ANTIBIOTIC SUSCEPTIBILITY AND PREVALENCE OF *CAMPYLOBACTER jejuni, ESCHERICHIA coli, SALMONELLA* AND *SHIGELLA* IN DIARRHOIC CHILDREN AT KAPSABET COUNTY REFERRAL HOSPITAL.

BY

HENRY ONGWAE ZACHARIAH

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DECLARATION

DECLARATION BY THE CANDIDATE

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Name of student: **Ongwae H. Zachariah**

SC/PGB/025/10

Signature_____ Date _____

DECLARATION BY SUPERVISORS

This thesis has been submitted with our approval as university supervisors.

Dr. Lizzy Mwamburi

University of Eldoret

School of Science.

Department of Biological Sciences

Signature _____ Date _____

Professor Rose Kakai

Maseno University

School of Medicine

Department of Medical Microbiology

Signature _____ Date _____

ABSTRACT

Diarrhoea causes significant morbidity and mortality among children in Kenya, but it is also a devious problem in children less than 5 years of age in most developing countries with substantial illness among rural sub-Saharan Africans. This cross sectional study was set out to determine the antibiotic susceptibility patterns and prevalence of *Campylobacter jejuni, Non-typhoid Salmonella* (NTS) and *Shigella* among diarrhoeic children admitted to the paediatric ward at Kapsabet County Hospital. Stool samples collected in a period of six months were cultured in selective media and biochemical tests done on suspect colonies for proper identification. The Kirby-Bauer disk diffusion method was used for susceptibility testing on eleven commonly used antibiotic agents. One hundred and thirty-nine (97%) specimens were investigated, which yielded a total of 72 bacterial isolates, with 12.9% of *C. jejuni, Salmonella* species-(NTS) 9.4% and *Shigella* (20.5%). Further evaluation of samples yielded enterogenic *E. coli* (9.4%) and on differential diagnosis, *Giardia lamblia* (2.9%) and *Entamoeba histolytica* (0.7%) were identified. *C. jejuni* and *Salmonella* (NTS) and *Shigella* spp. were found to be highly susceptible to ciprofloxacin at (70.4%), (100%) and (73.3%) respectively. *Salmonella* was the single most highly susceptible isolate to Nalidixic acid at 13(86.7%; p=.016) while *E.coli* was highly susceptible to ciprofloxacin 11(100.0%) and minocyclin 8(88.9%). Among the 28 isolates of *Shigella* spp. 82.1%, were resistant to ampicillin. The least potent drugs were erythromycin, doxycyclin, cotrimoxazole and ampicillin. All the 72 isolates were resistant to at least four antibiotic agents (p= .047) indicating that their use would not have reduced the illness. Low resistance to ciprofloxacin, norfloxacin, ceftriaxone and Cefuroxime indicates that these drugs may be more effective. Chi-square ($\chi 2$) value 252.4, p=0.0001 indicated significant difference in drug resistance patterns among the pathogens isolated. Nearly one-half of the patients who presented to the paediatric ward with diarrhoea had a definite or plausible pathogen in their stool specimens, with the overall prevalence of cases of diarrhoea in Kapsabet with a known aetiology of 52% during the period under study. This not only calls for a better health education, improved and sustainable food hygiene and provision of more functional water services but also a judicious use of antibiotics.

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ABBREVIATIONS

CDC – Centre for Disease Control

CDCP – Centre for disease control and prevention

CID – centre for information and documentation

CHERG - Child Health Epidemiology Research Group

CLSI – Clinical and Laboratory standards institute

DDP – District Development Plan

IMViC – Indole, Methyl red, Voges Proskauer's, Citrate

IREC – Institutional Research and Ethics Committee

KCH – Kapsabet County Hospital

MDR – Multi Drug Resistance

MOH – Ministry of Health

TSI – Triple sugar iron

WHO – World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Diarrhoea is one of the principal causes of morbidity and mortality among children in the developing world. Successive estimates of global diarrhoeal deaths have fallen from 4·6 million in 1980 through 3·3 million, 2·9 million, to 2·2 million in 2000(Curtis *et al.*, 2003; WHO, 2003).

Reviews of active surveillance data from studies conducted in the 1950s through the 1990s, estimated that children in the developing world experienced a median of between two and three episodes of diarrhoea every year. Therefore, every year there are approximately 1.5 billion diarrheic episodes and 4 million deaths in children less than five years of age (most from 6 months to 60 months) caused by this disease (WHO, 2000; Vargas *et al.*, 2004).While there may be other, underlying causes such as malnutrition, diarrhoea is often an immediate cause of death, and its prevention could avert mortality. In 2009 diarrhoea was estimated to have caused 1.1 million deaths in people aged 5 and over and 1.5 million deaths in children under the age of five (5) years. Reports indicated that infant mortality rate for children in Benin under 5 (five) remains one of the world's highest and that diarrhoea still remains the second leading killer of children. Nearly one in five children under the age of five dies as a result of dehydration, weakened immunity or malnutrition associated with diarrhoea. Similarly an estimated 800,000 – 1.5 million children succumb to diarrhoea in the developed world each year (UNICEF, 2009/2010).

While mortality from diarrhoea among children under five has dwindled over the past two decades, overall incidence had remained steady at 2.5 billion cases per year. South Asia and Sub-Saharan African continue to shoulder the heaviest burden (UNICEF 2009/10; WHO 2009).

Diarrhoea is defined by the World health Organization as having 3 or more loose or liquid stools per day, or as having more stools than is normal for that person. Diarrhoea causes significant morbidity and mortality among children in Kenya, but it is also a devious problem in children less than 5 years of age in most developing countries with substantial illness among rural sub-Saharan Africans (WHO, 1992).

The main aetiology of the diarrhoea is related to a wide range of bacteria (such as *Campylobacter jejuni*, *Escherichia coli*, *Salmonella* spp., *Vibrio cholerae*, *Yersinia enterocolitica*, and *Aeromonas* spp.), enteroparasites (*Giardia* spp., *Cryptosporidium* spp., and *Entamoeba histolytica*), and viruses (*adenovirus*, *Norwalk virus*, and *rotavirus*). In many hospitals in developing countries lacking clinical microbiology laboratories, the cause of diarrhoea in children is unknown (WHO, 2000; Vargas *et al.*, 2004)

This episode of diarrhoea even if not deadly seem to have a profound impact on a child's immune system, nutritional states and cognitive development, and at this stage it is important to use effective anti-diarrhoeal agents, where indicative to appropriately manage diarrhoea (Malakooti *et al.*, 1997). Antibiotics are recommended for treating watery and bloody diarrhoea to shorten the duration of illness, decrease morbidity and mortality, and reduce the duration of bacterial shedding. Antibiotic resistance among the major bacterial causes of bloody diarrhoea is increasing worldwide such that in

sub-Saharan Africa, repeated prolonged outbreaks of dysentery with high case fatality rates have increased the demand for antibiotics; consequently, causative pathogens such as *Shigella* developed resistance to locally affordable and available antibiotics (WHO, 1999; Jorgensen, 2007).

World Health Organization (WHO) identified Tetracycline, trimethoprim – sulphamethoxazole and Nalidixic acid as some of the drugs of choice for treatment of specific causes of diarrhoea in children. Effectiveness of aminoglycosides, cephalosporin and cotrimoxazole has also been established on bacterial isolates from children with diarrhoea in the Indian-subcontinent specifically in Indonesia and Pakistan. Streptomycin was found to be effective against most aerobic gram negative bacteria. It was however, noted that extensive use of streptomycin was associated with development of widespread antibiotic resistance (WHO, 2000).

Treatment of dysentery with antibiotics to which the etiologic agent is resistant may prolong illness and increase risk of haemolytic uremic syndrome and death. This condition is caused by an array of pathogens and more specifically the enteric bacteria and other related ones, which continue to reflect in the high incidence rate of diarrhoea among children under 5 years of age in the last 20 years. Due to this increased rate, there has been increasing recommendation for self-initiated therapy and use of prophylactic drugs for infant diarrhoea, resulting in development of resistance by some of the pathogens (Ahmed *et al.*, 2000; Lalitha 2002).

First line antibiotics such as Ampicillin, erythromycin and chloramphenicol continue to show high level of resistance. Even the current first line antibiotic agents including the fluoroquinolones and erythromycin show significantly high minimum inhibition

concentration (MICs) above the cut offs for resistance (Doern, 1995; Ndungu *et al.*, 2002). Accordingly microbial resistance to common antibiotics has increased recently and hence anti-diarrhoeal drugs have a limited role in the treatment of diarrhoea and should only be used if diarrhoea continues for more than 24 hours. The effective anti-diarrhoea antibiotics can only be selected after the agent has been identified by a technique of stool culture, but should not be given routinely to patients with diarrhoea (WHO report 1999/2000; Lalitha, 2002).

1.2 Statement of the problem

Nearly half of deaths from diarrhoea among young children occur in Africa where diarrhoea is the single largest cause of death among under 5-year-olds and a major cause of childhood illness. Unfortunately, Sub-Saharan Africa continues to experience repeated outbreaks of diarrhoeal diseases with high case fatality rates, which has led to an increased demand for antibiotics (Jorgensen, 2007).

Clinicians rarely rely on empirical evidence from ASTs for selection of antimicrobial drugs in the appropriate management of their patients who suffer from diarrhoeal illnesses.

Surveillance reports from the Kapsabet County Referral Hospital (MOH, 2010) indicates that not less than two to three cases of diarrhoea were recorded per day at the paediatrics filter clinic, and half of these cases were admitted for hospital based management. An earlier report indicates that diarrhoeal disease ranked fourth among the most common diseases diagnosed at the hospital (Development plan, 2007). At the same time most of the cases of diarrhoea are clinically managed and thus the etiological agents are not known.

The purpose of this study was to isolate *C. jejuni, E. coli,* Non-typhoid *Salmonella* and *Shigella* species as aetiological agents of diarrhoea and their antibiotic susceptibility in children aged below five years.

1.3 Justification

Diarrhoea still remains a health risk factor to children under five years, and thus knowledge of types of common causal organisms and their antibiotic susceptibility is important to guide policy in selecting the appropriate therapy and control measures. *C. jejuni, Shigella* (NTS) and *Salmonella* species among others continue to be a major source of morbidity and mortality in the developing world due to poor sanitation and unsafe practices in the food chain. Antibiotic resistance constitutes an important element of disease burden and the patterns of antibiotic resistance, which vary considerably from place to place and which are in a continuous state of evolution, must also be updated (WHO, 1999; Wilson 2005).

The fact that resistant bacteria can multiply and spread implies that they can also share their resistance with other kinds of bacteria to create new resistant bacterial strains. Most enteric infections are self-limiting, but antimicrobial agents are essential to treat severe illness, however if pathogens are resistant to antimicrobial agents, illness may be prolonged or more severe and thus determining antimicrobial resistance in bacteria isolated from people is central to understanding and preventing transmission of antimicrobial resistant infections. Antibiotic resistance patterns may vary locally and regionally, so surveillance data needs to be collected from selected sentinel sources. Results of *in-vitro* antibiotic susceptibility testing, will guide clinicians in the

appropriate selection of initial empiric regimens and, drugs used for individual patients in management of specific pathogens (Lalitha, 2007).

1.4 Broad Objective

Determine the bacterial aetiology and associated causal factors for diarrhoea and evaluate the effect of different families of antibiotics used in the clinical therapy of the enteric bacterial pathogens in children below 5 years of age admitted with diarrhoea at Kapsabet County Hospital.

1.4.1 Specific Objective

1. To determine the prevalence of *C.jejuni,* Non-typhoid *Salmonella, E. coli* and *Shigella* spp. in children aged five years and below admitted with diarrhoea at Kapsabet County Hospital.
2. To determine the *in vitro* antibiotic susceptibility of *C. jejuni,* Non-typhoid *Salmonella, E. coli* and *Shigella* in children aged five and below.
3. To establish possible sources of *C. jejuni*, Non-typhoid *Salmonella*, *E. coli* and *Shigella* infection among the affected children in the study population.

1.5 Research Questions

1. What are the prevalence rates of *Campylobacter jejuni*, Non-typhoid *Salmonella*, *E. coli* and *Shigella* in children aged five years and below admitted with diarrhoea at Kapsabet County Hospital?
2. What are the patterns of antimicrobial susceptibility testing of *Campylobacter jejuni*, Non-typhoid *Salmonella, E. coli* and *Shigella* in children aged five and below in the sample population?

3. What are the possible sources of *Campylobacter jejuni*, Non-typhoid *Salmonella*, *E. coli* and *Shigella* in children aged five and below in the sample population?

CHAPTER TWO

LITERATURE REVIEW

2.1 Epidemiology of Diarrhoea

Diarrheal diseases constitute a major burden of disease in the world, especially in low- and middle-income countries, more so in sub-Saharan Africa it is responsible for substantial illness among the rural sub-Saharan Africans, where visibly bloody diarrhoea causes proportional greater morbidity and mortality. Of all medical conditions, diarrhoea is the second leading cause of time lost to illness, 72.8 million disability adjusted life years (DALYs). Diarrheal illnesses are particularly dangerous for young children who are more susceptible to dehydration and nutritional losses during an episode of acute diarrhoea. Around 90% of diarrhoea-related deaths occur among under-five children living in low- and middle-income countries (Tafa*et al.,* 2013). Over 1.8 million under-five children die of diarrheal disease, these accounts for 19% of all childhood deaths. Diarrhoeal diseases are amongst the top three killers of children in the world today. At least 20 viral (such as *Adenovirus*, *Rotavirus*, and *Norwalk virus*) are the most common cause of diarrhoea bacterial, while, *E. coli, Clostridium difficile, Campylobacter, Vibrio cholerae*, *Salmonella* spp. and *Shigella spp.* are common bacterial causes. *Entamoeba histolytica* and *Giardia, Cryptosporidium,* and *Cyclospora* species are parasitic or protozoan agents that cause diarrhoea (Kubota *et al.,* 2008). These pathogens multiply in the human gut, exit in excreta, and transit through the environment, causing diarrhoea in new hosts (Curtis *et al.,* 2003; Ramaswany *et al.,* 2001).

According to the World Health Organization (WHO) fact sheet diarrhoea occurs worldwide and causes 4% of all deaths and 5% of health loss to disability. It is most commonly caused by gastrointestinal infections, which kill around 2.2 million people globally each year, mostly children in developing countries. At the time data from Kenya reveals that diarrhoea prevalence varied from high rates of about 20% in Kakamega, Siaya, Homabay, Migori and Kilifi counties to low rates of below 10% in TaitaTaveta, Uasin Gishu, Muranga, Nyeri and Nandi counties (WHO, 2000).

It is estimated that more than 10 million children younger than 5 years of age die each year worldwide, with only six countries accounting for half of these deaths .Although most mortality under 5 years of age occurs in India, Nigeria, and China, of the 20 countries with the highest mortality rates for individuals under 5 years of age, 19 are in Africa. The Child Health Epidemiology Research Group (CHERG), created by the WHO in 2001, has used various methods to determine specific causes of mortality. Based on older data, the CHERG estimated that the syndrome of diarrhoea accounted for 18% of all deaths in children under the age of 5, with malnutrition as a comorbid condition in 53% of all deaths (William *et al.,* 2008)

Worldwide diarrhoea constitutes a major cause of morbidity and mortality, especially among the very young, very old, and infants. Most cases are caused by enteric infections; Food- and waterborne outbreaks involving a relatively small subset of population and recurrent bouts of illness in others comprise most cases. Diarrhoea is more prevalent among adults who are exposed to children and non-toilet-trained infants, particularly in a day-care setting. It is also more prevalent in people living in nonhygienic environments, with exposure to contaminated water or foods (Boyd, 1981; WHO Weekly Report, 2004; William *et al.,* 2008). Although a large proportion

of the population resides in rural areas, where poor water quality, unhygienic disposal of human waste, and other established risks for bacterial diarrhoea are encountered frequently, surveillance has generally been limited to major urban areas, where laboratory capacity, trained health care personnel, and resources are concentrated (Brooks *et al*., 2003).

Fatalities from acute diarrhoea are overall globally declining but remains high. Most estimates have diarrhoea as the second cause of childhood mortality, with 18% of the 10.6 million yearly deaths in children younger than age 5 years (Wilson, 2005). Despite a progressive reduction in global diarrhoeal disease mortality over the past 2 decades, diarrhoea morbidity in published reports from 1990-2000 slightly increased worldwide compared with previous reports (WHO, 2008). Furthermore, in countries where the toll of diarrhoea is highest, poverty also adds an enormous additional burden, and long-term consequences of the Acute diarrhoea and dehydration is a serious problem in children less than 5 years of age in developing countries, more so in sub-Saharan Africa and in the Indian sub-continent (Burgess *et al.,* 2005).. Worldwide in 2004 approximately 2.5 billion cases of diarrhoea occurred which resulted in 1.5 million deaths among children under the age of five. Greater than half of these were in Africa and South Asia, down from a death rate of 5 million per year two decades ago (WHO, 1999; Burgess *et al.,* 2005).

Estimation assumes a cumulative incidence of 1 hospitalization for diarrhoea per 23-27 children by age 5 years, with more than 50,000 hospitalizations in 2000. Furthermore, acute diarrhoea is responsible for 20% of physician referrals in children younger than 2 years and for 10% in children younger than 3 years (CDC, 2000; WHO, 2008). In developing countries, an average of 3 episodes per child per year in

children younger than 5 years is reported; however, some areas report 6-8 episodes per year per child. In these settings, malnutrition is an important additional risk factor for diarrhoea, and recurrent episodes of diarrhoea lead to growth faltering (Lalitha, 2002). Childhood mortality associated with diarrhoea has constantly but slowly declined during the past 2 decades, mostly because of the widespread use of oral rehydration solutions; however, it appears to have plateaued over the past few years (Behrman *et al.,* 1996). Mortality vicious cycle of enteric infections, diarrhoea, and malnutrition are devastating (WHO, 2002).

Diarrhoea causing pathogens are usually transmitted through the faecal-oral route, and the risk factors for this type of transmission include improper disposal of faeces and lack of proper hand washing following defecation and faeces contact before handling food. Other risk factors include improper food hygiene, inadequate food refrigeration, food exposure to flies, and consumption of contaminated water (Brooks *et al.,* 2003). Epidemiologic risk factors for certain diarrhoeal diseases and their spread include recent travel to an underdeveloped area, day-care centre exposure, consumption of raw meat, eggs, shellfish, and unpasteurized milk products, contact with reptiles or pets with diarrhoea, a history of other ill people in a shared dormitory facility, recent antibiotic use, and a history of HIV or medically induced immunosuppression (Kariuki *et al.,* 1994; Loren *et al.,* 2006).

2.1.1 Pre-disposing factors for diarrhoea

Although some viruses are geographically ubiquitous, such as rotavirus, which is estimated to infect 90% of the population of the world younger than 5 years of age, most enteric infections are environmentally determined, with restricted geographical

and seasonal patterns related to the degree of sanitation and hygiene as well as access to clean drinking water. As sanitation, hygiene, and safe drinking water are directly related to economic development, over time this has effectively defined the incidence and prevalence of many of the bacterial agents of enteric infections. For example, cholera, shigellosis, and typhoid are most common in the most underserved populations, with greater incidence at times of limited water supply and flooding (during which water supplies can be contaminated by sewage) (Arvelo *et al.*,2009).

Other factors reported to be indirectly associated with occurrence of diarrhoea, include; Host susceptibility, Gut immunity, Biomarkers of inflammation and barrier disruption among others**.** Individuals are not equally susceptible to infection by different microbes; if infected, possible outcomes range from asymptomatic colonization to death. There are many reasons why individuals differ in their susceptibility to infection with enteric pathogens, including their genetic makeup and their ability to mount potent immune responses in the gut (William *et al.*, 2008).

2.1.2 Pathophysiology

The pathophysiology of infectious diarrhoea elucidates fundamental processes of cell signalling and transport and has several mechanistic paradigms by which infectious agents interact with intestinal mucosa. At the onset approximately eight to nine litre of fluid enter the intestines daily. One to two litres represents food and liquid intake, and the rest is from endogenous sources such as salivary, gastric, pancreatic, biliary, and intestinal secretions. Most of the fluid, about 6 to 7 litres, is absorbed in the small intestine, and only about 1 to 2 L is presented to the colon. Most of this is absorbed as it passes through the colon, leaving a stool output of about 100 to 200 g/day. Although

many organisms simply impair the normal absorptive processes in the small intestine and colon, others, organisms, such as *Vibrio cholerae*, secrete a toxin that causes the colonic mucosa to secrete, rather than absorb, fluid and electrolytes and thus voluminous diarrhoea may result (William *et al.*, 2008).

For *C. jejuni* the sites of tissue injury include the jejunum, the ileum, and the colon, where it appears to invade and destroy epithelial cells. *C. jejuni* are attracted to mucus and fucose in bile, and the flagella may be important in both chemotaxis and adherence to epithelial cells or mucus. Mucosal invasion is mediated by bacterial surface proteins and occurs after specific binding, and seems to be the most important virulence trait. Low levels of cytotoxins that damage mammalian cells are produced by some isolates and may help in the invasion process (Michael *et al.*, 2001; Simango *et al.*, 1997; Benaissa, 2007). Some strains of *C. jejuni* produce a heat-labile, cholera like enterotoxins, which is important in the watery diarrhoea observed in infections. Infection with the organism produces diffuse, bloody, oedematous, and exudative enteritis (Coker *et al.*, 1994). It produces an inflammatory, sometimes bloody, diarrhoea, or dysentery syndrome, mostly including cramps, fever and pain (Karmali *et al.*, 2009). Most strains of *C.jejuni* produce enterotoxins (cytolethal distending toxin) that hinder the cells from dividing and activating the immune system. This helps the bacteria to evade the immune system and survive for a limited time in the cells (Butzler, 2000; Jenifer *et al.*, 2013).

Bacteria that destroy enterocytes such as *Shigella, enteroinvasive E. Coli* and bacteria that penetrate the mucosa such as *Salmonella, C. jejuni* and *Y. enterocolitica* result in moderate to severe inflammation with or without ulceration just as does exposure to enterotoxins (exotoxins that elicit secretion by increasing an intracellular second

messenger) or cytotoxins. They may also trigger release of cytokines (CT) attracting inflammatory cells, which, in turn, contribute to the activated secretion by inducing the release of agents such as prostaglandins or platelet-activating factor. The enterotoxins from the bacterial pathogens may also strongly activate adenylyl cyclase, causing a prolonged increase in intracellular concentration of cyclic AMP within crypt enterocytes, which stimulates the active Cl^- secretion by activating or inserting Cl^- channels into the apical membrane of crypt cells and inhibit electroneutral NaCl absorption by decreasing the activity of parallel apical membrane Na/H and Cl/HCO_3 exchange in villous cells, but they do not alter apical membrane glucose-stimulated Na absorption. This change results in prolonged opening of the chloride channels that are instrumental in secretion of water from the crypts, allowing uncontrolled secretion of water (Behrman *et al.,* 1996; Bowen, 2006). By contrast, ST activates guanylate cyclase, resulting in increased mucosal cyclic GMP, which has similar, but not identical, effects on ion transport as cAMP (William *et al.,* 2008).

Additionally, these toxins affect the enteric nervous system, resulting in an independent stimulus of secretion. Disruption of the epithelium of the intestine due to microbial pathogens is a very common cause of diarrhoea in all species, such that it's destruction results not only in exudation of serum and blood into the lumen but often is associated with widespread destruction of absorptive epithelium. In such cases, absorption of water occurs very inefficiently and diarrhoea results (Bowen, 2006; William *et al.,* 2008).

The immune response to inflammatory conditions in the bowel contributes substantively to development of diarrhoea. Activation of white blood cells leads them to secrete inflammatory mediators and cytokines which can stimulate secretion, in

effect imposing a secretory component on top of inflammatory diarrhoea. Reactive oxygen species from leukocytes can damage or kill intestinal epithelial cells, which are replaced with immature cells that typically are deficient in the brush border enzymes and transporters necessary for absorption of nutrients and water (Ndungu *et al.,* 2001).

Ingestion of preformed toxin produced by bacteria such as *B. cereus, S. aureus*, and *C. perfringens* can result in acute jejunitis. *Aeromonas, Shigella, and Vibrio* spp. (e.g., *V. parahaemolyticus)* produce enterotoxins and also invade the intestinal mucosa. Patients therefore often present with watery diarrhoea, followed within hours or days by bloody diarrhoea. Bacteria that produce inflammation from cytotoxins include *C. difficile* and hemorrhagic *E. coli* O157:H7 (Kubota *et al.,* 2008; William *et al.,* 2008).

Exudative diarrhoea results from extensive injury of the small bowel or colon mucosa as a result of inflammation or ulceration, leading to a loss of mucus, serum proteins, and blood into the bowel lumen. Increased faecal water and electrolyte excretion results from impaired water and electrolyte absorption by the inflamed intestine rather than from secretion of water and electrolytes into the exudates. Non-infectious causes of diarrhoea include inflammatory bowel disease, irritable bowel syndrome, ischemic bowel disease, partial small bowel obstruction, pelvic abscess in the recto-sigmoid area, faecal impaction, and the ingestion of poorly absorbable sugars, such as lactulose and acute alcohol ingestion. Diarrhoea is one of the most frequent adverse effects of prescription medications; it is important to note that drug-related diarrhoea usually occurs after a new drug is initiated or the dosage increased (Naik, 2006; Huttly*et al.,* 2007).

Diarrhoea caused by either infectious or non-infectious aetiologies is invariably the result of changes in fluid and electrolyte transport in the small and/or large intestine Although diarrhoea represents increased fluid loss through the stool, intestinal fluid movement is secondary to solute movement, so that solute absorption and secretion are the driving forces for net fluid absorption and secretion, respectively. Thus, an understanding of diarrhoea requires delineation of the regulation of ion transport in the epithelial cells of the small and large intestine. Net fluid secretion is secondary to stimulation of Cl^- secretion in crypt cells and/or inhibition of electroneutral NaCl absorption in villous surface epithelial cells (William *et al.,* 2008).

2.1.3 Signs and Symptoms

Symptoms of *Campylobacter* infection begin after an incubation period of up to a week. As the infection is usually self-limiting in most cases, symptomatic treatment by reposition of liquid and electrolyte replacement is enough in human infections. Symptoms typically last for 5–7 days (Behrman *et al.,* 1996; Laforce *et al.,* 1999).

Campylobacteriosis (the illness associated with infection with *Campylobacter* spp.), lacks very specific clinical features therefore it is difficult to distinguish gastrointestinal problems associated with *Campylobacter* spp. from other acute gastrointestinal infections. A definitive diagnosis can be made only by detecting *Campylobacter* spp. in clinical specimens. Clinical disease following infection with *Campylobacter* spp. varies from a mild self limiting enterocolitis lasting 24 hours to severe illness lasting up to 10 days. A relapse occurs in10 to 20% of patients and is usually less severe than the original episode of illness. The great majority of persons infected with *Campylobacter* spp. will recover without any specific treatment.

Infection may be more common and more severe in HIV-infected patients and in other patients suffering from immunosuppression. In the immunocompromised and in those with more severe illness, antimicrobial agents may be required, and can shorten the duration of symptoms if they are given early in the illness. Mortality is rare (Food authority, 2002).

Shigella spp. requires very low inocula to cause illness. Ingestion of as few as 10 *Sh.dysenteriae* serotype 1 organisms can cause dysentery in some susceptible individuals (Brooks *et al.,* 2003). This is in contrast to organisms such as *Vibrio cholerae,* which require ingestion of 10^8-10^{10} organisms to cause illness. The inoculum effect explains the ease of person-to-person transmission of *Shigella* in contrast to *Vibrio cholera* (Lalitha, 2002; Benaissa *et al.,* 2007).

From the incubation period of between 12 to 50 hours, *Shigella* symptoms usually develop about 1 to 7 days (average 3 days) after you come in contact with the bacteria. The disease is self-limiting, usually resolving within 5 to 7 days without antibiotics. In severe cases, the infection may last 3 to 6 weeks, but severe cases, particularly when dehydration is a concern, may require antibiotic therapy. Acute, life-threatening complications are most often seen in malnourished infants and young children living in developing countries (Hohmann, 2001; Boyd, 1981).

The onset is acute, with fever, abdominal pain and watery diarrhoea. As the disease increases in intensity, bloody diarrhoea with mucous, faecal urgency and severe cramping abdominal pain occur. Nausea, vomiting, headache and convulsions (in children) may occur. Persons with diarrhoea usually recover completely, although it may be several months before their bowel habits are entirely normal. About 3% of

persons who are infected with one type of *Shigella*, *Shigella flexneri*, will later develop pains in their joints, irritation of the eyes, and painful urination, this is called Reiter's syndrome. It can last for months or years, and can lead to chronic arthritis which is difficult to treat. Reiter's syndrome is caused by a reaction to *Shigella* infection that happens only in people who are genetically predisposed to it (Kottlof *et al.,* 2000). Severe infections result in mucosal ulceration, rectal bleeding and dehydration. The fatality may be as high as 10-15% with some strains (Pornthip *et al.,* 2006).

Patients with severe infection may pass more than 20 dysenteric stools in one day. With repeated infections by the same serotype, illness is absent or attenuated and excretion is diminished. Rarely, *Shigella* disseminates to distant sites causing focal infections such as meningitis, arthritis, spleenic abscess, and osteomyelitis (Stutman, 1999; WHO, 1995).

All age groups are susceptible to Non typhoid *Salmonella*, but symptoms are most severe in the elderly, infants, and the infirm. AIDS patients suffer salmonellosis frequently (20 fold greater than the general population) and suffer from recurrent episodes. Typically, the illness begins 12 to 48 hours after the ingestion and consists of nausea and vomiting, with abdominal pain and diarrhoea. Fever is present in about half the patients and a mild headache may be present. Diarrhoea persists as the prominent symptom for 3 or 4 days. Salmonellosis may be complicated by reactive arthritis and Reiter's syndrome. The infective dose is small: as few as 15-20 cells (Hellene *et al.,* 2010).

In cases of extra intestinal manifestations the acute gastroenteritis caused by many *Salmonella* serotypes is also associated with transient bacteraemia. In humans, *S. choleraesuis* often presents as a focal infection without any obvious GI manifestations (William *et al.,* 2008). About one-half of infected persons (carriers) continue to excrete *Salmonellae* 1 month after the symptoms have disappeared and 1 in 20 persons still do 5 months later. An unknown fraction of people become carriers after asymptomatic infection; the median carriage rate of *Salmonella* among healthy persons in developed countries is 0.13% (Hellene *et al.,* 2010).

2.2 Microbiology of *C. jejuni,* NTS, *E. coli and Shigella* spp.

2.2.1*Campylobacter jejuni*

Campylobacter species have been the focus of growing attention for the past 30 years because of the increasing frequency with which they have been isolated from man, animals, food and water. Although several Campylobacter species (*C. jejuni, C. coli, C.upsaliensis, C. lari, C. concisus, C. fetus* sub-sp. *fetus, C. jejuni sub-sp.doylei*, and C.*hyointestinalis*) have been shown to cause diarrhoea, Campylobacter jejuni is by far the most frequent species isolated from man. *C. jejuni* is a frequent cause of morbidity, in both industrialized and developing countries, and represents a considerable drain on economic and public health resources (Michael *et al.,* 2001; Butzler, 2004).

The genomes of several *Campylobacter* species have been sequenced, providing insights into their mechanisms of pathogenesis (Poisson *et al.,* 1992; Chen *et al.,* 1995). The first *Campylobacter* genome to be sequenced was *C. jejuni* in 2000.*Campylobacter* species contain two flagellin genes in tandem for motility, flaA

and flaB. These genes undergo intergenic recombination; further contributing to their virulence; however, non-motile mutants do not colonize (Michael *et al.,* 2001). *Campylobacter* that typically infects the bowels is now the leading cause of bacterial gastroenteritis and of systemic infections, primarily in children and compromised patients. *Campylobacter* (meaning 'twisted bacteria') is a genus of bacteria that are gram-negative, spiral or thin, short and S-shaped or long multispiralled, and filamentous, non-spore forming and microaerophilic(Behrman *et al.*,1996).They are motile with either unipolar or bi-polar flagella, the organisms have a characteristic spiral/corkscrew appearance and are both oxidase-and catalase test positive, measuring 0.5-1mm slightly raised, smooth colonies on solid media. Organisms from young cultures have a Vibrio-like appearance, but, after 48 hours of incubation, organisms appear coccoid. *C. jejuni* colony morphology may appear as small, mucoid, greyish, flat colonies with irregular edges and non-haemolytic patterns at 24-48 hours. They may also appear as round, convex, entire, glistening colonies 1-2mm in diameter. Certain strains of *C. jejuni* may appear lightly pink or tan in colour (Janet, 1999).

Campylobacter organisms require reduced oxygen (5-10%) and increased carbon dioxide (3-10%). The organisms grow slowly, with 3-4 days required for primary isolation from stool samples, and even longer from blood. Most members of this group are partially anaerobic, and most undergo transformation into coccoid forms when exposed to adverse conditions, especially oxidation (Vandamme, 2000).These appear to be degenerative forms, but some believe they are potentially dormant forms capable of long survival. Although some *Campylobacter* can survive in cold water for several weeks, even months, they are not necessarily in coccal form. In general, these

bacteria are fragile and easily destroyed by heat, desiccation, acidity, irradiation, and disinfectants (Behrman *et al.*,, 1996).

2.2.2 *Salmonella*

According to contemporary classification, the genus *Salmonella* contains only two species, *Salmonella bongori* and *Salmonella enterica*, but there are more than 2,500 serovars of S. enterica. Traditionally, *Salmonella* species were named in accordance with the Kaufmann-White typing system, defined by different combinations of somatic O, surface Vi, and flagella H antigens. In 2005, *Salmonella enterica* finally gained official approval as the type species of the genus *Salmonella*. The genus *Salmonella* also contains the species *Salmonella subterranean*, which was recognized in 2005.Currently, *Salmonella* species have the serologically defined names appended as serovars or serotypes. For instance, the current nomenclature of *S. typhi* is *S.enterica* serovar Typhi. *S. enterica* is preferred over confusing name *S.choleraesuis*, which is also the name of a commonly isolated serotype (Brenner *et al.*, 2000; Su *et al.,* 2007). Although no longer prevalent in the developed world, *S. enterica* serovars Typhi and Paratyphi continue to cause enteric fever in many parts of the developing world, especially in Asia and northern regions of Africa (Gordon, 2009) These agents are still estimated to cause approximately 22 million cases of disease and 200,000 deaths each year, primarily in regions where sanitation is poor and clean water is inaccessible (Hellene *et al.,* 2009)

The genus *Salmonella,* which belongs to the family Enterobacteriaceae, was named after Daniel E. Salmon, an American veterinarian who first isolated *S.choleraesuis* from pigs with hog cholera in 1884. As with the closely related bacterium *E. coli*,

salmonellae are potential enteric pathogens and a leading cause of bacterial foodborne illness. In addition, *Salmonella* species have been implicated in a spectrum of other diseases, including enteric (primarily *S. typhi* and *S. paratyphi*), endovascular infections, focal infections (e.g., osteomyelitis), and enterocolitis (typically caused by *S. typhimurium, S. enteritidis,* and *S. Heidelberg*) (Brenner *at al*., 2000).

Salmonellae are oxidase-negative and predominantly lactose-negative. Less than 1% of nontyphoidal *Salmonella* isolates are lactose-positive (pink on MacConkey agar), but most produce hydrogen sulphide. *Salmonella* is a group of closely related slender, fimbriated, non-sporing and non-encapsulated Gram negative motile rods with flagella. They are resistant to many physical agents but can be killed by heating at 54.4^0C for 1 hour or 60^0 C for 15 minutes. They remain viable at ambient or reduced temperatures for days and may survive for weeks in sewage, dried foodstuffs, pharmaceutical agents and faecal material (Ackers *et al.,* 2000; Behrman *et al.,* 1996).

Salmonella posses' somatic O antigens that are the heat-stable lipopolysaccharides components of the cell wall, and the H antigens that are Heat- labile proteins. They also posses another antigen, the virulence (Vi) capsular polysaccharide. There are different types of *Salmonella*, but they all produce a similar clinical picture to other forms of infective gastroenteritis (Saida, 2007). Serogroups A to E are the ones that usually cause disease in humans. Serogroups B, C, and D are responsible for most infections. *S. enteritidis* is serogroup D and is the most common cause of salmonella gastroenteritis. The other epidemiologically important species is *S.typhimurium* (JohnDT, 1999; Boyd, 1981).

2.2.3 *Shigella*

The *Shigella* species are the principal agents of bacterial dysentery. *Shigella* was discovered over more than a century ago by a Japanese scientist named Shiga, after whom they are named. *Shigella* was adopted as a genus in the 1950s and is a member of the family Enterobacteriaceae and tribe Escherichieae. The genus *Shigella* is subdivided into four groups (Group A - *Sh.dysenteriae*, Group B - *Sh.flexneri*, Group C- *Sh.boydii*, Group D - *Sh.Sonnei*) on the basis of O-specific polysaccharide of the LPS and their biochemical and serological reactions (Boyde, *et al.*, 1981). These organisms were recognized as the etiologic agents of bacillary dysentery or shigellosis in the 1890s. They are a group of gram-negative, facultative intracellular pathogens, long, slender non-motile and non-encapsulated. Group A has 13 serotypes, group B has 6 serotypes, group C has 18 serotypes, and group D has 1 serotype. Geographic distribution and antibiotic susceptibility varies with different species. *Sh.dysenteriae* serotype 1 cause deadly epidemics, *Sh.boydii* is restricted to the Indian subcontinent, and *Sh.flexneri* and *Sh.Sonnei* are prevalent in developing and developed countries, respectively. *Sh.flexneri*, enteroinvasive gram-negative bacteria, is responsible for the worldwide endemic form of bacillary dysentery (Boyde, *et al.*, 1981; WHO, 1992). It has Aerobic and facultative anaerobic cultural characteristics with optimum growth temperature at 38˚c and pH range of 6.4 -7.8.The organisms grew well on ordinary media, forming small shinny; regular, convex raised dry colonies on DCA/MacConkey-colonies appear pale due to Non-lactose fermentation (Cheesbrough, 1989; Jorgensen, 2007).

2.2.3 Disease burden and epidemiology

2.2.3.1 *Campylobacter jejuni*

Nowadays, *Campylobacter* is the most common cause of bacterial gastroenteritis in developed and developing countries. It is responsible for 400–500 million cases of diarrhoea each year, the number of cases often exceed those of salmonellosis and shigellosis. The common routes of transmission are faecal-oral, ingestion of contaminated food or water, and the eating of raw meat. It is thought that the infective dose of *C. jejuni* ranges from 500 - 10,000 cells, depending on the strain, damage to cells from environmental stresses, and the susceptibility of the host. Infants and young children are the most susceptible (Vandamme, 2000; Engberg *et al.,* 2000; Butzler, 2004). However, a dose of less than 10,000 organisms is not a common cause of illness (Behrman *et al.,* 1996; Laforce *et al.,* 1999).

This bacterium is most often spread by contact with raw or undercooked poultry. A single drop of juice from a contaminated chicken is enough to make someone sick. A common way to become infected is to cut poultry meat on a cutting board and then use the unwashed cutting board or utensil to prepare vegetables or other raw or highly cooked foods (Lalitha, 2002; Behrman *et al.,* 1996).

Outbreaks are associated with ingestion of contaminated milk and water. Ingestion of improperly handled or under cooked food, primarily poultry products, raw milk, or contaminated water are common sources for human infections. Both in the developing and the developed world, young children remain most susceptible (Loppez *et al.,* 1999). Although disease is generally mild and self-limiting, severe post-infectious

complications such as Gullain-Barré syndrome may occur (Behrman *et al.,* 1996; WHO, 2010).

This bacterium continues to be the most common enteric pathogen isolated from patients with diarrhoea (Mandomando *et al.,* 2007), and its infections are usually sporadic and tend to occur in the summer and early fall. In developed countries *C.jejuni* is usually more common than *Shigella* or Salmonellae infections. New Zealand reported the highest national Campylobacteriosis rate, which peaked in May 2006 at 400 per 100,000 populations. Asymptomatic infection is also more common (Tauxe, 1992). In Bangladesh, up to 39% of all children younger than 2 years have asymptomatic infection (Poisson *et al.,* 1992).

An estimated 2 million cases of *Campylobacter* enteritis occur annually in the USA, accounting for 5-7% of cases of common gastroenteritis. *Campylobacter* organisms have a large animal reservoir, with up to 100% of poultry, including chickens, turkeys, and waterfowl having asymptomatic intestinal infections. Changes in the incidence of culture-confirmed Campylobacter infections have been monitored by the Foodborne Diseases Active Surveillance Network (Food-Net) since 1996, with frequent infections reported from Europe, Africa, South East Asia and China. The prevalence of infection is much greater in developing countries than in the developed world (CDC, 2000; Burgess *et al.,* 2005).

Among the studies carried out in Europe 17.5% isolation rate has been reported from France, 12% from Czechoslovakia and 8.3% from Turkey. In the African countries 18% isolation rate has been reported from Tanzania, 16.5% from Nigeria and 9.3% from Zimbabwe. In India a study carried out among the rural population in Calcutta

revealed a frequency of 5% while in another study from Northern India an isolation rate of 5% was reported (Behrman *et al.,* 1996; Luber *et al.,* 2002).

Studies from Bangladesh and China revealed an isolation rate of 25.5 % and 11.8%. A study carried out in Lahore, Pakistan during reflected an isolation rate of 12% the frequency reported from Tanzania and France is little higher than what was reported from Lahore, Pakistan. The difference in frequency of *C. jejuni* in different parts of the world is probably due to varying standards of living conditions, water supply and feeding habits as the infection occurs through water and food especially the consumption of poultry and even association with animals is a significant factor in the acquisition of disease (Vandamme,2000).

Poultry is the primary food vehicle for transmission of *C. jejuni*. Some case control studies indicate that up to 70% of sporadic cases of Campylobacteriosis are associated with eating chicken. Domestic and wild animals have been considered to be the most common reservoir of Campylobacter for human infection. *C. jejuni* is commonly present in the GIT of healthy chicken, cattle, pigs, turkeys, ducks and geese and direct animal exposure can lead to infection. Pets that may carry Campylobacter include birds, cats, dogs, hamsters and turtles. The organism is occasionally isolated from streams, lakes and ponds. In United States, poultry is the most common source of sporadic infection. Epidemiological investigation have implicated raw milk, eggs, beef contaminated water and contact with infected animals including cats and puppies (Fitch, 2005).

In Africa, a few studies have indicated that Campylobacteriosis is mostly common among children of young age. In Nigeria, for example, *C. jejuni* was found to be an

important agent of diarrhoea in Ile-Ife and should be considered strongly in children with diarrhoea. Studies in Addis Ababa, Ethiopia, also indicated that *Campylobacter* spp were important bacterial agents of diarrhoea in adults and children and should be considered routinely in the diagnosis of patients with diarrhoea. In Durban, South Africa, *Campylobacter* were found in 21% of diarrhoeal cases among children aged less than five years (WHO, 1999). The age distribution of *Campylobacter* gastroenteritis in developed countries is bimodal, with one peak in children younger than four years of age and a second in adolescents and young adults. The highest incidence occurs in the first year of life. In developing countries, infections occur early in life, usually below the age of five years. Many infections are asymptomatic in older children (Behrman *et al.,* 1996).

Campylobacter enteritis is a worldwide zoonosis and as such the gastrointestinal tract of many domestic and wild animals is the main reservoir of infection. *C. jejuni* has been isolated from the faeces of 30-100% of chickens, turkeys and water fowl. Most farm animals, meat sources and pets can harbour the organisms. Transmission of *C. jejuni* from animals to persons occurs most often by the faecal-oral route by ingestion of contaminated food, especially undercooked poultry, unpasteurized milk and untreated water. Outbreaks of *Campylobacter* are reported in nurseries and day-care centres. Communicability is highest during the acute phase of the illness and can last as long as 2-3 weeks, but appropriate antibiotic treatment can shorten this period to 2-3 days. Chronic carriage is uncommon because *Campylobacter* has so many reservoirs in the environment; food products (especially poultry, beef, and pork) are at-risk of contamination during processing (Tauxe, 1992; Behrma *et al.,* 1996).

Raw milk surveys have shown that *Campylobacter* occurs in about 0.5%to 12% of samples from the bulk tank. *Campylobacter* was the most common pathogen found in raw milk during a survey conducted in the upper Midwest. *The* agent is readily destroyed by pasteurization of *dairy* products. Produce may also become contaminated with *Campylobacter* if exposed to raw meat or poultry products/juices in the kitchen or if contaminated by animal faeces in the fields where raw fruits and vegetables are grown (Boyd 1981; Michael *et al.,* 2001).

2.2.3.2 *Salmonella*

Shigella spp. causes dysentery by invading the colonic mucosa. *Shigella* bacteria multiply within colonic epithelial cells, cause cell death and spread laterally to infect and kill adjacent epithelial cells, causing mucosal ulceration, inflammation and bleeding (Kipkemboi *et al*., 2012). Although all *Shigella* species elaborate varying quantities of exotoxins, only *Sh.dysenteriae* 1 produces the highly potent cytotoxin called Shiga toxin. Shiga toxin production is not required for virulence of invasive *Sh.dysenteriae* strains, but may increase the severity of disease by destroying the endothelium in the local capillary loops and causing ischemia in the intestinal tissue (Benaissa *et al.,* 2007).

Salmonellosis is due to the invasion of the GI tract by the *Salmonella* organism. There is evidence that enterotoxins may be produced. This pathogenicity is conferred due to the ability to invade intestinal mucosa and ileum crypts and production of toxins. The host-Salmonella interaction is dominated by the broad array of sophisticated weaponry used by *Salmonella* to overcome host defences. Direct interactions of *S. typhimurium* with host cells initiate the production of proinflammatory cytokines in

tissue, which drive a rapid recruitment of neutrophils, the pathological hallmark of gastroenteritis caused by nontyphoidal *Salmonella* serovars (Hellene *et al.,* 2010).

The only significant reservoir of these bacteria is humans. Faecal-oral route is the most common mode of transmission of the disease. Infection may also occur after ingestion of contaminated food and water. Food handlers who demonstrate poor personal hygiene are often responsible for outbreaks. Outbreaks are also common in conditions of overcrowding and poor sanitation such as prisons, refugee camps, as well as, third world nations with poor sewage disposal (Boyd, 1981).

Salmonellosis remains a significant threat to the health of individuals in developing countries. Although its prevalence varies across regions, the incidence and prevalence of typhoid fever has declined greatly with the provision of clean water and good sewage systems in Europe and the USA since the early 20th century (Pathucheary *et al.,* 2004). The infection though, remains a serious public-health problem in developing countries (Crump, *et al.,* 2004).

Salmonellosis is estimated to have caused 21·6 million illnesses and 216 500 deaths globally in 2000(Crump, *et al.,* 2004). These data represent the tip of the iceberg since the real burden of infection remains almost unknown in different developing countries where the disease is a serious public health problem. The incidence of typhoid was high (>100 cases per 100 000 population per year) in south-central Asia, southeast Asia, and possibly southern Africa, medium (10–100 cases per 100 000) in the rest of Asia, Africa, Latin America, and Oceania, except for Australia and New Zealand, and low in the other parts of the world (<10 cases per 100 000) (Gideon, 2008).

These estimates are based on stool-culture-positive cases in 22 population-based studies, many of which were done after publication of the previous global estimates. Where the infection is endemic, most cases in health facilities are children aged 5–19 years and young adults. Recent population based studies from India, Indonesia, and Vietnam suggest that in some settings fever and diarrhoea are common in 1–5 year-old children (Guechi, 1992; WHO, 1992). Data from hospital-based studies in Bangladesh and Thailand support these findings. Surveillance data from developed countries indicate that the proportion of cases of *Salmonella* species infection is constant over the first 25 years of life (Crump, *et al.,* 2004). Studies from two rural settings in Nepal and Vietnam also found a high disease burden (Acharya, *et al.,* 1987; Bhan, 2005).

Most cases in developed countries arise in travellers, but domestically acquired disease is still reported (Ackers, *et al.,* 2000). A total of 1393 Salmonella cases were reported between 1994 and 1999 in the USA, 74% of which were related to travel and the rest of which were acquired domestically; 7% of total cases were part of recognized outbreaks (Olsen, *et al.,* 2003).

The epidemiology of *Salmonella* infections is changing. It is reported that there is a dramatic decline in the overall incidence of gastroenteritis due to *S. typhi* in recent years, suggesting that eradication of this disease might be within reach. This reduction is not the result of a specific control program but rather a consequence of economic development and improved sanitation, paralleling the decline of the disease in the developed countries during the early 20th century. *S. typhimurium* and *Salmonella enteritidis* are the most common serotypes of NTS causing human disease in sub-Saharan Africa (Susan *et al.,* 2009).

Non-Typhoid *Salmonella* (NTS) is among the 3 most common pathogens causing bacterial infections in adults and children in sub-Saharan Africa. Children <3 years old and human immunodeficiency virus (HIV)-infected adults carry most of the burden of invasive disease, and mortality among these groups is high. This contrasts with developed countries, where NTS disease is usually self-limited diarrhoea, and mortality is lower. The burden of mortality due to childhood bacterial disease may be greater than that due to childhood malaria in some African communities. In rural Kenya, the estimated minimum incidence of infection due to NTS was 505 cases per 100,000 person-years in the age group of <5 years old, of which 88 cases per 100,000 person-years were NTS infections (Kipkemboi *et al.*, 2012).

Most studies, particularly in industrialized countries, have implicated farm animals as the main reservoirs of NTS, and infections have often been associated with food contamination, either in homes or in the food production industry (Tauxe, 1992). However, the sources and transmission dynamics of NTS in Africa have remained unknown, and it was only speculated that person-to-person transmission, in addition to sporadic foodborne outbreaks, played a significant role in community-acquired NTS bacteraemia in children. Studies from two hospitals in Nairobi have reported observations that a significant number (a prevalence of 6·9%) of NTS from siblings and parents of index cases were of the same serotype and antibiotic-susceptibility profiles, and were clonal in origin (Kariuki *et al.*, 2006). In this portion of the community, NTS behaved in a similar manner to typhoid in terms of the characteristics of asymptomatic carriage. It is probable that the finding of NTS in family contacts of an index case represents either a situation in which all family members acquired the bacterium at the same time or that the index case became

infected from other family members or vice versa. NTS may also precipitate serious infection in these carriers if malnutrition, underlying illness and immunosuppression are present, and the carriers may also shed NTS into the environment, providing a possible source of infection to other children in the home. It is possible that transmission may occur through food, water or contamination of surfaces within the home (Lalitha, 2002; Kariuki *et al.*, 2006).

In rural Mozambique, the incidence of childhood infection was 425 cases per 100,000 person-years among children aged <15 years, and within this category, NTS incidence accounted for 120 cases per 100,000 person-years. In Uganda, NTS was reported to be rare among adults with $CD4^+$T-lymphocyte counts (CD4 cell counts) >500 cells/mm^3, but the incidence was 500 cases per 100,000 person-years among adults with CD4 cell counts of 200–500 cells/$mm^3$and was 7500 cases per 100,000 person-years among adults with CD4 cell counts <200 cells/mm^3. In sub-Saharan Africa, cases of nontyphoidal salmonellosis, frequently complicated by bacteremia, are now more numerous than cases of enteric fever (Gordon, 2009).

The incidence of NTS has risen in association with predisposing infections with HIV and *Plasmodium falciparum*. NTS isolates are now among the most common enteric culture isolates in many parts of Africa, comprising as much as 50% of cases of bacterial infections including bacteremia (Gordon *et al.,* 2008). It has been documented that in Malawi NTS isolates are the leading cause of bacteremia in children and that 75% of these isolates are *Salmonella enterica* serovar Typhimurium (Brent *et al.,* 2006). Robert Kingsley and colleagues at the Sanger Institute have used subgenomic assays and whole-genome sequencing to characterize a specific invasive multidrug-resistant NTS clone designated ST313, which is now dominant in Malawi

and Kenya. ST313 clusters most closely with the host-specific *S.* Typhimurium strain ST128 (Kingsley *et al.,* 2009).

Since the reported 14,465 cases of all Non-typhoidal *Salmonella* species in the year 2000, there has been a downward trend in the subsequent years (provisional figures for 2008 show a total of 9,867 cases). The majority of cases were *S. enteritidis.* The highest rate of infection is in those over 60 and under 20 months, especially infants. The number of *Salmonella,* reported to the HPA for England and Wales for 2000 were 4,719 and 3,549 respectively and provisional figures for 2008 were 912 and 3,278 respectively (CDC, 2008). Although *Salmonella* infections due to *S. typhi* are usually regarded as an illness of school-age (>5 years of age) children and young adults, there is considerable heterogeneity; some sites in Asia report high incidences of *Salmonella* diarrhoea among children <5 years of age (Ochiai *et al.,* 2008). In India, this continues to be a major health problem (Bhan, 2005).

Later reports indicate an increasing prevalence in this age group (Gideon, 2008). In 1995 a retrospective study was undertaken to find out its prevalence among hospitalized children and the clinical profile in children below 2 years of age. A total of 409 cases were admitted to the paediatric ward during these 5 years, of which 42 (10.9%) were below 2 years of age. Of these 42 children, a majority 36 (85.7%) were between 13 to 20 months of age. Only 6 (14.3%) cases were seen in infancy, all of whom were more than 6 months of age. *S.typhi* was isolated from 18 (42.9%) cases (Oundo *et al.,* 1996). According to a recent surveillance supported by the World Health Organization (WHO, 2000-2002), *S. typhi* ranked as the third (8%) most prominent *Salmonella* spp. isolated in Africa after *S. typhimurium* (26%), and *S. enteritidis* (25%) . In Libya, a comprehensive study was conducted on 30,165 patients

who were hospitalized with acute diarrhoea during 1975-1980 (El, 1998). A low prevalence of *S. typhi* and *S. paratyphi* in stool samples of patients was shown. Among different serotypes of Salmonella identified in 7,560 patients, 81 were identified as S. typhi, 3 *S. paratyphi* A and 4 *S. paratyphi* B. In recent years, an increased rate of *Salmonella* infections has been observed, for example, according to information resources of the Libyan Center for Information and Documentation (CID) of the Secretary of Health and Environment, the incidence rate for the years 2004, 2005 and 2006 were seven, 21 and 16/100,000 persons/year respectively (CID, 2006). The significant increase in the incidence over this period is probably due to the improvement of the data reporting system and improved infrastructure in local health care facilities (Ghengesh, *et al.*, 2009).

Several studies have been conducted to establish the etiology of enteric infections with a particular emphasis on the identification of *Salmonella spp.* and *S.typhi* in Egypt on stool samples collected from 6,278 young patients (mean age of 5.1 years) admitted to the Abbassia Fever Hospital in Cairo from 1986 to 1993 with acute enteric infections (CDC, 2009). Reportedly, *Salmonella* spp was isolated from 465 patients the cohort. The majority of the isolated strains (53%) were *S. typhi*, while only 3% were *S. paratyphi* A and *S. enteritidis* and *S. typhimurium.* The peak of cases was observed during February-March, June-July, and October-November. Further on, an outbreak of bacterial gastroenteritis caused by a chloramphenicol resistant strain of *Salmonella* spp. occurred in Gharbeya Governorate in 1990, involving 90 children and 43 adults (Battikhi *et al.*, 2003). Although, the source of infection was not confirmed, consumption of contaminated food was hypothesized as the main cause of infection

due to the involvement of children and young adults in urban area (Wasfy *et al.,* 2000).

In 1977 and 1982, the National Centre of Salmonella at the Institute Pasteur Tunis, identified 1,715 *Salmonella* isolates from throughout Tunisia; with the *Salmonella* spp. being the most predominant (99.6%) isolates (Pandey *et al.,* 1990). As predicted, contaminated drinking water was the main source of the disease's transmission (Ahmed, *et al.,* 1987/2000). In 1989, an incidence of about 6 cases per 100,000 per year was reported (Gideon, *et al.,* 2008). Salmonella gastroenteritis is endemic in Morocco (Bouallegue *et al.,* 2004). Recent studies from national and international agencies such as the National Institute of Hygiene (Rabat) and the WHO Global *Salmonella* Surveillance Program have provided useful epidemiological data regarding the prevalence of *S. typhi* and non-typhoidal *Salmonella* in Morocco (WHO, 2008).

The prevalence of *Salmonella* infections in Algeria was first reported by Guechi and Hamza (Guechi *et al.,* 1992), covering the years from 1986 to 1990 claiming 98% of the 3,340 clinical isolates of *Salmonella* spp recovered from children <5 years old. However, from 1985 to 2005, the National Institute of Public Health reported only 3-22 cases per 100,000, which is similar to what has been reported from Middle-Eastern countries (Battikhi *et al*., 2003). In Algeria, the highest incidence rates are reported during the hot summer months and among school-aged children (from 5 to less than 20 years of age) (Guechi *et al.,* 1992).

Salmonellosis erupted in Kenya in 1997-99 due to *S. enterica* serovar Typhi, resistant to ampicillin, tetracycline, chloramphenicol, streptomycin, and cotrimoxazole. Multi

drug resistant *S. typhi* were identified in people suffering from salmonellosis in Nairobi, Kenya during 1997-99 study. In a study to determine the prevalence of *Salmonella* in provincial and selected district hospitals in Kenya, specimens were examined by culturing to isolate *Salmonella* species. All samples came from patients who were clinical suspects of food poisoning and presenting with fever and diarrhoea. Comparative analysis of *Salmonella* specific morbidity showed that some parts of the country namely Nyanza, Eastern and Rift Valley were most affected (Oundo *et al.,* 1996). The results showed no significant statistical difference between the sexes infected (c2=0.56; df=1;p=0.454). It demonstrated that there was no difference in infectivity among different age groups. The data showed a proportion of 1:20 for confirmed cases to those that were clinically diagnosed. There was a statistical difference between serology tests and culture confirmed cases. (c2=36.851; p=0.000; df=1). It was also demonstrated that there were other infections (Brucellosis, *E.coli,* shigellosis), which were clinically similar to salmonellosis in presentation (Gikunju, 2003).

The advent of chloramphenicol treatment changed the perception of gastroenteritis due to *Salmonella* species from a severe, often fatal, illness to a common, readily manageable infection (Chen *et al.,* 1995). Outbreaks of chloramphenicol-resistant typhoid were reported in 1972. At this time, the isolates were still sensitive to cotrimoxazole and ampicillin or amoxicillin. In the late 1980s and 1990s, outbreaks of Salmonellosis caused by organisms resistant to chloramphenicol, co-trimoxazole, ampicillin, and amoxicillin were reported (Mirza *et al.,*, 1996). Currently, fluoroquinolones and third-generation cephalosporins are the drugs of choice for treatment of typhoid fever, but decreased susceptibility to these antibiotics has been

reported (Linfy *et al.*, 2000; Olsen, 2003). There is an urgent need to keep the possible emergence of untreatable strains to a minimum, by prudent use of existing drugs and by resisting the temptation to use yet more antibiotics (Ochiai *et al.*, 2008).

2.2.4.3 *Shigella*

This organism remains a burden in developing countries; sensitization of the population and practitioners to this lethal infection would contribute to limiting its severity and prevalence (Alexandre *et al.,* 2010). Infections caused by *Shigella* species are an important cause of diarrhoeal disease, in both developing and developed countries. Worldwide, it is estimated that shigellosis causes around 600,000 deaths per year, two-thirds of the deceased being children under 10 years of age. *Shigella dysenteriae* and *Shigella flexneri* are the predominant species in the tropics, while *Shigella sonnei* is the predominant species in industrialized countries (CDC, 2000; Loren *et al.,* 2006).

Shigella species remain important pathogens responsible for diarrhoeal diseases and dysentery occurring all over the world, especially in developing states. The morbidity and mortality due to shigellosis are especially high among children in developing countries (Kotloff *et al.,* 2000). Of the estimated 165 million cases of *Shigella* diarrhoea that occur annually, 99% occur in developing countries, and in developing countries 69% of episodes occur in children under five years of age. Moreover, of the 1.1 million deaths attributed to *Shigella* infections in developing countries, 60% of deaths occur in the under-five age group. Travellers from developed to developing regions and soldiers serving under field conditions are also at an increased risk to develop shigellosis (Lian *et al.,* 2012). The median percentages of isolates of *Sh.flexneri*, *Sh.sonnei*, *Sh.boydii*, and *Sh.dysenteriae* were, respectively, 60%,

15%,6%, and 6% (30% of *Sh.dysenteriae* cases were type 1) in developing countries; and 16%, 77%, 2%, and 1% in industrialized countries. In developing countries, the predominant serotype of *Sh.flexneri* is 2a, followed by 1b, 3a, 4a, and 6. In industrialized countries, most isolates are *Sh.flexneri* 2a or other unspecified type 2 strains (Kotloff *et al.*, 2000).

Shigella is spread in three main ways, one is person-to-person. Any infected person can infect others by failing to properly wash their hands before handling food or coming into close contact with another person. Infections in households, pre-schools, child care facilities, and elderly and developmentally disabled living facilities commonly spread in this manner. In the Foodborne situation, flies can potentially spread the bacteria by landing on contaminated faeces and then food. The third mode of spread is Waterborne; faecal contaminated recreational water, such as fill and drain wading pools, can be a source for spread (Pornthhip *et al.*, 2006).

An estimate of *Shigella*-associated mortality among 0±4-year-olds among patients admitted to the inpatient unit of the International centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR, B) over the period 1988-1998, indicated that13.9% of infants and 9.4% of 1±4-year-olds who are hospitalized with shigellosis die each year. Studies performed in the 1980s in both rural and urban settings have provided evidence that many additional diarrhoeal deaths occur at home for reasons that include family preference, access to care, and long-term complications of the illness. A one-year census-based survey of deaths among children younger than 7 years in a rural area of the Gambia found that only 12% of deaths occurred in a hospital or health centre (Bhan *et al*., 2005; Loren, 2006). Only 17.8% of deaths detected during the 3 months following admission for shigellosis to the rural Diarrhoea Treatment Centre in

Matlab, Bangladesh, occurred in the treatment centre. The mortality rate among 2±5-year-old children who had received medical treatment for diarrhoea during the preceding 4 months was slightly lower among those residing in urban Bangladesh than in the Gambia (Loren, 2006).

Among the older children and adults, each year approximately6 744 075 episodes of shigellosis among older children and adults living in developing countries are evaluated in treatment centres. It is estimated that 11% of outpatients with *Shigella* infection are admitted to a hospital. At the ICDDR, B over the period 1974±88, 8.2% of patients older than 5 years who were hospitalized with *Shigella* infection died in the hospital (60), making 60 830 deaths each year for this age group. A correction for out-of-hospital deaths, similar to that used for children younger than 5 years of age, resulted in an estimated 425,810 *Shigella* deaths among older children and adults living in developing countries (Lian *et al.*, 2012).

Shigella is of public health importance because it causes especially severe infections and may occur in explosive epidemics. Large epidemics had been reported from many parts of the world including Central America, Central Africa, and the Indian subcontinent. Although there are more than 30 recognized *Shigella* serotypes, only a few predominate in any geographic area. *Sh.dysenteriae* type 1, also known as Shiga bacillus, has been recognized as the major cause of epidemic dysentery for nearly 100 years. During the past 30 years, pandemics of Shiga dysentery have spread across Central America, Bangladesh, South Asia and Central and East Africa, particularly affecting populations in areas of political upheaval and natural disaster. This devastating form of shigellosis is associated with high rates of illness (attack rates have ranged from 1.2% in El Salvador to 32.9% during an outbreak on St. Martins

Island) and case-fatality (ranging from0.6% during an epidemic in Burma to 7.4% in the Guatemalan epidemic). The pandemic that began in Central Africa in 1979 progressed to East Africa and has since become particularly problematic among refugee populations. The pandemic strains often exhibit multiple antibiotic resistances and induce severe illness with high case fatality in all age groups .In general, both the incidence and the fatality rates are highest among the very young and the elderly. However, available data only permit an estimation of deaths that occur during the acute or sub acute phase of shigellosis (WHO, 1995).

Deaths that result after extended periods of persistent diarrhoea, intestinal protein loss, and chronic malnutrition following shigellosis could not be measured. Although epidemic Shiga dysentery is the most dramatic manifestation of *Shigella* infection in developing countries, the majority of *Shigella* infections are due to endemic shigellosis. Endemic *Shigella* is responsible for approximately 10% of all diarrhoeal episodes among children younger than five years living in developing countries and up to 75% of diarrhoeal deaths (CDC, 2005). In Bangladesh alone, *Shigella* dysentery causes 75 000 deaths among children in this age group annually during peak epidemic years and an estimated 35 000 deaths in non-epidemic years. A recent review of the literature concluded that, of the estimated 165 million cases of *Shigella* diarrhoea that occur annually, 99% occur in developing countries, and in developing countries 69% of episodes occur in children under five years of age. Moreover, of the cases 1.1 million deaths attributed to *Shigella* infections in developing countries, 60% of deaths occur in the under-five age group (WHO, 1995).

This picture contrasts with that for the other main causes of infectious diarrhoea in developing countries— rotavirus and enterotoxigenic Escherichia coli- for which the

overwhelming burden of morbidity and mortality occurs in children under three years of age. Although generally instructive, these conclusions are limited by several features. First, although estimates of the burden are based on multiple studies for children, data are limited for adults, who may account for a substantial fraction of *Shigella* morbidity and mortality and who may thus constitute an important target group for future vaccines. Second, it is appreciated that in developing countries a substantial fraction of *Shigella* deaths occur in persons who never seek medical care or who die after discharge from the hospital. Indeed, it has been estimated that the absolute number of persons who die from *Shigella* infections out of hospital may outnumber the absolute number of *Shigella* deaths in the hospital by six-fold. Yet, estimates of out-of-hospital *Shigella* mortality are based on very few studies (CDC, 2000; Ochiai *et al.*, 2008).

High numbers of organisms are excreted throughout the acute phase of the disease and smaller numbers are shed during convalescence and by asymptomatic carriers. Because the infectious dose is low (10-100 organisms), person-to-person transmission readily occurs, particularly among children. While reported cases of *Shigella* range between 14,000 and 20,000 annually, *Shigella Sonnei* is the most common type of *Shigella*. It accounts for over two-thirds of cases of shigellosis in the United States. Foods that come into contact with human or animal waste can transmit *Shigella*. Thus, handling toddlers' diapers, eating vegetables from a field contaminated with sewage, or drinking pool water are all activities that are commonly implicated in food borne illness and diarrhoeal diseases (WHO, 2005). An example of the devastation that can be produced by Shiga occurred among the 500,000-800,000 Rwandan refugees who fled into the North Kivu region of Congo-DRC in 1994. During the first month alone,

approximately 20,000 persons died from dysentery caused by a strain of *Shigella dysenteriae* type 1 that was resistant to all of the commonly used antibiotics (Lancet, 1995).

2.2.3.4 *E. coli*

Diarrheogenic *Escherichia coli* (DEC) strains are pathogens of public health importance affecting both adults and children worldwide. Most diarrhoeal cases in children under 5 years of age have been shown to be due to diarrheogenic *E. coli*. DEC are recognized as being associated with diarrheal disease, and have been classified into six groups based on clinical, epidemiologic, and molecular criteria: enteropathogenic *E. coli* (EPEC); enterotoxigenic *E. coli* (ETEC); Shiga toxin–producing *E. coli* (STEC), also known as enterohemorrhagic *E. coli* (EHEC) or verotoxin-producing *E. coli* (VTEC); enteroinvasive *E. coli* (EIEC); enteroaggregative *E. coli* (EAEC or EAggEC); diffusely adherent *E. coli* (DAEC), and among the DEC categories, EAEC appears to have been increasingly recognized as an emerging pathogen causing diarrhoea in both developing and industrialized countries (Okeke *et al*., 2001; Nataro *et al*., 2006).

2.3 Overview of Antibiotics and Antibiotic Susceptibility

Control of faecal–orally transmitted pathogens is inadequate in many developing countries, in particular, in sub-Saharan Africa. Acquired resistance to antimicrobial drugs is becoming more prevalent among *Shigella species*, *Salmonella enteritidis*, diarrheogenic *Escherichia coli, Campylobacter* spp. and other pathogens in this region. The poor, who experience most of the infections caused by these organisms, bear the brunt of extended illness and exacerbated proportion of deaths brought about

by resistance. Improved antimicrobial drug stewardship is an often cited, but inadequately implemented, intervention for resistance control (UNICEF/WHO, 2009).

Antibiotics, also known as antibacterial, are types of medications that destroy or slow down the growth of bacteria and are thus used to treat infections caused by bacteria. (Lalitha, 2002). The sensitivity of microorganisms to antibiotics varies based on many and varied factors. Antibiotic that acts upon both Gram positive and Gram negative bacteria is termed a broad spectrum antibiotics, while a narrow spectrum antibiotics, will act on only a single group of organisms, either Gram positive or Gram negative bacteria, and those of limited spectrum are only effective against a single organisms (Cavallo *et al.*,1993; Lalitha, 2002).

Antibiotics are among the most commonly used drugs. For example, 30% or more hospitalized patients are treated with one or more courses of antibiotic therapy. However, antibiotics are also among the drugs commonly misused by physicians, for example usage of antibiotic agents in viral respiratory tract infections and also in most cases of bacterial gastroenteritis which does not require antibiotic treatment, antibiotic use is widespread. The inevitable consequence of widespread and injudicious use of antibiotics has been the emergence of antibiotic-resistant pathogens, resulting in a serious threat to global public health. The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. Paralleled to all this there has been an alarming increase in bacterial resistance to existing agents, resulting to concern worldwide that antibiotics are being overused. Antibiotic overuse is one of the factors that contribute towards the growing number of bacterial infections which are becoming resistant to antibacterial medications (Ndungu *et al.,* 2002). Emergence of resistance to multiple

antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria (Tafa *et al.*, 2013).

There are several modern types of antibiotics, which mainly include; Penicillins such as penicillin and amoxilin, Cephalosporins such as cephalexin, Macrolides such as erythromycin and Azithromycin/zitromax), Fluoroquinolones such as ciprofloxacin, Sulphonamides such as co-trimoxazole and trimethoprim, Tetracycline such as tetracycline and Aminoglycosides such as gentamicin (Ford, 1999; Lalitha, 2002; Gauwitz, 2011).

A recent survey of eight Asian countries revealed nalidixic acid resistance in 5 to 51% of *S. typhi* isolates, and many nalidixic acid-resistant strains are resistant to multiple antibiotics (Lalitha, 2002). Isolates resistant to nalidixic acid are less responsive to fluoroquinolone. Nalidixic acid-susceptible *Salmonella* isolates with reduced susceptibility to fluoroquinolones are also increasingly being recognized and the presence of these isolates may require changes in current clinical microbiology practices used to detect non-susceptible strains. The latter resistance pattern is caused by plasmid-borne *qnr* resistance determinants, which raise MICs for fluoroquinolones but not nalidixic acid. An unexpected recent finding is that mutations conferring antibiotic resistance do not necessarily appear to reduce *Salmonella* fitness in the absence of antibiotic selection (Hellene *et al.*, 2010).

There is the growing problem of antibiotic resistance, which has now become a global problem affecting anyone at any age. Over the years, sulphonamides, tetracycline, Ampicillin and trimethoprim/sulphamethoxazole initially appeared as highly

efficacious drugs, only to become impotent in the face of emerging resistance. In the 1990s, few reliable options exist to treat multi-resistant *Shigella* infections, particularly in developing countries where cost and practicality are important considerations. Multidrug-resistant nontyphoidal *Salmonella* spp. (NTS) have emerged as a global public health threat. In industrialized countries, they are most commonly associated with foodborne gastroenteritis. In parts of sub-Saharan Africa, however, NTS are important causes of life-threatening bacteremia. Other studies in Kenya have found that community-acquired NTS are among the top 3 causes of death among children <5 years of age. In a recent study, children from poor slums of Kenya were significantly more likely to be infected with multidrug-resistant NTS than were children from middle-income families (Brooks et al., 2003; Udo *et al.,* 2004).

The patterns of resistance among these strains suggest that third-generation cephalosporins should be the drug of choice for empiric management of these infections, but in most cases, these drugs are too expensive (Cavallo *et al.*, 1993; Udo *et al.,* 2004).

Use of these drugs for infections other than gastroenteritis and self medications is often the cause of resistance in developing countries (WHO, 2004). Although most diarrheal diseases are self-resolving and should not be treated with antimicrobial agents, invasive or protracted infections require chemotherapy and are typically managed empirically. Recent data from within the country, Gabon, Nigeria, Senegal, and Tanzania suggest that resistance among causative organisms of these infections, such as enterotoxigenic, enteropathogenic, and enteroaggregative *Escherichia coli,* is high and appears to be rising (UNICEF/WHO, 2009).

Although oral rehydration therapy has drastically reduced deaths from the disease, prolonged infectious bouts of diarrhoea have long-term consequences for physical and cognitive development (Ndungu *et al*., 2001; Brent *et al*., 2006).

2.4. Factors associated with spread of enteric pathogens

Campylobacter infection is a Zoonotic disease that can be hyperendemic, linked to outbreaks and sporadic infections. Foods of animal origin, particularly poultry, are significant sources of *C. jejuni,* especially when eaten raw and undercooked or decontaminated following cooking (CDC, 2000).

Salmonella are found in a great many animals, domestic, agricultural and wild. Intensive farming methods are thought to be behind its initial rise to importance. Contamination occurs from animal faeces, and infected foods usually look and smell normal. Many cases arise from outbreaks for example in weddings. The source is usually of animal origin; such as beef, poultry, unpasteurized milk or eggs, but all food, including vegetables may be contaminated. Eggs continue to be a source of infection across the globe but majorly in developed countries (CDC, 2008).

Organisms multiply rapidly in warm humid conditions, and cross contamination between surfaces and tools used in cooked and infected uncooked food areas is a potential source. Inadequate thawing from freezing is a common source. Heat readily kills Salmonella, but it can survive spit and oven roasting if not properly defrosted (Crump, 2003; Crump *et al.,* 2004). *Salmonella* infection can also be spread by the faecal-oral route if a carrier does not wash hands after using the toilet. Gastric acidity gives some protection, and thus large inoculums are required. Conversely those with loss of acidity, including those on acid suppressing drugs, are more at risk. Also

liquids which pass through the stomach quickly, or milk and cheese that raise the pH, enable smaller inoculums to be infective (Behrman *et al.*, 1996; Oundo *et al.*, 1996).

Salmonella gastroenteritis usually follows the ingestion of food or drinking water contaminated by faces and accounts for 15% of foodborne infection in the developed nations. Associated foods include raw meats, eggs, milk and *dairy* products, shrimps, yeast, coconut, sauces and salad dressing, cake mixes, cream-filled desserts and toppings, peanut butter and chocolate. Various salmonella species have long been isolated from the outside of egg shells (Huttly *et al.,* 2007). The present situation with *S. enteritidis* is complicated by the presence of the organism inside the egg. Therefore, raw eggs may cause salmonella infection. Foods other than eggs have also caused outbreaks of *S.enteriditis* disease. It is estimated that 2 to 4 million cases of salmonellosis occur in the US annually. Risk factors for NTS infection in Africa have not been well characterized; consequently, evidence-based prevention interventions are limited (Vargas *et al.,* 2004).

2.4.1 Environmental Risk Factors

Food and water. Seasonal peaks of NTS disease occur with the rainy season among both adults and children suggesting that environmental risk factors are important. Faecal organisms are found at the highest concentrations in drinking water sources in Africa at the onset of the wet season and this may correspond to increased risk of waterborne NTS. Protection of source water; increased access to centrally treated safe water; strategies such as the use of narrow-mouthed, spigoted containers for water storage and treatment of water at home by chlorination, solar disinfection, filtration, flocculation, or a combination of measures may reduce the risk of diarrhoea(Susan *et*

al.,2009). Identification and management of hazards, facilitated by microbiological sampling at critical control points from farm to fork, have been used in both developing and industrialized countries to improve food safety and to control NTS disease (Kariuki *et al.,* 1994; Susan *et al.,* 2007).

2.4.2 Zoonotic Transmission and transmission between Humans

Animal contact, particularly handling of young chickens by children, is a well-established risk factor for acquisition of NTS disease in industrialized countries .Although not often considered a common risk factor for NTS infection in studies of developed countries, apparent transmission between humans has been suggested to be relatively more important in Africa demonstrated carriage of identical strains of NTS in the stool of human household contacts of children with invasive disease and a lack of such strains in environmental and domestic animal sampling from the households, although a common source from food or water could not be ruled out. Asymptomatic carriers of NTS have been described in Africa. A Kenyan study of NTS carriage at admission to the hospital found that 20 (3.6%) of 556 children but none of 111 adults carried NTS (Paton *et al.,* 1991; CDCP, 2007).

2.4.3 Hospital – Acquired Infection

Outbreaks of hospital-acquired NTS can be particularly severe on paediatric wards in developing countries, where children may be malnourished and have other host risk factors. In African hospitals, food is often provided by a patient's family. Although few studies have examined risk factors for infection in hospital outbreaks, contaminated food and person-to-person transmission have been considered. Of 360 adult and paediatric patients with hospital-acquired diarrhoea in a Kenyan hospital in

1988, 10% of cases were due to *Salmonella* species and 2.5% to *Shigella* species (CDC, 2000). Among children aged 6 months to 6 years, recent antibiotic use and crowded living conditions at home were associated with hospital-acquired diarrhoea due to Salmonella or *Shigella* species. Among adults, sharing a hospital room with somebody who has diarrhoea and a history of previous hospitalization were associated with hospital-acquired *Salmonella* or *Shigella* diarrhoea (CDC, 2000; WHO, 2009).

2.4.4 Host Factors

Age. Children and infants <3 years old are particularly at risk for invasive NTS disease. Worldwide, the incidence of shigellosis is highest among children 1 to 4 years old. Any age group can become infected with enteric pathogens. The most commonly affected groups of people are; young children, especially those in child care centres and pre-schools, People living in crowded conditions, People living in long-term care facilities , Men who have sex with men, The elderly, the debilitated, and the malnourished of all ages are particularly susceptible to severe disease and death (Paton *et al.,* 1991).

Foods and water consumed: Nontyphoidal infections can cause gastroenteritis, and are usually due to contaminated food or water and can be transmitted by animals or humans. These infections cause one of the largest communicable bacterial diseases in the United States. They are found in contaminated animal products such as beef, pork, poultry, and raw chicken eggs. As a result, any food product that uses raw eggs, such as mayonnaise, homemade ice cream, or Caesar salad, could carry these bacteria. Non-typhoid *Salmonella* infections are also found in contaminated food and water (Susan *et al.,* 2009).

Shigellosis is known to be acquired by ingestion. Because of its high infectivity, person to person spread is of great significance although food and water do play a role. Associated foods include salads, raw vegetables, milk and dairy products, and poultry. Contamination of these foods is usually through the faecal-oral route. Faecal contaminated water and unsanitary handling by food handlers are the most common causes of contamination. *Sh.Sonnei* is usually involved in food borne outbreaks, whereas *Sh.* dysenteriae is usually associated with contaminated water. Water transmission is documented, either from recreational exposure to faecally contaminated surface water and swimming pools, or by cross-connections between the sanitary sewer and the potable water supply (Behrman *et al.,* 1996).

Foods which are served without heating may become contaminated during preparation by food handlers with dirty fingers. However, person-to-person transmission is probably the major mechanism of infection in most areas of the world. Spread within families, custodial institutions, and day care centres demonstrates the ability of low numbers of organisms to cause a person-to-person basis (Bouallegue *et al.,* 2004).

The predominant mode of transmission of shigellosis among children 1 to 4 years old is by faecal-oral contact, and the low infectious inoculum (as few as10 organisms) renders *Shigella* highly contagious (Behrman *et al.,* 1996). Persons symptomatic with diarrhoea are primarily responsible for transmission. Less commonly, transmission is related to contaminated food and water or fomites; however, the organism generally survives poorly in the environment. In certain settings where disposal of human faeces is inadequate, houseflies can serve as a mechanical vector for transmission. *Shigella* is one of the most easily transmitted bacterial diarrhoeas; since it can occur after fewer than 100 bacteria are ingested (Kotloff *et al.,* 2000).

Surprisingly other findings show drinking bottled water increases the risk for shigellosis. Explanations include contamination of the bottled water or that those households having only access to poor quality water make use of bottled water (Arvelo *et al.*, 2009).

Earlier studies isolated enteric pathogens including *Shigella* species from houseflies, *Musca domestica*, the predominant species in 53% of the study households. Environmental factors such as rubbish in front of the household and latrines without water for flushing may contribute to the presence of flies and were a confounder for the effect of flies in this analysis (Kotloff *et al.*, 2000).

Shigella bacteria pass from one infected person to the next. *Shigella* are present in the diarrhoeal stools of infected persons while they are sick and for a week or two afterwards. Most *Shigella* infections are the result of the bacterium passing from stools or soiled fingers of one person to the mouth of another person. This happens when basic hygiene and hand washing habits are inadequate. It is particularly likely to occur among toddlers who are not fully toilet-trained. Family members and playmates of such children are at high risk of becoming infected (Udo *et al.*, 2004; Loren, 2006).

2.5 Prevention and Control of Diarrhoeal Diseases

Because diarrhoeal diseases are of faecal origin, interventions that prevent faecal material entering the domestic environment of the susceptible child are likely to be of greatest significance for public health. The key primary barriers to the transmission of enteric pathogens are safe stool disposal and adequate hand washing especially after contact with faecal material during anal cleansing of adults and children. Hands serve as vectors, transmitting pathogens to foodstuffs and drinks and to the mouths of

susceptible hosts. In a 1997 review, five studies quoted on hand washing with a median reduction in diarrhoea incidence of 35 % (Curtis *et al.,* 2003).

The thorough washing of hands with soap and running warm water should be for no less than 15 seconds. This should be done every time people use the toilet, change diapers, or before they eat or prepare any food. Infants and children should have their hands washed as above after a diaper change, after using the toilet, or before eating. Infected people should stay away from school, child care, or work while they have diarrhoea. Food handlers, child care workers, or health care workers should consider treatment. Water in the latrine and garbage disposal should be improved. The avoidance of dumping or burning rubbish around the household and the control of flies in the household should be essential steps in the future prevention of shigellosis (Behrman *et al.,* 1996; CDC, 2004).

Control of the transmission of *Salmonella* infections to humans requires control of infection in the animal reservoir, judicious use of the antibiotics in *dairy* and livestock farming, prevention of contamination of food stuffs prepared from animals and use of appropriate standards in food processing in commercial and private kitchens. Whenever cooking practices prevent food from reaching a temperature greater than 65.5^0 C for more than twelve (12) minutes, salmonellosis may be transmitted (Acharya *et al.,* 1997). Because large outbreaks are often related to mass food production, it should be recognized that contamination of just one piece of machinery used in food processing may cause an outbreak; meticulous cleaning of the equipment is essential. No vaccine against non-typhoidal salmonella infections is available (Ackers *et al.,* 2000).

If a child in diapers has shigellosis, hands should be washed after changing the diaper and wiping down the changing area with disinfectant. People with *Shigella* should not prepare food for others for at least two days after diarrhoea has stopped. It is recommended to only swim in pools maintaining a chlorine level of 0.5 parts per million and stay clear of pools where children not yet toilet trained are swimming. Frequent hand washing is key to preventing *Shigella*, since individuals can carry *Shigella* without noticing symptoms, and *Shigella* bacteria can remain active for weeks after illness (Hohmann, 2001).

There are, broadly speaking two approaches to reducing opportunities for human infection with *Campylobacter*, elimination or reduction of *Campylobacter jejuni* and treatment of the end product to eliminate *Campylobacter jejuni* prior to consumption. These approaches are not mutually exclusive. The value of elimination of *Campylobacter* spp. from contaminated food prior to retail sale is well illustrated by the impact of required pasteurization of all milk on rates of infection with *Campylobacter* spp. in Kenya (Kariuki et al., 2006).

In the absence of measures to ensure *Campylobacter*-free raw meat products, food preparation must proceed on the basis that meat, particularly poultry meat, entering the commercial or domestic kitchen is frequently contaminated with *Campylobacter* spp. and unless properly handled and prepared may act as a direct vehicle of infection and as a source of cross contamination of other foods in preparation.

The following key recommendations are made; During poultry production, the practice of thinning and point-selling should be avoided, where possible, by completely de-stocking the flock in each house at one time; The meat and poultry

meat industries should develop and implement evidence-based standard operating procedures, in order to prevent or minimize product contamination with *Campylobacter* spp; Food businesses should document and implement a food safety management system; All staff involved in catering and food production generally, should be appropriately trained in food safety to a level commensurate with their work activities; Cook all poultry products thoroughly. Make sure that the meat is cooked throughout (no longer pink) and any juices run clear. All poultry should be cooked to reach a minimum internal temperature of 110^0 C (Boyd, 1981; CDC 2000). A supply of potable water must be available for the preparation of food; Wash hands with soap before preparing food .Wash hands with soap after handling raw foods of animal origin and before touching anything else; Prevent cross-contamination in the kitchen by using separate cutting boards for foods of animal origin and other foods and by thoroughly cleaning all cutting boards, countertops, and utensils with soap and hot water after preparing raw food of animal origin; Ensure that persons with diarrhea, especially children, wash their hands carefully and frequently with soap to reduce the risk of spreading the infection; Physicians who diagnose campylobacteriosis and clinical laboratories that identify this organism should report their findings to the local health department (Curtis *et al*., 2003). As with many enteric infections prolonged breast-feeding decreases the risk of symptomatic campylobacter infections (Behrman *et al.,* 1996; CDC, 2000, WHO, 2009).

An important remaining challenge for the eradication of bacterial enteritis is the development and implementation of a rapid, accurate, and affordable diagnostic assay that can be used in areas of endemicity. Health education should be given to families in hand washing techniques especially after defecation and before food preparation

and consumption. The promotion of these approaches suggests that effective health messages may be provided through schools and communities especially on the provision of safe water, sanitation, and fly control in all households (Behrman *et al.*, 1996; CDC, 2004; Huttly *et al.*, 2007).

Prevention of secondary transmission through education and promotion of hand washing and strict hygiene practices in affected households remains the mainstay of control (CDC, 2000; Brooks *et al.*, 2003). To identify symptomatic and asymptomatic cases of enteric diarrhoea, all household contacts of children (<5 years old) and contacts with diarrhoea should be screened. Exclusion of cases and contacts with diarrhoea from work, school and day-care remains important to prevent spread, but that should not include asymptomatic child contacts under five years (Brooks *et al.*, 2003; Kipkemboi *et al.*, 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was conducted at the Kapsabet County Referral Hospital of Nandi Central district in the County of Nandi in the republic of Kenya (**Appendix 1&2**). The hospital records about 11000 children annually with 5-10 percent of these cases presenting with acute diarrhoea (MoH 2010). The hospital also functions as the primary health facility for the surrounding population. The hospital serves mainly the peri-urban and rural populations (23,990 peoples) of Nandi Central district. The site serves almost 90% of the infants and children under the age of five within the Kapsabet Municipality and its immediate environs. The study area is situated about 300 km south-west of Nairobi on the Eldoret-Kisumu highway, and 41 Km from Eldoret town. The area borders Nandi East district to the East, Nandi North district to the North, Nandi South district to the south and Vihiga County to the West. The area lies at 0, 2000 (012’0.000”N) latitude and 35100 (356’.000”E) Longitude. The area is served by a number of private clinics and 32 government health facilities provide support health services besides the district hospital. The area currently lacks adequate water connections, but with exception of a few households with tap water, borehole water is the major source of water supply (MoH 2010/Development Plan 2006).

3.2 Study Design

A cross sectional study design was employed (Hennekens *et al*., 2007; Olson *et al*., 2011).

3.3 Study Population

The population of study was composed of 144 children aged five years and below admitted with diarrhoea at Kapsabet County Hospital.

3.4 Sample Size Determination

From previous research elsewhere in Mozambique and Tanzania the proportion of the population with the requisite characteristics is estimated at 59.5percent (Mandomando *et al*., 2009; Vargas *et al*., 2004), the sample size was determined from children under five years of age using the formula;

$$\mathbf{n} = \frac{Z^2pq}{D^2}$$

Where; **n**= the minimum sample required

Z= the score corresponding to the level of confidence with which it is desired to be sure that the true population lies between + D percentage points of the sample estimate. Chosen value from the standard normal distribution 1.96

P= the proportion in the target population estimated to have characteristics being measured, expected population estimate to have a particular characteristic-(89.5%)

q= 1-p thus 1- 0.595= 0.105

D= the level of statistical significance set / maximum tolerable error (Degree of accuracy estimates at 0.05). (Fisher *et al.,* 1991),

$$\mathbf{n} = \frac{(1.96^2).(0.595).(0.105)}{(0.05^2)}$$

$$= \frac{(3.8416).(0.093975)}{(0.0025)}$$

n= 144, the minimum number required for the study.

3.5 Inclusion and Exclusion Criteria

3.5.1 Inclusion Criteria

- All children aged 5yrs and below admitted with diarrhoea at the paediatric ward and whose consent was granted by their parents/guardians for the use of their stool in the study.

3.5.1 Exclusion Criteria

1. All other children attending other units within the study area
2. Children unable to obtain stool sample within 4-6 hours of admission and/or whose parental/guardian consent was not granted.
3. Children on antibiotic treatment

3.6 Sampling Procedure

Purposive sampling was used to allow researcher to use cases that present to the hospital for care of diarrhoea. Children admitted in paediatric ward 5 were screened for diarrhoea by a nurse. (Diarrhoea was considered as the passage of watery or loose motions of stool three or more times in a 24 hours and lasting less than 14 days). The study was explained to the caregivers (either the biological parent or any other primary carer/guardian) from whom a written/signed informed consent was obtained **(Appendix 3).** The children were recruited by the nurse or the principal investigator or the research assistant. Children aged 5years and below with diarrhoea and where a stool sample was obtained within four to six hours of hospitalization were recruited into the study.

3.7 Data Collection

Data were collected using a pre-coded data collection tool. After each participant provided written informed consent, primary demographic information, symptoms and medication taken before admission were gathered (**Appendix 3&4**). Stool specimens were collected, assigned laboratory numbers/codes, recorded into a clinical log book, which was constructed as a line list, with each line corresponding to a single client/patient and with the columns for the entry of relevant information. Samples of stool were collected every day of the week consecutively from Monday to Friday.

A structured (closed-ended questions) questionnaire was administered to elicit responses from mothers/ (parents) or guardians of the children admitted to the Paediatric ward to establish possible sources of infection such as water, food sources, and general sanitation at home (**Appendix 4B).** The questionnaire captured information related to environmental risk factors, such as source/type of drinking water, hand washing practices, and dietary habits. Up to an average of four children with diarrhoea daily were enrolled into the study. The information from the patients was entered in a log book from where it was transferred for computerized entry. For every microbiological outcome, patient's results were dispatched to the ward and possibly used to guide therapy.

3.8 Stool Sample's pre-analytical phase

Phases of laboratory processing of the faecal specimens consisted of the following: specimen accession to the laboratory; primary isolation, biochemical testing of isolates and antibiotic susceptibility testing of identified isolates; recording of laboratory findings; and storage or autoclaving of isolates.

Suitable disposable polypots with screw caps were issued in the ward, into which freshly voided stool specimen was collected from children below five years presenting with diarrhoea. The screw caps were replaced tightly and securely closed and the specimen sent to the Laboratory within one hour after voidance.

The stool samples were coded or labelled to correspond with every individual patient. Each specimen was assigned a code corresponding to date of collection, patient's serial number in the clinical log book and Laboratory initials.

3.8.1 Isolation and Identification of pathogens

Upon receipt in the laboratory each specimen was examined macroscopically for colour, texture and presence of any extraneous material, (blood, mucus or watery) or shreds of epithelium and these findings were recorded in the log forms corresponding to individual participant.

Media, reagents, bacterial isolation techniques and antibiotic susceptibility testing were routinely quality-controlled.

After each round of sample inoculation into media, a direct wet mount was done on each sample for microscopic examination to rule out ova/cysts presence.

A selective media Enriched campylobacter agar **(Appendix 12)** was used in this study for the isolation of *C. jejuni* (Fitch *et al.*, 2005). The inoculum for *C.jejuni* was done onto the medium and incubated in candle jar oxygen, so as to provide microeraerophily, at 42^0C for at least 24 to 48 hours (Lalitha *et al.*, 2002).

After each round of inoculation and incubation, each plate was viewed for growth of colonies. Up to three suspect colonies from the primary isolation media were selected and applied onto oxidase strips, catalase testing and Hippurate detection. From the

isolates gram staining was carried out; counter staining with carbol-fuschin rather than safranin was used to give *Campylobacter* cells a more intense colour (Cheesbrough, 2002). Wet mounts were prepared from the cultures to study the motility features of the isolate.

Catalase activity- A single colony was selected from the agar medium, and placed onto a clean glass slide. One to two drops of 3% hydrogen peroxide were added to the colony of suspected *C.jejuni* isolate, and a reaction observed within 60 seconds. The slides were examined macroscopically to detect the formation of bubbles of gas. Positive catalase reactions were observed through bubbles of gas formation,

Oxidase test- A fresh colony growth was selected with a clean wooden applicator stick, and smeared across the commercial oxidase disc (HIMEDIA). A positive reaction was indicated by the bacterial growth turning dark purple in colour within ten (10) seconds (Fitch *et al.*, 2005; Lalitha, 2002).

Hippurate Activity-Suspected colony isolate was routinely smeared onto a commercially obtained Hippurate impregnated paper discs. A positive hydrolysis of Hippurate was observed by the colour change from pink to purple (Finegold *et al*., 1978; Lalitha, 2002).

3.8.2 Isolation and Identification of NTS, *Shigella* spp. *and E. coli*

MacConkey agar, Deoxycholate Citrate Agar and Shigella-Salmonella (SS) agar were used for the recovery of *Salmonella* and *Shigella* (Finegold *et al*., 1978; Ackers *et al.*, 2000; Lalitha, 2002).

After overnight incubation, at 37^0C the cultures were examined macroscopically for characteristic growth of *Shigella* and/or *Salmonella* colonies and results recorded into the appropriate log book.

A loopful of each specific sample was cultured in 10 ml Selenite Faeces (SF) broth for enrichment, incubated at 37^0C for two to six hours and then subcultured into MacConkey agar and/or DCA, and SS agar media by the streak method, using sterile wire loop all the time. Culture plates were incubated aerobically in the incubator at 37^0C for 18hours and then examined for the typical colonies and lactose fermentation. A select colony from each of the respective plates was used to carry out gram staining (Finegold *et al*., 1978; Cheesbrough, 2002).

The organisms that produced characteristic discrete colonies after 24 hours of incubation at 37°C were identified by conventional biochemical IMViC reactions (Indole, Methyl red, Voges Proskauer, Citrate, Urease tests) (Cheesbrough, 2002). The isolates that were positive to Indole and methyl red tests but negative to voges proskauer, citrate and urease tests were identified as NTS, *Shigella* spp. or *E. coli*. These biochemical tests included Triple Sugar Iron (TSI), Hi-IMViC/HiASSORTED tests (KB001 and KB002 [HIMEDIA-INDIA]). Biochemical Test Kits were inoculated as per the manufacturer's instruction, and resulting colour reactions viewed after additional of supplied reagents to appropriate wells and further reactions observed macroscopically thereon. Results were interpreted as per the indicators given in the result interpretation standards (Lalitha, 2002).

TSI inoculation-This test was based on the fermentation of the three sugars contained in the Triple Sugar Iron agar (TSI) medium, and also confirmed for Hydrogen

sulphide production by the suspect culture isolates. A pH indicator (phenol red) changed the colour of the media in response to fermentation, while the changes that occurred in the slant tube indicated what sugar or sugars had undergone fermentation. Presence of black colour indicated production of H_2S (Ackers *et al.,* 2002; Lalitha, 2002). The media was inoculated with select bacterial isolates using a sterile needle to stab the agar Butt and a sterile wire loop to streak the top slant region. The TSI tubes were incubated at 37^0C for 12-18 hours, and then the slants and the butt examined for characteristic fermentation, gas production and H_2S production.

3.9 Antibiotic Susceptibility Testing (AST)

The Kirby- Bauer Disk diffusion method was used for testing antibiotic sensitivity. It is a qualitative test, in which a plate of suitable culture medium was inoculated by evenly spreading a sample of culture across the agar surface. Two to three colonies were selected and suspended in 2 ml of 0.9% sterile saline. This dilution was shown to result in confluent growth on the agar plates used for disk diffusion testing, besides showing good batch-to-batch uniformity due to its low levels in tetracycline and sulphonamide inhibitors (Lalitha, 2002; CLSI, 2010).

The concentration of each segment of the disk is specified so that zone diameters of the appropriate size developed to indicate sensitivity or resistance. The Müeller-Hinton agar (HIMEDIA) plate (60ml) for each isolated organism was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60^0each time to ensure an even distribution of inoculum. Filter paper disk impregnated with known concentrations of different antibiotic agents were placed in the agar plate. With an

alcohol-flamed, fine pointed forceps, the selected discs were laid on the inoculated plate and firmly pressed onto the agar to ensure complete contact with the agar surface. The disks were distributed evenly in such a manner as no closer than 24 mm from centre to centre and no less than 15 mm from the edge of the plate (a maximum of six were firmly laid on the surface of each plate, and on a repeat plate to ensure all placement of all the eleven select discs). Once a disc was placed, it was not removed since some diffusion of the drugs occurs instantaneously; Plates were inverted and incubated at 37^0C and 42^0C for 18 – 24 and 48 hours for *Shigella*/*Salmonella* and *C. jejuni* respectively (Lalitha, 2002; CLSI, 2010).

Antibiotic susceptibility testing was conducted using the following antibiotic discs; Cotrimoxazole (CoT, 25µg), Erythromycin (ERY, 15µg), Doxycycline (DCN, 30µg), Minocycline (MI, 30µg), Nalidixic acid (NA, 15µg), Cefuroxime (CXM, 30µg), Ampicillin (AMP, 10µg), Ceftriaxone (CTX, 30 µg), Ciprofloxacin (CIP, 10 µg), Norfloxacin (NOR, 10 µg), and Gentamicin (GEN, 10 µg).

Reference organisms were set along with each test batch on a weekly basis following the same procedure precisely so as to obtain reliable results and review the completeness and consistency of the results (Yen-Hsu Chen *et al.,* 2011). The Quality control strains of *E. coli* ATCC 35218 were investigated weekly from pure subcultures and the inhibition zones were within the limits as defined by the CLSI (CLSI, 2009).

3.9.1 Reading Plates and Interpreting Results

During incubation the antibiotic produced a gradient of antibiotic concentration and the further it got from the disc, the smaller the concentration of the agent. Each plate

was examined to check on the resulting zones of inhibition which was uniformly circular on the confluent lawn of growth. The diameters of the zones of complete inhibition (as judged by the unaided eye) were measured, including the diameter of the disc. The zone margin was taken as the area showing no obvious, visible growth that was detected with the unaided eye. Zones were measured to the nearest whole millimetre, using a ruler, held on the back of the inverted Petri plate. The size of this area of suppressed growth, the zone of inhibition, was determined by the concentration of the antibiotic agent in the area; thus the diameter of the zones of complete inhibition (including the diameter of the disk) was measured and recorded in millimetres (CLSI, 2009). The zones of growth inhibition were compared to a current zone size on the interpretation table (with the standard cut off measurements for susceptibility resistance) (CLSI, 2010), and results was reported either as R (Resistance) or S (sensitive). All intermediate zones of inhibition (zones less than 12 mm diameter were ignored for susceptibility and reported as resistant). The term sensitive (S) implied that infection caused by the strain tested responded favourably to the indicated antibiotic for that type of infection and pathogens. Resistance (R) strains were not inhibited completely by the therapeutic concentrations (Lalitha, 2002; Jorgensen *et al.*, 2007; CLSI, 2009).

3.10 Statistical Methods

Data were entered in the computer, according to the codes given and analyzed using SPSS Version 20.0. The isolated pathogens were subjected to descriptive statistics using frequency tables and charts. Measure of central tendency and dispersion like mean, skewness, and standard deviation were also applied to bring out the characteristics of the data. The data were then subjected to a statistical inferential

analysis where pearson correlation was used to determine presence of any relationship between diarrhoea and causative agents at a level of significance level, p = 0.05 or 95%. The chi-square - test and Fisher exact tests were used to perform and establish any statistical difference among the variables of interest.

3.11 Ethical Consideration

The collection and processing of the stool samples was carried out by trained Laboratory and Clinical personnel, who were well versed with the practice of bioethics and biosafety/biosecurity precautions.

Informed consent was obtained from the parents/guardians of all clients before entering the research by signing a consent form (**Appendix 3**). Research authorization (**Appendix 8**) was obtained via approval certificate number 00762 from Institutional Research &Ethics Committee (IREC), based at Moi Teaching &Referral Hospital (MTRH)/Moi University, College of Health Sciences- School of Medicine.

3.11.1Confidentiality

The participants were protected by non-disclosure of results or/and clinical information to the other people, the information they gave and that no record bore any name of the participants but codes. Institution managers, Unit managers, and others involved in the recruitment of research candidates maintained and adhered to confidentiality practice.

CHAPTER FOUR

RESULTS

4.1 Demographic Characteristics

A total of 144 children were recruited and accepted into the study, of which a few were discarded due to incompleteness and invalid/mismatched information. Socio-demographic characteristics of the respondents were noted entirely with several factors like gender and age summarized.

During the period of study, a total of 139 clients were investigated; this represented 97% of the total cases observed in the areas of study. Age category was generally categorized into five groups where the variable of interest was months. These were 0 to 11 months, 12 to 23 months, 24 to 35 months, 36 to 47 months and 48-59 months (Table 1).

A further description of distribution of ages per category with gender was done in the study. Distribution of age by gender was generally the same in both gender but lowest in males at 45-59 age categories. Similarly there were fewer cases of females observed above 36 months of age (Table 1).

Proportion of cases aged 12-23 months with diarrhoea was greater (31.7%) as compared to others. Overall, about 25.8% were aged between 0-11 months, 23% were aged 24-35 months while 36-47 months and 48-59 months had 12.2% and 7.2% of cases of diarrhoea. About 59% of all cases were females while 42% of the cases being males.

Table 1: Distribution of Age by Gender among the cases under study

Distribution of age by gender							
		Age category (months), (%)					Total
		0-11	**12-23**	**24-35**	**36-47**	**48-59**	
Gender of the child	**Male**	15 (26.3)	19 (33.3)	10 (17.5)	10 (17.5)	3 (5.4)	57
	Female	21 (25.6)	25 (30.5)	22 (26.8)	7 (8.5)	7 (8.5)	82
Total		**36 (25.8)**	**44 (31.7)**	**32 (23)**	**17 (12.2)**	**10 (7.2)**	**139**

4.2 History and Clinical Observation of the patients

History and observation of the patient provided valuable information regarding the infection in terms of duration, episodes and presence or absence of bloody/mucoid diarrhoea. Predisposing factors like previous meals, waste disposal, general hygiene and other general factors were also indicated. Other observation on presentation showed that, 62 (45%) of the cases exhibited dehydration, 75 (54%) had no dehydration while 2(1%) reported recent dehydration.

Ninety four (94) cases of diarrhoea cases were mucoid, 19 were bloodied while 26 cases were watery. This accounted for 68%, 14% and 19% respectively. Data in (Table 2) shows most of bacteria isolation was found in mucoid and bloodied stool with 37 (26.6%) and 21 (15.1%) cases being isolated respectively. *Shigella* was prominent in bloody diarrhoea while *Campylobacter jejuni*, NTS and *E. coli* were more prominent in mucoid diarrhoea. Most of the children passing mucoid stools had *C. jejuni* infection, but none from children with bloody diarrhoea, while about 50% of diarrhoeal stool that yielded *Shigella* spp. had blood and 25% had mucus. Most

children with mucoid diarrhoea yielded 53.8% of NTS organisms, while highest level of *E. coli* was found in children with mucoid diarrhoea.

A further tabulation of types of diarrhoea against bacteria isolated was done. Using the above categories - Watery, mucoid and bloody, the results were summarized as indicated in table 2 below;

Table 2: Diarrhoea Types and bacterial pathogen isolated

		Bacteria Isolated Frequency & percentage (%)				
		Shigella spp.	*C. jejuni*	*Salmonella* (NTS)	*E. coli*	**TOTAL**
Diarrhoea	Watery	7 (25)	4 (22.2)	2 (15.4)	1 (7.7)	14/**26**
type	Bloody	14 (50)	0 (0)	4 (30.8)	3 (23.1)	21/**19**
	Mucus+	7 (25)	14 (77.8)	7 (53.8)	9 (69.2)	37/**94**
Total isolates		**28(20.1)**	**18(12.9)**	**13(9.4)**	**13(9.4)**	**2/139**

Duration of diarrhoea was categorized into 1 day, 2 days, 3 days and 4 or more day's duration. The results were summarized in the table 3 below. Frequencies of diarrhoea duration were concentrated on and above 3 days showing that more than three days of diarrhoea incidence were highly reported. More cases of diarrhoea were captured in over three days. A mean of 3.06 was obtained. A skewness of -0.438 was obtained indicating a slight negative skewness of the duration.

In the associations between isolates and diarrhoeal episodes, higher diarrhoea episodes were noted with 71 (51.1%) of the cases having more than three diarrhoea episodes per day. Cases with three episodes were 49 (35.2%) while those with two episodes were 19 (13.7%). Further analysis of the episodes considered the distribution characteristics which observed a mean of 3.42. Frequency of diarrhoea episodes was

concentrated on and above 3 episodes, indicating thereof that more than three episodes of diarrhoea incidence were commonly reported (Table 3).

Table 3: Diarrhoeic cases based on duration and episodes among the children with bacterial pathogens

Description of diarrhoea		Frequency	Percent
D**uration(days)**	1	2	2.8
	2	15	20.8
	3	32	44.4
	4 and above	23	31.9
	Total	72	100.0
Episodes of diarrhoea	twice	19	13.7
	thrice	49	35.2
	>thrice	71	51.1
		139	100

4.3 Differential Diagnosis

Microscopic examination was carried out on the stool samples to determine presence of other possible causes of diarrhoea. The examination established absence of ova or cysts in 134 cases representing 96.4%. Among the parasitic protozoan agents of diarrhoea *E. histolytica* was detected in a single case each while *G. lambia* was observed in 4 cases (2.9%). The results are presented in the table 4 below.

Table 4: Microscopy examination of stool for ova and protozoa among the children under study.

Microscopy sample testing		
	Frequency	Percent
No ova or cyst seen	134	96.4
G. lamblia	4	2.9
E. histolytica	1	.7
Total	139	100.0

4.4 Pathogenic Bacterial isolates

The present study shows that *Shigella* spp. is the predominant enteric pathogen causing diarrhoea in children less than five years old in Kapsabet, although *C. jejuni* may equally account for a large fraction of illnesses in children within this age group. Moreover, *E.coli and* Non-typhoidal *Salmonella* spp. are other causal microbes worth worrying about. Children under five years of age are a high-risk group for diarrhoea.

A total of 72 pathogens were isolated in all cases. No bacterial pathogen was isolated from 56 specimens (48%), while 11 specimens (7.9%) produced at least single but non-pathogenic type of bacterial isolate. *Shigella* spp. were the most common pathogen isolated with a frequency accounting for 28 isolates (38.8%) followed by *C. jejuni* with a frequency of 18 (25.0%), while 13 (18.1%) cases of *Salmonella* (NTS) were isolated. A few cases of *E. coli* 13 (18.1%) were identified in the course of culture isolations.

The distribution of pathogens varied according to the type of diarrhoeas. A further cross tabulation of age category against bacteria pathogens isolated in the study, controlling for gender, the distribution of pathogens isolated according to age and gender are all summarized in **table 5** hereunder. A total of 33(45.8%) cases of intestinal pathogen were isolated in males while 39 (54.2%) of cases were isolated in females. *Shigella* spp. was more prevalent in males at 24-35 months old and females at the age category of 24-35 months and 12-23 months with 9 and 10 cases isolated in each group respectively. These results also indicated the consistency of infection by *Shigella* spp. among the various age categories, with higher rates in the 12-35 months age groups in either gender.

In this study peak incidence of *C. jejuni* infection (33.3%) associated with diarrhoea was observed in age categories of 12-23 months, while *Shigella*'s peak infection (39.3%) was in the age category of 24-35 months. *Salmonella* (NTS) highest frequency was observed in the age category of 36-47 months (53.9%).

Campylobacter jejuni was more prevalent at 0-11 months of age in males but in 12 to 35 months of age in female. Non- typhoid *Salmonella* was prominent at up to 23 months old in males but more prevalence in females in 0-11 months of age. *E.coli* was also found to be more prevalent in both male and female below 23 months of age with no cases isolated in the rest of the ages categories.

The study further established a strong negative significance relationship (r=-0.502, p=0.003) between males and bacteria pathogen isolated among the age category. This showed that as the age increases, prevalence decreases synchronously with required hygiene. In female, no significance relationship between age category and pathogen isolated was observed (r=-0.110, p=0.505). This indicated no observable difference in age category and pathogen isolated. Overall, a significance relationship of r=-0.289 was observed which was significant at 95% level with p= 0.014. This indicated that, as the age increases, the number of isolates decreases significantly.

It was also observed that females predominated for all the agents isolated except for *C. jejuni* where more isolates were in males than in females. A further study of this involved a correlation of sex with the diarrhoea etiological agent. In this instance female dominated for all agents (r= 0.51) indicating a lack of significant relationship between gender and presence of bacteria agents isolated (p=0.592). While this may

have had no any significant effects on the results, more female were diagnosed with diarrhoea cases than male.

The isolates depicted different colony morphological characteristics, which were used for selection of colonies for biochemical identification/differentiation (Figures 1 & 2 below).

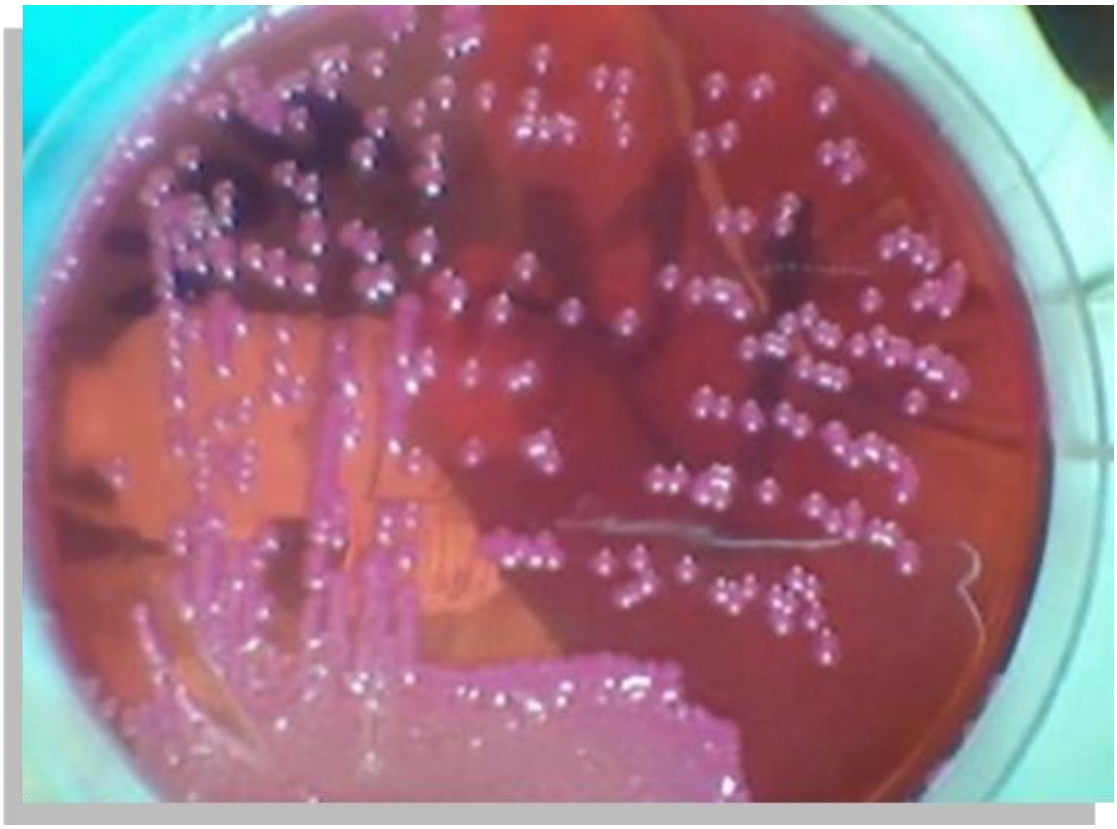


Figure 1: Bacterial colonies/isolates (Lactose Fermenters) on MacConkey Agar at KCH Microbiology Lab. (Source: Author, 2014).

Table 5: Proportional distribution of pathogens in relation with age and gender category

PROPORTIONAL DISTRIBUTION OF PATHOGENS ISOLATED AGAINST AGE AND GENDER							
Gender of the child			**Bacteria Isolated - frequency and (%)**				
			Shigella spp.	*C.jejuni*	*Salmonella* (NTS)	*E.coli*	**Total**
Male	age	0-11	1(8.3)	5 (50)	0 (0)	2 (40)	**8**
		12-23	3 (25)	3 (30)	0(0)	3 (60)	**9**
		24-35	6 (50)	1 (10)	3 (50)	0 (0)	**10**
		36-47	1 (8.3)	1 (10)	3(50)	0 (0)	**5**
		45-59	1 (8.3)	0 (0)	0 (0)	0 (0)	**1**
	Sub-Total		12	10	6	5	**33**
Female	age	0-11	3 (18.8)	0 (0)	0 (0)	2(25)	**5**
		12-23	5(31.2)	3 (37.5)	0(0)	4 (50)	**12**
		24-35	5 (31.2)	3 (37.5)	2 (28.7)	2 (25)	**12**
		36-47	1 (6.3)	2 (40)	4 (57.1)	0 (0)	**7**
		45-59	2 (12.5)	0 (0)	1 (14.2)	0 (0)	**3**
	Sub-Total		16	8	7	8	**39**
Total	age	0-11	4 (14.3)	5 (27.8)	0 (0)	4 (38.5)	**13**
		12-23	8 (28.4)	6 (33.3)	0 (0)	7 (61.5)	**21**
		24-35	11 (39.3)	4 (22.2)	5 (38.5)	2 (15.4)	**22**
		36-47	2 (7.1)	3 (16.7)	7 (53.9)	0 (0)	**12**
		45-59	3(10.7)	0 (0)	1 (7.6)	0 (0)	**4**
	Total		**28(20.1)**	**18(12.9**	**13(9.4)**	**13(9.4)**	**72**

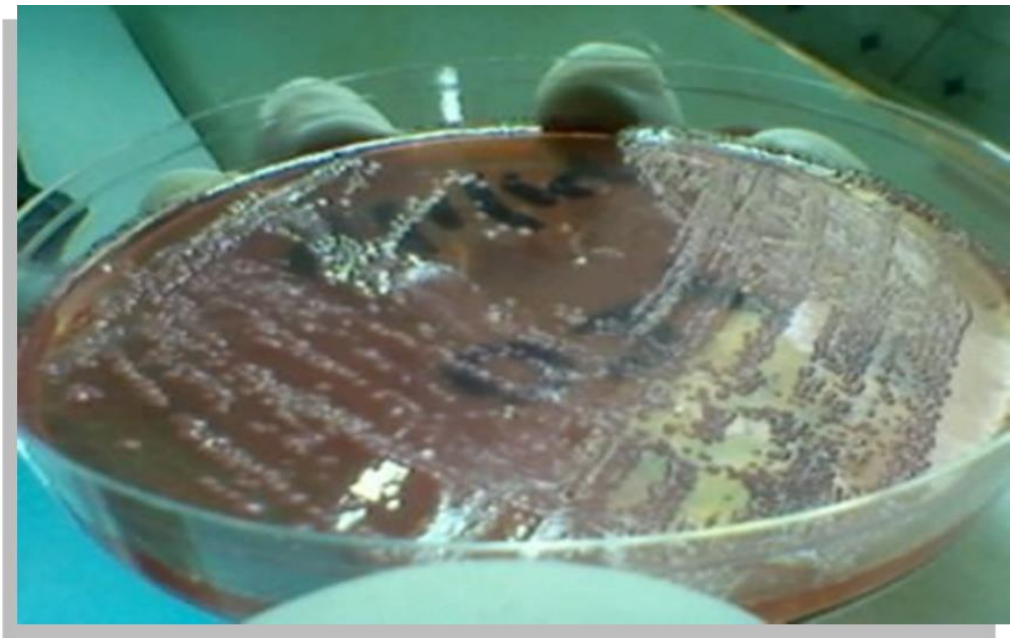


Figure 2: Colonies of *Campylobacter* on CS agar plate at the KCH microbiology Lab. (Source: Author, 2014).

4.5 Antibiotic sensitivity testing

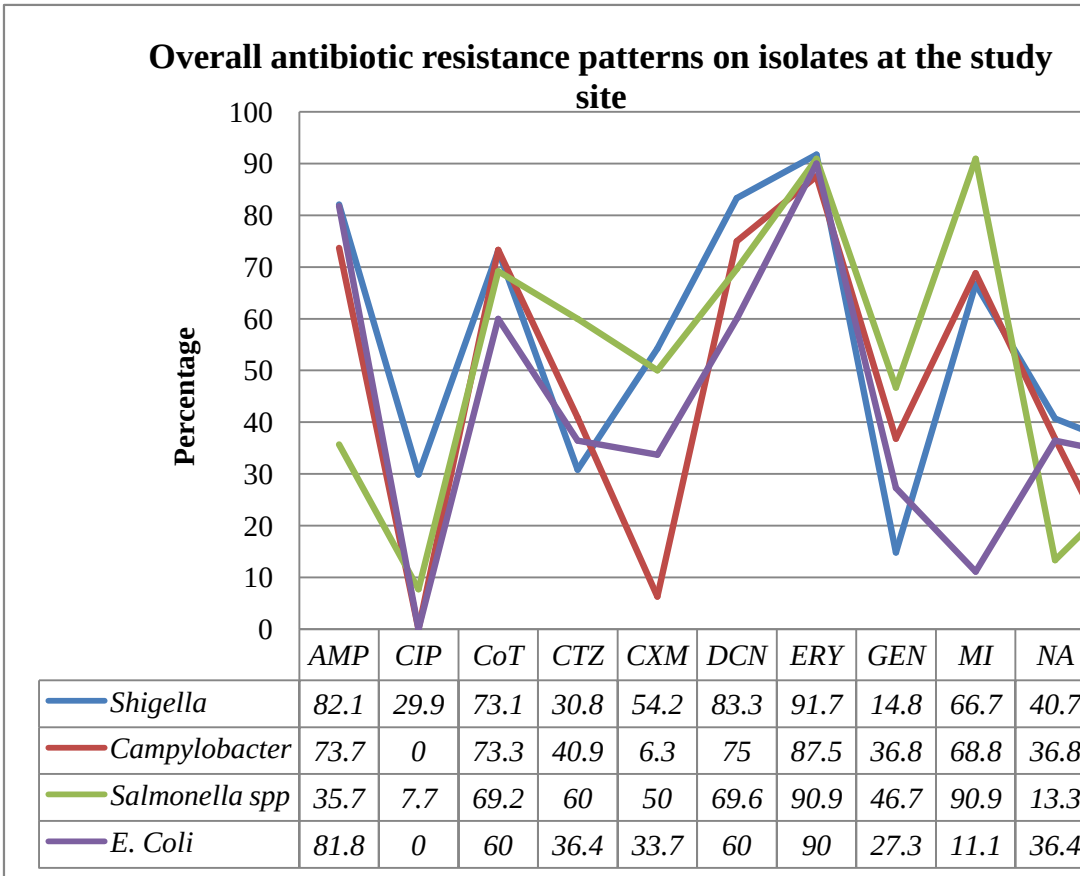
The *in vitro* antibiotic sensitivity test results showed varying patterns of susceptibility (Figures 4a & 4b below). The highest level of potency was detected for ciprofloxacin in which all 11(100%). *E. coli* isolates were found to be sensitive. *E. coli* was found to be highly susceptible to Minocyclin with 8(33.9%) of the cases isolates. *C. jejuni* was highly susceptible to ciprofloxacin in which all 100% of isolates were found to be susceptible. Highest level of susceptibility was also detected for gentamicin and ciprofloxacin, 85.2% and 72.7% for *Shigella* spp. and *E. coli* isolates respectively. *Salmonella* (NTS) were equally found to be highly susceptible to ciprofloxacin for 73.3%.

The patterns indicated that isolates of *Shigella*, *C. jejuni* and *E. coli* and were generally resistant to cotrimoxazole, doxycyclin, gentamicin, minocyclin, erythromycin, ampicillin and ceftriaxone, just as *C. jejuni* was highly resistant to

Cotrimoxazole (73.3%), doxycyclin (75%), minocyclin, (68.8%), erythromycin (87.5%), ampicillin (73.7%) and thus an indication of Multi drug resistance (MDR) as shown in **APPENDIX VI.**

In *Shigella* spp. high resistance levels were observed for ampicillin, cotrimoxazole, Erythromycin, Streptomycin, Minocyclin and Doxycyclin (82%, 73%, 91.7% and 54.2 %, 66.7 % and 83.3% respectively), thus showing 11 multiresistant patterns. *Salmonella* spp. (NTS) was also found to be highly susceptible to Nalidixic acid at 13 (86.7%) while *E. coli* was highly susceptible to Minocyclin 8 (88.9%). *C. jejuni* and *Salmonella* spp. (NTS) were also found to be susceptible to norfloxacin 15 (93.8%) and 10 (71.4%) respectively. The most potent drug was ciprofloxacin followed by norfloxacin, gentamycin, Cefuroxime and ceftriaxone in that order at 90.7%, 74.3%, 68.6%, 58.9% and 57.9% respectively, while the least potent antibiotic agents were erythromycin (90%), doxycyclin (70.5%) and cotrimoxazole (68.9%) as shown in figure 5. Resistance to antibiotics ranged from as low rate of 6.2 % for norfloxacin to high one of 91.7% for erythromycin as seen in *Shigella* spp. and *C. jejuni* respectively.

To determine whether there was any significance difference in the antibiotic susceptibility among the isolated pathogens, Chi-square ($\chi 2$) value of 252.4, p=0.0001 indicated a very strong significant difference in susceptibility patterns in relation to pathogens isolated at the study site.



	AMP	CIP	CoT	CTZ	CXM	DCN	ERY	GEN	MI	NA
Shigella	82.1	29.9	73.1	30.8	54.2	83.3	91.7	14.8	66.7	40.7
Campylobacter	73.7	0	73.3	40.9	6.3	75	87.5	36.8	68.8	36.8
Salmonella spp	35.7	7.7	69.2	60	50	69.6	90.9	46.7	90.9	13.3
E. Coli	81.8	0	60	36.4	33.7	60	90	27.3	11.1	36.4

Figure 3: Proportion (%) of isolates resistant to 11 antimicrobials. AMP-**ampicillin, CIP- ciprofloxacin, CoT-cotrimoxazole, CTZ- ceftriaxone, CXM-cefuroxime, DCN-doxycyclin, ERY-erythromycin, GEN-gentamycin, Mi-minocyclin, NA-nalidixic acid, NOR-norfloxacin.**

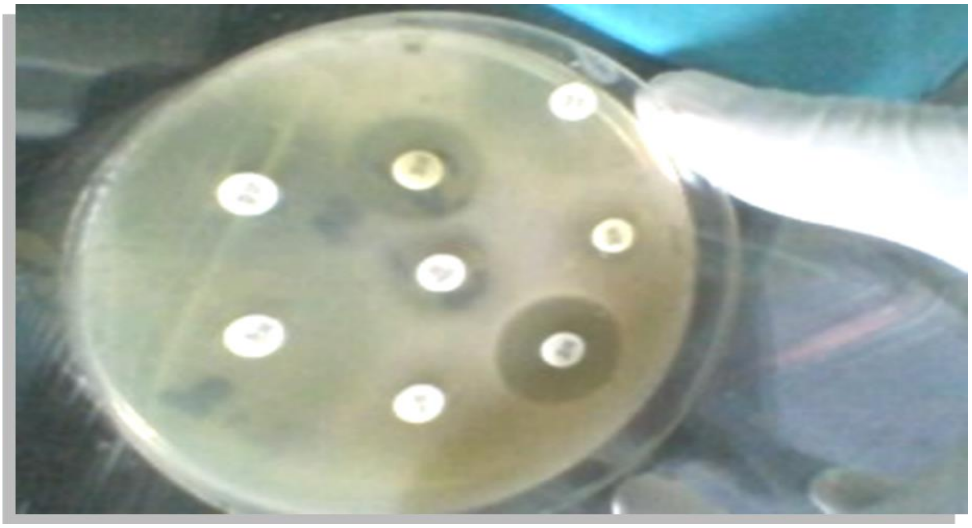


Figure 4a: AST patterns on MHA at KCH microbiology lab. (Source: Author, 2014).

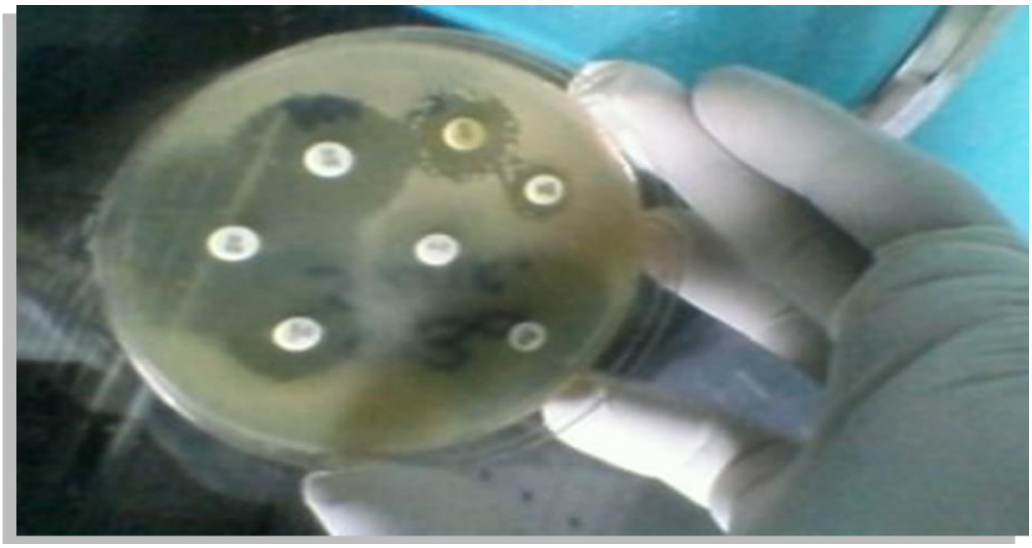


Figure 4 b: AST patterns on MHA- showing antibiotic synergism at KCH microbiology laboratory. (Source: Author, 2014).

4.6 Empirical treatment of diarrhoea post positive culture results

The antimicrobial agents prescribed most frequently for diarrhoea post culture results was gentamicin (the first choice therapy) in 40.3% of the cases, ceftriaxone, 29.2%; and Sulphonamides such as co-trimoxazole and trimethoprim in 23.6% of the cases, as ampicillin was prescribed for another 6.9%, who had salmonellosis, while metronidazole was prescribed for only 5% of the illnesses. Susceptibilities to cefuroxime, norfloxacin, and ciprofloxacin were > 80%; however all were less frequently prescribed or were unavailable in the health facility and or inaccessible to the community.

Using this data from the study population from whom a pathogen was isolated, for which antimicrobial susceptibility was determined , and who were prescribed an antimicrobial, 15% received only antibiotics to which their isolate was not susceptible

4.7 Assessment of sources associated with diarrhoeal illness

To determine sources/causes of diarrhoea, drinking water, last meal before illness and general hygiene were analyzed for any correlation with pathogens isolated during the study period. Major water source indicated included borehole, tap water, stream, and others like rain water. Table 7 gives descriptive characteristics of source of water for all the cases. Majority of cases investigated obtained their water from stream with a proportion of 48.9%. Borehole source was second with 26.6% while tap water and others had a 19.4% and 5% proportion respectively. For the cases with bacterial isolations, 36.1% used boiled water while 22.2% used boiled water occasionally while the majority did not use boiled drinking water at all. A further correlation with different pathogens observed gave a negative correlation (r= -0.97) which was significant at 5% level (p=0.016). This indicated there was some relation between cases isolated and source of water. Many of the children were not using boiled water, with only 30.6% boiling water always, 44.3% did not use boiled water at any time while 25.2% used boiled water occasionally. A further correlation between drinking boiled water and presence of bacteria pathogen indicated a negative relationship (r=-0.79) which was significant at 0.05 level of significant (p=0.047). This indicated that there was some significant relation between bacterial incidences and use of unboiled drinking water, as the data would reveal more cases of bacterial incidence for the group that were given unboiled water than the group that used boiled water for drinking.

Latrine as a waste disposal method was generally reported for all the participants. However 15.5% of the patients acknowledged washing hands after visiting toilet with a bigger proportion (69.4%) of the cases not washing their hands while 15.1% did so

occasionally. Forty-four percent of the parents/guardians washed hands before preparing food with a respective 33% and 23% not washing hands or doing so occasionally. Higher proportion was found not to wash hands before feeding the baby (57%) with 12% doing so before feeding the baby and 31% did so occasionally (Table 6). The feeding on previously cooked food by the patient was reported as an important pre-disposal factor for the diarrhoeal pathogens. Milk was observed as the main previously served meal in most cases of diarrhoea diagnosed with 48.9% of the cases. Chicken and chicken products had a total of 6.4% of cases while other meals which included ugali, and mashed bananas had 44% of the cases reported as indicated in table 6 below.

A correlation between bacteria agent isolated and feeding on previously cooked food for the subject under study indicated a weak negative relationship between the two variables (r= -0.065). A two tailed significant test at 5% level of significance for all other pathogens except for *C. jejuni* and 10% NTS isolates, indicated lack of significance relationship between the previous food and bacterial agent isolated with a p-value of 0.445.

Table 6: Sources associated with diarrhoeal illness among the population under study

Description	Frequency	Bacterial cases (%)	p value
Hand washing with soap before feeding child			
✓ **Yes**	17(12)	5(3.6)	0.049
✓ **No**	49(57)	45(62.5)	0.002
✓ **Occasionally**	73(31)	22(30.6)	0.035
Use of boiled water			
✓ **Yes**	43(30.6)	14(19.4)	**0.047**
✓ **No**	62(44.3)	40(55.6)	0.027
✓ **occasionally**	35(25.2	18(25.0	0.061
Water sources			
Borehole	37(26.6)	**18(25)**	**0.005**
Tap	27(19.4)	17(23.6)	0.048
Stream	68(48.9)	**33(45.8)**	**0.016**
other	7(5.0)	4(5.6)	0.064
Child ate food cooked previous day			
✓ **Yes**	68(48.9)	28(38.8)	0.445
✓ **No**	9(6.8)	9(12.5)	0. 740
✓ **occasionally**	62(44.6)	35(48.6)	0.035

CHAPTER FIVE

DISCUSSION

5.1 Discussions of the Findings

The aetiological patterns of diarrhoea in Children under five years of age seem to have changed as this category are at a high-risk for diarrhoea. While *Campylobacte*r emerged as the second leading pathogen, *Shigella* spp. still remains a major cause of diarrhoeal illness even among the children under five years of age at study site. The results from this study revealed a prevalence rate of *C. jejuni, Shigella*, *E. coli* and *Salmonella* (NTS) at 38.8, 25.0, 18.1 and 18.1 percent respectively in the study area. This represented an overall prevalence rate of 52% of all cases. *Salmonella* (NTS) recorded a low prevalence compared to other pathogenic enterics across the age categories. Diarrhoea in children in developing countries other than Kenya has been reported in 50–60% of the diagnosed cases (Sang *et al.*, 1985; Malakooti *et al.,* 1997; Vargas *et al.,* 2004).

No bacterial aetiological agent was identified in 48.2% of patients with diarrhoea in this study. This may be explained by the fact other diarrhoeal related agents such as viruses were not considered during the study, and or the individual parents/guardians may not have disclosed children who had been treated with antibiotics prior to enrolment/participation in the study.

The main difference between these reports and the present work is the inclusion of diarrheogenic *E. coli*, which increased the frequency of cases of diarrhoea with known aetiology (Vargas *et al.,* 2004). In the present study *C. jejuni* was isolated at almost half as frequently as *Shigella*. In comparable studies of semi-urban Bolivian children

less than five years old with bloody diarrhoea *C. jejuni* was isolated at least half as the proportion of *Shigella* (Vargas *et al.,* 2004).

Shigella spp. was isolated at a higher frequency 28 (38.8%). This result is consistent with a previous report that showed that shigellosis was more prevalent in rural communities (Vargas *et al.,* 2004). *Shigella* spp. is a major cause, as reported in Senegal, Djibouti and Zambia, where it represents respectively 11%, 15% and 10.2% of enteropathogenic agents. As reported in Senegal, Djibouti and Zambia, where it represents respectively 11%, 15% and 10.2% of enteropathogenic agents, *Shigella* is a major cause of diarrhoeal enteric illness. Such an increase in prevalence is supported by World Health Organization data (Manirakiza *et al.,* 2010; WHO, 2003).

A previous study in Bangui in 1983 found only 2.2% of diarrhoea attributable to *Shigella*. Such an increased trend in prevalence is supported by World Health Organization data, which demonstrates a regular increasing trend of *Shigella* spp. in sub-Saharan countries in the last ten years (Manirakiza *et al.,* 2010). The result also revealed a high prevalence of *Shigella* spp. (38.8%) among children within the age group (24-35 months) which compares well with an earlier report of (44%) prevalence rate in rural western Kenya(Brooks *et al.,* 2003). This finding is similarly in agreement with other reports from developing nations, that *Shigella* spp. is still a major isolate in many developing countries (Kotloff *et al.,* 1999).

The isolation rates for *Shigella* spp. increased with increasing age of the patient and reached significant percentages in children 0-11 (14.3%), 12-23 (28.6%) and ≤ 24-35 months (39.3%). These isolation rates are in agreement with those reported from

Kolkata, Algeria during 1986-1990 (Guechi *et al.*,1992) and Boukina-Faso in 2009 (Sangare *et al.*, 2012).

The lower shigellosis rate among children under age 1year old may be attributed to the protective immune properties of breast milk or the exclusion of *Shigella*-infected foods from their diet (Ahmed *et al.,* 1997). The increased number of shigellosis cases seen after age 1 year, when children are no longer breast feeding likely reflects the lack of natural anti- *Shigella* immunity of recently weaned children (Elly *et al.,* 2010).

C. jejuni was more prevalent at 0-11 months of age in males but in 12 to 35 months of age in female. This result was comparable to those found as well in other developing countries where enteritis due to *Campylobacter* have been studied (Vargas *et al.,* 2004; Sobel *et al.,* 2004; Mshana *et al.,* 2009; Adabare *et al.,* 2012;Sangare *et al.,* 2012).

The study found the proportion of children with *Campylobacter* infection among children with acute diarrhoea to be 18.0 %. This rate is comparable to the rate in developing countries which ranges from 5–20 %. The rates reported elsewhere in Egypt (9.0%) and Brazil (9.9%) are lower when compared to Tanzania (18%) and another study elsewhere in Kenya (11%). This can be explained by the fact that this study was done in sub-urban setting where people live in close proximity with animals hence facilitating transmission and perhaps use of contaminated poultry products (Kipkemboi *et al.,* 2012). Outbreaks of *C. jejuni* are commonly associated with ingestion of contaminated milk and water. Ingestion of improperly handled or under cooked food, primarily poultry products, raw milk, or contaminated water are common sources for human infections. Domestic animals have been considered to be

the most common reservoir of *Campylobacter* for human infection, and considering the socio-cultural backgrounds of the community in the study area, there was a high possibility of close association with domestic animals. It is worth to take cognizance that the pathogen is occasionally isolated from streams, and ponds water and hence most probably the reason for transmission of *C. jejuni* from such sources (Vandamme, 2000).

The infection rate in males was higher than in females, although this difference was not statistically significant. In some developed countries, it has been demonstrated that the rate of *Campylobacter* enteritis was higher in males than in females. The association between gender and campylobacteriosis may vary according to the behaviour, geographical area and the population (Karmali *et al*., 1999). By comparison, the high recovery rate of *Campylobacter* species in children is common worldwide, and the study area was no exception. In other studies elsewhere it was observed that neither schooling, nor the level of education were risk factors to enteritis due to *Campylobacter*, contrary to data reported in a study in Jordan where education was a risk factor that correlated significantly with diarrhoea due to *Campylobacter* enteritis(Adabare *et al.*, 2012).

Non-typhoid *Salmonella* spp. was prominent in the age up to 23 months old in males but more prevalent in females in 0-11 months of age. As many as 40% of nontyphoidal *Salmonella* (NTS) isolates in the United States are multidrug resistant, with increasing resistance to all *Salmonella* strains worldwide. In particular parts of the world (for example India, Pakistan and Egypt), multiply antibiotic-resistant strains of *S. typhi* are reported. Decreased ciprofloxacin susceptibility and ceftriaxone

resistance has been reported in developing countries. These rates are reflective of the outcomes in another work in Malawi by Gordon and colleagues (Gordon *et al.,* 2008).

We found that, as a percentage of diarrheal patients, NTS positivity in stools has decreased much comparing other studies elsewhere in the world. The reason for this observation is unclear, although it may be because of a combination of factors that could include changing socioeconomic conditions, better food handling practices, and increasing empiric antibiotic use in the community.

The percentage of stools positive for NTS peaked in the months of March, a month that is marked by high amounts of rainfall and higher ambient temperature, and these conditions may be associated with higher rates of contamination of water and food. This seasonal peak during the rainy month corresponds to reports from other Asian countries (Pathucheary *et al*., 1994; Ochiai *et al*., 2008). Similarly, studies in Malawi (Gordon et al., 2008) and elsewhere in Kenya (Brooks *et al*., 2003; Kipkemboi et al., 2012) describe peaks of NTS disease associated with the rainy season, which correlates with our data suggesting that rainfall-associated contamination of water may be associated with this disease transmission, it is likely that both environmental and host behavioral factors play a role in the seasonal variation of NTS, as they do for other enteric infections in Kenya (Brooks *et al.,* 2003; Gordon *et al.,* 2008). In this study, we show that approximately eighteen percent, of all diarrhoea cases associated with NTS were in children aged 24-47 months, but none in children < 1 year of age. This finding is likely a reflection of the demographic profile of patients presenting to our institution, of patients being < 5 years.

Although NTS is a known cause of gastroenteritis in resource-rich environments, the data are incomplete regarding how its isolation from stool relates to presence of symptoms in low-income households with frequent faecal–oral contamination. As shown in our study and others, NTS are often isolated alongside other pathogenic organisms, a finding common for other enteropathogens. These data suggest pathogenicity of NTS in our study population.

We hypothesize that these socioeconomic associations may reflect differences in sanitation levels. Although in our all-diarrhoea analysis, NTS patients had significantly fewer numbers of family members using the same water source, i.e. there were no differences in type of water source. Combined, these data may suggest that, in Nandi transmission of NTS may occur largely through ingestion of contaminated food and water rather than through direct contact with animals.

E.coli was also found to be more prevalent in male for up to 23 months of age but generally distributed across age in females. Overall, the proportion of diarrheogenic *E. coli* was high (18%) which is consistent with previous reports from developing countries. However, in some cases, differences were found in comparison with reports in the literature. Our results differ with those analyzing the prevalence of *E. coli* in children from Bangladesh in whom a higher prevalence of *E. coli* was found, although these infections peaked during the dry, warm months (Orn-Anong *et al.,* 2004). Similar studies have reported high infections in children from Egypt. Many reports have demonstrated the association of *E. coli* with diarrhoea in children in developing countries (Rao *et al.,* 2001). In our study females predominated for all the agents isolated except for *E.coli* where an equal number of bacteria was observed in both gender. A further study of this involved a correlation of sex with the diarrhoea

etiological agent. Even in this instances female dominated for all agents (r= 0.51) indicating lack of association between gender and presence of bacteria agents isolated.

There was no difference between infection in cases under 12 months and those above 12 months of age, (p=0.176). This can be explained by the fact that our study population was in children 5 years and below, this age group is almost equally affected in developing countries (Kipkemboi *et al.,* 2012). This finding is similar to the 46.0% prevalence rate earlier reported in Ifakara, Tanzania (Vargas *et al.,* 2004) and Mulago, Kampala (Mshana *et al*., 2009) both of which agree with the position of the World Health Organization that the vast majority of enteric diarrhoeal cases occur in Asia, Africa and Latin America where water borne diseases are highly prevalent because of inadequate supply of potable water to the public with concomitant poor environmental and personal hygiene (PHLS, 2009; WHO, 2010).

In general the infection rate was high in children below two years (63.8%) old. This has been found in other developing countries such as Uganda, Nigeria, Tanzania, and Thailand (Mshana *et al.,* 2009). In our study it was observed that the infection rate was decreasing with age as shown from the age of 2 years but the difference was not significant p=0.85. This can be explained by the fact that our study population was under five which can be equally affected.

Regardless of the type of diarrhoea all the 72 isolates were resistant to at least four antibiotic agents. Also found was a moderate level of resistance to the antibiotics most commonly prescribed, the most being gentamicin and Ampicillin at 37% and 68.1% respectively. This finding illustrates that gentamicin had a degree of potency against a

fairly large number of enteric pathogens as opposed to ampicillin which at that rate is not suitable for culture untested cases.

Patterns of susceptibility of bacterial pathogen against antibiotics, from the study established that antibiotics such as cotrimoxazole, Doxycyclin, Erythromycin, ampicillin and minocyclin showed high resistance to all pathogen isolated. High level of antibiotic resistance by isolates was observed to the routinely used antibiotics in the study area. This is in agreement with the earlier report of a worldwide occurrence of multidrug resistance enteric pathogens. This development as observed may be traceable to wrong and inaccurate diagnosis and abusive use of the available antibiotics resulting in the development and spread of multidrug resistance (CDCP, 2007).

In this study over eighty percent of isolates were sensitive to Ciprofloxacin using disc susceptibility; thus differing from the study in Nigeria where 54 % were resistant, this would be explained by the fact that resistance of *Campylobacter* to macrolides, including erythromycin is resulting from inappropriate use of these drugs in treating human infection (Adabara *et al.,* 2012). *C. jejuni* was at a resistance rate of more than 50% for over five of the drugs used in the study, indicating a high degree of MDR. Approximately 6.2% of *Campylobacter* isolates were resistant to Norfloxacin. This is very low compared to Thailand and Nigeria where resistance to cephalosporins increased from 0% to 84% (1991–1996) as result of inappropriate use of that class of drugs. Resistance may be contributed to by the indiscriminate use of drug before culture (Orn-Anong *et al.,* 2004; Mshana *et al.,* 2009).

In *Shigella* spp. high resistance levels were observed for ampicillin, cotrimoxazole, Erythromycin, Minocyclin and Doxycyclin, thus showing high multiresistant pattern, and thereby may not be drugs of choice in the management of bacillary diarrhoea and the specific enteric bacterial illness. The study identified minimal resistance to ciprofloxacin and minimal resistance to Nalidixic acid in *Shigella* spp. The increased resistance frequency of *Shigella* to ampicillin and cotrimoxazole has been reported worldwide (CDCP, 2007; UNICEF/WHO, 2009).

Other studies have also found low resistance to Nalidixic acid and/or no resistance to ciprofloxacin among *Shigella* in East Africa and in other African countries. Ciprofloxacin, followed by norfloxacin, and Cefuroxime were found to be most sensitive to *Salmonella*, *Shigella* and *C. jejuni* which agreed with other studies (Ahmed, 2007). It was however observed that there was emerging resistance towards ciprofloxacin by the other isolates except *E. coli.* The use of ciprofloxacin has been limited in Kenya and this could explain its current effectiveness (Kariuki *et al.*, 2004). The multi drug resistance shown in this study agrees with other studies (Cavallo *et al.*, 1993; Zhi-Dong *et al.*, 2002; Udo *et al.*, 2004; Alexandre *et al.*, 2010).

A high frequency of resistance was seen in *Shigella* spp. to cotrimoxazole, ampicillin, and doxycyclin; therefore these drugs may be assumed to be no longer effective for the treatment of *Shigella* infections in the study area and by extension Kenya. The reason might be due to the argument that these organisms harbour an R plasmid encoding resistance to several antibiotics and thus because of increasing evidence of multi-drug resistance, cotrimoxazole and ampicillin can no longer be used empirically to treat dysentery. Nalidixic acid, Cephalosporin and Fluoroquinolones remain

effective, however; the choice of antibiotic presupposes knowledge of the antibiotic susceptibility pattern of local strains (Mirza *et al.*,, 1996; Udo *et al.,* 2004).

Salmonella spp. (NTS) showed a resistance pattern that illustrates a similar trend. Most resistance was Erythromycin and Minocyclin at (90.9%), Cotrimoxazole (69.2%), Doxycyclin (63.6%), while the lowest level of resistance was observed at 7.7% and 13.3% for gentamicin and Nalidixic acid respectively. Potency of drugs used was most prominent for Nalidixic acid (86.7%), ciprofloxacin (73%), Norfloxacin (71%), and gentamicin (64%). Gentamicin was susceptible at the rate of 53%, indicating that it is still active against this particular spp. However the pattern is worrying in the results reveals that this organism exhibited resistance across the entire panel of drugs albeit at varying low levels.

The study has demonstrated that among non-typhoid *Salmonella* causing enteric infection in Kapsabet, antibiotic resistance to the traditional first-line antibiotic agents ampicillin, Gentamicin and cotrimoxazole occurs in a substantial proportion of isolates but that susceptibility to Ciprofloxacin and extended-spectrum drugs is largely but not completely preserved. Although resistance to ciprofloxacin was minimally identified among NTS isolates, the occurrence of Nalidixic acid resistance (13.3%) however minimal is cause for concern. This kind of resistance may usually result from a single chromosomal point mutation in the DNA gyrase gene, and is considered to be an initial step toward the development of ciprofloxacin resistance, which is usually associated with two or more chromosomal point mutations (Crump *et al.,* 2011). Consequently, the occurrence of Nalidixic acid resistance among NTS isolates warrants vigilance for the emergence of ciprofloxacin resistance. While ceftriaxone resistance remains uncommon, it is important that clinicians be aware that resistance

to extended-spectrum cephalosporins may occur among enteric NTS, particularly since outbreaks of multidrug-resistant *Salmonella* (Lalitha, 2002).

In this study most isolates were resistant to more three (3) drugs indicating that their use would not have reduced illness and subsequent treatment in their community of residence/setting. Low resistance to ciprofloxacin, Norfloxacin, ceftriaxone and Cefuroxime indicates that these drugs may be more effective; and confirms further the current evidence which supports the use of ciprofloxacin, ceftriaxone, and Nalidixic acid for treatment of bloody diarrhoea. However they may be neither readily available nor avoidable at the primary healthcare level (which is cause for concern), neither are they routinely recommended in the protocols for management of diarrhoeal illnesses in Kenya (Brooks *et al.,* 2003; Ochiai *et al.,* 2008; Sang *et al.*, 2012).

In our study, most isolates were resistant to co-trimoxazole and the other available antibiotic drugs, indicating that their use would not have reduced illness and subsequent transmission in this setting. We found a marked increase in MDR to first line drugs and decreased resistance to the newer regimens, ciprofloxacin and norfloxacin at 7.7% and 28.6% respectively. This result may be because of a decreasing use of non-fluoroquinolones agents, especially nalidixic acid, in favour of fluoroquinolones for the treatment of both gastroenteritis and undefined febrile illnesses. However, the study established an increased resistance to ceftriaxone, which is in contrast with increasing rates of ceftriaxone resistance and emergence of extended spectrum β-lactamase–resistant (ESBL) organisms in Pakistan (Ochiai *et al.,* 2008).

Resistance to ampicillin in Gram-negative bacteria can be explained by the fact that it is primarily mediated by β-lactamases, which are predominant in Gram-negative bacteria and are associated with primary resistances that hydrolyse the β-lactam ring and thereby inactivate the antibiotic (Ashraf *et al.,* 2006). It also explained that the spread of antibiotic resistance determinants by integrons underlies the rapid evolution of MDR phenotypes among diverse gram-negative clinical isolates (White *et al.,* 2001).

As identified in this study a large proportion of participants using stream (48.9%) and borehole water (26.6%), might have resulted in the high prevalence rates for microbial isolates. It is highly likely that such waters were not well treated or boiled to render them microbe free and thus posed a great threat to public health at community level. The number of enteric pathogens associated with water borne diseases (stream and borehole water at 45.8 and 25.0 percent respectively) suggested an increased lack of access to safe water and it would appear to remain a challenge especially among rural communities today. The study established that even tap water can be unsafe, considering that 23.6 % of the isolates were associated with this source. It was determined that children with a history of where water was never boiled before use yielded 41.7% of the positive cultures, and even where boiling took place occasionally 22.2% still yielded a positive culture. This perhaps demonstrates lack of hygienic practices in the handling, storage and usage of the water. Indeed significance at 5% level ($p=0.016$) provides the evidence that unsafe water remains a major source of enteric transmission.

It is biologically plausible that hand washing prevents the transmission of diarrhoeal pathogens. This becomes complicated in areas where animals and humans use waters

at similar points, making it easier for the introduction of extraneous organisms (Curtis *et al.,* 2003). Observation from this study illustrates the importance of hand washing. Hands can carry pathogens from faeces to surfaces, to foods, and to future hosts, and hand washing with soap is effective in removing pathogens. A high proportion of cases had a poor history of hand washing practices, with 57% not washing hands before feeding the baby and 31% doing so occasionally. From among this 62.5 % of the isolates were directly associated with lack of hand washing after toilet contact. This confirms that hand washing after stool contact is relatively rare in developing countries as cited in nine studies reporting rates of hand washing with soap after toilet contact in developing countries with median rate of hand washing with soap after cleaning up a child at 13% (range 0–20%) and for the guardian/attendant after defecation was 14% [range 1–20%] (WHO, 2009).

The guardian/parents, who did wash their hands occasionally before feeding their babies, still posed a threat to their children as determined in this study, with 30.6% of the isolates being associated with such a partial hygiene practice. Children are an important reservoir of diarrhoeal pathogens and the guardian/parent who clean the child is often the main cook for the household (Curtis *et al.,* 2003). It has been estimated that the attributable risk for dysentery from not washing hands before preparing food in rural African communities is as high as 30%. Hand washing, especially if soap is used, substantially reduces both primary enteric infections and secondary transmission (Curtis *et al*., 2003; Vargas *et al.*, 2004).

It is reasoned that children form the most vulnerable group in environments where inadequate water supply and poor environmental hygiene are problems because of their high level of ignorance, for they are usually quick to satisfy their thirst

irrespective of the water source especially if the water is apparently clean and without colour (Mates *et al.,* 2000). While there is much discussion on how to improve hand washing habits in health-care settings, the importance of hand washing in homes, particularly in developing countries, receives scant attention (CDC, 2007). Whereas major new initiatives to combat malaria, HIV and tuberculosis have been announced, interest in research and intervention in the diarrhoeal diseases has waned. Modern methods of promoting hand washing can be effective and cost-effective on a large scale (Brooks *et al.,* 2003; Mshana *et al.,* 2012).

According to this study, latrine use did not offer any significant risk to cases of diarrhoea.

The fact that diarrhoeal patients with Salmonellosis or Shigellosis presented with mucoid and bloody diarrhoea was an interesting finding that may have an impact on the way practitioners treat diarrhoea, and protocols of diarrhoic cultures in the study area.

Providing access to safe drinking water and to latrines, and promoting hand washing could substantially reduce the incidence of diarrhoea, the resultant need for antibiotics, and the pressures favouring increased antibiotic resistance (Curtis *et al*., 2003; Vargas *et al.,* 2004).

The study established that young children are still vulnerable to consumption of unsafe waters, yet safe drinking water is essential to stopping deaths from diarrheal disease, although safe drinking water still eludes more than 780 million people. If everyone had access to safe water, almost 90% of diarrheal deaths could be prevented (UNICEF/WHO, 2012)

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6:1 Conclusions

1. *Campylobacter jejuni,* Non-typhoid *Salmonella, E. coli* and *Shigella* spp. were found to be most responsible for diarrhoea amongst the children at the study site.
2. There was significance difference in terms of drug resistance and pathogen isolated as all pathogens were highly susceptible to ciprofloxacin, ceftriaxone, cefuroxime, Norfloxacin and Nalidixic acid.
3. The *in vitro* AST found an overall prevalence of high resistance to minocyclin, cotrimoxazole, ampicillin, doxycyclin, and erythromycin among/across all pathogens (a phenomenon of multidrug resistance).
4. Individual sanitation challenges- the washing of hands with soap before feeding and/or food preparation and water storage/handling are critical public health concerns.

6.2 Recommendations

✓ Since *C. jejuni* and *Shigella* spp. are some of the most important enteric pathogens, further studies are still needed in order to reveal the geographic prevalence and resistance distributions of these microorganisms in Nandi county.

✓ Ciprofloxacin, ceftriaxone, cefuroxime, Norfloxacin and Nalidixic are recommended as drugs of choice for treatment of bacterial diarrhoea in KCH and its environs. The findings of high rates of resistance of *Shigella* spp. to co-trimoxazole, which is the first line drug recommended treatment for bloody diarrhoea in sub-

Saharan Africa, requires the strengthening of national surveillance for antibiotic drug resistance, which would provide the evidence to better inform policy decision makers

✓ For the threat associated with MDR pathogens, there is need for clinical laboratories to carry out surveillance programs to monitor antibiotic resistance patterns, but also innovative therapeutic strategies need to be developed; to involve the enhancement or potentiation of existing antibiotics against resistant strains of bacteria.

✓ Provision of portable water, public education, simple targeted interventions, such as hand washing after defecation and before food preparation, can interrupt the faecal-oral transmission route.

6.3 Recommendation for further Research

In the future rigorous intervention trials are needed to explore the impact of hand washing on diarrhoea and other infections, in a variety of settings.

Since there is an established strong correlation between the biophysical properties of bacterial communities called biofilms and resulting antibiotic penetration and the fact that the biophysical properties are directly linked to chemicals present in the matrix material of these bacterial biofilms, bacterial metabolism be explored and used as a targeted response based on its chemistry, using biophysical properties to enhance current antibiotics in the market.

Research for a new strategy for antibiotic agents to determine what are the bacterial receptor sites that convert normal flora bacteria to pathogens in the GIT (for example *E. coli* in the GI tract to pathogenic invaders in children under two years) by using protein modelling as an attempt to displace the "pathogenic factor" with an inhibitor.

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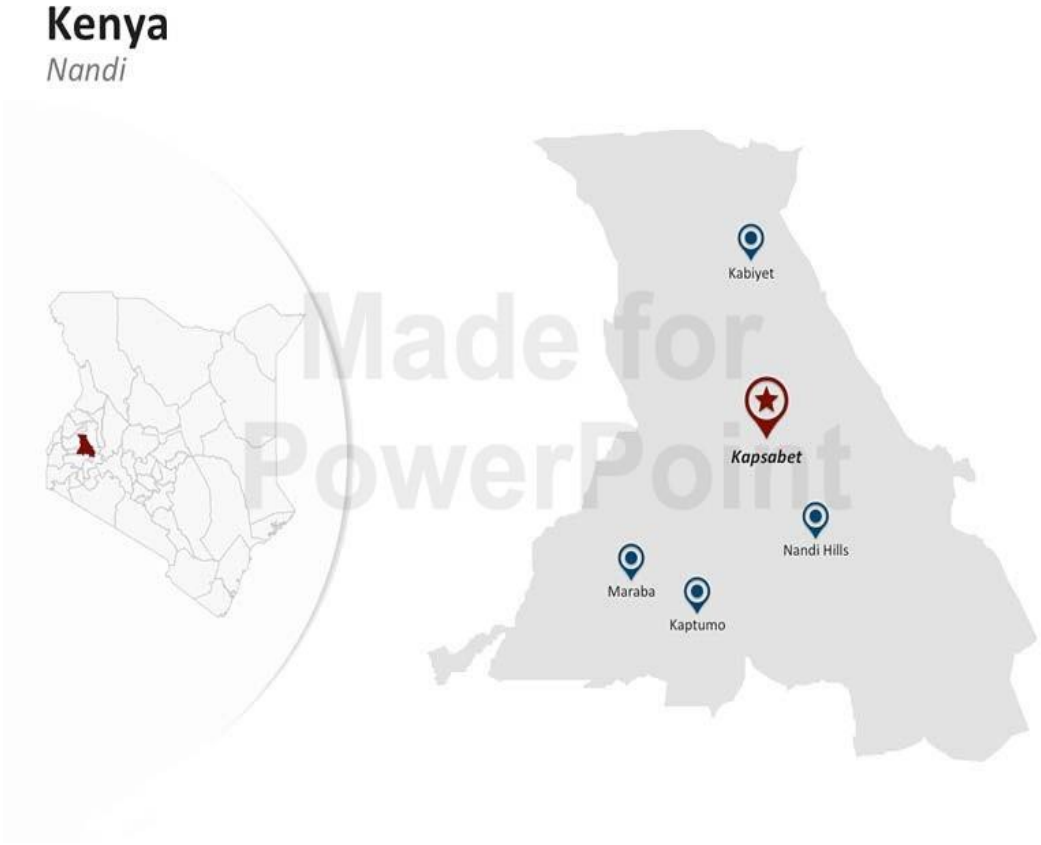
APPENDICES

APPENDIX I: KENYA'S ADMINISTRATIVE COUNTIES

APPENDIX II: NANDI COUNTY SHOWING KAPSABET LOCATION



Source: KNBS, 2000

APPENDIX III: INFORMED CONSENT AGREEMENT FORM FOR PARENTS/GUARDIANS OF CHILDREN RECRUITED FOR THE RESEARCH

CODE NUMBER …… HOSPITALNUMBER……

I,Mr/Mrs./Miss…….....the parent/guardian of…….....male/female…….....
Aged…….....months/years (Name and Age of Child), do hereby give permission/consent for my child to be included in the approved research entitled "**ANTIBIOTIC SUSCEPTIBILITY AND PREVALENCE OF *CAMPYLOBACTER jejuni, E. Coli, SALMONELLA* AND *SHIGELLA*, AMONG DIARRHOIC CHILDREN AT KAPSABET COUNTY REFERRAL HOSPITAL**." I have fully understood what the study is all about, the procedures to be used, the benefits, risks, hazards and discomforts, associated with the procedures to be done. I have been given an opportunity to ask questions and seek clarification on this investigational study, and the questions have been answered to my full and complete satisfaction. However, I have been made to understand that should I have any other questions concerning the rights of my child/the child under my care during the current research session, as a research subject, I may contact the Chairman, Secretary or any other official of the Institutional Research and Ethics Committee (IREC) of Moi University/Moi Teaching and Referral Hospital, P.O Box 3 or 4606, Eldoret, Tel. (053)33471/2/3. I accept that the investigator(s) may take stool sample(s) from my child for the investigation they need to do. I understand that I may, at any time during the course of this study, revoke my consent and withdraw from the study without any penalty or loss of treatment benefits to my child/the child under my care during the current research session.

All the issues concerning this research have been explained to me in.....…….language, which I clearly understand.

Name and Signature of
Parent/Guardian……Date……

Name of Principal Investigator: Henry O. Zachariah

Signature of Principal
Investigator…….....Date……

APPENDIX IV: QUESTIONNAIRE SCHEDULE FOR THE SUBJECT

"ANTIBIOTIC SUSCEPTIBILITY AND PREVALENCE OF *CAMPYLOBACTER jejuni*, *SALMONELLA* AND *SHIGELLA*, AMONG DIARRHOIC CHILDREN BELOW FIVE YEARS AT KAPSABET HOSPITAL"

CODE NUMBER …….

HOSPITAL NUMBER ……

SECTION A: History and observations (Select/Tick suitable response below)

1. Child's gender Male [ ] Female [ ]

2. Child's age is (Months/years)

a) 0-11 [ ] **b)** 12-23 [ ] **c)** 24-35 [ ] **d)** 36-47 [ ] **e)** 46 -60 [ ]

3. Duration of diarrhoea

a) 1 day [ ] **b)** 2 days [ ] **c)** 3 days [ ] **d)** more than 3 days [ ]

4. Episodes of diarrhoea in a day

a) Once [ ] **b)** Twice [ ] **c)** Thrice [ ] **d)** more than Thrice [ ]

5. Type of diarhhoea

a) Watery [ ] **b)** Bloody [ ] **c)** Mucoid [ ]

SECTION B: Pre-disposing factors of diarrhoea

INSTRUCTIONS: Please select/tick the best response to the following questions by giving your response as YES, NO, Sometimes, Always. Ensure that you respond to all questions.

		yes	no	rarely	always
6	The Domestic water supply/source is from:- b) Borehole c) Rain water d) River/ Spring e) Tap water f) Other- Specify Boiling of water before drinking/use				
7	Hand washing is carried out after toilet visit? Washing of hands before food preparation? Hand washing is carried out before feeding the baby?				
8	Consumption of undercooked food by baby/child Childs meal before current illness:- a) Milk b) Chicken c) Chicken product d) Other- (specify)…				
9	Child on medication/Antibiotics				
10	Recent diarrhoea with some dehydration				
11	Specimen obtainable?				

APPENDIX V: LABORATORY RESULTS ENTRY DATASHEET FOR THE SUBJECT

CODE NUMBER: ……

HOSPITAL NUMBER …….

1. Blood/mucus/ detected in stool sample? Yes No (tick appropriately)

2. Sample suitable for culture? Yes No

3. Microscopy results: ……..…

4. Gram stainreactions ….....

5.Culture results (Colony description) ……

6.0 BIOCHEMICAL TESTING

Samples	Identification index for culture isolates (Enteric bacteria) (Indicate +/--)							
	Catalase	Oxidase	Hippurate	HI- IMVIC/ASSORTERD				

6.1 TSI reaction

Sample	Typical Reactions patterns observed on TSI agar							
	Slant	Butt	Gas	H_2S				

KEY for TSI: R=Red Y=Yellow + = positive --- = Negative

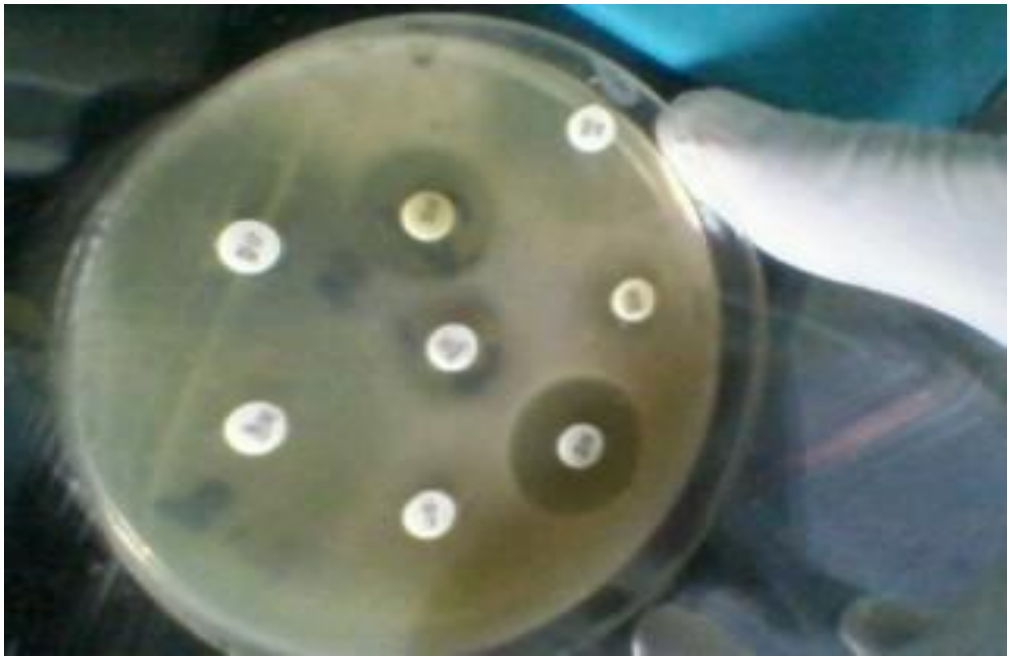
7.0 Sensitivity reactions

samples	Discs reactions: indicate as R or S or I										
	CoT.	NA	DCN	MI	ERY	NRF	CIP	CXM	CTX	AMP	GEN

APPENDIX VI: Antibiotic susceptibility patterns of *Campylobacter jejuni, E.coli, Salmonella and Shigella* spp. isolates among diarrheic patients at the study site (N=72)

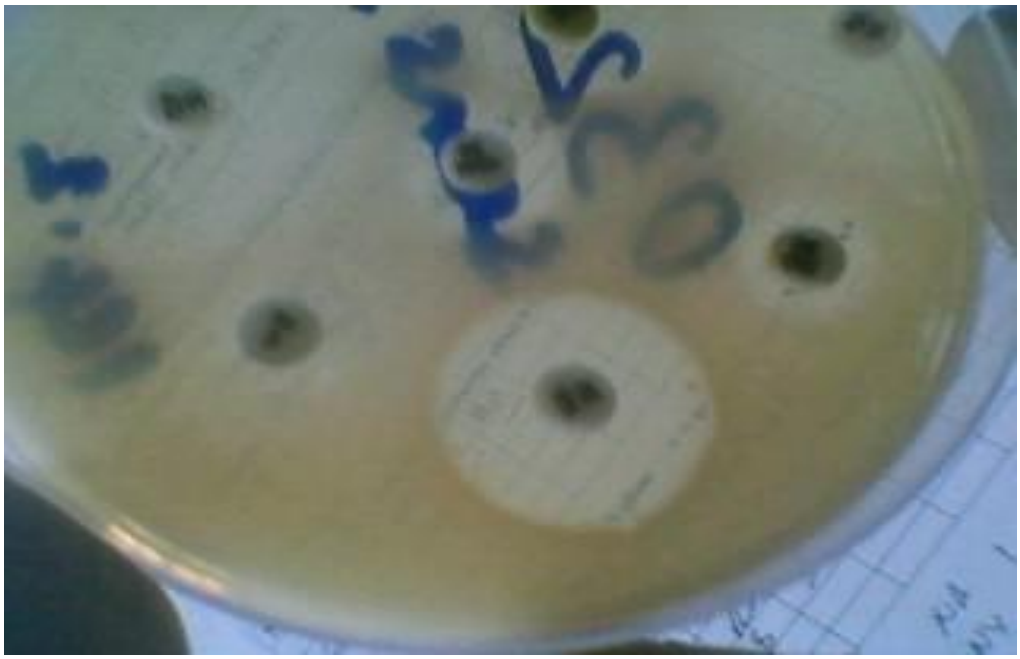
Organism isolated	Susceptible (n, %)	Resistant (n, %)
Shigella (N=28)		
CoT	9 (26.9)	19(73.1)
NA	17(59.3)	11(40.7)
DCN	8(16.7)	20(83.3)
MI	8(33.3)	20(66.7)
ERY	2(8.3)	26(91.7)
NOR	19(65.4)	9(34.6)
CIP	20(70.4)	8(29.6)
CXM	15(45.8)	13(54.2)
CTZ	20(69.2)	8(30.8)
GEN	24(85.2)	4(14.8)
AMP	5(17.9)	23(82.1)
Campylobacter jejuni (N=18)		
CoT	7(21.1)	11(73.3)
NA	12(63.2)	6(36.8)
DCN	6(25.0)	12(75.0)
MI	7(31.2	11(68.8)
ERY	4(12.5)	14(87.5)
NOR	17(93.8)	1(6.2)
CIP	18(100.0)	0(0.0)
CXM	14(73.7)	5(6.3)
CTZ	13(59.1)	9(40.9)
GEN	12(63.2)	6(36.8)
AMP	4(26.3)	14(73.7)
Salmonella spp-NTS (N=13)		
CoT	4(30.8)	9(69.2)
NA	11(86.7)	2(13.3)
DCN	6(36.4)	7(63.6)
MI	3(9.1)	10(90.9)
ERY	3(9.1)	10(90.9)
NOR	10(71.4)	5(28.6)
CIP	11(73.3)	3(7.7)
CXM	7(50.0)	6(50.0)
CTZ	4(40.0)	9(60.0)
GEN	6(53.3)	7(46.7)
AMP	8(64.3)	5(35.7)
Escherichia coli (N=13)		
CoT	4(40.0)	9(60.0)
NA	9(63.6)	4(36.4)
DCN	4(40.0)	9(60.0)
MI	12(88.9)	1(11.1)
ERY	1(10.0)	12(90.0)
NOR	12(66.7)	1(33.3)
CIP	13(100.0)	0(0.0)
CXM	10(66.7)	3(33.7)
CTZ	9(63.6)	4(36.4)
GEN	10(72.7)	3(27.3)
AMP	4(18.2)	9(81.8)

APPENDIX VII (a): AST Results on MHA plate



Source: Author, 2014

APPENDIX VII(b): MHA agar showing high antibiotic senstivity



Source: Author, 2014

APPENDIX VIII: UOE Introductory Letter

University of Eldoret
flame of knowledge and innovation

P.O.Box 1125 - 30100, Eldoret, Kenya
Tel: +254 0208008143 Ext. 364/365/366
Fax: +254 53 2031299
E-mail: biolscdept@yahoo.com
Website: www.uoeld.ac.ke

DEPARTMENT OF BIOLOGICAL SCIENCES

Our Ref: UOE/BIOLSC/PG/88

Date: 5th September, 2013

Medical Superintendent
Kapsabet District Hospital
P.O Box 5
KAPSABET

10/9/13
Approved:- AH- 2nd yr
- 40% - wk 5
Dr Seron

Dear Sir/Madam,

RE: INTRODUCTION LETTER FOR ONGWAE HENRY ZACHARIA –REG. NO. SC/PGB/025/10

This is to certify that the above named is a student of University of Eldoret in the Department of Biological Sciences, School of Science. He is undertaking a Master of Science degree in Microbiology.

Mr. Ongwae completed his 1st year Coursework and successfully defended his M.Sc Research Proposal. He is now carrying out a research project on "***Antibiotic Susceptibility and Prevalence of Campylobacter Salmonela and Shigella, in Children below Five Years with Diarrhea at Kapsabet District Hospital".***

Mr. Ongwae is a hardworking, honest and dedicated person. Any assistance given to him will be highly appreciated.

Yours faithfully,

04 SEP 2013

fw **Dr. Elizabeth W. Njenga**
Head, Department of Biological Sciences

APPENDIX IX: IREC Approval Certificate

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

Reference: IREC/2011/198
Approval Number: 000762

27th January, 2013

Ongwae Henry Zacharia,
Chepkoilel University,
School of Sciences,
P.O. Box 1125-30100,
ELDORET-KENYA.

Dear Mr. Ongwae,

RE: CONTINUING APPROVAL

The Institutional Research and Ethics Committee has reviewed your request for continuing approval for your study titled:-

"Antibiotic Susceptibility and Prevalence of Campylobacter Salmonela and Shigella in Children below Five Years with Diarrhea at Kapsabet District Hospital".

Your request has been granted Approval with effect from 27th February, 2013. You are therefore permitted to continue with your study.

Note that this approval is for 1 year; it will thus expire on 26th February, 2014. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

17/01/2013
DR. W. ARUASA
VICE-CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc: Director - MTRH
Dean - SOM
Dean - SPH
Dean - SOD
Dean - SON

APPENDIX X: MOH Approval

REPUBLIC OF KENYA

MINISTRY OF HEALTH

The Medical Superintendent
County Referral Hospital.
P.O Box 5 - 30300
KAPSABET

Telegrams; MEDICAL;
Telephone; 52081, 52623
When replying please quote

10/9/ 2013

Ref: A.16/VOL. III/8

TO: ONGWAE HENRY ZACHARIA

RE: RESEARCH ON ANTIBIOTIC

Your request to conduct a research on Antibiotic susceptibility and prevalence of campylobacter salmonella and shigella in children below five years with diarrhea at Kapsabet hospital has been approved.

You are instructed to observe research medical ethics during the process. The ~~direction~~ duration of the research is 3 months from the date of this letter.

DR. SEREM EDWARD
MEDICAL SUPERINTENDENT
KAPSABET DISTRICT HOSPITAL