygiene and sanitation conditions have continually been a burden among communities in developed as well as developing countries. These infections are either viral or bacterial in nature. Viral infections associated with gastro-enteritis include infections with rotavirus, norwalk virus, calicivirus, astovirus and enteric adenoviruses while bacterial pathogens include S. Typhi, Shigella species, enteropathogenic E. coli among others. These infections cause high morbidity in young children unlike in adults (Dennehy, 2000).

The main transmission route for these infections is from person-to-person via the fecal-oral route (Kothari et al., 2008). The infections are acquired when surfaces, food, water or hands are contaminated with the above named pathogens. The airborne route has also been shown to facilitate transmission of the rotavirus (Prince et al., 1986). Varying amounts of the pathogen are required for the establishment of disease. For instance, high amounts of inoculum for S. Typhi are required to cause typhoid fever unlike Shigella which requires a small inoculum (Dennehy, 2000).

2.1.1 Klebsiella pneumonia

*Klebsiella pneumonia* is a gram negative bacterium that causes significant blood stream infections that result to morbidity and mortality if not treated on
time. It is one of the most common causes of bacteremia associated with gram negative bacteria. It is also a causative agent of urinary tract infections, nosocomical pneumonia and intra-abdominal infections (Lin et al., 2010). The bacterial pathogen can be acquired in the hospital during hospital admissions and visits or within the community. *K. pneumonia* is mainly found in the environment; it is found in water, sewage, soil and plants and on the mucosal surface of mammals (Bagley, 1985). The bacterium inhabits the nasopharynx and the intestinal tract of humans with higher detection rates being in the latter via the stool cultures (Podschun and Ullmann, 1998). Underlying conditions commonly associated with *K. pneumonia* bacteremia include diabetes mellitus, neoplastic disease, chronic lung disease, hepatobiliary diseases and alcoholism (Ko et al., 2002; Tsay et al., 2002; Lin et al., 2010). It is worth noting that the rates of *K. pneumoniae* bloodstream infections differ significantly in different temperature and humid conditions. High rates of *K. pneumoniae* infections occur during the warm months of the year (Deverick et al., 2008).

Most clinical isolates of *K. pneumoniae* have developed resistance to most antibiotics used for empirical treatment thereby reducing the treatment options to third generation cephalosporins and quinolones to which resistance is also slowly emerging (Ko et al., 2002). Resistance has been associated with the increasing prevalence of extended-spectrum β-lactamase producing strains (Podschun and Ullmann, 1998). Prevention strategies put in place by the public health ministries in Kenya and around the world include proper hand washing and proper hygiene conditions and access to sanitation facilities.
2.1.2 *Staphylococcus aureus*

*Staphylococcus aureus* is a gram positive bacteria frequently associated with community acquired-invasive bacterial infections. These include Ritter disease, folliculitis, furuncle and carbuncle, septic arthritis, endocarditis, toxic shock syndrome, pneumonia and thrombophlebitis. The bacterium is also responsible for *Staphylococcal* food poisoning that arises from ingestion of food contaminated with the organism. Contamination occurs when food is prepared and stored in areas of poor hygiene where the bacterium is present. Most resource-poor settings lack clean water services and sanitation facilities therefore with the high numbers of people in these congested areas, contamination of water and food with pathogens including *S. aureus* normally occurs and is responsible for significant morbidity and mortality.

Strains of *S. aureus* which are resistant to methicillin are commonly referred to as methicillin-resistant *S. aureus* (MRSA) and continue to cause diseases among populations. In addition, multidrug resistant *S. aureus* strains have emerged, a trend that continually exhausts the available treatment options available (Appelbaum, 2006). *Staphylococcus aureus* infections cause fatal disease in patients whose immune system are impaired or weakened as the bacterium is highly aggressive and is able to invade and destroy tissues (Owais et al., 2010).

2.1.3 *Escherichia coli*

*Escherichia coli* is a gram negative bacterium that inhabits many parts of the body. It is the most prevalent normal flora bacteria in the body and is usually
non-pathogenic in the areas it colonizes (Nataro and Kaper, 1998). However, in immuno-compromised hosts or when the gastrointestinal barriers are violated, the non-pathogenic strains of *E. coli* can cause infection. There have emerged *E. coli* strains that produce entero toxins that are responsible for traveler’s diarrhea and infant diarrhea (Nataro and Kaper, 1998). These strains of *E. coli* mostly invade the mucosal surfaces but could at times spread to other parts of the body. Diseases that are as a result of *E. coli* bacteremia include urinary tract infection, meningitis and diarrheal disease (Nataro and Kaper 1998; Pathak *et al*., 2012). The infection rates of *E. coli* are varied with considerably high incidences being recorded in the warmest months of the year (Al-Hasan *et al*., 2009). Studies have reported that *E. coli* strains have shown high resistance to beta-lactam antibitiocs than other classes of antimicrobials (Alam *et al*, 2011). The β-lactam antibiotics including penicillin, cephalosporins and related compounds, are commonly given to patients presenting with bacterial infections.

### 2.1.4 *Salmonella enterica* serovar Typhi

*Salmonella enterica* serovar Typhi are gram-negative rods, non-spore forming, motile and microscopic living creatures. They are oxidase-negative, catalase-positive, non-lactose fermenters, producing acid from D-glucose usually at times accompanied with the production of carbon dioxide and some utilize citrate as a sole carbon source (Cabral, 2010). The bacteria is characterized by its flagella antigen, H, its lipopolysaccharidic (LPS) O antigen as well as its polysaccharide (PS) capsular virulence (Vi) antigen, found at the surface of
freshly isolated strains (WHO, 2006). The Vi antigen has been considered important in enabling the organism to cause disease. Despite Vi-positive *S. Typhi* being the main cause of typhoid fever over the years, recent studies indicate the emergence of Vi-negative variants of *S. Typhi* and *S. Paratyphi A* as significant causes of typhoidal disease (Ali *et al*., 2008).

Untreated typhoid fever persists for two weeks or more and convalescence may last for 3-4 months. In most cases, if an appropriate antibiotic (chloramphenicol, ampicillin, co-trimoxazole) is given, the fever gradually falls over 3-4 days. However, resistance posed to the first-line antimicrobial agents ampicillin, chloramphenicol and co-trimoxazole which defines multi-drug resistance has been on the rise. The situation has even worsened with the emergence of multidrug resistant serovar Typhi resistant to nalidixic acid and the floroquinolones (Kariuki *et al*., 2004).

### 2.2 Typhoid fever Epidemiology

Typhoid fever in the past was not recognized as a separate clinical entity and was often confused with other prolonged febrile syndromes (Singh, 2001). The global estimates of typhoid fever range from 17 to 22 million cases per year with an associated 216,000 to 600,000 deaths annually (Steele, 2008). In Kenya, typhoid incidence at rates of 39/100,000 have been reported, however, these figures may be an underestimate due to under reporting (Kariuki *et al*., 2010). In endemic areas, the incidences of typhoid fever are considered to be low in the first few years of life, peaking in school-aged children and young
adults and then falling in the middle aged. The peak incidence of typhoid fever is reported during July- September which coincides with the rainy season and a substantial increase in house fly population.

2.3 Transmission and risk factors of typhoid fever

Typhoid fever is human-restricted; humans are the only natural host and reservoir of S. Typhi (WHO, 2003). S. Typhi can survive for prolonged periods in water, ice, dust and dried sewage which become sources of infection. Salmonella Typhi is mainly transmitted by ingestion of food or water contaminated with faeces from an infected person. Ice cream, contaminated water supply, flavoured iced drinks or food from street vendors, shellfish taken from contaminated water and raw fruit and vegetables fertilized with sewage are significant risk factors for transmission of typhoid fever (WHO, 2003; Bhan et al., 2005). The above named sources can also harbor other bacteria associated with poor hygiene thus serve as transmission routes.

Typhoid is introduced and spread mainly within the household by convalescent cases excreting the bacterium. The risk factors associated with the intra-household spread are poor hand washing hygiene and sharing of food from the same plate. The extra-household factors associated with acquisition of typhoid fever include public toilets which are usually dirty, lack maintenance, lack water for cleaning the toilets as well as for hand-washing (Vollard et al., 2005). Several studies have emphasized the importance of the use of soap when washing hands for the reduction of diarrheal diseases. This has led to massive
public health campaigns on the importance of hand-washing and proper hand-washing hygiene using soap in a bid to reduce the burden of infectious diseases.

There is need for follow-up of enteric fever cases, especially among food vendors, in order to reduce the role of chronic carriers in food borne transmission (Vollard et al., 2005). The host genetic factors also play a major role in susceptibility or resistance to infection with typhoid fever. This explains the variation in the incidence of typhoid fever among the developing countries with similar standards of public health and hygiene (Kothari et al., 2008). Helicobacter pylori infection is also associated with increased risk of typhoid fever (Bhan et al., 2005). Helicobacter. pylori infections cause hypochlorhydria thus compromise the gastric-acid barrier which has been shown to be an important protective mechanism against Salmonella infections (Giannella et al., 1973).

2.4 Clinical features of diseases caused by bacteria normally isolated alongside S. Typhi

The presence of bacterial pathogens in blood is referred to as bacteremia. The magnitude of bacteremia is dependent on the age of the patient, immune status, virulence of the bacterial strain and geographical location. The general symptoms displayed during bacteremic episodes include fever over (38.3°C), chills, malaise, abdominal pain, nausea, vomiting, diarrhea, anxiety, shortness
of breath and confusion. It is essential to note that not all these symptoms are present in any one particular patient.

The clinical presentation of typhoid fever ranges from an asymptomatic infection to a severe form which is dependent on a number of factors. These factors include the duration of illness before initiation of appropriate therapy, the choice of antimicrobial treatment, age of the patient, the previous exposure or vaccination history, the virulence of the bacterial strain, host’s health status and whether the individual was taking other medications such as H$_2$ blockers or antacids (WHO, 2003). Fever and myalgia mark the onset of bacteremia but are mild and patients do not usually present to hospital until towards the end of the first week of symptoms. With progression of the disease, other symptoms characteristic of typhoid fever including headache, abdominal pain, relative bradycardia, splenomegally and leucopenia begin to manifest (Uneke, 2008).

2.5 Laboratory diagnosis of typhoid fever

A specific diagnosis of typhoid fever requires access to a competent laboratory that can process blood and bone marrow aspirate culture samples. Direct culture of blood samples followed by microbiological identification is considered as the gold standard for the diagnosis of typhoid fever (Baker et al., 2010; Ley et al., 2011). Although this method is recommended for best results it has several limitations. Even under the best culture conditions, the organisms may not be isolated from blood, especially after antimicrobial treatment is commenced (Arjunan and Al-Salamah, 2010). The bactericidal activity of the
antibiotics suppresses the growth of the organisms in the culture medium. It is important to also note that the sensitivity of the blood culture decreases with the duration of illness.

Isolation of S. Typhi alone from stool is insufficient for diagnosis and only marginally improves diagnosis by blood culture. This is because S. Typhi is obtained from stool cultures during the second and third weeks of the illness. In most cases stool culture is normally used to assess the carrier status of the patient (Bhan et al., 2005). The likelihood of obtaining positive results increases with the quantity of stools collected. Cultures of bone marrow aspirates and intestinal secretions have a higher sensitivity than blood culturing but they require more invasive procedures thus are not commonly done (Rubin et al., 1990; House et al., 2001). These methods are highly sensitive as they have been shown to isolate organisms even after antibiotics have already been given (Singh, 2001). Although several urine assays have been developed for the rapid diagnosis of acute typhoid fever, none has proved optimal (Olsen et al., 2004). Isolation of the organism from urine is less frequent but can be used to assess the carrier state of the patient as is in cases of chronic carriage. The above culture methods, though expensive, are most preferred as the organisms obtained are subjected to antibiotic susceptibility testing and the most effective antibiotic against the organism is used to clear away the infection.
Serologic tests based on agglutination detection have been commonly used in areas where the disease is endemic since they are rapid, easy to perform and affordable. These tests include both antibody and antigen detection techniques. For the antibody detection methods, the most widely used is the Widal test. It is based on a macroscopically visible serum – mediated agglutination reaction between S. Typhi somatic lipopolysaccharide O antigens (TO test) and flagellar H antigens (TH test). Usually, two specimens of serum are required at an interval of 2-3 weeks and a four-fold rise in the titer of H (flagellar) or O (somatic) agglutinins indicates a strong likelihood of the disease (Olopoenia and King, 2000). There is delayed diagnosis due to the fact that a second specimen is required at a later date.

There has been controversy on the use of a single serum specimen which is widely used in most clinical facilities to define treatment options. Some reports from studies in developing countries suggest that a single Widal test is sufficient to make diagnosis of typhoid fever while others have dismissed its usefulness indicating the high rates of false-positive results (Wicks et al., 1974; Aquino et al., 1991). In one of the study, the use of a single serum specimen has been shown to be useful in unvaccinated subjects from non-endemic regions and in children residing in typhoid fever endemic areas and are less than 10 years (Singh, 2001). Nevertheless, the Widal test, which has been banned in Kenya, is widely performed when children and adults present with fever to treatment centers since it is cheap, easy to perform and readily available unlike the gold standard, blood culture, which is costly and many
health amenities in endemic areas lack these facilities (Ley et al., 2011; KMLTTB, 2012).

The polymerase chain reaction (PCR) has also been shown to detect S. Typhi DNA in the blood. Because of its specific nature, PCR has been of great use in patients with suspected clinical signs and symptoms of typhoid fever but have negative culture results. In addition, it has been shown to detect S. Typhi in patients who have been on antibiotics prior to diagnosis. Generally, PCR can reinforce the clinical diagnosis of typhoid fever in culture-negative cases and thus avoid other unnecessary treatment options, emergence of drug resistance occasioned by the improper use of drugs and high costs in treating ailments (Song et al., 1993). However, it is rarely used since most laboratories lack equipment, the high costs of running the test and there is also lack of skilled manpower to run the tests.

The recent emergence of Salmonella species other than S. Typhi that causes typhoid fever and widespread drug resistance has led to improved diagnostic methods like nested multiplex PCR technique that facilitates definitive differential diagnosis of typhoid fever. This method is rapid and sensitive and specific diagnosis of typhoidal pathogens is made directly from blood samples (Ali et al., 2008).
2.6 Treatment and control of diseases associated with poor hygiene

Most of bacterial infections associated with poor hygiene are cleared using beta-lactam antibiotics, cephalosporins and related compounds. Treatment normally begins with a broad spectrum antimicrobial agent after which a narrow spectrum antibiotic is given if the illness persists. The susceptibility of these organisms to antibiotics differs geographically depending on the selection pressures present for each region.

Prevention strategies against illnesses associated with poor hygiene mainly focus on improving sanitation, ensuring safety of food and water supplies and use of vaccines. Typhoid fever control should entail follow-up on enteric fever cases especially among food handlers in order to reduce the role of transient or chronic carriers in the food-borne transmission (Vollard et al., 2005). The use of vaccine as a means of control of typhoid fever requires an individualized approach which involves vaccination of children living with the identified typhoid fever cases (Luxemburger et al., 2001).

Currently, there are two safe and efficacious typhoid vaccines that have been licensed; the injectable Vi polysaccharide and the oral Ty21a. The Vi polysaccharide vaccine given in a single subcutaneous or intramuscular dose is licensed for use in individuals older than two years and is normally effective for about three years after vaccination. Ty21a vaccine is a live oral vaccine usually available in enteric-coated or liquid formulation. It is approved for use in people 6 years of age and above and remains moderately effective for three years after vaccination. Both vaccines require a booster dose every three years.
The use of typhoid fever vaccines has not been implemented as a routine public health measure in most typhoid-endemic countries despite the low price of the vaccine and high cost of treating the disease (WHO, 2003; Ochiai et al., 2008). This is due to the uncertainty of the burden of typhoid disease as it is confused with other febrile illnesses, a preference among policymakers for water and sanitation improvements over vaccination to control many enteric diseases, lack of awareness among policymakers about the new-generation vaccines and uncertainty of the logistic feasibility of mass vaccination of children outside the infant expanded programme on immunization (EPI) schedule in their countries (Steele, 2008).

2.6.1 Drug resistance

The increasing resistance observed against anti-microbials used to treat bacterial infections is causing panic amongst health providers. Resistance among the gram negative has been attributed to the presence of extended-spectrum beta-lactamases (ESBL), a family of plasmid-mediated enzymes that hydrolyse and cause resistance to most of the beta-lactam antibiotics, including penicillins, monobactams and most cephalosporins. Resistance has also been attributed to the increasing rate of over-the-counter sale of antibiotics without a prescription. Poor diagnosis occasioned by use of inadequate tests, poor interpretation of laboratory results as a result of visiting some privately owned clinics found in most poor informal settlements that lack expertise is also to blame for the high resistance observed (Kariuki et al., 2010). Different combinations of antimicrobials are being assessed to provide more affordable
options for treatment of most of these resistant bacteria for instance quinolone-resistant typhoid fever (Bhan et al., 2005).
CHAPTER THREE: MATERIALS AND METHODS

3.1 Ethical approval

This study was part of a parent project for which scientific and ethics approval was granted by Kenya Medical Research Institute, Scientific Steering Committee No. 1698. Informed consent was also obtained from study patients and guardians for those below age 16.

3.2 Study sites

The study sites were Alupe district hospital and Alupe KEMRI clinic. These health facilities, located in the same compound, are situated 6 km north of Busia, along the Busia-Malaba road in western Kenya. The facilities lie in Busia County, South Teso district. Surrounding community members practice subsistence farming as their main economic activity. Most of the homes lack proper human waste disposal facilities and at times, inhabitants use bushes. Main sources of water include boreholes, wells, and seasonal shallow rivers. Samples were only obtained from consenting patients attending the two health facilities. These health facilities receive on average a total of five patients with febrile illness daily. Figure 1, shows the district in which the facilities are located as well as surrounding districts from which patients are received.
Figure 1. Map of Kenya and Busia County (flickr © Albert Kenyani Inima)
3.3 Inclusion criteria

Participants who consented to the study were selected on the basis of the following criteria:

(i) Patients presenting with fever $\geq 37.5^\circ\text{C}$ and confirmed to have been having fever for at least three consecutive days.

(ii) Patients presenting with fever $\geq 37.5^\circ\text{C}$ for three consecutive days and had a negative blood smear preparation for malaria parasites.

(iii) Patients who had been off antibiotics for at least 14 days prior to hospital visit.

(iv) Patients presenting with signs and symptoms of typhoid fever (myalgia, diarrhea, vomiting, abdominal pain, relative bradycardia, palpable masses (hepatomegally and splenomegally), anorexia and headache).

3.4 Exclusion criteria

Patients were excluded from the study based on the following traits:

(i) Patients who had been on antibiotics for at least two weeks prior to the hospital visit.

(ii) Patients presenting with fever $\geq 37.5^\circ\text{C}$ for three consecutive days and had a positive blood smear preparation for malaria parasites.

(iii) Patients who declined to participate in the study.

(iv) Patients presenting without signs and symptoms of typhoid fever (myalgia, diarrhea, vomiting, abdominal pain, relative bradycardia,
palpable masses (hepatomegally and splenomegally), anorexia and headache).

3.5 Sample size determination

Assuming *Salmonella* isolation rate of 9.17% by blood culture (Cheesbrough *et al*., 1997) and a level of significance of 5%, sample size was calculated using Fishers formula as below:

\[
n = \frac{z^2 P Q}{d^2}
\]

Where:-

\( n \) = minimum sample size required

\( z = 1.96 \) (standard normal deviate at the required confidence level)

\( P = 0.0917 \) isolation rate of *Salmonella* by the Widal test

\( Q = 1-P \)

\( d = 0.05 \) (level of significance)

\[
n = 1.96^2 \times 0.0917(1-0.0917) / 0.05^2 = 127 \text{ blood samples. The figure was then rounded off to 150 blood samples.}
\]

3.6 Sampling procedures

Patients attending KEMRI-CIPDCR clinic and Alupe district hospital were examined clinically for signs and symptoms of typhoid fever by a clinician. Systematic sampling method was used to recruit patients whose clinical outcomes suggested possibility of typhoid fever. The objectives of the study
were then explained to the patients who met the inclusion criteria and an informed consent obtained. A unique patient identification study number was given to the participants, recorded on the patient’s record book, consent form (Appendix IIA/IIB) and the structured questionnaire (Appendix III/IV) used to obtain socio-demographic and clinical data. From this point onward, only the patient identification number was used for sample collection, sample processing and results retrieval.

Upon registration, socio-demographic and clinical data were collected by the clinician using a standardized questionnaire (Appendix III/IV respectively). The patient’s samples were then collected at the KEMRI-CIPDCR laboratory. The sample collection materials were clearly labeled with the patient’s identification number. Depending on the age of the patient 5-7 or 10-12 mls of blood specimens was collected with a syringe and needle through vein puncture. About 2 mls was transferred into commercially prepared sterile ethylenediaminetetraacetic acid (EDTA) bottles for separation of serum for use in Widal test and about 5 mls or 10 mls into Hi-Combi dual performance medium. In addition, a stool sample was taken from each patient in order to assess their carrier status for typhoid fever. The specimens collected in the hospital were carried in a cool box to KEMRI-CIPDCR for laboratory investigations.
3.7 Laboratory analysis

3.7.1 Widal test
Standardized *Salmonella* Typhi somatic and flagella antigens Widal test kit (EME, UK) was used for the rapid and semi-quantification of anti-typhoid antibodies in blood samples. Two milliliters of blood were transferred into commercially prepared sterile EDTA bottles and centrifuged at 3,000 revolutions per minute for 5 minutes. Thereafter, one drop of serum was put onto 4 separate slides, H antigen was added to one slide, O antigen to another and a positive and negative control were put onto the remaining two slides. The contents were mixed thoroughly and slides were rocked side to side for 15 minutes. Agglutination indicates a positive result for the Widal test. The results obtained were recorded in the outcome table and were later used for comparison with blood culture outcomes.

3.7.2 Culture of samples

3.7.2.1 Preparation of culture media
The media used for culture was prepared according to the manufacturer’s instructions (Oxoid Ltd., UK).

3.7.2.2 Blood Cultures
The blood culture media used was commercially prepared and was stored in temperature ranges of between 6°C and 18°C according to manufacturer’s instructions. After inoculation, the blood cultures were incubated at 37°C. Turbidity and haemolysis of cultures was then examined daily for up to 21 days. The appearance of turbidity in the bottles indicated growth. Once it occurred, about 1ml of the suspension was drawn and put in a sterile bijou
bottle and the culture bottle returned into the incubator. The suspension was then subcultured onto the different agars.

3.7.2.2.1 Sub-cultures onto agars
Positive cultures were sub-cultured onto blood agar, MacConkey agar and *Salmonella Shigella* (S.S) agar and then incubated overnight at 37°C. An optochin disk was placed onto the blood agar plate to detect any *Streptococcus pneumonia*. The plates were then examined for growth after 24 hours and if no growth was seen, the plate was further incubated for an additional 24 hours. The colonies that grew were subjected to standard identification procedures as described below.

3.7.2.3 Stool cultures
This was done by inoculating approximately 1 gram of stool into 10ml selenite F broth, which was prepared according to the manufacturer’s instructions and incubated at 37°C for 18-48 hours. After turbidity was observed, a subculture of the selenite F broth was made on MacConkey and *Salmonella Shigella* agar. Using a sterile wire loop, a stab was made on the surface of the broth without disturbing the sediment and then inoculated onto the agar plates. The plates were incubated at 37°C for 18-48 hours. On observation of growth, the phenotypic characteristics of the colonies were described and recorded in the patient’s record book before standard identification procedures were performed.

3.7.3 Identification of Salmonella and other bacterial pathogens
Standard identification procedures included colony morphology and gram stain. Briefly, the key characteristics investigated to identify the isolates included
lactose fermentation on MacConkey’s and Salmonella Shigella agar (Oxoid Ltd., UK) and hemolysis on blood agar (Oxoid Ltd., UK). A gram stain was then performed on a single colony as described by Cheesbrough (2000) to differentiate between gram positive and gram negative bacteria and the results recorded. The bacteria isolates were subsequently subjected to sensitivity testing and further identification tests.

3.7.3.1 Catalase test

This test was only done on the gram positive bacteria. Briefly, a drop of hydrogen peroxide was placed on a slide. Using an applicator stick, a colony was picked and then smeared into the hydrogen peroxide drop. The rapid appearance of sustained gas bubbles indicated a positive reaction.

3.7.3.2 Coagulase test

This was done by using rabbit plasma that had been inoculated with a staphylococcal colony; gram positive bacteria. The tube was then incubated at 37°C for one and a half hours. If negative, the incubation was continued for up to 18 hours. Agglutination of the suspension indicated a positive reaction.

3.7.3.3 Oxidase test

This test was done on the gram negative bacteria identified by the gram stain technique. The procedure was carried out according to Barrow and Feltham, 2003 protocol. Briefly, the oxidase reagent discs were placed on a sterile slide using sterile forceps. A drop of normal saline was added to moisten the disc. The colony of the test organism on a wooden stick was rubbed against the oxidase disc. Formation of a purple colour within 20 seconds indicated a
positive oxidase test. The oxidase positive isolates were subjected to sensitivity testing. Subsequently, four tube biochemical tests were done to further identify the oxidase negative, gram negative rods.

3.7.3.4 Biochemical tests
The agars used for the biochemical tests included triple sugar iron, urea agar, Simmon’s citrate agar and motility-indole-lysine medium (Oxoid Ltd., UK). These agars were prepared under sterile conditions according to the manufacturer’s instructions and dispensed into 1 ml test tubes. The colony of interest was picked and emulsified using normal saline in a sterile bijou bottle. The suspension was then inoculated into the four different tubes for the biochemical tests.

Inoculation of the colonies in the triple sugar iron (TSI) agar was done by picking a single colony from the agar plate using a sterilized inoculating needle. A sterile TSI tube was then picked from a rack, the cap removed and the neck of the tube flamed to clear contaminants. A stab was then made up to the butt of the TSI tube and the surface of the slant streaked back and forth. The tube was then loosely capped and returned to the rack. In a similar aseptic technique, inoculation of the motility-indole-lysine medium (MIL) was done by stabbing in a single up and down motion in the centre of the agar going three-fourths of the way down the tube and keeping the wire as vertical as possible. The tube was then loosely capped and returned to the rack.
Simmon’s citrate tube was inoculated by streaking the slanted surface of the agar after which the tube was loosely capped. Inoculation of the urea agar was done by stabbing 2-3 times into the agar and the surface of the slant streaked back and forth before loosely capping the tube and returning it to the rack. The biochemical test tubes were incubated at 37°C for 18-24 hours. They were checked the next day for the different colour changes. The changes were recorded in the patient’s record book and identification of the different organisms followed thereafter.

3.7.4 Antimicrobial susceptibility testing
Antimicrobial susceptibility for all the isolates was performed as described by Scrascia et al. (2003) and Dalsgaard et al. (1996). Commercially prepared single antibiotic discs used included ampicillin-cloxacillin (10µg), amoxicillin-clavulanic acid (30µg), ceftazidine (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), gentamicin (10µg), cefuroximine (30µg), tetracycline (30µg) and co-trimoxazole (25µg). Muller Hinton agar (Oxoid Ltd., UK) was used for sensitivity testing. Briefly, to prepare the inoculum; a colony of the test organism was picked using a sterile wire loop and emulsified in a sterile bijou bottle using normal saline before streaking on Muller Hinton agar plate. A sterile non-toxic cotton swab on a wooden applicator was dipped into the inoculum and the soaked swab rotated to drain excess liquid. The entire agar surface of the plate was streaked with the swab and the inoculum allowed to dry for 5-10 minutes with the lid in place. Then using sterile forceps, the single antimicrobial discs were then placed on the agar making sure they are at least two millimeters away from each other. The plate was then incubated at 37°C
for 18 to 24 hours. After overnight incubation, the plates were examined for growth. Those with no growth were further incubated until the 24 hour limit. Using a ruler on the underside of the plate, the radius of each inhibition zone was measured in millimeters and the value doubled to get the diameter. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the Muller Hinton agar medium. The zones of inhibition were read and results interpreted using standardized thresholds for defining susceptibility as described by NCCLS (1998). The results were reported as sensitive, resistant or intermediate. *Escherichia coli* ATCC 25922 was used as a control strain.

### 3.8 Data analysis

The types of variables generated included: growth of the colonies indicated by color changes, colour changes for the four test tube biochemical tests and susceptibility to antimicrobial agents. Laboratory data was managed using Microsoft access software. All analysis was conducted using STATA version 9.1. Categorical variables were presented as percentages and continuous variables as means followed by standard deviation. The distribution of enteropathogens amongst the population was analysed using the Chi-square test. The relationship between the Widal test outcome and the age and gender was analysed using the Chi-square test. The comparison of antimicrobial resistance patterns of the isolates was analyzed using ANOVA. A p-value of <0.05 was considered as significant.
CHAPTER FOUR: RESULTS

4.1 Patients demographics

A total of 150 patients were recruited to the study at two health facilities from November 2011 to February 2012. One hundred and seven patients (71%) were enrolled at KEMRI-CIPDCR clinic and 43 (28%) at ADH. Ninety three (62%) of the patients were female while 57 (38%) were males. The patient’s age ranged from 0.83 years to 82 years with a mean of 38.7 and standard deviation of 19 as shown in Figure 2 and Table 1. The mean age of the females was significantly different from the mean age of the males. There was no significant difference in the age of the participants across gender ($X^2 = 0.57$ df =144). Majority of the study participants were self-employed (33%) most of them engaging in small scale farming. The other groups included the unemployed 32%, employed 18%, 15% were school going children and 2% were young children as shown in Figure 3.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Standard Deviation</th>
<th>Kurtosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients(All)</td>
<td>38.7</td>
<td>40</td>
<td>0.83</td>
<td>82</td>
<td>19</td>
<td>2.479</td>
</tr>
<tr>
<td>Male</td>
<td>39.857</td>
<td>39</td>
<td>7</td>
<td>82</td>
<td>19.21</td>
<td>2.516</td>
</tr>
<tr>
<td>Female</td>
<td>37.987</td>
<td>40</td>
<td>0.83</td>
<td>80</td>
<td>18.947</td>
<td>2.358</td>
</tr>
</tbody>
</table>

Table 1. Patient’s age statistics

Majority of the patients recruited to the study came from South Teso district (60%) and Busia district (24%) both in Busia County. This could be explained
by the proximity of these areas to the two health facilities. An overview of the geographical location from which the study participants resided is outlined in Table 2. From the information obtained from the study participants, 12% had taken analgesics prior to the hospital visit while none had taken antibiotics two weeks prior to consultation. Drug management during the hospital visit included 91% antibiotics, 95% analgesics and 19% anti-malarials.

![Age distribution](image)

**Figure 2. Age distribution**

![Occupation of the patients enrolled to the study](image)
Figure 3. Distribution of occupation of the participants enrolled into the study

<table>
<thead>
<tr>
<th>District</th>
<th>No. of Males</th>
<th>(%)</th>
<th>No. of females</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Busia</td>
<td>16</td>
<td>28.07</td>
<td>21</td>
<td>22.58</td>
</tr>
<tr>
<td>Kakamega</td>
<td>1</td>
<td>1.75</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>North Teso</td>
<td>3</td>
<td>5.26</td>
<td>5</td>
<td>5.38</td>
</tr>
<tr>
<td>Samia</td>
<td>2</td>
<td>3.51</td>
<td>2</td>
<td>2.15</td>
</tr>
<tr>
<td>Siaya</td>
<td>1</td>
<td>1.75</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>South Teso</td>
<td>32</td>
<td>56.14</td>
<td>59</td>
<td>63.44</td>
</tr>
<tr>
<td>Bugiri</td>
<td>0</td>
<td>-</td>
<td>1</td>
<td>1.08</td>
</tr>
<tr>
<td>Chakol</td>
<td>0</td>
<td>-</td>
<td>1</td>
<td>1.08</td>
</tr>
<tr>
<td>Funyula</td>
<td>0</td>
<td>-</td>
<td>2</td>
<td>2.15</td>
</tr>
<tr>
<td>Nyakach</td>
<td>0</td>
<td>-</td>
<td>1</td>
<td>1.08</td>
</tr>
<tr>
<td>Nambale</td>
<td>2</td>
<td>3.51</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2. Geographical location of study participants

4.2 Most common symptoms presented

The prominent signs and symptoms presented by all the study participants included fever, myalgia, abdominal pains, mild headache and severe headache as illustrated in Table 3. The clinical presentation of bacterial infections caused by *Proteus mirabilis* included fever, myalgia and severe headache. Likewise, those caused by *Escherichia coli* included fever, myalgia, rhonchi, bronchial breathing, abdominal pain, diarrhea, mild and severe headache.
*Staphylococcus aureus* disease presentation included fever, myalgia, abdominal pain, diarrhea and mild headache while that of *Shigella dysentriae* included fever, myalgia, palpable masses, abdominal pain, diarrhea and mild headache.

<table>
<thead>
<tr>
<th>Clinical signs and Symptoms</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever ≥37.5°C</td>
<td>150 (100%)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>106 (71%)</td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>100 (67%)</td>
</tr>
<tr>
<td>Mild Headache</td>
<td>96 (64%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>27 (18%)</td>
</tr>
<tr>
<td>Severe Headache</td>
<td>26 (17%)</td>
</tr>
<tr>
<td>Palpable masses</td>
<td>13 (9%)</td>
</tr>
<tr>
<td>Bronchial breathing</td>
<td>6 (4%)</td>
</tr>
<tr>
<td>Rhonchi</td>
<td>5 (3%)</td>
</tr>
<tr>
<td>Pallor</td>
<td>3 (2%)</td>
</tr>
<tr>
<td>Detention</td>
<td>4 (3%)</td>
</tr>
<tr>
<td>Dehydration</td>
<td>3 (2%)</td>
</tr>
</tbody>
</table>

Table 3. Clinical presentation by study participants

4.3 Typhoid fever diagnosis

Blood samples were collected from 99% (149) of the patients while stool samples were collected from 94% (140) of the patients. Of the 150 patients that presented with fever, 50% (73) showed a positive reaction for the Widal test. There was no significant correlation between the Widal test results and the age and gender of the patients (P=0.341 and P= 0.196, respectively). Of
these seventy three patients, 97% were treated with antibiotics including chloramphenicol, ciprofloxacin and gentamicin.

The prevalence of bacteria isolated from patient’s blood was 4%. It should be noted that only one pathogenic bacterium was isolated from each of the positive cultures for blood and stool. There was no S. Typhi isolated from the blood cultures or stool cultures of all patients who mainly presented with signs and symptoms of typhoid fever. Bacteria of clinical significance isolated from blood include *Escherichia coli* (33.33%), *Proteus mirabilis* (16.67%) and *Staphylococcus aureus* (50%). Pathogenic bacteria isolated from stool cultures included *Escherichia coli* (80 %) and *Shigella dysenteriae* (20%).

**4.4 Bacterial pathogens isolated**

A total of eleven isolates were obtained from the cultures. Bacteria of clinical significance isolated from blood include 27.3% gram positive bacteria and 72.7% gram negative bacteria. The specific species of bacteria isolated are as follows: *Escherichia coli* (33.33%), *Proteus mirabilis* (16.67%) and *Staphylococcus aureus* (50%). Pathogenic bacteria isolated from stool cultures were all gram negative bacteria and included *Escherichia coli* (80%) and *Shigella dysenteriae* (20%). The most prevalent bacteria isolated in blood and stool was *S. aureus* (3 isolates) and *E. coli* (4 isolates) respectively.
### 4.5 Antibiotic susceptibility patterns

The isolates obtained from both stool and blood cultures were subjected to sensitivity testing using several antibiotics. An interpretive chart as shown in Table 4 was used to determine whether the organism was resistant, intermediate or sensitive to the different antibiotics used.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Resistant(mm)</th>
<th>Intermediate(mm)</th>
<th>Sensitive(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (10µg)</td>
<td>≤28¹</td>
<td>-</td>
<td>≥29¹</td>
</tr>
<tr>
<td></td>
<td>≤11²</td>
<td>12-13²</td>
<td>≥14²</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid (30µg)</td>
<td>&lt;13¹</td>
<td>13-18¹</td>
<td>&gt;18¹</td>
</tr>
<tr>
<td></td>
<td>&lt;19²</td>
<td>19-20²</td>
<td>&gt;20²</td>
</tr>
<tr>
<td>Ceftazidine (30µg)ᵃ</td>
<td>≤14</td>
<td>15-17</td>
<td>≥18</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>≤12</td>
<td>13-17</td>
<td>≥18</td>
</tr>
<tr>
<td>(30µg)ᵃ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin (5µg)ᵃ</td>
<td>≤15</td>
<td>16-20</td>
<td>≥21</td>
</tr>
<tr>
<td>Gentamicin (10µg)ᵃ</td>
<td>≤12</td>
<td>13-14</td>
<td>≥15</td>
</tr>
<tr>
<td>Cefuroxime (30µg)ᵃ</td>
<td>≤14</td>
<td>15-17</td>
<td>≥18</td>
</tr>
<tr>
<td>Tetracycline (30µg)</td>
<td>≤14¹</td>
<td>15-18¹</td>
<td>≥19¹</td>
</tr>
<tr>
<td></td>
<td>≤10²</td>
<td>11-18²</td>
<td>≥19²</td>
</tr>
<tr>
<td>Co-trimoxazole (25µg)ᵃ</td>
<td>&lt;10</td>
<td>11-15</td>
<td>&gt;16</td>
</tr>
</tbody>
</table>

¹ zone size for gram negative bacteria  
² zone size for gram positive bacteria  
ᵃ Value for both gram negative and positive bacteria  

**Table 4. The zone size interpretive chart**
Figure 4: *Staphylococcus aureus* isolates on an agar plate

Figure 5: Antibiotic susceptibility test

4.5.1 Overall antibiotic profile of the isolates
All the eleven isolates from both blood and stool were highly susceptible to chloramphenicol and gentamicin. There was also high resistance to ampicillin-cloxacillin, cefuroxime and tetracycline. There is no isolate that was susceptible to ampicillin-cloxacillin. There was no significant difference in the susceptibility patterns to the antibiotics used (F=0.52). The antibiotics used were as follows: Ax- Ampicillin-cloxacillin, CXM- Cefuroxime, Te- Teracyline, C- Chloramphenicol, AMC – Amoxicillin-clavulanic acid, CAZ- Ceftazidine, CIP- Ciprofloxacin, COT- Cotrimoxazole, GEN- Gentamicin.

4.5.2 Antibiotic susceptibility profiles of the individual species

4.5.2.1 Blood isolates

4.5.2.1.1 Staphylococcus aureus

There was high resistance towards ampicillin-cloxacillin and cefuroxime, commonly used in the area for treatment while moderate resistance towards tetracyline and cotrimoxazole was observed. High susceptibility towards gentamicin, ciprofloxacin and chloramphenicol was also observed in the same species. The species showed relative susceptibility to cotrimoxazole, amoxicillin-clavulanic acid and ceftazidine. However no S. aureus isolate was susceptible to ampicillin-cloxacillin and cefuroxime as illustrated in Figure 5 below.
Figure 6. Susceptibility profile for *Staphylococcus aureus* isolated in blood

4.5.2.1.2 *Proteus mirabilis*

The isolate showed high resistance to ampicillin-cloxacillin, cefuroxime, tetracycline, amoxicillin-clavulanic acid, ceftazidine and ciprofloxacin. High susceptibility to gentamicin, cotrimoxazole and chloramphenicol was observed.

Figure 7. Susceptibility profile for *Proteus mirabilis* isolated in blood
4.5.2.1.3 *Escherichia coli*

The isolates were moderately resistant to ampicillin-cloxacillin, cefuroxime, tetracycline and amoxicillin-clavulanic acid while high susceptibility was observed in chloramphenicol, ciprofloxacin, co-trimoxazole and gentamicin.

![Figure 8. Susceptibility profile for *Escherichia coli* isolated in blood](image)

4.5.2.2 Stool isolates

4.5.2.2.1 *Escherichia coli*

Moderate resistance towards ampicillin-cloxacillin, cefuroxime and cotrimoxazole was observed in the isolates. However, the isolates were highly susceptible to chloramphenicol, ceftazidine, ciprofloxacin and gentamicin.
Figure 9. Susceptibility profile for *Escherichia coli* isolated in stool

4.5.2.2.1 *Shigella dysentiae*

The isolate was susceptible to chloramphenicol, ciprofloxacin, gentamicin, ceftazidine, amoxicillin-clavulanic acid, co-trimoxazole, cefuroxime while high resistance was observed towards ampicillin-cloxacillin and tetracycline.

Figure 10. Susceptibility profile for *Shigella dysentiae* isolated in stool
4.6 Distribution of enteropathogens

There were six pathogenic isolates obtained from stool cultures and they include pathogenic *Escherichia coli* (80%) and *Shigella dysenteriae* (20%). The *E. coli* species were considered pathogenic as they were isolated from children below the age of 5 years. These isolates were from patients residing in Busia (44.44%), North Teso (11.11%) and South Teso (44.44%) districts. There were no significant difference in the gender, occupation and location of the patients from which these isolates were obtained as illustrated in Table 5.

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Table 5. Distribution of enteropathogens.
CHAPTER FIVE: DISCUSSION, CONCLUSION AND
RECOMMENDATIONS

5.1 DISCUSSION

Typhoid fever continues to be a burden in most developing countries. Poor
diagnosis has contributed to this, as typhoid fever presents with signs and
symptoms similar to those of other common febrile illnesses. The Widal test,
blood culture and stool culture which are methods commonly used for the
diagnosis of typhoid fever were done simultaneously to increase the probability
and accuracy of detecting typhoid fever cases. It is necessary to note that the
use of the Widal test has been banned in Kenya but it is still widely used in
most health centers (KMLTTB, 2012). Malaria parasite screening was also
done to all patients to eliminate malaria as the primary cause of fever as the
area where the study was carried out is considered malaria endemic.

The findings indicate that the typhoid fever cases reported were diagnosed
using the Widal test. However the same patient’s blood or stool culture did not
confirm S. Typhi isolate. In these particular patients whose Widal test was
reactive, other bacterial organisms were isolated thus emphasizing the
unspecific nature of the Widal test. A single Widal test, using the slide test
technique, is commonly used in the two facilities where the study was carried
out, for the diagnosis of typhoid. Though widely used in such resource limited
settings, it is not reliable and may produce false-positive results thus leading to
over-diagnosis of typhoid fever. Its performance is affected by cross-reactions with non-typhoidal Salmonella, previous immunization with Salmonella antigen, non-bacterial infections such as malaria, dengue, hepatitis A and infectious mononucleosis that lead to an increase in the O antibodies (Ley et al., 2011).

Both gram negative and gram positive bacteria were obtained from the clinical samples. All the isolates obtained from stool samples were all gram negative bacteria while those from blood samples were a mixture of both gram positive and negative bacteria. There were no blood cultures or stool cultures that confirmed positive results for S. Typhi from the study participants who presented with signs and symptoms of typhoid fever infection. The specific bacterial pathogens isolated from the blood cultures include S. aureus, P. mirabilis and E. coli. These results are in agreement with studies done in Tanzania and Kenya where similar bacterial pathogens have been shown to cause bacteremia (Moyo et al., 2010; Crump et al., 2011). The study results further illustrate the importance of gram negative bacteria in causing bacteremia which was also observed in South Korea, where increasing incidences of blood stream infections as a result of antibiotic resistance gram-negative bacilli were noted (Kang et al., 2005).

The cultures done in this study yielded very few organisms. Blood cultures were performed using commercially prepared media, incubated at the right temperature and routinely observed for growth. Therefore, the low yield of blood cultures could not be attributed to technical laboratory errors during
media preparation. The media used was also stored under the right conditions as indicated by the manufacturer. It should also be noted that before the study began, a run of quality control check was performed on all the media used. All of them supported growth of the control strains, namely *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* serovar Typhi, *Salmonella enterica* serovar Typhimurium and *Escherichia coli* ATCC 25922; all obtained from KEMRI-Centre for microbiology research, Kenya (CMR). With this aspect considered, it is in order to report confidently that there were no contaminants present in the media that could have inhibited growth of bacteria pathogens present in both blood and stool.

The low yield of the blood cultures as observed could have resulted from a number of reasons. Firstly, all study participants were outpatients who were relatively strong thus it is assumed that bacteremia had not taken a toll on them. In most studies where high levels of bacteremia are reported, the study population most often consists of in-patients who are admitted to hospital with significant disease level (Berkley *et al.*, 2005). Additionally, there could have been delayed diagnosis of bacteremia including typhoid fever. Essentially, blood cultures produce best results when performed in the first week of infection. This requires keen diagnosis by the clinician and early presentation by the patients to the hospital. In the area where the study was conducted, there are delayed hospital visits by the patients until the disease has progressed. Also, most of the patients are peasant farmers, who may not afford the cost of treatment in hospital. It was observed that most of these patients have several
pre-treatment options usually with available local concoctions (Aloe vera) and analgesics with the hope that the infection will clear away. This is a move to cut costs incurred during hospital visits as well as purchasing of medicine prescribed. Some of the local herbs have bactericidal effects which could in-turn affect the blood culture yields since the bactericidal compounds suppress the growth of bacteria.

Bacteremia at times could be caused by very few counts of bacteria for instance typhoid fever infection is at times caused by very low levels of bacteremia; 10 bacteria/ml (Werner et al., 1967). These few numbers could be missed out during cultures thus leading to misdiagnosis and under reporting of bacteremia. The use of antibiotic prior to hospital visit has been shown to have a bactericidal effect on the bacteria thus preventing their growth in the culture medium (Hosoglu and Wain, 2008). In this study it was difficult to ascertain whether the information given regarding lack of use of antibiotics was true or not. Although the use of Qiagen stool kit would have helped to check for use of antibiotics in the stool samples, this was not possible due to lack of resources to procure this kit.

Another reason for the low yield of cultures could have been due to the reliance on a single blood and stool cultures results as opposed to multiple cultures that are known to increase culture yields. It was not possible to perform multiple cultures due to resource constraints. Additionally, follow up of the patients was
difficult since all the patients recruited in the study were outpatients. Though costly, there is dire need to employ the use of automatic culture systems and the addition of resins in the culture specimens to absorb antibiotics present so as to increase the yield in blood culture outcomes for improved diagnostic methods (Smith et al., 2011).

There was remarkable resistance to some of the empirical formulas used to treat bacterial infections. Most of the isolates both in stool and blood were resistant to ampicillin-cloxacillin, tetracycline and cefuroxime. The high levels of resistance of the blood isolates to cefuroxime could have been attributed to the increasing high usage of orally administered cefuroxime. Resistance for orally administered drugs occurs when patients fail to adhere to the specified dosages or when they fail to clear the drugs prescribed. The isolates showed moderate resistance to tetracycline (33.3%) which is commonly used in adults and these results concur with a study done in Tanzania to establish the causative agents of blood stream infections and their antimicrobial susceptibility. In the study, resistance to tetracycline was reported to be 63.8% (Moyo et al., 2010). *Staphylococcus aureus* was resistant to ampicillin-cloxacillin, tetracycline and cefuroxime. This partly disagrees with the findings of a study in Nigeria in which *S. aureus* isolates showed high susceptibility to ampicillin-cloxacillin and tetracycline (Nkang et al., 2009). The differences in the susceptibility profiles of *S. aureus* in the two studies could be attributed to the observed alterations of resistance and susceptibility of *S. aureus* at relatively high temperatures (Obiazi et al., 2007).
The *E. coli* strains were moderately resistant to amoxicillin-clavulanic acid, cefuroxime and tetracycline. This is in agreement with a study done in Delhi to outline the resistance patterns of bacteria isolated from blood stream infections, where resistance of *E. coli* to cefuroxime and amoxicillin-clavulanic acid was observed (Alam *et al.*, 2011). In the same study relative susceptibility to chloramphenicol, ciprofloxacin and gentamicin was high, a case similar to this study findings. However, this data disagrees with findings in the UK and Ireland where resistance of the *E. coli* isolates to ceftazidime, cefotaxime, ciprofloxacin and gentamicin has been reported and this has been attributed to the upsurge of CTX-M extended-spectrum β-lactamases (ESBLs) (Livermore *et al.*, 2008). The susceptibility patterns shown by the blood and stool isolate of *E. coli* were slightly different probably due to mutation changes that occur as the organisms colonize the different habitats. *Proteus mirabilis* is a common bacterial pathogen isolated in Kenya as reported by a study done to assess bacteremia in children admitted to a rural hospital in Kenya where seven isolates of *P. mirabilis* were obtained (Berkley *et al.*, 2005). The isolate showed relative susceptibility to the antimicrobials used against the organism. However, there was marked high resistance to ciprofloxacin similar to what has been observed in UK and Ireland. In the study, Livermore *et al.* (2008) observed that *P. mirabilis* isolates were non-susceptible to ciprofloxacin.

The enteropathogens isolated were *E. coli* and *Shigella dysenteriae* bacteria pathogens similar to those isolated in stool of patients in a study done in Vientiane (Yamashiro *et al.*, 1998). The antibiogram shows that all the
enteropathogens were susceptible to chloramphenicol, ciprofloxacin and gentamicin. The high susceptibility to the above antibiotics is in agreement with the findings of studies done in Nigeria and Kenya in which high susceptibility to the above antibiotics was also observed (Kariuki et al., 2006; Nkang et al., 2009). This is a welcome relief as there is increasingly widespread multi-drug resistance to the empirical formulas used to clear away bacterial infection. Other antibiotics with relative susceptibilities and useful in clearing bacterial infections in the study area include amoxyclovulin acid, ceftazidine, and co-trimoxazole. It is highly unlikely that the resistance patterns observed could have been as a result of self medication with antibiotics. From the study findings, it is evident that the communities self medicate with anti-malarials which is in part useful as it serves to reduce the widespread multi-drug resistance to antibiotics.

Although the study had several limitations, as outlined below, it still provided useful information. First, by screening patients for malaria and thereafter not including those that had malaria parasites eliminated the cases of co-infections. This was necessary as the study site is a malaria endemic region. Another limitation was screening for invasive bacterial pathogens using a single stool and blood culture. Multiple cultures are highly recommended since the likelihood of obtaining positive results increases with the quantity of stools or blood collected. It was also not possible to collect convalescent sera for the Widal test. All the study participants were outpatients and majority are low income earners; some had to walk long distances to the hospital so they would
not consider a follow up visit to the hospital necessary. It was even observed that only a few came back for the culture results on time. Therefore, it should be noted that it is likely that the study underestimated the true prevalence of typhoid fever within the study population.

It is plausible that misdiagnosis of typhoid fever using the Widal test and subsequent inappropriate use of traditional antibiotics is responsible for the increased antibiotic resistance observed. It is common practice in the two facilities, for the clinicians to request a blood smear for malaria and Widal test simultaneously to those patients presenting with typhoid fever like symptoms. Elevated levels of the O antigen in the plasma are observed during infections such as malaria and typhoid fever. Therefore, when the Widal test is done cross reactions could occur and as a result false positive results are relayed. Cross reactions must have been responsible for the elevated number of positive results for the Widal test observed in the study.

In order to avoid such cases of misdiagnosis, the clinicians should request for a blood smear for microscopy for the diagnosis of malaria and then request a blood culture test if no *Plasmodium* parasites are seen. This measure will also save on the treatment costs incurred by the patient. In such resource limited areas where blood culturing facilities are expensive or lacking, an immunoassay based on detecting of anti-LPS IgM or the prototype anti-serotype Typhi
IgM dipstick test would be of greater diagnostic use than the Widal test (House et al., 2001).

5.2 CONCLUSION

Patients who present with typhoid like symptoms should be screened for other bacteria pathogens in addition to *Salmonella* species. The diagnosis of typhoid fever should not be made solely from clinical presentation of the patient. The above should always be backed up by serial blood cultures considered as the gold standard method or the rapid agglutination kits. Proper and sufficient sanitation facilities should also be provided for areas that lack these, especially those areas facing rural to urban migration. Global campaigns on good hygiene and sanitation like the hand washing campaign should be emphasized in order to reduce morbidity and mortality caused by bacteria transmitted via the fecal oral route. There should also be deployment of vaccines to the masses to curb these diseases. Moreover, there is need for extensive research on novel diagnostic kits for the specific diagnosis of the different bacterial pathogens.

5.3 RECOMMENDATIONS

- The use of gold standard methods for diagnosis of typhoid fever should be encouraged by the health officials.
- The indiscriminate use of antibiotics should be discouraged in order to reduce the cost of treatment and emergence of resistant strains.
There is need for improved hygiene in the living environments to discourage multiplication of these pathogenic bacteria that thrive in poor hygiene conditions.
REFERENCES


Appendix I

KENYA MEDICAL RESEARCH INSTITUTE

Appendix I: Novel Approaches to the Diagnosis, Characterization, and Surveillance of Priority Infectious Diseases

This is to inform you that during the 12th meeting of KEMRI/National Ethics Review Committee held on Tuesday 13th October, 2009, the abovementioned study was reviewed.

The Committee commends you for this timely and useful study/program that will enable the capacity building of the Centre for Infectious and Parasitic Disease Control and Research and wishes you the best in your endeavor.

Due consideration has been given to ethical issues and the study is hereby granted approval for implementation effective the 15th day of October 2009, for a period of twelve (12) months.

Please note that authorization to conduct this study will automatically expire on Thursday, 14th October 2010. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by Thursday, 2nd September 2010.

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the ERC prior to initiation. You may embark on the study.

Yours sincerely,

R. C. Ketherei
FOR: SECRETARY
KEMRI/NATIONAL ETHICS REVIEW COMMITTEE

ESACIPACIISC/497

Maitha Mwai

To:

Director, CIPDCM
P.O. Box 3
KUSA (K)


I am pleased to inform you that the above mentioned proposal, in which you are the PI, was discussed by the KEMRI Scientific Steering Committee (SSC), during its 16th meeting held on Tuesday 8th September, 2009 and has since been approved for implementation by the BSC.

The SSC however, advise that work on this project can only start when ERC approval is received.

Yours sincerely,

C. Mwenda,
PhD
SSC SECRETARY

In Search of Better Health
Appendix IIA

KENYA MEDICAL RESEARCH INSTITUTE

INFORMED CONSENT FORM (TYPHOID STUDY)

AGE≥16 YEARS

Scientists from Kenya Medical Research Institute (KEMRI) are conducting a study to come up with appropriate test kit for typhoid fever. In order to do this, we are asking you to give a small sample of your 12 mls of blood, which will be used for analysis. The results will be availed to your doctor and will be used in the management of your illness. The process of sample collection is harmless though mild pain might be experienced during blood collection. You are free to choose to participate and withdraw anytime. Your refusal to participate or withdrawal from this study at any stage, will not affect the quality of health care given to you. Incase of complaints from this study, you are free to seek advice from Director CIPDCR, KEMRI-Busia (Tel 055-22232 )or Ms. Makwaga Olipher (cell phone 0720406703)

DECLARATION

I understand that, the study is investigating the above mentioned phenomenon that will lead to development of appropriate test kit for diagnosis and management of typhoid fever. I am being asked voluntarily to participate. My participation will involve taking samples of my 10 mls of blood. I understand sample collection is harmless and being conducted by qualified personnel. I further understand that my participation in this study is voluntarily and I may withdraw whenever I choose to anytime unconditionally.

I have read and understood the information stated above.

I sign this consent form willingly:

Name of the patient……………………..Sign……………………….Date……..

Witness………………………………….Date…………………………

Name of officer/s………………………..Sign………………….Date………

NB: Translation to local languages will be done if need be.
Kiambatisho IIA

TAASIS YA UTAFITI YA UTABIBU YA KENYA(KEMRI)

FOMU YA KUPATA KIBALI (UTAFITI WA HOMA YA MATUMBO)

UMRI≥miaka16
Wanasayansi kutoka Kenya Medical Research Institute (KEMRI) wanafanya utafiti kuchunguza njia mwafaka ya utambuzi wa homa ya matumbo. Ili kufanya hivyo, tunachukua sampuli ya 12mls ya damu yako, itakayotumika kwa ajili ya uchambuzi. Matokeo itapewa daktari wako na itatumika katika usimamizi wa ugonjwa wako. Mchakato wa kukusanya sampuli ni wa upole ingawa unaweza hisi maumivu kidogo wakati wa ukusanyaji wa damu. Wewe uko huru kuchagua kushiriki na kuondoka wakati wowote. Kukataa kushiriki au kujiondoa kutoka utafiti huu katika hatua yoyote, hakutathiri huduma za afya utakayopewa. Iwapo utakuwa na malalamiko kutoka kwa utafiti huu uko huru kutafuta ushauri kutoka kwa Mkurugenzi CIPDCR, KEMRI-Busia (Tel 055-22,232) au Bi Makwaga Olipher (simu ya mkononi 0720406703)

TANGAZO

Jina la mgonjwa ......................... Sign .......................... Tarehe ........

Shahidi .................................................. Tarehe .................................

Jina la afisa / s ........................................ Sign ......................... Tarehe ........

NB: Tafsiri kwa lugha za kienyeji itafanyika.
Appendix IIB

KENYA MEDICAL RESEARCH INSTITUTE

INFORMED CONSENT FORM (TYPHOID STUDY)

AGE<16 YEARS

Scientists from Kenya Medical Research Institute (KEMRI) are conducting a study to come up with appropriate test kit for typhoid fever. In order to do this, we are asking you to allow your child to give a small sample of his/her 7 ml of blood, which will be used for analysis. The results will be availed to his/her doctor and will be used in the management of his/her illness. The process of sample collection is harmless though mild pain might be experienced during blood collection. You are free to choose your child to participate and withdraw anytime. Your refusal for your child to participate or withdrawal from this study at any stage, will not affect the quality of health care given to him/her. Incase of complaints from this study, you are free to seek advice from Director CIPDCR, KEMRI-Busia (Tel 055-22232) or Ms. Makwaga Olipher (cell phone 0720406703)

DECLARATION

I understand that, the study is investigating the above mentioned phenomenon that will lead to development of appropriate test kit for diagnosis and management of typhoid fever. I am being asked voluntarily to allow my child to participate. My child’s participation will involve taking samples of his/her 5 ml of blood. I understand sample collection is harmless and being conducted by qualified personnel. I further understand that my child’s participation in this study is voluntarily and I may allow him/her withdraw whenever I choose to anytime unconditionally.

I have read and understood the information stated above.

I sign this consent form willingly:

Name of the parent/Guardian…………….Sign……….Date………………

Witness………………………..Date………………………………

Name of officer/s……………………….Sign………………..Date………

NB: Translation to local languages will be done if need be.
KiAMBATISHO IIB

TAASIS YA UTAFITI YA UTABIBU YA KENYA(KEMRI)

FOMU YA KUPATA KIBALI (UTAFITI WA HOMA YA MATUMBO)

UMRI < miaka 16

Wanasayansi kutoka Kenya Medical Research Institute (KEMRI) wanafanya utafiti kuchunguza njia mwafaka ya utambuzi wa homa ya matumbo. Ili kufanya hivyo, tunachukua sampuli ya 7 mls ya damu yako, itakayotumika kwa ajili ya uchambuzi. Matookeo itapewa daktari wako na itatumika katika usimamizi wa ugonjwa wako. Mchakato wa kukusanya sampuli ni wa upole ingawa unaweza hisi maumivu kidogo wakati wa ukusanyaji wa damu. Wewe(au mwanao) uko huru kuchagua kushiriki na kuondoka wakati wowote. Kukataa kushiriki au kujiondoa kutoka utafiti huu katika hatua yoyote, hakutathiri huduma za afya utakayopewa. Iwapo utakuwa na malalamiko kutoka kwa utafiti huu, uko huru kutafuta ushauri kutoka kwa Mkurugenzi CIPDCR, KEMRI-Busia (Tel 055-22,232) au Bi Makwaga Olipher (simu ya mkononi 0720406703)

TANGAZO


Mimi nimeweka sahihi fomu hii kwa hiari yangu:

Jina la mgonjwa ... ... ... ... ... ... Sign ... ... ... ... ... ... Tarehe ... ... ..... Shahidi ... ... ... ... ... ... Tarehe ... ... ... ... ... ... Tarehe ...

Jina la afisa / s ... ... ... ... ... ... Sign ... ... ... ... ... ... Tarehe ... ...

NB: Tafsiri kwa lugha za kienyeji itafanyika.
Appendix III

KENYA MEDICAL RESEARCH INSTITUTE

PERSONAL DATA (TYPHOID STUDY)

1. Study No…………………………Date…………………………

2. Study site ………………………………………

   KEMRI =1 □  Alupe district Hospital = 2 □

3. Sex: Male=1                Female=2

4. Age: Years  Months  Days

   □□□□

5. Place of residence
   a) Village……………………………………
   b) Sub-location……………………………
   c) Location………………………………
   d) Division………………………………
   e) District………………………………

6. Socio-medical data
   a) Occupation (specify)…………………………

   b) If child, Father`s/Mother`s/Guardian`s occupation
Appendix IV

KENYA MEDICAL RESEARCH INSTITUTE

CLINICAL EXAMINATION (TYPHOID STUDY)

Date ..........................

Health facility........................Study No...........................................

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Observed clinical signs on examination


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## Drug Management

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### Drug management offered during this visit (if any)

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Examined
by…………………………………Sign………………………….Date
Appendix VA

KENYA MEDICAL RESEARCH INSTITUTE

LABORATORY INVESTIGATION FORM (TYPHOID STUDY)

1. BLOOD

Date…………………………

Sex……………………..Age……………………Study No……………………

Type of specimen;

a) Blood…………………………………………………………………………………

Investigation requested
Blood culture…………………………. Widal test……………………………………

Quality of specimen
Good……………………Insufficient (request for another)………………………….

Laboratory report
Widal test…………………………………………………………………………………
Gram stain…………………………………………………………………………………
Bacterial culture……………………………………………………………………………
Antibiotic sensitivity………………………………………………………………………
………………………………………………………………………………………………
Report by………………………………
Sign……………………….Date……………………

Authorized for issue………Sign………………….Date…………………………
Appendix VB

KENYA MEDICAL RESEARCH INSTITUTE

LABORATORY INVESTIGATION FORM (TYPHOID STUDY)

1. STOOL

Date……………………………………

Sex……………………………Age……………..….Study No……………………………………

Type of specimen;

a) Stool……………………………………………………………………

Investigation requested
Stool culture……………………………………

Quality of specimen
Good…………………………….Insufficient (request for another)……………………

Laboratory report
Gram stain……………………………………………………………
Bacterial culture…………………………………………………………
Antibiotic sensitivity………………………………………………
………………………………………………………………………………

Report by………………….. Sign……………………Date……………………

Authorized for issue…………Sign…………………..Date……………………
Appendix VI

Biochemical tests used in the study

Catalase test
Catalase is an enzyme that decomposes hydrogen peroxide into oxygen and water. Hydrogen peroxide forms as one of the oxidative end products of aerobic carbohydrate metabolism. Hydrogen peroxide is a potent oxidizing agent that can destroy the cell if not metabolized. This test is commonly used to differentiate *Streptococci* (negative) for *Staphylococci* (positive).

Coagulase test
This test identifies whether an organism produces the exoenzyme coagulase which causes the fibrin of blood plasma to clot. When coagulase is present, it reacts with fibrinogen in plasma, causing the fibrinogen to precipitate. This causes the cells to agglutinate, or clump together, which creates the lumpy look of a positive coagulase test. *S. aureus* produces free coagulase while *S. epidermidis* does not. This test is also useful for differentiating potentially pathogenic *Staphylococci* such as *S. aureus* from other gram positive, catalase-positive cocci.

Simmons Citrate
It is used to distinguish members of the enterobacteriaceae and other gram-negative rods on the basis of citrate utilization as their sole carbon source and ammonium as the sole source of nitrogen. The medium contains citrate, ammonium ions and bromotymol blue indicator. The colour of the indicator is green but when the pH rises as a result of citrate utilization it turns to a blue colour. Positive result is indicated by blue color formation initially on the agar slant and eventually spread to the rest of the agar with time.
**Motility-Indole lysine medium**

It is utilized to demonstrate motility, lysine decarboxylase activity and indole ring production. In tubes with a motile organism, visible growth is seen extending away from the stab line. The agar becomes visibly turbid. For negative results, growth is only seen along the stab line and the agar remains clear. The colour of the agar in the tubes in which the organisms utilize the amino acid turns to a light shade of the purple colour usually distinct from un-inoculated tubes with purple colour. Negative results are observed when the agar in the middle of the tube turns yellow.

For the Indol reaction, a second reagent is added after recording the motility and lysine decarboxylation reaction characteristics. About 3-4 drops of Kovac’s reagent are added to the surface of the tube. For appositive reaction, the Kovac’s reagent turn pink-red while for a negative reaction no colour change is observed and the Kovac’s reagent remains orange-yellow.

**Triple Sugar Iron**

It is used to differentiate enterocactericeae according to their ability to ferment lactose, glucose and glucose with or without gas production. The ability of the organism to produce hydrogen sulphide from thiosulphate in an acid environment is also tested. The TSI agar contains three sugar; glucose, lactose and sucrose, pH indicator phenol red and ferrous sulfate. Fermentation of glucose alone will show as a yellow colour in the butt of the medium while the slant retains the colour of the indicator as there is an alkaline reaction due to utilization of peptones. The fermentation of sucrose and/or lactose will cause both butt and slant to be yellow. The slant and butt colour changes are recorded. Acid production results in a yellow colour because the phenol red indicator turns yellow at low pH. Hydrogen sulphide production from thiosulphate is seen as black areas in the medium due to ferrous sulphate production. Air production is usually seen as air bubbles in the medium or cracking of the medium. Interpretation of the characteristics is as follows;
Carbohydrate fermentation
Alkaline slant/alkaline butt-no sugars fermented
Alkaline slant/acid butt-only glucose fermented
Acid slant/acid butt-glucose fermented along with lactose and/or sucrose

Gas production
Positive-gas bubbles in agar or splitting of agar
Negative-no bubbles or splitting of agar

H₂S
Positive-black colour along the streak or throughout the medium
Negative-no black colour.

Urea Agar
It differentiates organisms based on urease activity. Organisms which produce urease split urea into carbon dioxide and ammonia. The ammonia then combines with water to form ammonium carbonate which raises the pH of the medium leading to a shift of the pH as detected by the phenol red indicator. An intense pink colour is observed on the slant which later spreads to the whole agar indicates a positive result.

Oxidase
This test assays for the presence of cytochrome oxidase which catalyses the transfer of electrons from the reduced cytochrome c to molecular oxygen. The test reagent, N,N,N’,N’-tetramethyl-p-phenylenediamine dihydrochloride, acts as an artificial electron donor for the enzyme oxidase. Oxidase reagent is colourless in its reduced state and dark purple in its oxidized state.