MOLECULAR EPIDEMIOLOGY OF *SALMONELLA TYPHI* AMONG PATIENTS ATTENDING GARISSA PROVINCIAL GENERAL HOSPITAL

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Molecular Epidemiology of *Salmonella Typhi* among Patients Attending Garissa Provincial General Hospital

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DECLARATION

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

Signature……..Date……

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This thesis has been submitted for examination with our approval as the University Supervisors

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DEDICATION

I dedicate this project to the Mighty God, for giving me strength, power of mind, protection and skills to persevere through to the end of this process. I also dedicate this work to my mother who has been the source of my inspiration, guide strength, moral, spiritual and emotional support throughout the years of this study.

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ABBREVIATIONS AND ACRONYMS

CDC	Centre for Disease Control and Prevention
DCA	Desoxycholate Citrate Agar
ERC	Ethical Review Committee
HIV	Human Immunodeficiency Virus
IPs	Intestinal Perforations
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
KIA	Kliger Iron Agar
LIA	Lysine Iron Agar
MAC	MacConkey
MDR	Multidrug Resistant
PCR	Polymerase Chain Reaction
SIM	Sulfide Indole Motility
SSC	Scientific Steering Committee
TCV	Typhoid Conjugate Vaccine
UI	Uncertainty Interval
UN	United Nation
WHO	World Health Organization

ABSTRACT

Typhoid and paratyphoid fever a food and water-borne disease is caused by *Salmonella enterica* serotype *Typhi* (*S. Typhi*) and *S. enterica* serotype *Paratyphi*. The disease is an important cause of illness and death especially among children in developing countries majorly due to poor sanitation and unsafe food and water. Quantification of *salmonella* burden is therefore crucial not only for management but also for preventative measures. This cross-sectional study therefore determined the epidemiology and antimicrobial resistance patterns in bacterial etiologies of enteric fever among patients attending Garissa County Referral Hospital, (GCRH) located in a semi-arid region of North Eastern Kenya. Blood and stool samples were obtained from 379 consenting patients and a detailed sociodemographic questionnaire was administered. Isolation and identification of *Salmonella Typhi*, *S. Paratyphi* A and *S. Paratyphi* B were done by conventional culture, PCR and Vitek-2 compact detection method. Antimicrobial susceptibility testing was done using Kirby-Bauer's disc diffusion method. Multidrug resistance was defined as co-resistance to ampicillin, chloramphenicol and co- trimoxazole. The mean (± standard deviation - SD) age of the participants was 37.3 (±13.3) years ranging (18 to 95 years). The majority of the participant 54.4% were female, 58% married, 39.3% had secondary level education, 75.2% had body temperature >37.1ºC, while 58.8% had headache. There were 7.4% participants who had laboratory confirmation of typhoid while 35.1% had history of typhoid fever. Eight of the 379 (2.1%) participants were positive for *Salmonella* spp by Vitek and PCR *Salmonella*-specific gene (*inv*A). Of the 8 *Salmonella* isolates there were *S.* Typhi (n=2; 25%), *S. Paratyphi* A (n=2; 25%) and *S. Paratyphi* B (n=4; 50%). Resistance to ampicillin, tetracycline, gentamycin, chloramphenicol, nalidixic acid and trimethoprim-sulfamethoxazole was 100%, 87.5%, 75%, 50%, 25% and 25%, respectively. All isolates were susceptible to ciprofloxacin. Half of all *S. Typhi*, *S. Paratyphi* A and B were multidrug-resistant. In multivariate analysis, the factors associated with *Salmonella* infection included laboratory confirmation of typhoid (OR 66.6, 95% CI 5.8-757.2), often eating outside homestead (OR 5.3, 95% CI 1.4-12.4), family eating from a common plate (OR 6.1, 95% CI 1.2-21.2), taking locally prepared cold drinks (OR 6.9, 95% CI 1.4-32.3), family wash hands in common basin (OR 7.3, 95% CI 1.9-31.2) and monthly income Kshs <20,000.00 (<200USD) (OR 0.2, 95% CI 0.003-0.8). The isolation of a large proportion of MDR *S. Typhi, S. Paratyphi* A and B is worrying. Although these isolates were susceptible to fluoroquinolones, there is need for routine surveillance to monitor susceptibility to the initial first line antibiotics. Addressing issues of contaminated food, water, sanitation and hygiene and low socio-economic status is likely to prevent and reduce the burden on enteric fever in this region.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Enteric fever generally refers to Typhoid and paratyphoid fevers which are caused by systemic infection with *Salmonella enterica* subspecies serovars *Typhi* and *Paratyphi* A, B, and C (Harris & Brooks, 2020). While most non-typhoidal *Salmonella* spp. infections characteristically produce diarrheal illness and less commonly cause bloodstream infection, typhoid and paratyphoid infections are associated produce primarily bacteraemic febrile illnesses, with prolonged high fever, headache, and malaise being characteristic symptoms (GBD 2017 Typhoid and Paratyphoid Collaborators, 2019). Without effective treatment, typhoid and paratyphoid fevers can lead to altered mental states (termed the typhoid state), ileus, gastrointestinal bleeding, intestinal perforation, septic shock, and death (Harris and Brooks, 2020). Generally Typhoid fever is transmitted via the faeco-oral route through food or water, contaminated with urine or faeces of a patient or a chronic carrier (Nabarro *et al*., 2022). Consequently, clean water, hygiene and good sanitation are recommended avenues to prevent the spread of typhoid and paratyphoid diseases.

Typhoid and paratyphoid infections are relatively common in countries with poor water supply and sanitation, especially south Asia, southeast Asia, and sub-Saharan Africa, where they are a major cause of death and disability, especially among children (Mogasale *et al*., 2014; GBD 2017 Disease and Injury Incidence and Prevalence, 2018). Globally, 14·3 million (95% uncertainty interval [UI] 12·5–16·3) cases of typhoid and paratyphoid fevers occurred in 2017. Various incidence rate of typhoid and paratyphoid fevers have been reported in 2017: About 71·8% of global case representing for the highest age-standardized incidence rate (549 cases per 100 000 person-years) and the largest number of cases (10·3 million) were reported in south Asia (GBD 2017 Disease and Injury Incidence and Prevalence, 2019). The east Asia, and Oceania super-region accounted for 14·1% of global cases (2·02 million), and the sub-Saharan Africa super-region accounted for 12·1% (1·73 million) (GBD 2017 Disease and Injury Incidence and Prevalence, 2018). The GBD 2017 Disease and

Injury Incidence and Prevalence report of 2018 further shows that 76·3% of all global cases were due to *S. Typhi* accounting for 10·9 million cases of typhoid fever compared to 3·4 million cases of paratyphoid fever globally. Reports by Mogasale *et al*., (2014); Kim *et al*., (2017); suggest that incidence of Typhoid and paratyphoid fever is highest in south Asia, followed by southeast Asia, western sub-Saharan Africa, eastern sub-Saharan Africa, and Oceania. The estimates by Mogasale *et al*., (2014); Kim *et al*., (2017), further suggest that incidence rates in central and eastern sub-Saharan Africa are similar to those of south Asia. Some local estimates of typhoid incidences in different African regions have been made.

In Africa, incident rates of Typhoid and paratyphoid fever are as high as 200 to 500 per 100,000 in Burkina Faso and Ghana to 130 to 200 per 100,000 in Kenya, Ethiopia and other east African countries (Ng'eno *et al*., 2023). Lower incident rates 0 to 15 per 100,000 have been reported in Southern part of Africa (GBD 2017 Disease and Injury Incidence and Prevalence, 2018). Different prevalence rates have been reported in Kenya ranging from 38% positive for Typhoid in Moyale region (Galgallo *et al*., 2018), 6.4% S. *Typhi* from Kibera and 0.6% in rural Lwak region of Nyanza (Breiman *et al*., 2012). The incidence rates are generally highest among children and HIV-infected adults who have low CD4 T-lymphocyte counts with mortality rates of nearly 10–30% (Reddy *et al*., 2010; Ng'eno *et al*., 2023). Typhoid and paratyphoid fever are associated with high morbidity and mortality in Africa (Tabu *et al*., 2012) and has also been established to occur in high frequencies in urban settlements (Breiman *et al*., 2012). Globally, in 2017 about 135·9 thousand deaths were due to typhoid and paratyphoid fever. Similar to incidence rates, south Asia had the highest mortality rates representing 69·6% of global deaths, followed by the sub-Saharan Africa super-region accounting for 15·9% of global deaths. Mortality rates were highest among young children, with 17·2% of global deaths reported among children younger than 5 years of age (GBD 2017 Disease and Injury Incidence and Prevalence, 2019).

The risk of enteric fever is highest in infants, young children and young adults with underlying comorbidities, including severe anaemia, malaria, malnutrition and HIV infection (Church *et al*., 2014). The case fatality rate is high in those with HIV

infection and among those living in lower-income countries due to overpopulation and poor hygiene (Church *et al*., 2014; WHO, 2018).

Problems are also emerging with the clinical treatment of typhoid in resource-poor settings. For many years, the antibiotics chloramphenicol, ampicillin, and cotrimoxazole formed the mainstays of typhoid treatment. In the past two decades, multidrug-resistant (MDR) strains of *S. enterica* have emerged worldwide (Phan *et al*., 2009). Reduced susceptibility to fluoroquinolones has been reported in Kenya (Kariuki *et al*., 2019). Changing trends in antibiotic resistance among *S. enterica* has appeared against ampicillin, co-trimoxazole, and chloramphenicol have also been reported in Kenya (Kariuki *et al*., 2006; Kariuki *et al*., 2019). Critical therefore, understanding local and regional antimicrobial susceptibility trends is vital in guiding empiric therapy. Monitoring and reporting of antimicrobial susceptibility can guide public health decision-making on the need for control strategies including vaccination. While improvements in drinking water quality and sanitation have helped eradicat the disease from the majority of developed countries (Meiring *et al*., 2019; UN-Water, 2023), short- to medium-term control of the pathogen through vaccination is highly recommended in reducing disease burden in low-income settings (Meiring *et al*., 2017; Gibani *et al*., 2018). In 2017 the WHO recommended the use of typhoid conjugate vaccines (TCVs) in high-burden countries and the Gavi commitment for funding the introduction of TCVs into eligible countries (WHO, 2017).

In Kenya, MDR *S. Typhi* isolates from adults and school age children associated with sporadic outbreaks in resource-poor settings, especially in slum areas, have been reported (Kariuki *et al*., 2004). Unfortunately, however, data are scarce on the epidemiology and antimicrobial resistance pattern of *S. Typhi* and *S. Paratyphi* species in Garissa County. This geographical region is marked by lack of one or more of the following five conditions: access to improved water, access to sanitation, durable housing, sufficient living area, and secure tenure (UN-HABITAT, 2006) essentially presenting risk factors for transmission of *S. Typhi* and *Paratyphi* occurs through consumption of contaminated food or water via short-cycle or long-cycle transmission. Evidence from studies in Kenya, India, Egypt, and Bangladesh demonstrate that morbidity and mortality in such areas are much higher than the national averages

(Kimani-Murage *et al*., 2014; Cara *et al*., 2022). Against this backdrop, this study was undertaken to determine the epidemiology and antimicrobial Resistance Pattern of *S. enterica* serovars in cases of clinically suspected enteric fever among patients in Garissa County, a Semi-Arid Region of North Eastern Kenya.

1.2 Statement of the Problem

Typhoid and paratyphoid infections are prevalent in developing countries marked by poor water supply and sanitation and sub-Saharan Africa is among these countries (Mogasale *et al*., 2014; GBD 2017 Disease and Injury Incidence and Prevalence, 2018). Typhoid fever is an acute, life-threatening febrile illness caused by the bacterium *Salmonella enterica* serotype *typhi*. (Enteric fever) (CDC, 2008) and remains a major public health problem in developing countries even in the twenty first century. Globally, 14·3 million cases of typhoid and paratyphoid fevers were reported in 2018. Higher Typhoid and paratyphoid fever have been reported in Kenya at 100 to 200 per 100,000 (GBD 2017 Disease and Injury Incidence and Prevalence, 2018). High mortality due to typhoid and paratyphoid fever have been reported both globally at about 135·9 thousand deaths with sub-Saharan Africa super-region accounting for 15·9% of global deaths. Mortality rates were highest among young children, with 17·2% of global deaths reported among children younger than 5 years of age (GBD 2017 Disease and Injury Incidence and Prevalence, 2019). Intraregional difference prevalence rates have been reported in Kenya ranging from 0.6% to 38% (Galgallo *et al*., 2018). The government of Kenya and some private partners have directed their efforts and resources towards the control of water and sanitation related diseases such as typhoid, cholera among others. This was done through provision of improved water and sanitation, vaccination, environmental health education and prompt treatment (UN-HABITAT, 2006). Prevalence of typhoid fever in Garissa is not known and data on prevalence and risk factors of typhoid fever among patients and its molecular epidemiology in Kenya remains sparse despite the country being among the endemic regions for typhoid infection. There is need to determine the prevalence of antibiotic resistant typhoid fever in adult patients seeking medical attention evaluating the associated factors.

1.3 Justification

Typhoid and paratyphoid fever is an important cause of illness and death, particularly among children and adolescents in developing countries where enteric fever is associated with poor sanitation and unsafe food and water (GBD 2016 Disease and Injury Incidence and Prevalence, 2017). Quantification of disease burden is crucial for policy making about the deployment of enteric fever prevention measures and vaccines. The catchment area for the Garissa Provincial General Hospital includes Dadaab refugee camp (~400,000 refugee population), the world largest refugee camp which has challenges with provision of safe drinking water and other basic amenities. Further, the town is overpopulated due to the movement of refugees in and out of the town leading to poor sanitation and lack of hygiene resulting to frequent outbreaks of diarrheal diseases, intestinal worms and water borne diseases such as typhoid and dysentery (UN-HABITAT, 2006). The Garissa municipal water treatment process is also inadequate with water that is brown with silt running from the taps for most of the time.

Despite the public health burden, few reports exist about risk factors for transmission of typhoid fever in endemic areas (Breiman *et al*., 2012; Galgallo *et al*., 2018). Most epidemiological investigations of *S. typhi* transmission are conducted during acute typhoid fever outbreaks (CDC, 2008). However, given the limited generalizability of data derived from outbreaks, it is important to understand the risk factors for typhoid transmission for early detection, control and management of outbreaks. Equally the diagnostic test routinely used to make diagnosis of typhoid is Widal test which is no longer recommended by WHO and has been banned by the health authorities in Kenya (WHO, 2018). There is possibility of over/under diagnosis of typhoid fever in this area. In determining the prevalence this study will further provide evidence for the need for better methods for monitoring the emergence and spread of typhoid to enable better control and treatment.

The rise in MDR *S. Typhi* isolates is a concern in Kenya which have been associated with sporadic outbreaks in resource-poor settings, especially in Nairobi area (Kariuki, 2019), and among children <16years (Kariuki *et al.*, 2022). Data are however few on

the epidemiology and antimicrobial resistance pattern of *S. Typhi* and *S. Paratyphi* species in Garissa County. This study aimed at determining the prevalence of typhoid fever and associated risk factor among adult patients attending Garissa Provincial General Hospital. The findings from this study will be useful in establishing the prevalence of Salmonellosis in the region and the possible predisposing risk factors for infection and the rate at which it may be underreported. Additionally, the antibiotic susceptibility profiles would be useful as a guide for consideration during empirical treatment of Salmonellosis as well as in influencing policy change both in management and control of typhoid in areas the disease may be endemic.

1.3 Research Questions

1. What is the prevalence of typhoid fever among adult patients with febrile illness attending Garissa Provincial General Hospital?
2. What are the antibiotic susceptibility profiles of the *Salmonella typhi* isolated from adult patients with febrile illness attending Garissa Provincial General Hospital?
3. What are the virulence factors in isolated *Salmonella typhi*?
4. What are the risk factors associated with typhoid fever among adult patients with febrile illness attending Garissa Provincial General Hospital?

1.4 General Objective

To determine the molecular epidemiology of *Salmonella typhi* isolated from patients with febrile illness attending Garissa Provincial General Hospital

1.5 Specific Objectives

1. To determine the prevalence of *Salmonella typhi* from patients with febrile illness attending Garissa Provincial General Hospital.
2. To determine the antibiotic susceptibility profiles of *Salmonella typhi* isolated from adult patients with febrile illness attending Garissa Provincial General Hospital.

3. To detect virulence factors in *Salmonella typhi* from adult patients with febrile illness attending Garissa Provincial General Hospital.
4. To determine risk factors associated with the rate of *Salmonella typhi* infection among adult patients attending Garissa Provincial General Hospital outpatient clinic.

CHAPTER TWO

LITERATURE REVIEW

2.1 Typhoid Fever

Typhoid fever is an acute systemic infection caused by the bacterium *Salmonella enterica* serotype Typhi (*S. Typhi*) and *S. enterica* Paratyphi A, B, and C cause the clinically similar condition, paratyphoid fever (WHO, 2018). Typhoid and paratyphoid fevers are collectively referred to as enteric fevers. In most endemic areas, approximately 90% of enteric fever is typhoid. Typhoid is transmitted by the faecal-oral route via contaminated food and water and is therefore common where sanitary conditions are inadequate and access to clean water and proper sewage system is limited (Galgallo *et al*., 2018). Although typhoid fever was common in the United States and Europe in the 19th century, it is now encountered mostly throughout the developing world. In the last fifteen years, the emergence of resistance to the antibiotics used for treatment has led to large epidemics, and complicated the management of this serious disease (Ochiai *et al*., 2008).

2.2 Epidemiology of Typhoid Fever

With an estimated global incidence of 14·3 million illnesses and 135923 deaths annually (116815 and 19108 deaths typhoid and Paratyphoid, respectively), typhoid fever caused by *Salmonella typhi* remains an important public health problem in many tropical and sub-tropical countries (GBD 2016 Disease and Injury Incidence and Prevalence, 2017). Reports shows that Typhoid fever accounts for high morbidity and mortality rates mainly in developing countries (GBD 2016 Disease and Injury Incidence and Prevalence, 2017). In sub-Saharan Africa, the incidence of typhoid fever is greater than 100 per 100,000 persons per year resulting in 21,578 deaths accounting for 16% of global typhoid deaths in Africa (GBD 2017. Typhoid and Paratyphoid Collaborators, 2019). Incidence may vary from one country to another due to dynamics in risk factor exposure levels attributed to the disease; some countries like Egypt report low incidence rate (13/100,000–59/100,000 persons annually) while others like Kenya have reported high adjusted incidence rate of up to 247 cases per 100,000 persons in

an informal settlement (Mogasale *et al*., 2014; Kariuki *et al*., 2015; Lee *et al*., 2016; Mutai *et al*., 2018). These figures may, however, be overestimated based on the controlled risk factors (Mutai *et al*., 2018).

This problem is especially pronounced in the developing countries due to many interrelated factors that include among others variable efficacies of currently available vaccine preparations, unplanned urbanization with the growth of peri-urban slums lacking safe water supply and sanitation facilities, and increased regional movements of large numbers of migrant workers (CDC, 2008). Report by Kariuki *et al*., (2015) shown the high incidence of typhoid fever in urban informal settlements. In many typhoid endemic areas, human immunodeficiency virus (HIV) infection is a serious public health concern (Gordon *et al*., 2003). Although not reported from other endemic areas, a study in Peru has indicated that typhoid fever was 60 times more frequent in HIV-infected individuals as compared to the general population, presumably due to HIV-induced impairment of host's natural antibacterial activity against *S. typhi* and direct fecal-oral transmission of *Salmonella* within the homosexual population (Gotuzzo *et al*., 1991).

2.3 Distribution of Typhoid Fever

Enteric fever is a global major public health problem (GBD 2017. Typhoid and Paratyphoid Collaborators, 2019). The real impact of typhoid fever is difficult to estimate because the clinical picture is confused with those of many other febrile infections. Additionally, the disease is underestimated because there are few bacteriology laboratories in most areas of developing countries. These factors are believed to result in many cases going undiagnosed. On the basis of the literature (CDC, 2014). The incidence of typhoid fever recorded in control groups in large vaccine field trials with good laboratory support has been estimated that approximately 17 million cases of typhoid fever and 600 000 associated deaths occur annually (Ivanoff *et al.*, 1994). However, the estimates have been biased because study populations have usually been in areas of high incidence (Figure 2.1). Furthermore, these estimates of burden relate to the clinical syndrome of typhoid fever but not to *S. typhi* exposure. Preliminary results from recent studies conducted in Kenya show that

in the urban site, the overall crude incidence of *Salmonella enterica* serovar *typhi* (*S. typhi*) bacteremia was 247 cases per 100,000 person-years of observation (pyo) with highest rates in children 5–9 years old (596 per 100,000 pyo) and 2–4 years old (521 per 100,000 pyo). Crude overall incidence in Lwak (a rural site) was 29 cases per 100,000 pyo with low rates in children 2–4 and 5–9 years old (28 and 18 cases per 100,000 pyo, respectively). Adjusted incidence rates were highest in 2 to 4-year-old urban children (2,243 per 100,000 person-years of observation) which were 15-fold higher than rates in the rural site for the same age group (Breiman *et al*., 2012).

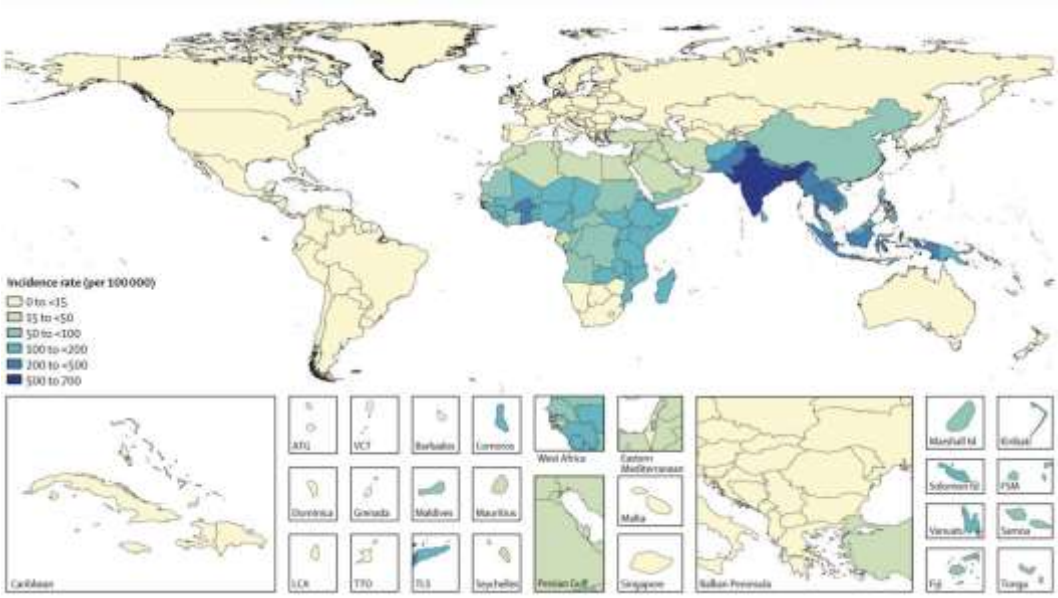

Figure 2.1: Incidence Rates (per 100 000) of Typhoid and Paratyphoid Fevers, by Country, in 2017

Key

Unfilled locations are those for which GBD does not produce estimates. The inset maps detail smaller locations. ATG=Antigua and Barbuda. FSM=Federated States of Micronesia. GBD=Global Burden of Diseases, Injuries, and Risk Factors Study. Isl=Islands. LCA=Saint Lucia. TLS=Timor-Leste. TTO=Trinidad and Tobago. VCT=Saint Vincent and the Grenadines. (Adopted from GBD 2017. Typhoid and Paratyphoid Collaborators, 2019)

Typhoid fever also has a very high social and economic impact because of the hospitalization of patients with acute disease, subsequent complications and loss of income (Kimani-Murage *et al*., 2014; Cara *et al*., 2022). Reports from some provinces in China and Pakistan have indicated more cases of paratyphoid fever caused by *S. paratyphi* A than by *S. typhi* (WHO, 2018). In areas of endemicity and in large outbreaks, most cases occur in persons aged between 3 and 19 years. In 1997, for example, this age range was reported during an epidemic of the disease in Tajikistan. Nevertheless, clinically apparent bacteraemic *S. typhi* infection in children aged under three years has been described in Bangladesh, India, Jordan, Nigeria and elsewhere (Saha *et al.*, 2001).

In Indonesia there is a mean of 900 000 cases per year with over 20 000 deaths. In Indonesia, people aged 3 to 19 years accounted for 91% of cases of typhoid fever and the attack rate of blood-culture-positive typhoid fever was 1026 per 100 000 per year. A similar situation was reported from Papua New Guinea (Antillo´n *et al*., 2018). When typhoid fever was highly endemic in certain countries in South America the incidence of clinical typhoid fever in children aged under 3 years was low. In Chile, however, single blood cultures for all children aged under 24 months who presented at health centers with fever, regardless of other clinical symptoms, showed that 3.5% had unrecognized bacteraemic infections caused by *S. typhi* or *S. paratyphi* (GBD 2017. Typhoid and Paratyphoid Collaborators, 2019).

Between 1% and 5% of patients with acute typhoid infection have been reported to become chronic carriers of the infection in the gall bladder, depending on age, sex and treatment regimen. The propensity to become a carrier follows the epidemiology of gall bladder disease, increase in age and being greater in females than in males (Saha *et al.*, 2001; Cara *et al*., 2022). The propensity to become a chronic carrier may have changed with availability and selection of antibiotics as well as with the antibiotic resistance of the prevalent strains. The role of chronic carriers as a reservoir of infection was studied in Santiago, Chile, where a crude rate of 694 carriers per 100 000 inhabitants was found (GBD 2017 Typhoid and Paratyphoid Collaborators, 2019).

2.4 Antigenic Characteristics of Typhoid Causing Bacilli

Salmonella typhi is a member of the *Salmonella* genus which belongs to the *Enterobactericeae* family of gram-negative bacteria. Other genera in this family include *Shigella*, *Escherichia*, and *Yersinia*, all of which include species that are important causes of intestinal infections and diarrhoeal diseases in human (Pegues and Miller, 2015). *S. typhi* is in group D *Salmonella* according to the classification by Kauffman and White (Ivanoff *et al.,* 1994). *S. typhi* is rod-shaped with a length of 2-3μm and a diameter of 0.4-0.6 μm. *S. typhi* is motile, with peritrichous flagella (H-d antigen), which is also encountered in 80 other bioserotypes of *Salmonella*. *S. typhi* contains three antigenic structures (Levine, 1999): somatic or O antigens, corresponding to bacterial endotoxin, are involved in the production of fever; H-d is a protein associated with flagella; and Vi-antigen (for virulence) is a polysaccharide on the exterior of the cell wall. In general, boiling of *S. typhi* cells destroys flagellar antigens because these are proteins (Levine, 1999).

Vi-antigen, which is also present in *Citrobacter freundii*, *S. para*typhi C, and *S. dublin* (Hashimoto *et al.*, 1995), interferes with the complement (C3b)-mediated opsonisation of *S. typhi* and thereby inhibits phagocytosis by preventing *S. typhi* from binding with the phagocytes (Harris and Brooks, 2020). Vi-antigen also determines phage susceptibility (Wain *et al*., 2002). Scattered along the conserved backbone of the *S. typhi* genome are the clusters of genes designated as "*Salmonella* Pathogenicity Islands" (SPI) that probably regulate the invasion of the intestinal wall by *S. typhi* (Wain *et al*., 2002). However, invA gene affects invasion but not attachment to cultured epithelial cells; sequence similarity to proteins for protein translocation.

2.5 Mode of Transmission of Typhoid

Humans are the only natural host and reservoir for *Salmonella enterica* serovar *typhi*. The infection is transmitted by ingestion of food or water contaminated with faeces (Mama and Alemu, 2016). The highest incidence of typhoid fever occurs where water supplies serving large populations are contaminated with faeces. Epidemiological data suggest that waterborne transmission of *S. typhi* usually involves small inocula, whereas foodborne transmission is associated with large inocula and high attack rates

over short periods (Mama and Alemu, 2016). The inoculum size and the type of vehicle in which the organisms are ingested greatly influence both the attack rate and the incubation period (Whitaker *et al*., 2009; WHO, 2018).

2.6 Clinical Signs and Symptoms of Typhoid Disease

Typhoid fever is an illness caused by *S. Typhi* bacterium and is associated with the following symptoms: High fever, flu-like symptoms and diarrhea (Whitaker *et al*., 2009). It should however be noted that Typhoid fever, does not always present with a distinct clinical picture. In fact, clinical manifestations of typhoid fever are extremely inconsistent, often non-specific, and clinically indistinguishable from other infections. The incubation period of typhoid fever is about 14 days, but it may be as short as seven days or longer than 21 days. This appears to vary inversely with the size of the infecting inoculum (Hornick, 1994; Whitaker *et al*., 2009).

The onset of bacteremia is marked by fever and malaise. Patients typically present to the hospital toward the end of the first week after the onset of symptoms with fever, influenza-like symptoms with chills (although rigors are rare), a dull frontal headache, malaise, anorexia, nausea, poorly localized abdominal discomfort, a dry cough, and myalgia, but with few physical signs (Sattar *et al*., 2016). A coated tongue, tender abdomen, hepatomegaly, and splenomegaly are common. Adults often have constipation, but in young children and in adults with HIV infection, diarrhea is more common (Vinh *et al*., 1996). It is unusual for a patient hospitalized with typhoid to have no abdominal symptoms and normal bowel movements. Initially the fever is low grade, but it rises progressively, and by the second week it is often high and sustained (39° to 40°C). A few rose spots, blanching erythematous maculopapular lesions approximately 2 to 4 mm in diameter, are reported in 5 to 30 percent of cases. They usually occur on the abdomen and chest and more rarely on the back, arms, and legs. These lesions are easily missed in dark-skinned patients. Convulsions may occur in children under five years of age (Butler *et al*., 1991). Complications occur in 10 to 15 percent of patients and are particularly likely in patients who have been ill for more than two weeks. Many complications have been described, of which gastrointestinal bleeding, intestinal perforation, and typhoid encephalopathy are the most important.

Intestinal (usually ileal) perforation is the most serious complication, occurring in 1 to 3 percent of hospitalized patients (Van Basten & Stockenbrugger, 1994).

Typhoid fever is among the water-borne infections characteristic of environments with poor sanitation and hygiene. Typhoid fever is a health problem that has been associated with development (Farooqui *et al*., 2009). Human infection with *Salmonella* is mainly by the oral route through ingestion of faecal contaminated food and water, unclean hands, flies and meat from infected animals (Hosoglu *et al*., 2006). Typhoid and paratyphoid germs are passed in the faeces and urine of infected people. People become infected after eating food or drinking beverages that have been handled by a person who is infected or by drinking water that has been contaminated by sewage containing the bacteria. Once the bacteria enter the person's body they multiply and spread from the intestines, into the bloodstream (WHO, 2012). Even after recovery from typhoid or paratyphoid, a small number of individuals (called carriers) continue to carry the bacteria. These people can be a source of infection for others.

The transmission of typhoid and paratyphoid in less-industrialized countries may be due to contaminated food or water (WHO, 2012). In some countries, shellfish taken from sewage-contaminated beds is an important route of infection. *Salmonella typhi* have somatic antigens and glycolipid microcapsule the vi or virulence antigen. Phage typing can distinguish different strains of the organism. Enteric fever caused by *Salmonella typhi* is often encountered in tropical countries including Kenya where they constitute serious sources of morbidities and mortalities (Galgallo *et al*., 2018).

Salmonella are divided into distinct serologic groups (A through E) on the basis of their somatic O antigens (Olopoenia *et al*., 2000). While all group D organisms, such as *S. typhi* possess O antigen 9, about 60 of the 78 groups D serotypes including *S. typhi* also have O antigen 12 (Olopoenia *et al*., 2000). Thus, infection by any of the group D serotypes can produce antibodies that can react with the O antigen used in the Widal reaction (Olopoenia *et al*., 2000). Also, since all groups A and B organisms possess O antigen 12, cross-reactions with O antibody of group D serotype can occur with any of the group A and B serotype O antigens. Depending on the relative quality and quantity of antigenicity of the O antigens 9 and 12 contained in other common

non-typhoidal *Salmonella serotypes*, cross-reaction may occur frequently enough to lessen considerably the diagnostic specificity of the Widal agglutination reaction (Olopoenia *et al*., 2000).

In endemic areas, most individuals are carriers. Thus, 35.9% of such apparently healthy persons have been detected with normal antibody titres of up to 1:40 and 1:80 for O and H *Salmonella antigens* (Tanyigna *et al*., 1999) and the levels reflected severity of infection with *Salmonella*.

Based on the immunology of *Salmonella* infection, serological diagnostic tests relying on *Salmonella antigens* as tentative evidence of Salmonellosis have been developed, notably, the widal agglutination test (Olopoenia *et al*., 2000). Agglutination is a classic serologic reaction that results in clumping of a cell suspension by a specific antibody, directed against a specific antigen. Such tests have been widely used for detection of antibodies against various disease-producing microorganisms in serum for a long time (Olopoenia *et al*., 2000). *S. typhi* can be identified in the laboratory by several biochemical and serological tests. One of the most specific is that of polysaccharide capsule Vi, which is present in about 90% of all freshly isolated *S. typhi* and has a protective effect against the bactericidal action of the serum of infected patients. This capsule provides the basis for one of the commercially available vaccines. Vi antigen is present in some other bacteria (*Citrobacter freundii, Salmonella paratyphi C* and *Salmonella dublin*) but not in exactly the same genetic context (WHO, 2003).

2.7 Laboratory Diagnosis of Typhoid Disease

Typhoid and paratyphoid fever most often present as clinically similar acute febrile illnesses, and accurate diagnosis relies on laboratory confirmation (Crump *et al.,* 2003). Bone marrow and blood culture remains the gold standard diagnostic test for enteric fever (Olsen *et al.,* 2004). Efforts to develop serologic methods for the diagnosis of typhoid fever that improve on the poor performance of the Widal test still suffer from substantial limitations of both sensitivity and specificity (Olsen *et al.,* 2004).

Serological approaches to the diagnosis of *S. para*typhi A, B, and C have been developed but have not been evaluated or adapted for field use (Chart *et al.*, 2007). Consequently, blood culture, a less sensitive method than bone marrow culture, is often the practical first choice test for both patient diagnosis and epidemiologic evaluation of *S. typhi* and *S. paratyphi* burden. However, most enteric fever occurs in low-and middle-income countries where blood cultures are often unavailable, unaffordable, or inconsistently applied (Archibald & Reller, 2001).

The most robust approach to the measurement of incidence of typhoid and paratyphoid fever is by regular, community-wide household visits to identify persons with febrile illness from whom blood samples for culture confirmation may be obtained. Alternatively, the results of surveys of health-seeking behavior and sentinel health care facility-based surveillance may be combined to estimate incidence (Crump *et al.*, 2003).

Because of the limited availability of blood culture services and the logistic challenges of enteric fever surveillance techniques capable of measuring disease incidence, the burden of typhoid and paratyphoid fever is poorly characterized in much of the world, particularly in sub-Saharan Africa (Crump *et al.*, 2008). To reduce gaps in the current understanding of typhoid fever incidence, complications, and case-fatality rate, large population-based studies using blood culture confirmation of cases are needed in representative sites, especially in low and medium human development index countries outside Asia (Crump *et al.*, 2008).

2.8 Treatment and Antimicrobial Resistance of *S. Typhi*

Appropriate antibiotic treatment (the right drug, dose, and duration) is critical to curing typhoid with minimal complications (Mills-Robertson *et al*., 2002). But problems are also emerging with the clinical treatment of typhoid in resource-poor settings. For many years, the antibiotics chloramphenicol, ampicillin, and cotrimoxazole formed the mainstays of typhoid treatment. However, outbreaks of multidrug-resistant (MDR) *S. typhi* (Phan *et al*., 2009) prompted the widespread use of fluoroquinolones, such as ciprofloxacin and ofloxacin. Fluoroquinolone usage was followed by the emergence of nalidixic acid-resistant *S. typhi* exhibiting reduced susceptibility to

fluoroquinolones in the early 1990s (Le, *et al*., 2007), and it has since become widespread (Phan *et al*., 2009; Kalckreuth *et al*., 2016; Kim *et al*., 2017). Thus, the spread of MDR and fluoroquinolone resistance in *S. typhi* presents significant clinical challenges. In Kenya, MDR *S. typhi* isolates from adults and school age children associated with sporadic outbreaks in resource-poor settings, especially in slum areas, have been reported (Kariuki *et al*., 2004; Baker *et al*., 2016). This resulted in the use of fluoroquinolones and third generation cephalosporins as alternatives for treatment of MDR *S. Typhi* cases (Dougan, 2017; Mutai *et al*., 2018).

2.9 Prevention and Control of Typhoid Disease

Humans are the only reservoir of *S. typhi* and typhoid fever is transmitted via the fecal-oral route through ingestion of contaminated food and drinks (Moorhead, 2002). Therefore, preventive strategies against typhoid fever should include provision of: safe water supply, disposal of human feces in a sanitary manner and maintaining fly-proof latrines, hygienic preparation of food and drinks, maintenance of cleanliness and hygiene during the preparation of food at home, adequate hand-washing facilities wherever food is handled (Hall *et al*., 2014).

Since the early recognition of the role of water in the transmission of typhoid fever, it has been demonstrated that improvements in access to clean water and improved sanitation result in dramatic reductions in typhoid fever–related death rates in many settings (Moorhead, 2002). Furthermore, safe drinking water and sanitation have been declared a human rights issue by the international community, due to their importance in human health (Hall *et al*., 2014). It is clear that as access to safe water and improved sanitation are being developed, this should dramatically reduce the exposure to *S. Typhi* and *S. Paratyphi* bacteria in the environment and, thus, enteric fever disease. However, global progress toward universal access to both safe water and improved sanitation at the household level where the health benefits are optimal are inadequate, and are likely compounded by several factors including inequity in coverage, where the most vulnerable populations have the poorest access; increasing urbanization; and increasing water scarcity in many regions (Cumming *et al*., 2014).

Exclusion of cases and carriers of typhoid fever from food-handling tasks, educating the public about the importance of hand-washing after visiting the toilet, others include: changing diapers/nappies and avoidance of foods and drinks that maybe contaminated by bacteria e.g. improperly cooked foods, snacks prepared by street vendors, and consumption of untreated tap water; and destruction of houseflies (Dyson et al., 2019).

2.10 Typhoid Vaccines

The old parenteral killed whole-cell vaccine was effective but produced strong side-effects because of lipopolysaccharide (Cara *et al*., 2022). Two safe and effective vaccines are now licensed and available. One is based on defined subunit antigens, the other on whole-cell live attenuated bacteria. The first of these vaccines, containing Vi polysaccharide, is given in a single dose subcutaneous or intra muscular. Protection begins seven days after injection, maximum (Steele *et al*., 2016; Cara *et al*., 2022). In 2008 the World Health Organization (WHO) recommended the consideration of two licensed typhoid vaccines (Vi-polysaccharide and Ty21a), for programmatic use by countries with high rates of typhoid fever for controlling endemic disease, as well as for use in outbreak settings and for travelers to endemic areas (WHO, 2012; Vij *et al.,* 2022). However, with the expectation of second-generation typhoid conjugate vaccines (TCV) becoming available in the near future Gavi, the Vaccine Alliance, deferred decisions on funding support until TCVs were licensed and prequalified by WHO (Steele *et al*., 2016). Proof-in-principle of TCV efficacy is derived from trials of the USNIH Vi-rEPA vaccine, which showed efficacy of up to 92% at 24 months and 89% at 46 months has been demonstrated in trials of the Vi-rEPA vaccine carried out in Vietnam, when given as a two-dose schedule in 2–5-year-olds (Vij et al., 2022). protection being reached 28 days after injection when the highest antibody concentration is obtained. The live oral vaccine Ty2la is available in enteric-coated capsule or liquid formulation. Travelers should be revaccinated annually. This vaccine is licensed in 56 countries in Africa, Asia, Europe, South America, and the USA (WHO, 2012). Several TCVs are currently in development, including Vi-CRM197and Vi-diptheria toxoid conjugates, many of which are in Phase 1and 2 trials (Mohan *et al*., 2016). There are currently two Vi-tetanus toxoid conjugates licensed on the basis

of immunogenicity. One such vaccine, PedaTyphTM (a Vi-polysaccharide tetanus toxoid conjugate manufactured by Bio-MedÒ) has been trialed in a school-based cluster randomized study in an urban slum setting of Kolkata, India, although published data are currently limited in size to allow for firm conclusions (Mitra *et al*., 2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Garissa Provincial General Hospital in Garissa town. Garissa County is in the North Eastern Province of Kenya and covers an area of 44,175 km^2. The total population of Garissa County is approximately 623, 060 with, males being 334,939 and females 288,121 respectively (http://garissaonne.usclargo.com). Garissa town is situated North East to the capital Nairobi and East of Somalia and about 100 km to the East of Garissa is Dadaab town, the world's largest refugee camp (Figure 3.1). The county is arid and semi- arid. The lifestyle in the county is nomadic but Garissa town has a more settled lifestyle. The landscape is flat with no mountains. The major physical features are seasonal laghas (seasonal stream) and the Tana-River basin in the western side of the county. The temperature ranges from 25^0C to 38^0C.

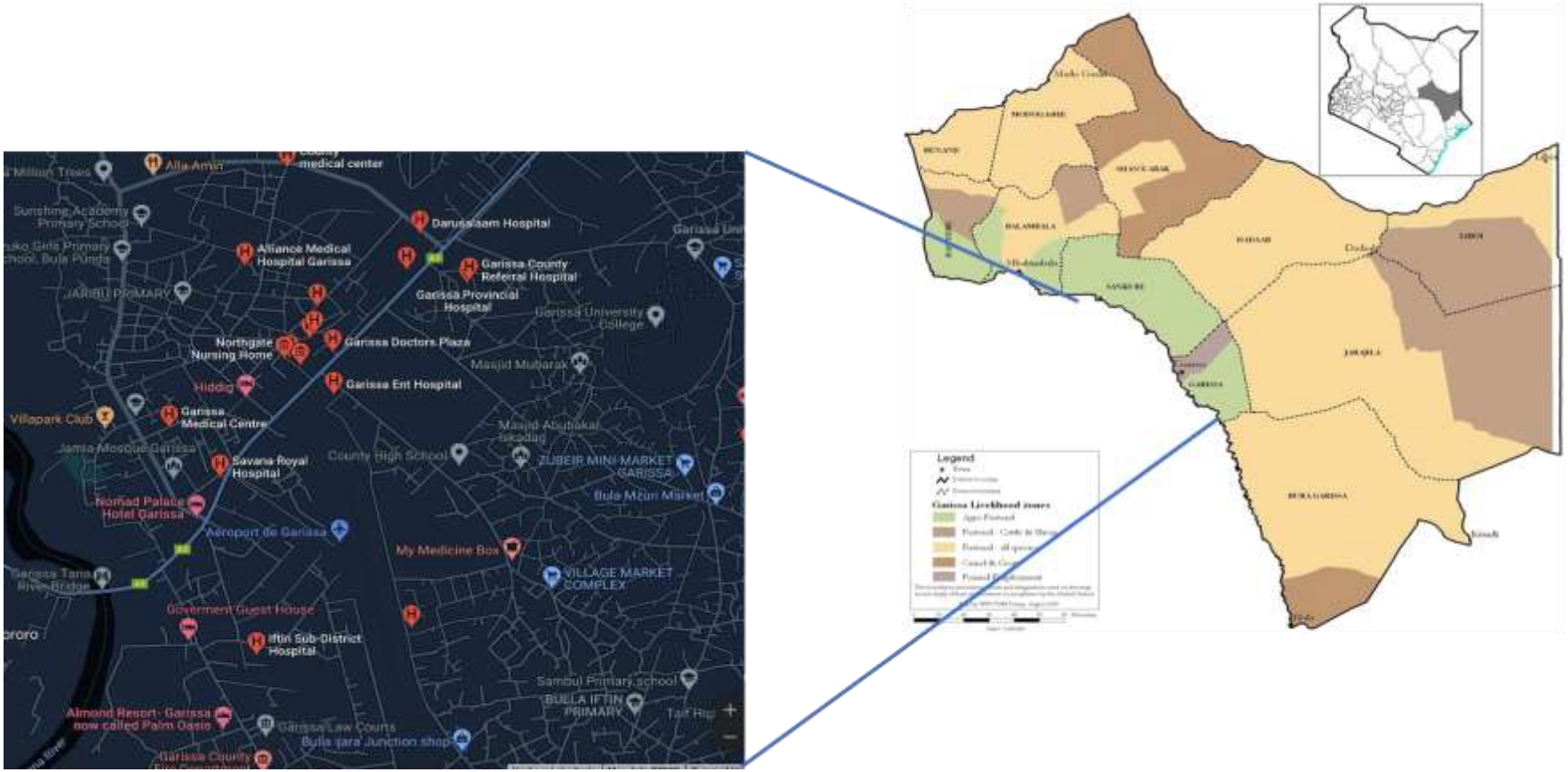

Figure 3.1: Map of Kenya Showing the Study Area (Garissa County).

(Source: www.mapsofworld.com100,000 pyo).

3.2 Study Design

This was a descriptive cross-sectional study. It was a hospital-based study including only patients with likely typhoid fever, and the proportion of these who would be confirmed to have typhoid fever.

3.3 Study Population

The study population comprised of adult (above 18 years of age) inpatient and outpatient with febrile illness attending Garissa Provincial General Hospital and clinic during the study period.

3.3.1 Inclusion Criteria

All consenting adults presenting at Garissa Provincial General Hospital with fever (38°C and above) that had lasted for at least three days and with either of the following symptoms abdominal pain, vomiting, diarrhea, constipation, headache, weakness, arthralgia or poor response to antimalarial medications.

3.3.2 Exclusion Criteria

Adults who came for treatment without the above symptoms, those on antibiotic and those who refused to give consent to participate in the study were excluded.

3.4 Sample Size Determination

The Lemeshow and Lwanga (1991) absolute precision formula was used to calculate the sample size. To describe the prevalence of typhoid fever, the sample size was calculated using the formula below which assumed a prevalence of 50%, since the prevalence of typhoid fever had not been documented from the study site. The other reason being that the sample size formula for this study was precision based, because the researcher was interested in finding out the confidence interval of the prevalence in the study area other than testing a hypothesis.

$n=Z^2P\ (1-P)\ /d^2$

n = Minimum sample size required

Z = 1.96 Standard error.

P = 0.50% (assumed prevalence of 50% will be used)

q = (1-p)

d = degree of accuracy desired; 0.05 the inverse of 95% confidence limit

$n = 1.96^2(0.5)\ (0.5)/\ 0.05^2 = 384$

A sample size of 384 was used.

3.5 Sampling

All patients meeting the inclusion criteria were recruited until the required sample size of 384 was reached. Informed consent for participation was sought and explained in the language understood by the participant. Patients were recruited consecutively from the outpatient department. Each suspected case of typhoid was reviewed by a qualified clinical officer.Vital parameters were taken and entered into a patient data sheet. At the outpatient laboratory qualified laboratory personnel administered the questionnaire. A venipuncture was done aseptically and 10ml of blood drawn from each patient and a stool sample was also collected from each patient. Samples were processed at Garissa provincial general hospital laboratory and only positive blood and stool specimens were subjected to further characterization for *S. typhi* strains at the Centre for Microbiology Research (CMR) Nairobi according to laboratory standard operating procedures.

3.6 Data Collection Tools

A pre-designed piloted interviewer administered questionnaire **(Appendix I)** that included socio-demographic data, information on age, gender, education level, family size and occupation was used for data collection. Residence past history of typhoid

and availability of toilets were also included. Desk review of Health records and key informants were also interviewed.

3.7 Study Setting and Design

This was a descriptive cross-sectional study design conducted between March and December, 2018 consented adult population attending Garissa Provincial General Hospital. This study was approved by Ethical Review Committee of Kenya Medical Research Institute (KEMRI/SSC No. 2464).

3.7.1 Culture of Blood

The blood was cultured in broth media containing brain heart infusion and para-aminobenzoic acid, incubated at 37°C, for up to 7 days and subculture when turbid onto sheep blood agar and MacConkey plates. All suspected *Salmonella* colonies were picked from the agar plates subjected to biochemical tests, PCR amplification of invA gene and subsequently serotyped using the VITEK 2 system, Version 0.8.01 (bioMe´rieux, Inc., Hazelwood, MO).

3.7.2 Culture of Stool

All stool samples were placed in Cary Blair media and then plated into MacConkey (MAC) and Deoxycholate Citrate Agar (DCA). Portions of whole stool were also placed into Selenite –F broth for subculture and incubated at 37ºC overnight. A subculture of Selenite broth on Mac Conkey agar, and xylose-lysine deoxycholate agar were made from the surface of the broth without disturbing the sediment. The plates were incubated at 37°C for 18-24 hours. All suspected *Salmonella* colonies were picked from the agar plates subjected to biochemical tests, PCR amplification of invA gene and subsequently serotyped using the VITEK 2 system, Version 0.8.01 (bioMe´rieux, Inc., Hazelwood, MO).

3.7.3 Biochemical Tests

All suspected *Salmonella* colonies were picked from the agar plates and inoculated into the following biochemical test tubes for confirmation as described by Kebede *et*

al., (2016): triple sugar iron (TSI) test (presumptive *Salmonella* colonies produce black colonies or colonies with black centers and red medium on TSI agar) (OXOID, England), citrate test (presumptive *Salmonella* colonies produce blue color for the citrate test), urease test (presumptive *Salmonella* colonies produce purple-red color for the urease test), lysine decarboxylase (LDC) agar (OXOID, England) test (presumptive *Salmonella* colonies produce purple-colored colonies on LDC agar), and indole test (presumptive *Salmonella* colonies produce violet-colored colonies for the indole test). Colonies were also tested for catalase production.

3.7.4 Serotyping of Salmonella Isolates

The bacterial isolates positive by the genus-specific PCR were serotyped by slide agglutination test targeting specific flagellar antigens. Further, serotyping was done using the VITEK 2 system, Version 0.8.01 (bioMe´rieux, Inc., Hazelwood, MO) according to the manufacturer's instruction

3.7.5 Molecular Detection of the Virulence Gene in *S. Typhi*

The boiling method was used to extract Salmonella plasmid or DNA template. Briefly, a single bacterial colony was picked from the Luria–Bertani (LB) agar plate, boiled in 50µl distilled water for 10 min and immediately cooled on ice for 5 min. After a short spin, 4µl of this solution was used as PCR template as described by Kebede *et al*., (2016). The bacterial DNA template was amplified using 0.5 µM primers specific for invA gene comprising of: forward primer GAG GAA GGG AAATGA AGC TTT T and reverse primer TAG CAA ACT GTCTCC CAC CAT AC, PCR buffer [10mM Tris/HCl (pH 8,3), 50mM KCl, 3mM MgCl, and 0.01% gelatin], 200 µM of each dNTP, and 1.0 U AmpliTaq Gold enzyme (Roche Molecular Systems, Inc, Brachburg, New Jersey, USA). The mixtures were amplified under the following cycling conditions: 40 cycles at 94ºC for 1 minute, 55ºC for 1 minute, and 72ºC for 2 minutes, with a final extension at 72ºC for 10 minutes in an automated thermal cycler (Control system PC- 710; Astec; Tokyo, Japan). An aliquot of 10µl of each amplified product was visualized in 2% (wt/vol) agarose gel electrophoresis.

3.7.6 Antimicrobial Susceptibility Testing of Isolates

Each isolate was tested for susceptibility to antimicrobials by a controlled disk diffusion technique on Diagnostic Sensitivity Testing (DST) agar (Oxoid Ltd., Basingstoke, United Kingdom) plates containing 5% lysed horse blood. *Salmonella* isolates were tested for susceptibility to the following 7 antimicrobials (all from OXOID, England): ampicillin (10μg), tetracycline (30μg), trimethoprim/sulfamethoxazole (30μg), chloramphenicol (30μg), gentamicin (10μg), ciprofloxacin (5μg), and nalidixic acid (30μg) using the disk diffusion method according to guidelines set by the Clinical Laboratory Standards Institute (CLSI, 2017). Antibiotic impregnated discs were dispensed on the surface of cultures of Muller-Hinton agar and incubated at 35°C for 20 hours. The diameters of the zones of inhibition were recorded to the nearest millimeter and classified as resistant, intermediate, or susceptible according to established interpretive chart (CLSI, 2017; CLSI, 2023).

3.7.7 Quality Control

Escherichia coli ATCC 25922 (with known MICs) was used as a reference strain in the disk diffusion susceptibility tests.

3.8 Data Collection, Entry and Cleaning

An informed consent to participate in the study was obtained from the participants using a standard consent form designed for this study (Appendix i). After the participant was interviewed, a questionnaire (Appendix ii) was administered by trained data collectors (Laboratory technologist) from PGH Garissa. All questionnaires were coded and validated daily during data collection. Data were entered in a computerized database designed in Epi Info data entry software to minimize data entry errors. Data cleaning and validation was done before analysis.

3.8.1 Data Analysis

Data were analyzed using Epi Info software version 3.3.2 (CDC, Atlanta, GA, USA, 2005). Descriptive statistics was carried out by calculating the mean, standard

deviation, 95% confidence interval and frequencies of different variables such as age, sex, and clinical history generated. Prevalence of typhoid fever was calculated by dividing the number of culture positive by the total number of the samples analyzed. Univariate analysis was performed to identify the possible risk factor for *S. typhi*. Chi-square with Yates correction test of statistical significance was used to determine any association between typhoid infection and various exposure variables. Results were considered significant at $P< 0.05$. Odds Ratio was used to compare positivity of typhoid infection with history of exposure to risk factors. Risk factor variables with $p<0.05$ were considered as having significant association with typhoid fever. Stratified analysis was used to identify socio-demographic factors to be included in unconditional logistic regression. Logistic regression analysis was performed with typhoid fever as the dependent variable to identify predictors and to control effects of potential confounding factors.

3.9 Ethical Considerations

3.9.1 Study Approval

Approval for the study was obtained from the Board of Postgraduate studies of Jomo Kenyatta University of Agriculture and Technology (JKUAT) and from the Kenya Medical Research Institute (KEMRI) Scientific Steering Committee (SSC) and Ethical Review Committee (ERC) before the study was initiated.

3.9.2 The Informed Consent Document

Informed consent to participate in the study was obtained from the participants using a standard consent form designed for the study. All participants required to take part in the study were requested to sign a consent form after study description given to them and the consequences of participating in the study explained to them prior to enrollment into the study.

3.9.3 Confidentiality of Records

Participant's confidentiality was ensured by use of unique identifiers only known to the principal investigator. Without their consent, no information that revealed their

identity was released or published to any unauthorized person. Records containing information about them were constantly kept under key and lock system and will be finally destroyed five years after the study period. Hard copies of questionnaires were stored at all times in lockable drawer accessible only to the principal investigator. Data were entered into a password protected database and subject consent was sought before removing blood and administering the questionnaire. Information obtained from patients for this study was kept confidential and was used only for the purposes of the study.

3.9.4 Risks or Discomfort to the Study Participants

No major risks were associated with this study but the participants may have experienced some discomfort when removing blood. Those found to be suffering from typhoid fever were referred for management and treatment at Garissa provincial general hospital or the nearest health facility.

3.9.5 Benefits to the Study Participants or Others

The study participants benefited from the care and management offered by the study clinician, but in the long run, the result of this study will be used to guide in the choice of typhoid vaccination as well as antibiotic strategy to implement, during empirical treatment of Salmonellosis as well as in influencing policy change both in preventing and management of typhoid infection in areas the disease may be endemic. The results obtained from antibiotic susceptibility were also beneficial to the patients as they were a guide to proper treatment.

3.9.6 Plans for Dissemination of the Research Findings

As part of the informed consent, all participants were made to understand that the findings of this study were to be presented to the university during my thesis defense and would be shared with the stakeholders at the two ministries of health and other policy decision makers through presentations at national and international

conferences. Findings of this study would also be published in peer reviewed journals without revealing the identity of the study participants. The results of this study will be used to give a health education package to the community at the health facilities through presentation in conferences and seminars, to enhance effective preventive interventions to typhoid infection in the study area.

CHAPTER FOUR

RESULTS

4.1 Socio-Demographic Characteristics of Study Participants

In this study, a total of 379 participants consented and responded to the structured questionnaire. Table 4.1 summarizes the baseline demographic characteristics of the study participants.

4.1.1 Gender

There were nearly equal distributions with regards to gender 206 (54.4%) female verses 173 (45.6%) male. There was no significant difference in the distribution of study participants with regards to gender ($\chi 2 = 2.873$; df = 1; P = 0.09) (Table 4.1).

4.1.2 Age

The mean (± standard deviation - SD) age of the participants was 37.3 (±13.3) years ranging from 18 to 95 years. The majority 151(39.8%) of the participants were aged between 18 to 30 years while the least 52 (13.7%) of participants were aged > 51 years. There was significant difference in the distribution of study participants with regards to age ($\chi 2 = 62.953$; df = 3; P = 0.0001) (Table 4.1).

4.1.3 Marital Status

The majority of the study participants 220 (58%) were married, 122(32.2%) were single while only 13(3.4%) were widowed. There was significant difference in the distribution of study participants with regards to marital status ($\chi 2 = 296.768$; df = 3; P = 0.0001) (Table 4.1).

4.1.4 Education level

The majority of the participants in this study 149 (39.3%) had secondary level of education followed by 112 (29.6%) who had none-formal education. Further, there were 49 (12.9%) who had primary level of education. There was significant difference

in the distribution of study participants with regards to education level ($\chi 2 = 63.29$; df = 3; P = 0.0001) (Figure 4.1).

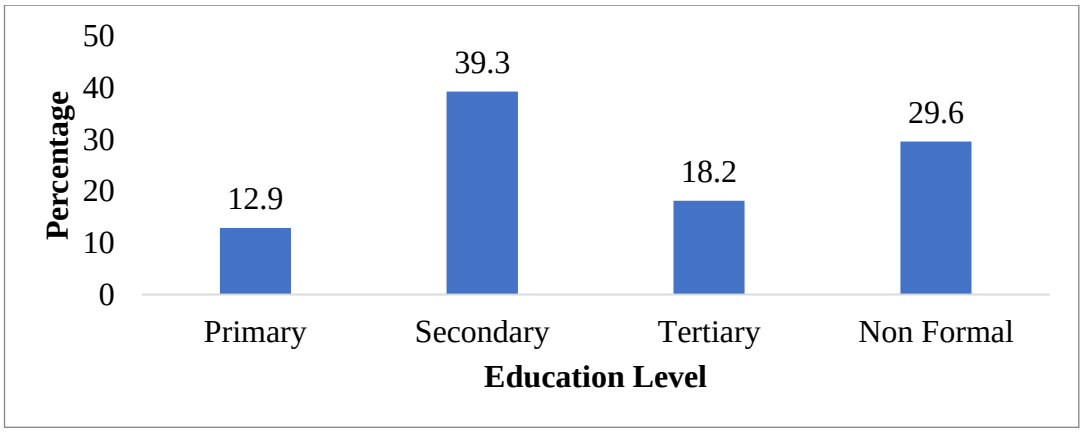

Figure 4.1: Distribution of Study Participants by Education Level

4.1.5 Occupation of Study Participants

The majority 177(45.1%) of the participants were in various businesses followed by 112 (29.6%) who were unemployed, 64 (16.9%) in various employment and the least 32 (8.4%) who were farmers. There was significant difference in the distribution of study participants with regards to occupation or cadre ($\chi 2 = 116.04$; df = 3; P = 0.0001) (Table 4.1).

4.1.6 Religion

The majority 303(79.9%) of the participants in this region were Muslims while 76(20.1%) were Christian. There was significant difference in the distribution of study participants by their religious affiliations ($\chi 2 = 135.96$; df = 1; P = 0.0001) (Table 4.1).

4.1.6 Household Size

The mean (± standard deviation - SD) household size was 5.25 (±3.5) ranging from 1 to 31 population. The majority 255(67.3%) of the participants were in from households with household size between 1 to 5 followed by 95(25.1%) household with 6 to 10 household size while the least 29(7.7%) household had more than 11. There was

significant difference in the distribution of study participants with regards to size of household ($\chi 2 = 213.805$; df = 2; P = 0.0001) (Table 4.1).

Table 4.1: Socio-Demographic Attributes of Study Participants

Variables	Frequency	Percentage	Chi-square	df	p value
Gender					
Male	173	45.6	2.873	1	0.09
Female	206	54.4			
Age (Years)					
Mean (± SD)	***37.3***	***(± 13.3)***			
Range	***77***	***(18-95)***			
18 - 30	151	39.8			
31 - 40	109	28.8	62.953	3	0.0001
41 - 50	67	17.7			
>51	52	13.7			
Marital status					
Single	122	32.2			
Married	220	58	296.768	3	0.0001
Separated\Divorced	24	6.3			
Widowed	13	3.4			
Education level					
Primary	49	12.9			
Secondary	149	39.3	63.29	3	0.0001
Tertiary	69	18.2			
Non Formal	112	29.6			
Occupation					
Business	171	45.1			
Employed	64	16.9	116.04	3	0.0001
Unemployed	112	29.6			
Farming	32	8.4			
Religion					
Christian	76	20.1	135.96	1	0.0001
Muslim	303	79.9			
Household population					
Mean (± SD)	***5.25***	***(± 3.5)***			
Range	***30***	***(1-31)***			
1 to 5	255	67.3	213.805	2	0.0001
6 to 10	95	25.1			
> 11	29	7.7			

4.2 Clinical Presentation of Patients

4.2.1 Body Weight

The mean (± standard deviation - SD) body weight of the participants was 63.7 (±11.1) Kg ranging from 27 to 91 Kgs. Close to all the participants 328 (86.5%) weighed more than 50Kgs versus only 15(13.5%) who weighed less than 50Kgs. There was significant difference in the distribution of study participants with regards to body weight ($\chi 2 = 202.451$; df = 1; P = 0.0001) (Table 4.2).

4.2.2 Body Temperature of Study Participants

The mean (±SD) body temperature of the participants was 37.9 (±1.3) degree Celsius ranging from 30 to 41 degree Celsius. The majority of the participants 285 (75.2%) had body temperature ≥ 37.1 degrees Celsius versus 94(24.8%) who had body temperature of ≤ 37 degrees Celsius. There was significant difference in the distribution of study participants with regards to the body temperature ($\chi 2 = 118.574$; df = 1; P = 0.0001) (Table 4.2).

4.2.3 Days with Fever Among Study Participants

The mean (± SD) number of days the participants' had fever was 3.2 (±2.1) days ranging from 1 to 28 days. Out of the 379 participants, the majority 300(79.2%) had fever for ≤ 3 days versus 79(20.8%) who had fever for ≥ 3 days. There was significant difference in the distribution of study participants with regards to the number of days with fever ($\chi 2 = 830.05$; df = 1; P = 0.0001) (Table 4.2).

4.2.4 Signs and Symptoms among Study Participants

When asked concerning typhoid related signs and symptoms, the majority 223(58.8%) had headache, followed by 41(10.8%) with both headache and diarrhea. The least 21(5.5%) participants had headache and body rash. There was significant difference in the distribution of study participants with regards to fever related signs and symptoms ($\chi 2 = 385.525$; df = 4; P = 0.0001) (Figure 4.2).

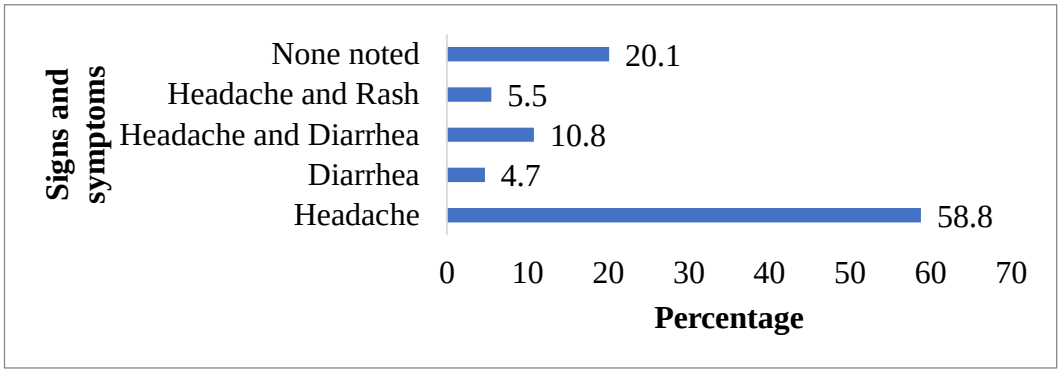

Figure 4.2: Distribution of Study Participants by Signs and Symptom

4.2.5 Fever for 3 Days

With regards to having fever for the last 3 days, there was near equal distribution between those who had fever 184 (48.5%) versus those who did not have 195(51.5%). There was no significant difference in the distribution of study participants with presence of fever in the last 3 days ($\chi 2 = 245.449$; df = 1; P = 0.572) (Table 4.2).

4.2.6 Received Treatment among Study Participants

The majority of the participants 342 (90.2%%) stated they did not receive any treatment for the fever or typhoid related symptoms they had versus only 37 (9.8%) who received treatment. There was significant difference in the distribution of study participants with regards to the receiving treatment ($\chi 2 = 245.449$; df = 1; P = 0.0001) (Table 4.2).

4.2.7 Type of Treatment among Study Participants

For those participants who received treatment, the majority of them 27(7.1%) received antibiotics while 10(2.6%) received non-antibiotic-based treatment type. There was significant difference in the distribution of study participants with regards to treatment type ($\chi 2 = 553.398$; df = 2; P = 0.0001) (Table 4.2).

4.2.8 Laboratory Confirmation of *Salmonella* Isolates

Only 28(7.4%) had laboratory confirmation done on the cause of fever compared to the majority 351(92.6%) who did not have the cause of fever confirmed. There was significant difference in the distribution of study participants with regards to laboratory confirmation ($\chi 2 = 275.274$; df = 1; P = 0.0001) (Table 4.2).

4.2.9 History of Typhoid Fever among Participants

Among the participants, majority 246 (64.9%) had no history of any typhoid fever compare to 133(35.1%) who had experienced such fever. There was significant

difference in the distribution of study participants with regards to history of typhoid fever ($\chi 2 = 33.691$; df = 1; P = 0.0001) (Table 4.2).

4.2.10 Contact with Typhoid Patient among Study Participants

Among the participants, the majority 341 (90%) reported not having any contact with any typhoid patient in the last one year while only 38(10%) reported such contact. There was significant difference in the distribution of study participants with regards to history of contact with any typhoid patient in the last one year ($\chi 2 = 242.24$; df = 1; P = 0.0001) (Table 4.2).

Table 4.2: Summary of the Clinical Characteristics of the Study Participants

Variables	Frequency	Percentage	Chi-square	df	p value
Weight					
Mean (± SD)	***63.7***	***(± 11.1)***			
Range	***64***	***(27-91)***			
< 50	51	13.5	202.451	1	0.0001
> 51	328	86.5			
Body Temperature					
Mean (± SD)	***37.9***	***(± 1.3)***			
Range	***11***	***(30-41)***			
< 37	94	24.8	96.256	1	0.0001
> 37.1	285	75.2			
Days with Fever					
Mean (± SD)	***3.2***	***(± 2.1)***			
Range	***27***	***(1-28)***			
< 3 Days	300	79.2	830.05	1	0.0001
> 3 Days	79	20.8			
Signs and symptoms					
Headache	223	58.8			
Diarrhea	18	4.7			
Headache and Diarrhea	41	10.8	385.525	4	0.0001
Headache and Rash	21	5.5			
None noted	76	20.1			
Fever for 3 Days					
Yes	184	48.5	0.319	1	0.572
No	195	51.5			
Receiving treatment					
Yes	37	9.8	245.449	1	0.0001
No	342	90.2			
Treatment Type					
Antibiotics	27	7.1			
Non-antibiotics based	10	2.6	553.398	2	0.0001
Not on treatment	342	90.2			
Laboratory confirmation of typhoid					
Yes	28	7.4	275.274	1	0.0001
No	351	92.6			
History of typhoid fever					
Yes	133	35.1	33.691	1	0.0001
No	246	64.9			
Had contact (work colleagues, school mates, relatives, neighbours) with typhoid patients within one year before					
Yes	38	10	242.24	1	0.0001
No	341	90			
Did you travel outside your community recently (three months ago)?					
Yes	31	8.2	265.142	1	0.0001
No	348	91.8			

4.3 Food as a Risk Factor for Enteric Fever

Concerning the food and eating habit of the study participants, the majority, 303 (79.9%) of the participants did no often eat outside in restaurants and other eateries, 236 (62.3%) often ate chicken 1 to 2 times per month, 166 (43.8%) often ate meat 1 to 2 times in a month, 153 (40.4%) often ate salad 1 to 2 times in a month and about 292(41.7%) of them reported having seldom or never ate ice-cream. Further, only 75(19.8%) of the participants ate from common plate while 50 (13.2%) of the participants took locally prepared cold drinks (Table 4.3).

Table 4.3: Food and Eating Related Characteristics of the Study Participants

Variables	Frequency	Percentage	Chi-square	df	p value
Do you often eat from outside					
Yes	76	20.1	135.96	1	0.0001
No	303	79.9			
How often do you eat chicken					
Everyday	2	0.5			
3 to 4 Times/Week	4	1.1			
1 to 2 Times/Week	22	5.8	536	4	0.0001
1 to 2 Times/Month	236	62.3			
Seldom\Never	115	30.3			
How often do you eat meat					
Everyday	81	21.4			
3 to 4 Times/Week	37	9.8			
1 to 2 Times/Week	47	12.4	148.691	4	0.0001
1 to 2 Times/Month	166	43.8			
Seldom\Never	48	12.7			
How often do you eat salad					
Everyday	56	14.8			
3 to 4 Times/Week	5	1.3			
1 to 2 Times/Week	18	4.7	260.881	4	0.0001
1 to 2 Times/Month	153	40.4			
Seldom\Never	147	38.8			
How often do you eat icecream					
Everyday	6	1.6			
3 to 4 Times/Week	17	4.5			
1 to 2 Times/Week	18	4.7	782.332	4	0.0001
1 to 2 Times/Month	46	12.1			
Seldom\Never	292	77			
Does the family eat from a common plate					
Yes	75	19.8	138.367	1	0.0001
No	304	80.2			
Do you take locally prepared cold drinks					
Yes	50	13.2	205.385	1	0.0001
No	329	86.8			

4.4 Sanitation and Hygiene Characteristics of Participants

The majority, 348 (91.8%) of the participants reported washing their hands, only 57 (15%) of the participants had their families washing their hands in a common basin. The majority 276 (72.8%) had the source of their water supply from wells, 274 (72.3%) used treated water for drinking and 146 (38.5%) reported treating their waters using chemicals. Further, 310 (81.8%) of the participants stored their foodstuffs in the kitchen, with 254 (67%) using modern toilets while 146 (38.5%) disposed their wastes using public sewage systems (Table 4.4).

Table 4.4: Sanitation and Hygiene Related Characteristics of the Study Participants

Variables	Frequency	Percentage	Chi-square	df	p value
Do you often wash your hands					
Usually	348	91.8			
Sometimes	10	2.6	583.889	2	0.0001
Never	21	5.5			
Does the family wash hands in common basin					
Yes	57	15	185.29	1	0.001
No	322	85			
Sources of water supplies in the family					
Municipal	35	9.2			
Wells	276	72.8	270.274	2	0.0001
River	68	17.9			
Drinking water treated					
Yes	274	72.3	75.359	1	0.035
No	105	27.7			
Method for water treatment					
Boiling	36	9.5			
Chemical	146	38.5	65.084	3	0.0001
Filtration	93	24.5			
None	104	27.4			
Food storage in your family					
Fridge	38	10			
Kitchen	310	81.8	400.723	2	0.0001
Garden	31	8.2			
Type of latrine do you have					
Modern Toilet	254	67			
Wet Latrine	31	8.2	366.298	3	0.0001
Dry Latrine	66	17.4			
Environment	28	7.4			
Where dispose your Sewage					
Public Sewage System	146	38.5	19.971	1	0.0001
Environment	233	61.5			

4.5 Socio-Economic Characteristics of Participants

The mean (± SD) number of people living in the participant's house was 6.2 (±4.9) ranging from 1 to 60 people. The majority, 199 (52.5%) of the participants lived with 1 to 5 persons in their houses, while 71 (18.7%), 88 (23.2%) and 63 (16.6%) owned cows, goats and chicken respectively. The mean (± SD) monthly family income of the participants was 24,170 (±16917) ranging from 10,000.00 to 200,000.00 Ksh with majority of the participants 256(67.5%) earning ≤ 20,000.00Ksh. The mean (± SD) numbers of household rooms of the participants were 3.6 (±1.9) ranging from 1 to 12 rooms with majority 318 (83.9%) having houses with ≤ 52 rooms. The majority 260 (68.6%) of the participants' houses/homes had electricity while 230(60.7%) were built using concrete (Table 4.5).

Table 4.5: Economic Related Characteristics of Study Participants

Variables	Frequency	Percentage	Chi-square	df	p value
Number of people living in the house					
Days with Fever					
Mean (± SD)	***6.2***	***(± 4.9)***			
Range	***59***	***(1-60)***			
1 to 5	199	52.5			
6 to 10	152	40.1	123.551	2	0.0001
> 11	28	7.4			
Family owns cows					
Yes	71	18.7	148.203	1	0.0001
No	308	81.3			
Family owns goats					
Yes	88	23.2	108.731	1	0.0001
No	291	76.8			
Family owns chicken					
Yes	63	16.6	168.889	1	0.0001
No	316	83.4			
Monthly income					
Days with Fever					
Mean (± SD)	***24170***	***(± 16917)***			
Range	***190,000***	***(10,000-200,000)***			
<20,000.00	256	67.5	46.673	1	0.0001
> 20,001.00	123	32.5			
Household Number of rooms					
Days with Fever					
Mean (± SD)	***3.6***	***(± 1.9)***			
Range	***11***	***(1-12)***			
< 5	318	83.9	174.272	1	0.0001
> 5	61	16.1			
Home/House has electricity					
Yes	260	68.6	52.456	1	0.0001
No	119	31.4			
House building material					
Concrete	230	60.7			
Bricks	107	28.2	313.391	3	0.0001
Concrete/Bricks	35	9.2			
Wood	7	1.8			

4.6 Prevalence of *Salmonella* Strains among Participants

Out of the 379 participants 8 (2.1%) were positive for Salmonella strains using both the VITEK 2 system, Version 0.8.01 (bioMerieux, Inc., Hazelwood, MO) and PCR. Out of the 8 salmonella positive strains (n=2; 25%) were *Salmonella enterica* subspecies enterica serovar typhi, (n=2; 25%) were *Salmonella paratyphi* A with the majority (n=4; 50%) being *Salmonella paratyphi* B as shown in figure 4.3.

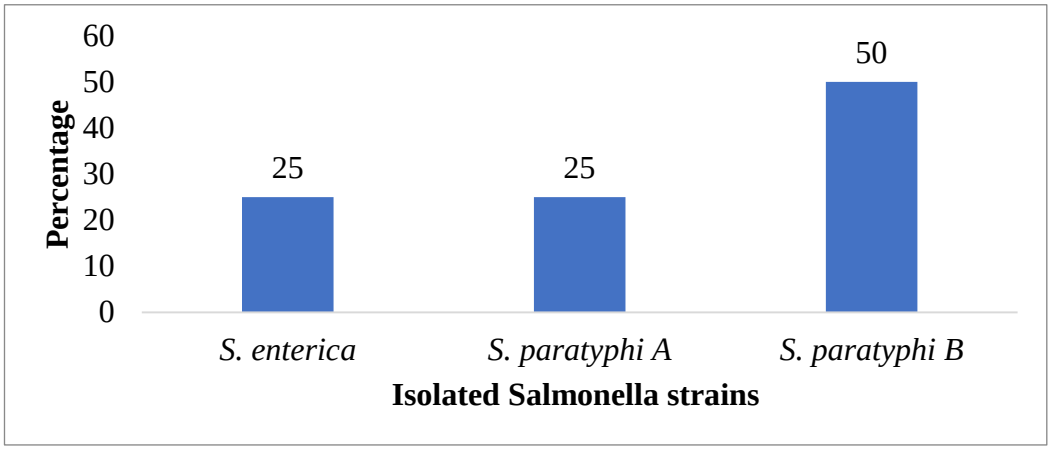

Figure 4.3: Distribution of Salmonella Strains Isolated from Study Participants

4.7 Occurrence of *Salmonella* Virulence genes

All the strains (2 *S. enterica*, 2 *S. paratyphi* A and 4 *S. paratyphi* B) in this study tested positive for Salmonella-specific gene (*inv*A) and were confirmed as *Salmonella* positive by the predicted product of 284-bp DNA fragment shown in figure 4.4.

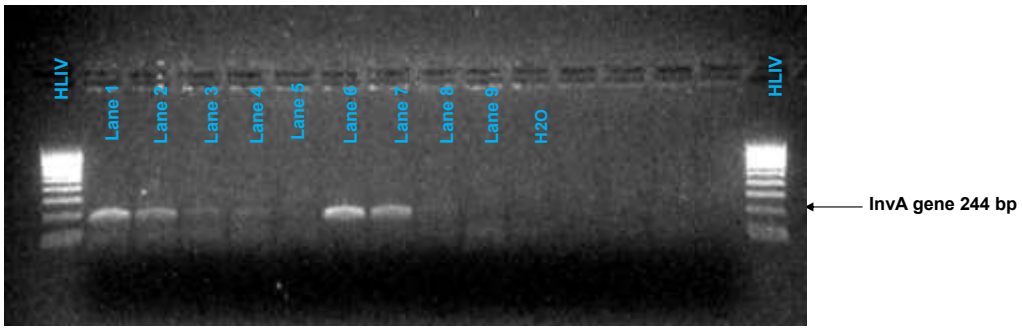

Figure 4.4: Agarose gel Showing PCR Amplification of *Salmonella* Strains

PCR amplification of S. typhimurium invA gene primers positive invA gene amplification for S. typhimurium ATCC 14028, S. enterica ATCC 13076 and S. enetritidis ATCC 13076 (Lane 1, Lane 2 and Lane 3) for 6 isolates (Lane 4-9). The DNA band at 248 bp shows marker presence, as visualized by gel electrophoresis using 1% agarose with an image analyzer and DNA marker (1 kb ladder)

4.8 Summary of Biochemistry and Molecular Results

All the strains (2 *S. enterica*, 2 *S. paratyphi* A and 4 *S. paratyphi* B) in this study were positive for the following biochemistry tests; Triple sugar iron, citrate, methyl red. The isolates negative for indole, urease, Voges Proskauer and oxidase tests. The identity was also confirmed as positive using both Viteck and PCR targeting the Salmonella-specific gene (invA) (Table 4.6)

Table 4.6: Summary of Biochemistry and Molecular Results for Salmonella Positive Isolates

Isolate	Biochemistry Tests									VITEK	PCR	Results
	Indole	Triple Sugar Iron	Citrate	Mortility	Urease	Methyl Red	Voges-Proskauer	Oxidase	Catalase		invA gene	
CMR_8530	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	*S. enterica*	Positive	*S. enterica*
CMR_6772	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	*S. paratyphi* B	Positive	*S. paratyphi* B
CMR_8548	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	*S. paratyphi* B	Positive	*S. paratyphi* B
CMR_8574	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	*S. paratyphi* A	Positive	*S. paratyphi* A
CMR_6704	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	*S. paratyphi* B	Positive	*S. paratyphi* B
CMR_5792	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	*S. enterica*	Positive	*S. enterica*
CMR_5722	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	*S. paratyphi* B	Positive	*S. paratyphi* B
CMR_5732	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	*S. paratyphi* A	Positive	*S. paratyphi* A

4.9 Antibiotic Susceptibility Profiles of *Salmonella* Strains

Single and multiple resistance to most of the antibiotics tested was observed. The highest level of resistance observed was to ampicillin (100%) with all 8 isolates being resistant. 7/8 isolates (87.5%) were resistant to tetracycline and 1 being intermediately resistant. There were 6/8(75%) isolates resistant to gentamycin with two isolates 1(CMR_5722) and 2(CMR_6704) being susceptible and intermediately resistant respectively. Ciprofloxacin was the most effective antibiotic. All isolates (100%) were sensitive. Nalidixic acid and Trimethoprim-sulfamethoxazole were the second most effective antibiotics, except that 2 (CMR_6772 and CMR_6704) and 2 (CMR_8548 and CMR_5732) isolates were resistant to the two antibiotics, respectively. Fifty percent (4/8) of the isolates were susceptible to chloramphenicol. The disk contents for all antibiotics used were the same as those described under "Zone Diameter and Minimal Inhibitory Concentration (MIC) Interpretive Standards" in the CLSI document (Table 4.7)

Table 4.7: Antibiotic Disk Diffusion Susceptibility Test Results for Salmonella Strains

Isolate	Strain	Antibiotic susceptibility profiles						
		AMP	NA	CHLOR	GEN	CIP	SXT	TET
CMR_8530	*S. paratyphi A*	R	S	S	R	S	S	R
CMR_6772	*S. paratyphi B*	R	R	S	R	S	S	R
CMR_8548	*S. paratyphi B*	R	S	R	R	S	R	R
CMR_8574	*S. paratyphi B*	R	S	S	R	S	S	R
CMR_6704	*S. paratyphi A*	R	R	R	I	S	S	R
CMR_5792	*S. paratyphi B*	R	S	R	R	S	S	R
CMR_5722	*S. enterica*	R	S	S	S	S	S	I
CMR_5732	*S. enterica*	R	S	R	R	S	R	R

S - Susceptible; R - Resistant; I - Intermediate-resistant; AMP-Ampicillin; NA - Nalidixic acid; CHLOR - Chloramphenicol; GEN - Gentamycin; CIP - Ciprofloxacin; SXT - Trimethoprim-sulfamethoxazole; TET - Tetracycline

4.10 Risk Factors Associated with Salmonella Infection among Participants

In this study, factors associated with pathogenic salmonella infection were evaluated for all the 379 participants.

4.10.1 Socio-Demographic Factors

Table 4.8 summarizes the demographic factors associated with pathogenic salmonella infection among study participants. In bivariate analysis, none of the demographic factors gender, age, marital status, education level, occupation, religion and household size were found to be associated with pathogenic salmonella infection among study participants.

Table 4.8: Demographic Factors Associated with Pathogenic Salmonella Infection

Variables	Total	Pathogenic Salmonella infection		P - value	Bivariate
		Frequency	Percentage		uOR (95% CI)
Gender					
Male	173	2	1.2	0.236	0.4(0.08-1.9)
Female	206	6	2.9	Reference	Reference
Age (Years)					
18 - 30	151	1	0.7	0.061	0.1(0.01-1.1)
31 - 40	109	2	1.8	0.21	0.3(0.05-1.9)
41 - 50	67	2	3	0.47	0.5(0.08 - 3.1)
>51	52	3	5.8	Reference	Reference
Marital status					
Single	122	3	2.5	0.999	
Married	220	5	2.3	0.999	ND
Separated\Divorced	24	0	0	0.999	
Widowed	13	0	0	Reference	
Education level					
Primary	49	1	2	0.622	0.6(0.06-5.2)
Secondary	149	2	1.3	0.266	0.4(0.07 - 2.1)
Tertiary	69	1	1.4	0.424	0.4(0.05 - 3.7)
Non Formal	112	4	3.6	Reference	Reference
Occupation					
Bussiness	171	4	2.3	0.306	3.5(0.3-38.6)
Employed	64	2	3.1	0.389	2.6(0.3-23.4)
Unemployed	112	1	0.9	0.376	3.5(0.2-55.9)
Farming	32	1	3.1	Reference	Reference
Religion					
Christian	76	2	2.6	0.728	1.3(0.3-6.6)
Muslim	303	6	2	Reference	Reference
Household population					
1 to 5	255	4	1.6	0.995	
6 to 10	95	4	4.2	0.994	ND
> 11	29	0	0	Reference	

OR - Odds ratio; CI - confidence interval; u - Unadjusted odds ratio; ND - Not done; P value- significant level

4.10.2 Clinical Factors Associated with Pathogenic Salmonella Infection

In bivariate analyses, only participants who had previous laboratory confirmation of typhoid (OR 87.7, 95% CI 10.8-713.2) to be infected with pathogenic strains of salmonella. The other clinical factors such as body weight, body temperature,

occurrence of fever for the last 3 days, treatment type, and history of typhoid fever, among others in table 4.9 were not associated with pathogenic salmonella infection among study participants.

Table 4.9: Clinical Related Factors Associated with Pathogenic Salmonella Infection

Variables	Total	Pathogenic Salmonella infection		P - value	Bivariate
		Frequency	Percentage		uOR (95% CI)
Weight					
< 50	51	0	0	0.998	ND
> 51	328	8	2.4	Reference	Reference
Body Temperature					
< 37	94	1	1.1	0.434	0.4(0.05 - 3.3)
> 37.1	285	7	2.5	Reference	Reference
Days with Fever					
< 3 Days	300	4	1.3	0.062	0.3(0.07 - 1.1)
> 3 Days	79	4	5.1	Reference	Reference
Signs and symptoms					
Headache	223	5	2.2	0.627	1.7(0.2-14.6)
Diarrhea	18	0	0	0.994	ND
Headache and Diarrhea	41	2	4.9	0.285	3.7(0.3 - 40.8)
Headache and Rash	21	0	0	0.994	ND
None noted	76	1	1.3	Reference	Reference
Fever for 3 Days					
Yes	184	7	3.8	0.061	7.4(0.9-60.3)
No	195	1	0.5	Reference	Reference
Receiving treatment					
Yes	37	0	0	0.999	ND
No	342	8	2.3	Reference	Reference
Treatment Type					
Antibiotics	27	0	0	0.99	
Non-antibiotics based	10	0	0	0.99	ND
Not on treatment	342	8	2.3	Reference	Reference
Laboratory confirmation of typhoid					
Yes	28	7	25	**0.001**	**87.7(10.8-713.2)**
No	351	1	0.3	Reference	Reference
History of typhoid fever					
Yes	133	2	1.5	0.563	0.6(0.1-3.1)
No	246	6	2.4	Reference	Reference
Had contact (work colleagues, school mates, relatives, neighbours) with typhoid patients within one year before					
Yes	38	1	2.6	0.816	1.2(0.2-10.4)
No	341	7	2.1	Reference	Reference
Did you travel outside your community recently (three months ago)?					
Yes	31	1	3.2	0.659	1.6(0.2-13.1)
No	348	7	2	Reference	Reference

OR - Odds ratio; CI - confidence interval; u - Unadjusted odds ratio; ND - Not done; P value- significant level

4.10.3 Food as a Risk Factor for Enteric Fever

In bivariate analysis, participants who often ate outside (OR 6.6, 95% CI 1.6-27.8), those whose family ate from a common plate (OR 6.7, 95% CI 1.6-28.3) and those

who took locally prepared cold drinks (OR 10.9, 95% CI 2.6-45.9) were more likely to be infected with pathogenic strains of salmonella (Table 4.10)

Table 4.10: Food and Feeding Related Factors Associated with Pathogenic Salmonella Infection

Variables	Total	Pathogenic Salmonella infection		P - value	Bivariate
		Frequency	Percentage		uOR (95% CI)
Do you often eat from outside					
Yes	76	5	6.6	**0.01**	**6.6(1.6-27.8)**
No	303	3	1	Reference	Reference
How often do you eat chicken					
Everyday	2	0	0	0.999	
3 to 4 Times/Week	4	0	0	1	ND
1 to 2 Times/Week	22	0	0	0.999	
1 to 2 Times/Month	236	5	2.1	0.999	
Seldom\Never	115	3	2.6	Reference	Reference
How often do you eat meat					
Everyday	81	3	3.7	0.999	
3 to 4 Times/Week	37	0	0	1	ND
1 to 2 Times/Week	47	3	6.4	0.999	
1 to 2 Times/Month	166	2	1.2	0.999	
Seldom\Never	48	0	0	Reference	Reference
How often do you eat salad					
Everyday	56	1	1.8	0.997	
3 to 4 Times/Week	5	0	0	1	
1 to 2 Times/Week	18	4	22.2	0.996	ND
1 to 2 Times/Month	153	1	0.7	0.997	
Seldom\Never	147	2	1.4	Reference	Reference
How often do you eat icecream					
Everyday	6	0	0	1	
3 to 4 Times/Week	17	1	5.9	0.999	
1 to 2 Times/Week	18	0	0	1	ND
1 to 2 Times/Month	46	2	4.3	0.999	
Seldom\Never	292	5	1.7	Reference	Reference
Does the family eat from a common plate					
Yes	75	5	6.7	**0.009**	**6.7(1.6 - 28.3)**
No	304	3	1	Reference	Reference
Do you take locally prepared cold drinks					
Yes	50	5	10	**0.001**	**10.96(2.6 - 45.9)**
No	329	3	0.9	Reference	Reference

OR - Odds ratio; CI - confidence interval; u - Unadjusted odds ratio; ND - Not done; P value- significant level

4.10.4 Sanitation and Hygiene Factors Associated with Pathogenic Salmonella Infection among Participants

In bivariate analysis, participants whose family washed hands in common basin (OR 9.4, 95% CI 2.3-39.4) were more likely to be infected with pathogenic strains of salmonella (Table 4.11)

Table 4.11: Sanitation and Hygiene Related Factors Associated with Pathogenic Salmonella Infection

Variables	Total	Pathogenic Salmonella infection		P - value	Bivariate uOR (95% CI)
		Frequency	Percentage		
Do you often wash your hands					
Usually	348	7	2	0.994	
Sometimes	10	1	10	0.994	ND
Never	21	0	0	Reference	Reference
Does the family wash hands in common basin					
Yes	57	5	8.8	**0.002**	**9.4(2.3-39.4)**
No	322	3	0.9	Reference	Reference
Sources of water supplies in the family					
Municipal	35	0	0	0.999	ND
Wells	276	7	2.5	0.61	1.7(0.2 - 14.1)
River	68	1	1.5	Reference	Reference
Drinking water treated					
Yes	274	7	2.6	0.356	27(0.3-21.8)
No	105	1	1	Reference	Reference
Method for water treatment					
Boiling	36	3	8.3	0.061	8.7(0.9 - 83.3)
Chemical	146	4	2.7	0.349	2.8(0.3 - 25.5)
Filtration	93	0	0	0.992	ND
None	104	1	0	Reference	Reference
Food storage in your family					
Fridge	38	2	5.3	0.689	1.6(0.1-17.9)
Kitchen	310	5	1.6	0.527	0.5(0.06-4.3)
Garden	31	1	3.2	Reference	Reference
Type of latrine do you have					
Modern Toilet	254	6	2.4	0.702	0.7(0.08-5.5)
Wet Latrine	31	0	0	0.993	ND
Dry Latrine	66	1	1.5	0.544	0.4(0.03-6.8)
Environment	28	1	3.6	Reference	Reference
Where dispose your Sewage					
Public Sewage System	146	4	2.7	0.509	1.6(0.4-6.4)
Environment	233	4	1.7	Reference	Reference

OR - Odds ratio; CI - confidence interval; u - Unadjusted odds ratio; ND - Not done; P value- significant level

4.10.5 Socio-Economic Factors Associated with Pathogenic Salmonella Infection

In bivariate analysis, participants whose household monthly income was <Kshs20,000.00 (< 200USD) (OR 0.2, 95% CI 0.03-0.8) were less likely to be infected with pathogenic strains of salmonella compared to those who had monthly income >Kshs20,001.00 (>200USD). (Table 4.12)

Table 4.12: Socio-Economic Factors Associated with Pathogenic Salmonella Infection

Variables	Total	Pathogenic Salmonella infection		P - value	Bivariate
		Frequency	Percentage		uOR (95% CI)
Number of people living in the house					
Days with Fever					
1 to 5	199	4	2	0.998	
6 to 10	152	4	2.6	0.998	ND
> 11	28	0	0	Reference	Reference
Family owns cows					
Yes	71	2	2.8	0.651	1.4(0.3-7.2)
No	308	6	1.9	Reference	Reference
Family owns goats					
Yes	88	3	3.4	0.348	1.9(0.5-8.3)
No	291	5	1.7	Reference	Reference
Family owns chicken					
Yes	63	1	1.6	0.755	0.7(0.09-5.8)
No	316	7	2.2	Reference	Reference
Monthly income					
<20,000.00	256	8	2.5	***0.025***	***0.2(0.03-0.8)***
> 20,001.00	123	0	0	Reference	Reference
Home/House has electricity					
Yes	260	5	1.9	0.711	0.8(0.2-3.2)
No	119	3	2.5	Reference	Reference

OR - Odds ratio; CI - confidence interval; u - Unadjusted odds ratio; ND - Not done; P value- significant level

4.10.6 Multivariate Analysis

After adjusting for confounders factors that remained independently associated with pathogenic salmonella infection included; laboratory confirmation of typhoid (OR 66.6, 95% CI 5.8-757.2), often eating outside homestead (OR 5.3, 95% CI 1.4-12.4) and family eating from a common plate (OR 6.1, 95% CI 1.2-21.2). Other factors included taking locally prepared cold drinks (OR 6.9, 95% CI 1.4-32.3), family washing hands in common basin (OR 7.3, 95% CI 1.9-31.2) and the participants who had monthly income <Kshs 20,000.00 (<200USD) (OR 0.2, 95% CI 0.003-0.8) (Table 4.13).

Table 4.13: Adjusted Factors Associated with Pathogenic Salmonella Infection

Variables	Total	Pathogenic Salmonella infection		P - value	Multivariate
		Frequency	Percentage		aOR (95% CI)
Laboratory confirmation of typhoid					
Yes	28	7	25	**0.001**	**66.6(5.8-757.2)**
No	351	1	0.3	Reference	Reference
Do you often eat from outside					
Yes	76	5	6.6	**0.03**	**5.3(1.4-12.4)**
No	303	3	1	Reference	Reference
Does the family eat from a common plate					
Yes	75	5	6.7	**0.039**	**6.1(1.2 - 21.2)**
No	304	3	1	Reference	Reference
Do you take locally prepared cold drinks					
Yes	50	5	10	**0.014**	**6.9(1.4 - 32.3)**
No	329	3	0.9	Reference	Reference
Does the family wash hands in common basin					
Yes	57	5	8.8	**0.022**	**7.3(1.9-31.2)**
No	322	3	0.9	Reference	Reference
Monthly income					
<20,000.00	256	8	2.5	***0.025***	***0.2(0.03-0.8)***
> 20,001.00	123	0	0		

OR - Odds ratio; CI - confidence interval; a - adjusted odds ratio; ND - Not done; P value- significant level

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Epidemiological studies are essential in preventing and managing any disease/condition. This study, the first of its kind in Garissa County was a buildup of growing need for data on enteric fever given the lack of access to clean water, sanitation, proper housing and sufficient food in in this Semi-Arid Region of North Eastern Kenya (UN-HABITAT, 2006). These attributes are essential risk factors for transmission of *S. typhi* and *Paratyphi.* Specifically, this study determined the serotypes, antimicrobial resistance and associated factors this region of Kenya. In this study, *S. Typhi* and S. Paratyphi A and B were isolated for a total of 25%, 25% and 50% of enteric-fever patients respectively. Thus, the ratio of isolation of *S. Typhi* and *S. Paratyphi* A and B was 1: 1: 2 which was in contrary to other studies conducted globally which reported ratio from 1.6: 1 to 4: 1 (Bhattacharya *et al*., 2011; WHO, 2012). Though the prevalence of *S. Typhi* remains high, several recent studies have highlighted the progressive increased proportion of *S. Paratyphi* A in the past decade (Dutta *et al*., 2014; Makkar *et al*., 2018).

Clinically, typhoid and paratyphoid fever are indistinguishable. Furthermore, many other acute febrile illnesses such as dengue, leptospirosis, and malaria may present a clinical picture similar to that of typhoid fever (Radhakrishnan *et al*., 2018). The results from this and other studies inevitably shows the importance for accurate and early diagnosis of typhoid and paratyphoid fever. The accurate diagnosis requires laboratory confirmation (Parry *et al*., 2011; Radhakrishnan *et al*., 2018). The development of practical, affordable, and accurate (i.e., both sensitive and specific) diagnostic tools is key to typhoid fever management and control.

5.2 Occurrence of *Salmonella* Virulence genes

Biochemical reactions are key for speciating the salmonella strains but it is recognized that genetic methods provide the correct identification (Sharma and Das, 2016). In the

study, using biochemistry test all the 8 isolates showed typical biochemical characteristics of *Salmonella* on the basis of Indole, Methyl Red, Voges-Proskauer, and Citrate tests (IMViC), gas production and sugar fermentation as per standard techniques. All the isolates tested negative oxidase, positive catalase, positive indole in typtone broth, positive methyl red, negative Voges-Proskauer, urease negative and citrate positive tests and fermented glucose, fructose and maltose.

This study strengthens the role molecular method targeting gene *inv*A to confirm the isolates as *Salmonella*. All PCR products of isolates including positive control, screened by PCR, resulted in 284 bp amplified fragment. No amplified DNA fragments were obtained from non- Salmonella species. The ability of *Salmonella* specific primers to detect *Salmonella* species rapidly and accurately is primarily due to the primer sequences that are selected from the gene *inv*A (Trafny *et al*., 2006; Sharma and Das, 2016). The *inv*A gene codes for protein in inner membrane of bacteria, which is necessary for invasion to epithelial cells (Shanmugasamy *et al*., 2011). The detection of the *inv*A genes in all the *Salmonella* isolates from human population is in agreement with the earlier reports (Trafny *et al*., 2006; Sharma and Das, 2016). Furthermore, it has been observed that this gene is involved in the invasion of the cells of the intestinal epithelium and is present in pathogenic *Salmonella* (Trafny *et al*., 2006; Sharma and Das, 2016). Therefore, for salmonellosis to occur it is important that a gene responsible for invasion must be present. This gene is essential for full virulence in *Salmonella* and is thought to trigger the internalization required for invasion of deeper tissue (Zahraei *et al*., 2006).

5.3 Antibiotic Susceptibility Profiles of *Salmonella* Strains

In this study MDR Salmonella resistant was observed to ampicillin, tetracycline, gentamycin and chloramphenicol which is similar to different studies. Increase in the incidence of MDR *Salmonella* resistant to ampicillin, chloramphenicol, cotrimoxazole, streptomycin, furazolidone and tetracyclines is an emerging problem and a matter of concern worldwide (Dutta *et al*., 2014; Makkar *et al*., 2018). Encouragingly, in this study ciprofloxacin, nalidixic acid and trimethoprim-sulfamethoxazole were still effective against *Salmonella* strain. This is contrary to

other studies such as in India Makkar *et al*., (2018), observed higher level of resistance to ciprofloxacin, co-trimoxazole, ampicillin, and third-generation cephalosporins. In this Indian study, this observation was a serious concern given that the hospital in which the study was conducted was the only tertiary care facility available in Garhwal region and the presence of antibiotic resistance in this area signals about indiscriminate usage of antibiotics by general practitioners working at rural or semi-urban areas (Makkar *et al*., 2018). Most typhoid fever infections diagnosed in the United States are fluoroquinolone nonsusceptible; therefore, health care providers should not use fluoroquinolones as empiric therapy, especially in returning travelers from South Asia (Date *et al*., 2016). Fluoroquinolone nonsusceptibility has been associated with treatment failure or delayed clinical response (Crump *et al*., 2015). The emergence of fluoroquinolone nonsusceptible strains that are resistant to third-generation cephalosporins, such as ceftriaxone has been observed in Pakistan and other countries (Ryan and Andrews, 2018). In recent data from Pakistan published as part of the surveillance for enteric fever in Asia project (SEAP), over half of all *S. Typhi* isolates were multidrug resistant. Fluoroquinolone resistance was noted in nearly 90% of *S. Typhi* and *S. Paratyphi* isolates (Qamar *et al*., 2018). A longitudinal study of typhoid fever trends at three large hospitals in India showed a fall in resistance rates for ampicillin, chloramphenicol, and co-trimoxazole between 2000 and 2014, as resistance to more widely used antibiotics has risen (Balaji *et al*., 2018). Near universal resistance to ciprofloxacin has been observed in recent isolates from India (Dahiya *et al*., 2017). In this study, the patients with suspected severe or complicated typhoid fever might need to be treated with a fluoroquinolone and carbapenem and the treatment regimens can be adjusted when culture and sensitivity results are available.

In this study, attributes related to water and food (such as often eating outside homestead, family eating from a common plate, taking locally prepared cold drinks, family wash hands in common basin) were found associated with *S. Typhi* and *S. Paratyphi* infection. As it is expected, Garissa is a semi-arid area marked by water shortage and poor sanitation hygiene. The association of food and water related behavior is not surprising. It should be noted that we did not distinguish if these factors were specific to either *S. Typhi* or *S. Paratyphi.* Similar to our study many authors investigating enteric fever do not distinguish factors coinciding either with typhoid or

paratyphoid (Vollaard *et al*., 2004). The assumption is that in paratyphoid fever, a higher dose of bacteria is required for infection than in typhoid fever; consequently, food is implicated as the major vehicle for transmission of paratyphoid fever, since *Salmonella* bacteria can multiply in food (Vollaard *et al*., 2004). Undoubtedly, risk factors for both typhoid and paratyphoid fever have been identified in several epidemiologic studies suggesting either waterborne or foodborne transmission (Sur *et al*., 2007; Anand *et al*., 2010; Khan *et al*., 2012; Mogasale *et al*., 2018). The odds of typhoid fever among those exposed to unsafe water ranged from 1.06 to 9.26 (Mogasale *et al*., 2018).

In our study, previous laboratory confirmation of typhoid was independently associated with being positive for enteric fever infection. According to WHO (2017), laboratory confirmation should always be sought for clinically suspected cases. Confirmation by culture (or validated molecular methods, as available) is essential as typhoid fever, paratyphoid fever and other invasive salmonellosis can present as a non-specific febrile illness, and current serological tests lack diagnostic specificity. It can be argued that those patients who sought laboratory confirmation had clinical signs and symptoms for enteric fever. Confirmation is essential to assess the proportion of enteric fever caused by these different organisms, determine antimicrobial susceptibility and perform molecular epidemiology studies (WHO, 2017).

Participant's low income was a key predictor for *S. Typhi* or *S. Paratyphi* infection. This was similar to other reports. Mogasale *et al*., (2018) besides water-related risk, identified other risk factors related to socioeconomic aspects, type of food consumption, knowledge and awareness about typhoid fever, and hygiene practices.

One of the major strengths of this study is the ability to contribute to the wealth of knowledge on the epidemiology and antimicrobial patterns of *S. typhi* or *S. paratyphi* infection in the semi-arid initially marginalized region of Kenya. The study also showed the potential association between food and feeding habit, hygiene and low socio-economic with *S. typhi* or *S. paratyphi* infection. However, some of the limitations need to pointed out: Firstly, the cross-sectional nature of our study only allowed us to describe associations between *S. typhi* or *S. paratyphi* infection with

food and hygienic related factors and not a causal conclusion. Such outcomes can be confirmed in a longitudinal study. Secondly, although we reported high carriage of MDR Salmonella, we cannot conclusively predict the source of exposure to these multidrug-resistant isolates; whether it is due to the modern food-animal production characterized by densely concentrated animals and routine antibiotic use or it's due to misuse of antibiotics in the human population, which is a common phenomenon in Kenya.

These limitations notwithstanding, our findings indicate the occurrence of enteric fever in this region facing climatic and economic hardship where majority of the population has limited access to diagnostic services. This is an indication that the disease burden is poorly quantified and policy makers have lacked the data needed to make decisions about the deployment of enteric fever prevention measures and vaccines. Further, isolation of the antimicrobial resistant *S. typhi* and *S. paratyphi* in this study points strongly to the need to establish hospital antimicrobial policy and antimicrobial prescribing guidelines. Periodic monitoring of the antibiogram pattern along with the implementation of strict antibiotic policies and patient education are crucial. Proper feeding, sanitation and hygiene practices are also likely to prevent and reduce the burdened on enteric fever in this region.

5.4 Conclusion

1. The prevalence of *S. Typhi* and S. *Paratyphi* A and B were reported among 25%, 25% and 50% of enteric-fever patients respectively. Thus, the ratio of isolation of *S. Typhi* and *S. Paratyphi* A and B was 1: 1: 2 which was in contrary to other studies conducted globally which reported ratio from 1.6: 1 to 4: 1
2. The MDR Salmonella resistant was observed to ampicillin, tetracycline, gentamycin and chloramphenicol. However, ciprofloxacin, nalidixic acid and trimethoprim-sulfamethoxazole were still effective against *Salmonella* strain
3. The PCR screening amplified gene *inv*A confirming the isolates as *Salmonella*.

4 Factors related to water and food (such as often eating outside homestead, family eating from a common plate, taking locally prepared cold drinks, family wash hands in common basin), low socio-economic status and availability of a previous laboratory confirmation of Typhoid were associated with *S. Typhi* and *S. Paratyphi* infection.

5.5 Recommendations

1 The increase isolation of S. *typhi*, *S. paratyphi A* and B among patients presenting with enteric fever is a indicator for health practitioners to explore and test for wider arrays of pathogens including salmonella among patients with these symptoms

2 The isolation of a large proportion of MDR *S. typhi*, *S. paratyphi A* and B is worrying. Although these isolates were susceptible to fluoroquinolone, there is need for routine surveillance to monitor susceptibility to the initial first line antibiotics in clinical settings since the MDR strains have lately shown increased resistance.

3 The association of the *Salmonella* strains with the gene *inv*A which encodes for protein in inner membrane of bacteria, which is necessary for invasion to epithelial cells, suggests that most of all the *salmonella* infections could be related to pathogenesis reiterating the need for the routine use of molecular methods not only to differentiate between *salmonella* strains but also as a tool for detection of pathogenesis

4 Addressing the food, water, sanitation and hygiene and low socio-economic status is likely to prevent and reduce the burdened on enteric fever in this region.

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APPENDICES

Appendix I: Questionnaire

1. Personal demographic information (Xogta shaqsiga ah ee dadka):

   Code number:…….

   Location (Goobta/Meesha): __ __ ___
2. Address (Ciwaan): …….
3. Sex (Jinsi): Male (rag)□ Female (dhumar)□
4. Marital status (Xalada gurka):

Single □
(Doob/Gabar)
Married (Xaas) □
Divorced (Garoob) □
Widowed □
Separated □

5. Date of birth (Taariikh dhalasho) …../……/……
6. Religion (Diinta):

Muslim □
Christian □
Hindu □
Others specify/ ……
(Waxi ka dheeri ah)

7. Education (Heer waxbarasho):

Illiteracy (Qorid/Akhrin) □
Primary school (Dugsi hoose dhexe) □
Secondary school (Dugsi Sare) □

Diploma □

Degree (Heer Jaamicadeed) □

8. Occupation (Meherad):

House wife (Guri joogto) □

Employed (Shaqaale) □

Business (Ganacsi) □

Farmer (Beeraley) □

Others specify (Howlo kale sheeg)----------

9. House hold size (Tirada qoyska) ……
10. CLINICAL DATA (Xogta caafimaad)

Weight (Culeyska) _____

Height (Joogga) _____

Temperature (Heerkulka)…….

Number of days with fever (Inta maalmood ee xummadda) ……

Rash/rose spots (Finan/ meelo gudguduudan).

Headache (Madax xanuun).

Diarrhoea (Shuban).

11. Have you had fever during the last 3 days (xummad ah miyaa ku qabtay saddexdii maalmood ee u dambeysay)? Yes (haa)□ No (maya)□ Unknown (mahubi)□

History (Wax daawo ah miyaad hadda qaadataa)? Yes(haa)□ No (maya)□ Unknown (mahubi)□

If yes what are you taking (Haddii haa maxaad qaadataa)? ……

12. LABORATORY INFORMATION (XOGTA SHEYBAARKA):

Confirmed (Xaqiijin) Yes (haa)No (maya)□

POSSIBLE SOURCE OF INFECTION DURING EXPOSURE PERIOD (Halka loo malaynayo inuu infekshanku ka yimid):

13. Patient with history of typhoid patient (<1 year) (Bukaanka la noolaa qof Tiifow qabay)

Yes (haa)	□
May be but not confirmed (lamahuboo)	□
No (maya)	□
Don't know (magaranayo)	□

14. Have you had contact (work colleagues, school mates, relatives, neighbours….) with typhoid patients within one year before (Ma la yeelatay xiriir taabasho qof (qof isku shaqo, isku iskuul, qaraabo, deris) qaba cudurka Tiifowga sanad gudihiii)?

Yes (haa)No (maya)□

If yes, where (xaggee)?

In household (Guriga) □ Neighbouring (Deriska) □ In hospital (Isbitaalka) □

Working place (Goobta shaqada) □ In the school (Iskoolka) □

15. Did you travel outside your community recently (three months ago)? (Ma u safartay meel ka baxsan beesha dhowaan (sadddex bilood ka hor)?

Yes (haa) □ No (maya) □

If Yes for how long (Haddii haa muddo intee la'eg)?……

If yes, Where (Xaggee)? ……

16. Do you often eat from outside (Badanaa bannanka wax ma ka soo cuntaa)?

Seldom/never (maya) □

Yes (haa) □

If yes, how many times per month (Aday jawabtu tahay haa,imisaa jeer)? ……

If yes where (xaggee)? In the restaurant. □ In the streets. □ In the pub.□

17. How often do you eat these types of food?

(Tick one box for each line), (t = times)

	Seldom/ never	1-2 t/month	1-2 t/ week	3-4 t/ week	Every day
Chicken	□	□	□	□	□
Meat	□	□	□	□	□
Salad	□	□	□	□	□
Ice-cream local seller	□	□	□	□	□

Ice cream small scale industry	□	□	□	□	□

18. Does the family eat from a common plate (unadha hal sixni miyad kucuntin)? Yes (haa) □ No (maya) □
19. Do you take locally prepared cold drinks (cabitanka melaha laguhagachiye macabta)? Yes (haa) □ No (maya) □
20. Do you often wash your hands (Marwaliba madaqata gacmahu)?

	never (maya marnaba)	Sometimes (Marmar)	Usually (Badanaa)
Before eating:	□	□	□
After visiting toilet:	□	□	□

21. Does the family wash hands in common basin (hal wel miyad kufaradaqatin)? Yes (haa) □ No (maya) □
22. What are the sources of water supplies in the family (Xage kahesha biyaha rerka istixmalu)?

Municipal water supply (biyaha tupada) Wells (celka)River(wowiga)□ Rainfall water (biyaha robka) □

23. Is the drinking water treated (biyaha lacabayo manadifisa)? Yes (haa) □ No (maya) □ Unknown □

If yes, by which method(s)? Boil (karkaris) □ Bleach/Chlorine (klorinta) □ Filter (kalamir) □

24. How do you keep your food in your family (halke unadha rerka digta)?

In refrigerator (firingerka) □

Kitchen (jikadha) □

Garden (berta) □

25. Which type of latrine do you have? (Musqul cenke qabta)

Modern toilet (musqul magala) □

Wet latrine (musqul qoyan)□

Dry latrine (musqul engagan)

Excrete directly in the field/bush (benanka miyad musqul uada mise dhurka)

26. Where do you dispose your Sewage (hagee wasaqda musqusha kudhathisa)?

Use public sewage system (waxan istixmala siwejka dhadwanaha) Yes. No. □

Disposal directly to the environment (waxan kudhadhiya binanaha) Yes. □ No. □

FAMILY ECONOMIC LEVEL.

27. How many people are living in your house (imisa qoof aya guriga kunol)? _____

28. How many of these animals does your family own? (imisa xolahan okale ayu rerka leyahay)?

Cow (saac) _____

Goat (rii) _____

Chicken (dhigag) _____

29. How much money does your family earn every month (bil walba rerkakina imisa laaq ey qatan)?.....

30. What type of Material is your house built with (gurigadha maxa lagudisey)?

Brick (dowa) □

Concrete (shamita) □

Wood (gedha)

31. How many rooms are in your house (Gurigadha imisa qol buleyahay)? □

32. Do you have electricity at home (Gurigina koronta maleyahay)?

Yes.(haa) □

No.(maya) □

Appendix II: Consent Form (English Version)

Title of Study: Molecular epidemiology of Salmonella Typhi among patients attending Garissa Provincial General Hospital

Investigator: Khatra Shariff Said

Institution: Jomo Kenyatta University of Agriculture and Technology/KEMRI

Sponsor: FELTP – Kenya

Request: We hereby request your participation in a research study. The research study involves 384 participants. The study aims to determine the rate of typhoid fever and factors associated with the rate and molecularly characterize *Salmonella typhi* isolates from patients attending Garissa Provincial General Hospital.

The study session with you will last about 30 minutes. During this time, you will be asked some questions about typhoid fever, personal hygiene practices, environmental health, waste disposal, and dietary. You will be asked to give a stool and blood samples for collection.

Risks: There are minimal risks involved in the study. You may only encounter some pain or discomfort when blood is being drawn. Your participation is voluntary. You are free to withdraw from the study at any time. If you choose not to participate, or to withdraw from the study, there will be no penalty. If you have any questions or concerns about the study, please contact **The Secretary, KEMRI Ethics Review Committee, P.O. Box 54840-00200 Nairobi; Telephone number**s **020-2722541, 0722205901, 0733400003; E-mail**

address: erc@kemri.org or Khatra Shariff Said, Principal **Investigator P.O. Box 20750 Nairobi (NPHLS); cell phone no. 0722506366; E-mail address: shariffkhatra @yahoo.co.uk**

Benefits: The study aims to improve knowledge on the causes and other factors involved with typhoid infection among the adults in Garissa, in order to reduce the incidence of typhoid infection in the study area. The stool and blood results will help in your diagnosis and treatment.

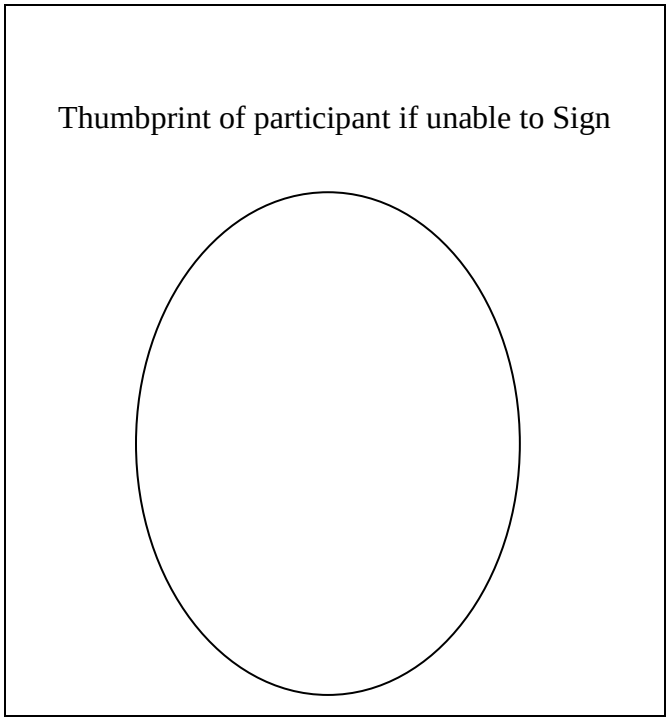
Confidentiality: Information obtained about you for this study will be kept confidential and will be used only for the purposes of the study. Your name will not be required. The results of the study may be published or disseminated without revealing your identity.

Consent: I have been explained and understood the information concerning the research study and voluntarily accept to be part of this study.

Participant

Name.....Signature.....

Date.....



Person Administering Consent

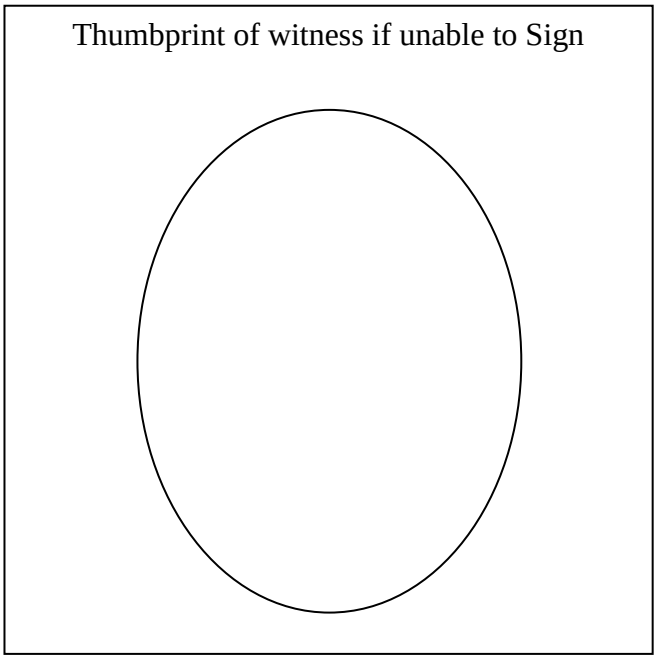
Name……. Signature…….

Date……

Witness

Name…….

Signature……Date……


Thumbprint of witness if unable to Sign

Appendix III: Consent Form (Somali Version)

Ciwaanka Cilmi barista: Astamaha lagu aqoonsado dhaliyaha cudurka tiifowga iyo tilmamaha laxiriiro dadka qabo cudurka oo yimaadan cisbataalka weeyn ee degmada Garissa

Baaraha: Qadra Shariif Saciid

Goobta wax barashada: Jomo Kenyatta University of Agriculture and Technology/KEMRI

Malgaliya: FELTP- Kinya

Codsi: Waxaan halkan kacodsanayaa kaqeeyb qadashadaada cilmi barista. Cilmi barista waxaay quseeysaa cudurka tiifowga iyo astaamaha laxariiro aqoonsiga waxa dhaliyo cudurka .

Qatarta iyo Faidooyinka:

Wax Qatar ah habayaraate majiro oo lalakulmay waqtigii cilmi barista. Cimi barista waxaay xoojineysaa aqoonta looleyahay cudurka tiifowga iyo sababaha ku dhaliya dadka waweeyn.Sheeybar lagu sameeyo saxada iyo dhiiga ayaa cadeeyni cudurka iyo daaweyntiisa.

Asturnaanta: Xugta lagaa helo oo laxiirira cilmi baaristaa waa mid lailaalinayo oona loo adegsani dono qasidka laga leeyahay kali ya.

Masuuliyad: Ka qeeb qadashadaada waa mid aan qasab aheeyn. Xor ayaa tahay waanad kabixi kartaa xiligaad doontid. Wax sameeyn ah kuguma yeelaneeyso hadaad dooratid inaadan kaqeeyb qaadan ama aad kabaxdid.

Fiiro gaar ah: Hadii aad kaqabtid wax suaal ah ama walaac quseeyso cilmi baarisata fadlan lasoo xiriir hafiska KEMRI Ethics Review Committee, Sanduuqa bosto 54840-00200 Nairobi; telefonadha 020-2722541, 0722205901, 0733400003;Imelka: erc@kemri.org mise Qadra Shariif Saciid. Sanduuqa bosto 20750 Nairobi (NPHLS) Telefonka gacanta. 0722506366, Imelka shariffkhatra @yahoo.co.uk

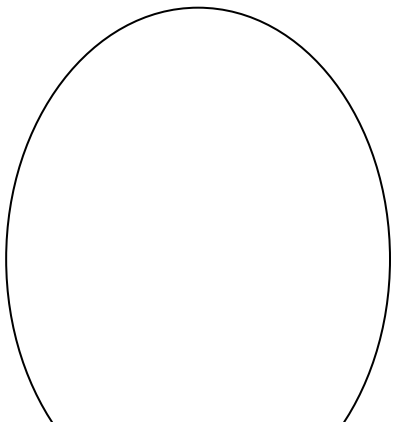
Waxaan xaqiijinyaa in laa laiga dhaadiciyay oonan fahmay xugta quseeysa cilmi barista oo an kaqeebqatay aniga oon laiqasbin.

Qofka kaqeebqadhanayo

Magaca……..Saxiixa……

Tariiiq……..

Sulka qofka kaqeebqadhanaya hadi uu saxixikarin



Qofka codsiga aqrinayo

Magaca …….. Saxiixa…….

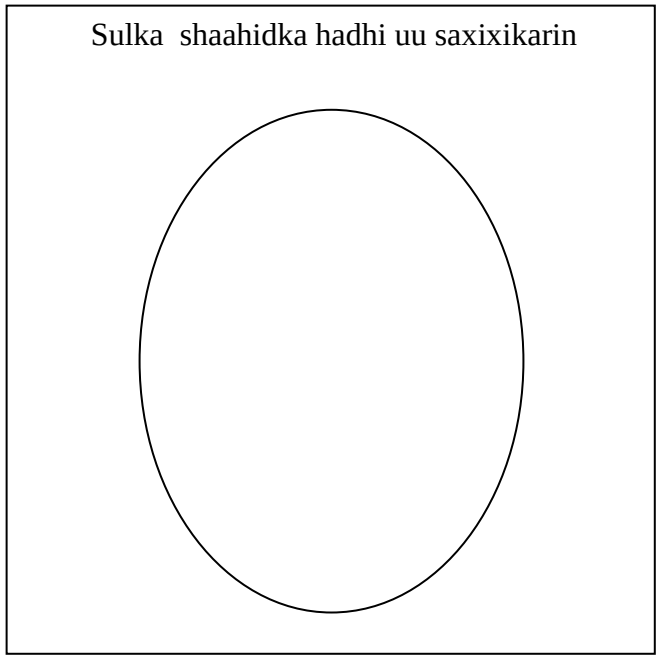
Tariiiq……..

Shaahidka

Magaca……Saxiix……..

Tariiq……

Sulka shaahidka hadhi uu saxixikarin



Appendix IV: SSC Approval

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-206901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

ESACIPAC/SSC/101364 22nd January, 2013

Khatra Said

Thro'
Director, CMR
NAIROBI

Forwarded
30/01/2013

REF: SSC No. 2464 (Revised) – Epidemiology of *Salmonella typhi* among patients attending Garissa Provincial General Hospital

Thank you for your letter received on 17th January, 2012 responding to the comments raised by the KEMRI SSC.

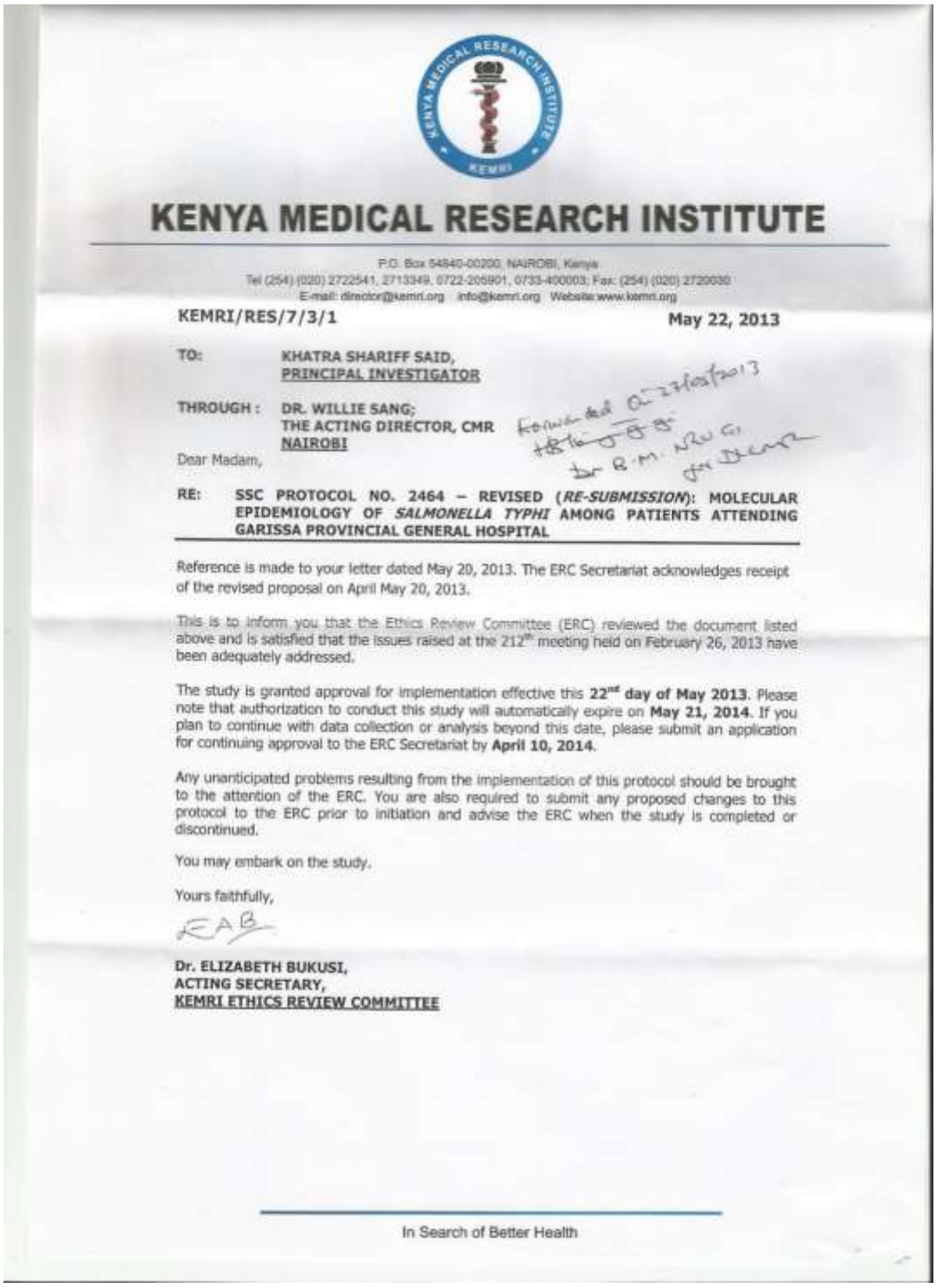
I am pleased to inform you that your protocol now has formal scientific approval from SSC.

The SSC however, advises that work on the proposed study can only start after ERC approval

FOR: Sammy Njenga, PhD
SECRETARY, SSC

In Search of Better Health

Appendix V: SRC Approval



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E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 May 22, 2013

TO: KHATRA SHARIFF SAID,
PRINCIPAL INVESTIGATOR

THROUGH : DR. WILLIE SANG;
THE ACTING DIRECTOR, CMR
NAIROBI

Dear Madam,

RE: SSC PROTOCOL NO. 2464 – REVISED (*RE-SUBMISSION*): MOLECULAR EPIDEMIOLOGY OF *SALMONELLA TYPHI* AMONG PATIENTS ATTENDING GARISSA PROVINCIAL GENERAL HOSPITAL

Reference is made to your letter dated May 20, 2013. The ERC Secretariat acknowledges receipt of the revised proposal on April May 20, 2013.

This is to inform you that the Ethics Review Committee (ERC) reviewed the document listed above and is satisfied that the issues raised at the 212th meeting held on February 26, 2013 have been adequately addressed.

The study is granted approval for implementation effective this **22nd day of May 2013**. Please note that authorization to conduct this study will automatically expire on **May 21, 2014**. 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 **April 10, 2014**.

Any unanticipated problems resulting from the implementation of this protocol should be brought to the attention of the ERC. You are also required to submit any proposed changes to this protocol to the ERC prior to initiation and advise the ERC when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

EAB

Dr. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE

In Search of Better Health