CHARACTERIZATION OF THERMOTOLERANT *ESCHERICHIA COLI* AND ASSOCIATED RISK FACTORS WITH CONTAMINATION OF SOURCE AND HOUSEHOLD DRINKING WATER IN KERICHO DISTRICT, KENYA

JOHANA KIPLAGAT TOO

MASTER OF SCIENCE

(Laboratory Management and Epidemiology)

JOMO KENYATTA UNIVERSITY

OF

AGRICULTURE AND TECHNOLOGY

2023

Characterization of Thermotolerant *Escherichia Coli* and Associated Risk Factors with Contamination of Source and Household Drinking Water in Kericho District, Kenya

Johana Kiplagat Too

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Laboratory Management and Epidemiology of the Jomo Kenyatta University of Agriculture and Technology

2023

DECLARATION

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

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

      Johana Kiplagat Too

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

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

      Prof. Zipporah Ng'ang'a, PhD

      JKUAT, Kenya

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

      Dr. Willy Sang, PhD

      KEMRI, Kenya

DEDICATION

I dedicate this project to God almighty, for all his mercies and strength to persevere through to the end of the road, and to my parents for inspiring me in all my years of study.

ACKNOWLEDGEMENT

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ACRONYMS AND ABBREVIATIONS

CDC   Centers for Disease Control and Prevention

CFU   Colony Forming Unit

CI   Confident Interval

DNA   Deoxyribonucleic acid

dNTPS   Deoxynucleotide triphosphates

EAEC   Enteroaggregative E. coli

EPEC   Enteropathogenic E. coli

ESBLs   Extended Spectrum Beta-Lactamases

ETEC   Enterotoxigenic E. coli

GDWQ   Guidelines for Drinking-water Quality

HPC   Heterotrophic plate count

LSA   Laurylsulphate agar

MDGs   Millennium Development Goals

NCCLS   Committee for Clinical Laboratory Standards

OR   Odds Ration

PCR   Polymerase Chain Reaction

TTC   Thermotolerant coliform

UN   United Nations

WHO   World Health Organization

WRP-KEMRI   Walter Reed Project – Kenya Medical Research Institute

ABSTRACT

Clean and plentiful water provides the foundation for prosperous communities. Changing climate patterns are threatening lakes and rivers, while key sources of drinking water are being overdrawn or tainted with pollution. Water can be contaminated with fecal material due to inadequate protection of the source, unhygienic practices of the community at the source and poor household handling practices. Water contamination exuberate its potential for greatly transmitting variety of enteric diseases. *Escherichia coli* is considered the most suitable index of fecal contamination. Thermotolerant (TTC) *E. coli* in water is used to monitor the quality of drinking water as well as disinfection indicators. Limited data exit in Kenya qualifying the safety (both from harmful bacteria and disinfectant byproducts) of drinking water for human consumption. In fact, with the poor water handling strategies both at the sources and within households especially in rural and socio-economically poor settings in Kenya calls for an urgent water quality survey to avert the water born infections epidemic waiting to occur. With this urgency, this cross-sectional study sought to determine the proportion of water (source and within the household) in Kericho district contaminated by TTC as well as evaluated factors associated with this contamination in the district. Water samples were collected aseptically using sterile sampling containers. About 100 mL of the water sample was collected and immediately analyzed for bacteriological qualities and physical chemical properties (pH, temperature, turbidity, and free chlorine) on site using a DelAgua water testing kit. Water source sampling (rivers, streams, or other surface waters) involved drawing water from 30cm below the surface. Sampling from wells and boreholes involved drawing water using a bucket and taking 100 mL into a sterile container. Water samples were filtered through a 0.45 μ m pore size membrane filter. Culture and multiplex polymerase chain reaction (PCR) techniques were used to characterize TTCs. The disk diffusion method was used for antibiotic susceptibility profiling of pathogenic *E. coli*. Structured questionnaire was adopted from the WHO/UNICEF Joint Monitoring Programme which was used to gather information related to water-extraction patterns, type of water transport, water treatment methods, hygiene and sanitation related issues. A total of 103 households consented and their household and source waters collected. Majority (30.1%) of the households were located within the Kericho Township, (68.9%) were in the rural locality, (95.1%) were female and (42.7%) were aged 21 to 30 years. The respondents mean age was 21.59 years, the range (18–29 years) was the majority. The main water source was river (36.9%) and only (33%) had piped or municipal supply. Most (43.7%) of the households had drinking water source within their premises while (13.6%) had to walk for 30 to 60 minutes to water source. Majority 60.2% of the household used clay pot for water storage; most of them (91.3%) did not treat their drinking water. The majority (83.5%) drew drinking water by dipping the cup into the water storage container. About (59.2%) did not wash their hands before drawing water while (96.1%) of them rinsed the water drawing utensils. There were 48/103 (46.6%) households whose drinking water was contaminated by Thermotolerant coliform (TTC) levels of >10cfu/100ml. Five (10.4%) of these 48 TTCs were toxigenic *E. coli* including 2/5 (40%) Enteroaggregative *E. coli* (EAEC), 2/5 (40%) Enterotoxigenic *E. coli* (ETEC) and 1/5 (20%) Enteropathogenic *E. coli* (EPEC). All of this pathogenic *E. coli* were resistant to commonly used antibiotics

such as Cephalothin, Ampicillin, Tetracycline, Ampicillin/sulbactam and Sulfamethoxazole/trimethoprim. Rural household locality (OR 2.01, 95% CI 1.09 to 4.12) and hand contact with drinking water during water withdrawal (OR 1.11, 95% CI 1.11 to 3.39) increased the likelihood of water TTC contamination. However, household whose main source of drinking water was from piped supply or from municipal (OR 0.38, (95%) CI 0.16 to 0.91), washed their drinking water storage containers (OR 0.58, 95% CI 0.31 to 0.99), washed hands before drawing drinking water (OR 0.33, 95% CI 0.15 to 0.67), households whose total coliforms count was less than 10 cfu/100ml (OR 0.45, 95% CI 0.26 to 0.81) and household water source temperatures was between 15 to 20°C (OR 0.39, 95% CI 0.16 to 0.96) were less likely to have household drinking water contaminated with TTC. This study shows that significant number of household drinking water in this study were contaminated with TTC including toxigenic multi-drug resistant *E. coli*. These strains are associated with great mortality and morbidity especially among children and immunocompromised population. The study recommends continuous monitoring of both water sources and household for contamination and that water treatment of any kind could reduce the level of TTC contamination. Further the study showed that by improving on hygiene and protecting water source are simple implementable steps household could adopt to improve the quality of drinking water in the district.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The importance of water to human health is encapsulated in the Human Right to Water and Sanitation, which entitles everyone to 'sufficient, safe, physically accessible and affordable water for personal and domestic uses' (Committee on Economic, Social and Cultural Rights 2002; United Nations, 2010). Unfortunately, over 275 million people in sub-Saharan Africa rely on unsafe drinking water sources from lakes, rivers, and open wells (WHO/UNICEF, 2010). Consequently, in this region, many waterborne-related diarrheal diseases are responsible for significant morbidity and mortality especially among children, the elderly, and immunosuppressed individuals (Kariuki *et al*., 2006; WWAP, 2006). Diarrhea, typhoid fever, cholera is among the most common health problems associated with unsafe drinking water in Kenya include (WWAP 2006).

The World Health Organization (WHO) guidelines for drinking water quality include criteria for assessing health risks and setting targets for improving water safety (WHO, 2011). The guidelines recommend using either thermotolerant coliforms (TTCs) or Escherichia coli in assessing fecal contamination of drinking water (WHO/UNICEF, 2010; WHO, 2011). A 100 mL water sample with <1 indicator organism is considered 'very low risk'; 1–10, 'low risk'; 10–100, 'medium risk'; >100, 'high risk' or 'very high risk' (WHO, 1997).

Studies have given varied total and fecal (*E. coli*) coliform contamination of water samples in different settings. Over a quarter of samples from improved water sources in China, the United Kingdom, France, Portugal and in selected low- and middle-income countries were shown to contain fecal contamination (Bain *et al*., 2014). About (95%) of water sources (dams, rivers, springs, and wells) in the informal settlements of Kisumu, Kenya had significant E. coli contamination (Opisa *et al.*, 2012). In another informal settlement in Nairobi, Kenya, Chemuliti *et al.* (2002) identified (35%) of standpipes and (95%) of in-house storage containers as being

contaminated with *E. coli* coliforms. Water source, storage practices, locality, poverty, hygiene, sanitary and environmental factors have been cited as sources for fecal water contamination (Gundry *et al.*, 2006).

The importance of safe storage of water within the household to maintain the cleanliness of drinking water is well established in studies worldwide (UN-WHO, 2021; Callaway *et al.*, 2021; Nyberg *et al.*, 2022). A combination of point-of-use treatment and safe storage, defined as a container with a narrow opening preventing contamination, achieve the greatest reduction in diarrheal disease relative to other interventions, including treatment without safe storage (Larson *et al.*, 2019; Kannan *et al.*, 2021; Larson *et al.*, 2023). However, other factors such as water storage may obscure the effects of water treatment on contamination levels (Larson *et al.*, 2023). Contamination of initially clean water through exposure to household storage has been shown in several studies including in Kenya (Oswald *et al.*, 2007; Too *et al.*, 2011; Hamzah *et al.*, 2020; Trajano Gomes da Silva *et al.*, 2020). Increased contamination in point-of-use water relative to source water has been demonstrated in studies globally (Wright *et al.*, 2004; Larson *et al.*, 2019). Furthermore, contamination of treated drinking water can occur at the point-of-consumption from drinking vessels (Larson *et al.*, 2019).

Gaps in clean, accessible water sources are pertinent for the transmission of antimicrobial resistance (AMR) and environmental contamination because inadequate drinking water systems can deliver antibiotic-resistant bacteria from local sources of contamination to humans (Kosek *et al.*, 2008; WHO, 2018). Infections with antibiotic-resistant bacteria is associated with greater duration of illness and mortality compared to disease with antibiotic-susceptible bacteria, as well as rising health care costs (WHO, 2018). Antibiotic resistance in *E. coli* has been globally identified in isolates from environmental, animal and human sources (Wose *et al.*, 2010). The Enterobacteriaceae family has been linked to well-known antibiotic-resistant gene pools. These genes are transferred into the normal flora of humans and animals, where they exert a strong selective pressure for the emergence and spread of resistance in both pathogenic and commensal bacteria (Lin *et al.*, 2005; Wose *et al.*, 2010). Eventually they find their way into the environment via wastewater, manure

and sewage sludge. Studies have reported different resistant patterns of E. coli from water source to commonly used antibiotics including tetracycline (5%−95%), ampicillin (10%−80%), chloramphenicol (5%−80%) and erythromycin (50%−100%) including multiple antibiotic resistant strains (Wose *et al*., 2010; Trajano Gomes da Silva *et al*., 2020). At the time of the current studies the prevalence of antibiotic resistant thermotolerant *E. coli* contamination of household source and drinking water in the study area was not available.

At the time of this study, however, Kericho District then, had unique feature warranting water contamination studies including; high population density about 901, 77, high population growth rate (3.6%); large urban and rural populations depending on piped water supply (township) and shallow wells, river, springs and rain water harvesting among the rural population (Kenya National Bureau of Statistics-KNBS, 2019); the water contamination studies have not been conducted. This study therefore, characterized and determined factors associated with thermotolerant *E. coli* contamination of source and household drinking water in Kericho county then district, in the Western part of Kenya.

1.2 Statement of the Problem

Diarrheal disease is the third leading cause of mortality among children <5 years of age globally (WHO, 2020). In 2019, 1 in 10 deaths were attributed to diarrhea with the greatest burden reported among children in South Asia and sub-Saharan Africa (Paulson *et al*., 2021). Over the last 3 decades, remarkable improvement has been seen in mortality rates associated with diarrheal disease in children <5 years old, attributed to declines in risks, such as unsafe water and sanitation and stunting, in association with social and economic development in low- and middle-income countries (Murray *et al*., 2020; Berendes *et al*., 2023). It is reasonable to expect that these ongoing shifts would have resulted in changes in the etiology, manifestations, and outcomes of diarrhea in young children. However, progress had not been distributed equitably, in particular pockets of sub-Saharan Africa had and continues to report high prevalence of risk factors and poor outcomes (Olofin *et al*., 2013). In Kenya, during the year 2019, 1,499,146 cases of diarrhea were reported among

children under five years (Guillaume *et al*., 2020). Among these diarrhea cases, Nairobi accounted for 136,028 cases while data were and are still skewed in Kericho county (Guillaume *et al*., 2020). Most of these diarrheal cases in Kenya have been associated with bacterial infections including pathogenic *E. coli* often being the most frequently isolated pathogen (over 50% of all reported cases). Other bacterial pathogens such as *Aeromonas*, *Shigella*, *Salmonella*, *Vibrio* and *Yersinia* species have been implicated (Guillaume *et al*., 2020). Mixed bacterial infection was observed. The occurrence of multidrug resistant strains of pathogenic *E. coli* to commonly used antibiotics have also been identified complicating further diarrhea management. The safety, handling practices and magnitude of contamination of water in Kericho District has not been studied. Identifying the Total and *E. coli* TT coliforms and possible factors associated with their contamination of the drinking water from the various sources was important in impacting the morbidity and mortality attributed to these waters borne enteropathogens in the district.

1.3 Justification of the Study

People living below poverty line in Kericho represent close to (60%) of the total population (KNBS, 2019). The most affected categories include unemployed youth, women and female-headed households, disabled, slum dwellers, the landless, the aged and street families/children (KNBS, 2019). The poor are scattered all over the district but most of them are in the urban areas in unplanned squatter settlements, which lack adequate and quality water supplies and sanitation facilities (Republic of Kenya Kericho District Strategic Plan 2005-2010, 2005). Although some of these areas may be served by a water distribution network, crowding, leaks, lack of sewerage systems and garbage disposal facilities exert great pressure on water quality. Water quality is further threatened by possibility of post collection contamination between communal water points and houses (Murray *et al*., 2020; Berendes *et al*., 2023). This type of contamination has been attributed to various water handling habits such as storage in open vessels or vessels that are not cleaned regularly, use of communal cups to draw water and hands touching water during collection and storage (Chemuliti *et al*., 2002). In view of the foregoing, the study was initiated with the objectives of assessing the bacteriological quality of water at

the source and household. This was important in guiding the authorities on the best approach to ensure the quality of drinking water is maintained at the required standard both at the source and household level.

In 2008 and 2009, there were 15 confirmed cases of cholera in Kericho District (Kericho District health information office- KDHIO, 2008). Kericho District hospital reports diarrheal diseases as a third cause of mortality after respiratory infections and malaria. According to surveillance report on diarrheal illness within the district conducted by Walter Reed Project (WRP)/KEMRI, various enteropathogens have been identified as etiologic agents for diarrheal diseases which include bacterial pathogens such as *Shigellas, Campylobacters, Escherichia coli* strains (ETEC and EAEC); parasites such as *Entamoeba histolytica, Giardia lamblia, Cryptosporidium parvum,* helminthes; and Rotaviruses, (Brett, *et al*., 2012). Ninety to hundred percent (90-100%) of the isolated bacterial enteropathogens were resistant to tetracycline and trimethoprim/ sulfamethoxazole while *E. coli* (ETEC and EAEC) were resistant to Ampicillin, Extended Spectrum Beta-Lactamases (ESBLs) (Brett, *et al*., 2012). Currently there are no studies done in Kericho District determining the total and fecal (*E. coli*) TT coliform which were the objectives of the current study.

1.4 Research Questions

1. What are the total and *E. coli* thermotolerant coliform contamination levels of source and household drinking water in Kericho district?
2. What are the characteristics (pathotypes, virulence, antibiotics profiles) of the thermotolerant *E. coli* isolated from source and household drinking water in Kericho district?
3. Which of the socio-demographic, hygienic and environmental factors are associated with the thermotolerant *E. coli* contamination of water utilized by the study participants in Kericho District?

1.5 Objectives

1.5.1 General Objective

To characterize the thermotolerant Escherichia coli and to determine the associated risk factors with contamination of source and household drinking water in Kericho District in Kenya

1.5.2 Specific Objectives

1. To determine the total and *E. coli* thermotolerant coliform contamination levels of source and household drinking water in Kericho district.
2. To characterize (pathotypes, virulence, antibiotics profiles) the thermotolerant *E. coli* isolated from source and household drinking water in Kericho district.
3. To determine the socio-demographic, hygienic and environmental factors associated with the thermotolerant *E. coli* contamination of water utilized by the study participants in Kericho district.

1.6 Significance of the study

This study was significant for the following reasons

1. By determining the contamination levels of thermotolerant *E. coli* of source and household drinking water in Kericho district was significant by informing authorities on potential fecal contamination and usability of these waters with an intention of providing preventive strategies.
2. The determination of pathotypes, virulence and antibiotics profiles of the thermotolerant *E. coli* isolated from source and household drinking water in the district aimed at showing the magnitude of the problem. Whether the isolated thermotolerant *E. coli* were pathogenic or part of normal flora. If pathogenic then the study aimed at informing the public and authorities on ways to prioritize water treatment strategies and formulating strategies of investigating the prevalence of household populations (if any) infected with these strains. This will help provide treatment and management strategies for those already infected

6

3. Analyzing predisposing factors associated with thermotolerant *E. coli* contamination of water utilized by the study participants in Kericho district, was key in providing a preventative strategy.

CHAPTER TWO

LITERATURE REVIEW

2.1 Water safety and human health

In developing countries, lack of access to safe drinking water especially in rural areas and among poor communities, obliges women to spend hours every day collecting water for their families' daily needs. This causes an enormous drain on their energy, productive potential and health (WHO, 2020). The quality of drinking water is a powerful environmental determinant of health. Drinking water quality management has been a key pillar of primary prevention for over one-and-a-half centuries and it continues to be the foundation for the prevention and control of waterborne diseases (Chan *et al*., 2021). Water is essential for life, but it can and does transmit disease in countries in all continents from the poorest to the wealthiest. The most predominant waterborne disease, diarrhea, has an estimated annual incidence of 4.6 billion episodes and causes 2.2 million deaths every year (WHO, 2010; UN-Water, 2014; UN-WHO, 2021).

Since the international drinking water sanitation decade (1981-1990) was launched significant progress was made in water and sanitation coverage (WHO, 2010). The proportion of people with access to adequate water and sanitation has not increased though, due to, population growth, insufficient continued investment on water quality management and lack of training (WHO, 2010). Only (61%) of people in developing countries are estimated to have access to a clean water supply (Cheesebrough, 2004). The Millennium Development Goal target 7c, calls for reducing by half the proportion of people without sustainable access to safe drinking water and basic sanitation by 2015, through tackling both the quantity (access, scarcity) and quality (safety) dimensions of drinking water provision (WHO, 2010; Murray *et al*., 2020; Berendes *et al*., 2023).

Diseases associated with lack of safe water and poor sanitation are major causes of poverty and death in Kenya especially in children and women (WWAP, 2006; Kariuki *et al.*, 2006; Murray *et al*., 2020). The most common health problems

associated with water in Kenya include: typhoid fever, cholera, diarrhea, dysentery, worms and bilharzias (WWAP, 2006; Berendes *et al*., 2023). The 2019 population census showed that a significant Kenyan population depends on lake, river, pond and dam water sources all of which are regarded as unsafe. Hence many people are exposed to serious health problems, (WWAP, 2006; Berendes *et al*., 2023).

2.2 Transmission of water borne diseases

Urbanization and industrialization increase the pressure on water supplies and systems of waste disposal, and by the middle of the 19th century, Britain was affected by major epidemics of cholera and endemic typhoid. John Snow and William Budd, provided incontrovertible evidence of the role of water in transmission of these two diseases. Snow's case rested very simply on a comparison of cholera incidence among the customers of three London water companies (Snow, 1855). After being excreted in feces from the body of their host, bacterial pathogens gradually lose viability and the ability to cause infection. The rate of decay varies with different bacteria, it is usually exponential, and after a certain period the pathogens will become undetectable (Cheesbrough, 2004**).** There are several variants of the fecal-oral pathway of water-borne disease transmission. These include contamination of drinking water catchments for example by human or animal feces, water within the distribution system, through leaky pipes or obsolete infrastructure or of stored household water as a result of unhygienic handling (WHO, 2010).

2.3 Diseases associated with quality of drinking water

2.3.1 Waterborne Enteropathogenic Bacteria

Diarrhea and gastroenteritis are major causes of death and ill health in many developing countries, especially in areas with inadequate water supplies, sanitation and little or no health education (Kalle, 2019). The common cause of acute and chronic diarrhea is bacterial and parasitic infections (WHO, 2009). High incidence of bacterial diarrhea constitutes one of the main health problems in Kenya (Osiemo *et al*., 2019). The poor sanitary facilities and the low standards of hygiene, which prevail in some parts of Kenya, make diarrhea due to *Salmonella, Shigella* and *E.*

coli likely to persist as endemic diseases (Vogel *et al*., 1982). Salmonellosis; a major problem in industrialized countries, is caused by the *Salmonella* bacteria and symptoms are fever, headache, nausea, vomiting, abdominal pain and diarrhea. Outbreak of salmonellosis has been associated with contaminated water and food such as eggs, poultry meats, raw milk and chocolate. Campylobacteriosis a widespread infection is caused by certain species of Campylobacter bacteria; in countries such as Denmark, Sweden, Finland, Norway, Netherlands, UK, its incidence surpasses that of salmonellosis (BGVV, 2001). Food-borne cases occur in raw milk, raw or undercooked poultry and drinking water. Acute health effects of campylobacteriosis include severe abdominal pain, fever, nausea and diarrhea. In (2-10%) of cases, the infection may lead to chronic health problems, including reactive arthritis and neurological disorders.

Pathogenic *E. coli* strains such as *E. coli* O157 which produce a potent (vero-) toxin, causing hemorrhagic infections in the colon, resulting in bloody diarrhea or life-threatening complications such as kidney failure. Such bacterial strains together with listeriosis, although having a low incidence, exhibit severe and sometimes fatal health consequences, particularly among infants, children and the elderly. Although E. coli O157 outbreaks have been mainly related to beef, sprouts, lettuce and fruit juice have also been linked (Cheesbrough, 2004). Cholera is a major public health problem in developing countries, caused by *Vibrio cholerae*, a bacterium. Both water and contaminated foods can be the vehicles of infection. Past outbreaks have involved different foods, including rice, vegetables, millet and various types of seafood. Symptoms, including abdominal pain, vomiting and profuse watery diarrhea, may cause severe dehydration and possibly death, unless fluid and salt are replaced. Management of diarrhea due to bacteria may require the use of antibiotics which shorten duration of diarrhea, decrease frequency of stool output and abrogate complications (Black, 1993).
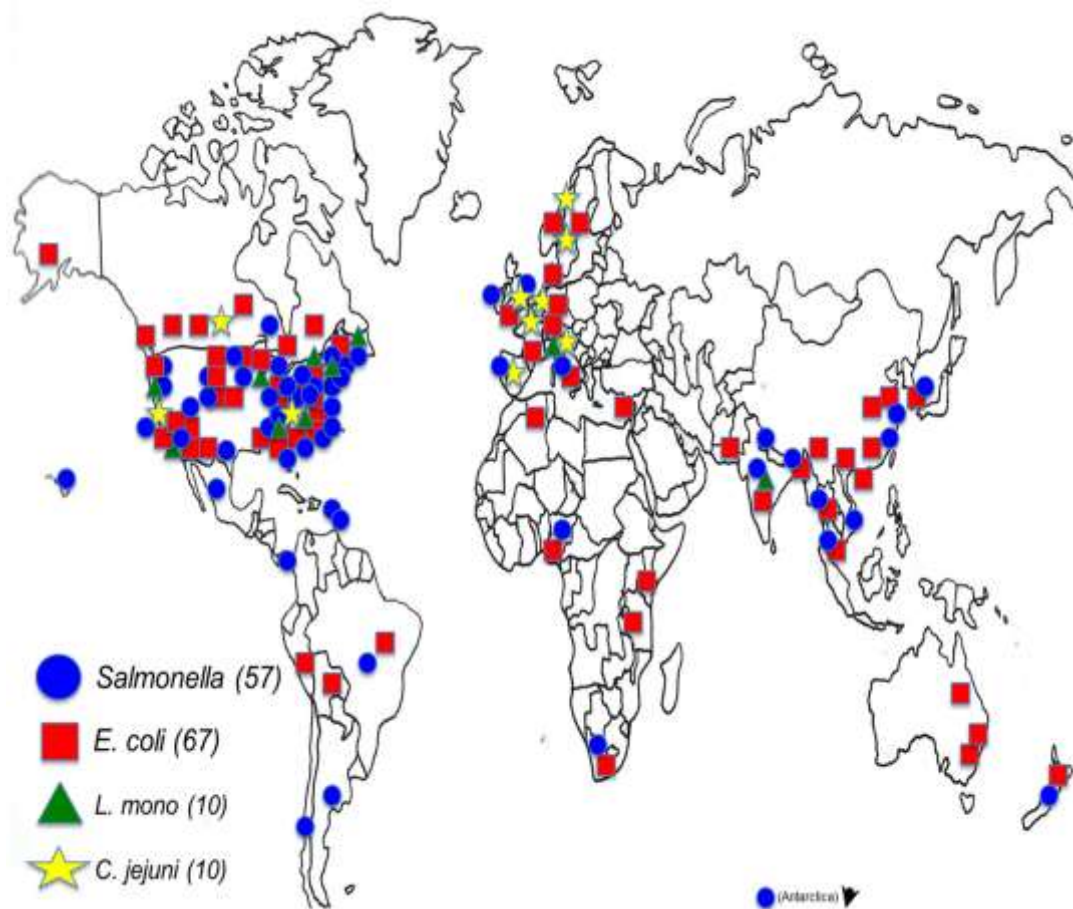
Figure 2.1: Global distributions of several foodborne bacterial species isolated from various water sources (Adopted from Bell et al., 2021)

Each separate shape represents one or more foodborne bacterial species from that given specific locale (country, province, or state). Shapes denote foodborne bacterial species as follows:circles, *Salmonella enterica*; squares, *E. coli/Shigella*; triangles, *L. monocytogenes*; and stars, *C. jejuni*. Parentheticals at the end of each species name denote the total number of pinpoints marked for the species indicated (Adopted from Bell *et al*., 2021).

2.4 Bacteriological Testing of Drinking Water

2.4.1 Microbiological Water Quality

The microbiological examination of water is used worldwide to monitor and control the quality and safety of various types of water. These include potable waters (water intended for drinking or use in food preparation), treated recreational waters (swimming pools, spa pools, and hydrotherapy pools), and untreated waters used for recreational purposes such as sea, river, and lake water. Microbiological indicators are bacteria shown to be associated with disease-causing organisms, but do not cause disease themselves. The three common microbiological indicators are: total coliform bacteria, fecal (thermotolerant) coliform bacteria, *E. coli.*

Surveillance of the bacteria quality of raw water is important not only in the assessment of the degree of pollution but also in the choice of the best source. Coliform organisms are suitable microbial indicator of drinking water quality because these organisms are easy to detect and enumerate in water. They are all Gram – negative, non- sporing rod – shaped bacteria capable of fermenting lactose at either 35°C or 37^0C with the production of acid, gas and aldehyde within 24 – 48 h (Cheesbrough, 2004). Currently, fecal coliform and *E. coli* are of great importance among bacterial indicators used in water quality definition and health risks (Giannoulis *et al.,* 2005).

Total coliform bacteria- Coliform organisms have long been recognized as a suitable microbial indicator of drinking-water quality, largely because they are easy to detect in sampled water. The term "coliform organisms" refers to Gram-negative, rod-shaped bacteria capable of growth in the presence of bile salts or other surface-active agents with similar growth-inhibiting properties and able to ferment lactose at 35–37°C with the production of acid, gas, and aldehyde within 24–48 hours. They are also oxidase-negative and non-spore-forming and display b-galactosidase activity, and include the following organisms *Escherichia*, *Citrobacter*, *Enterobacter*, and *Klebsiella.* Coliform group is not as specific indicator of fecal contamination as thermotolerant coliforms (WHO, 1997).

Thermotolerant coliform group is defined by the laboratory methods as gram negative rods, able to ferment lactose with gas production at $44^{O}C$ or $44.5^{O}C$ (Eijikmans test) indole positive. They include the genus *Escherichia* and to a lesser extent, occasional strains of *Enterobactor, Citrobactor,* and *Klebsiella* (Hurst *et al*., 2003). The *E. coli* colonize the gastrointestinal tract of humans and other mammal's shortly after birth and is considered part of our normal intestinal flora. Some types of *E. coli*, such as *E. coli* O157:H7 possess virulence factors and can cause diarrheal disease in humans, but most types of *E. coli* are harmless. In fresh feces it may attain concentrations of 10^9 per gram (WHO, 1997). The mammalian gut is the normal habitat for *E. coli*, and, unlike other coliform bacteria, they are not normally found in uncontaminated waters. This makes *E. coli* an ideal indicator for human health risk. WHO states, "The presence of *E. coli* in water indicates potentially dangerous contamination requiring immediate attention" (WHO, 1993). Due to its high prevalence and disease-causing properties, *E. coli* is a solid microbiological indicator. However, in some less contaminated environments, there is not enough *E. coli* present to calculate treatment process efficiency. When sampling for both human health risk and treatment efficiency a combined total coliform/fecal coliform bacteria test and *E. coli* test may need to be completed (CDC, 2010).

2.5 Microbiological identification of enteropathogens

2.5.1 Microscopy

Methods used by microbiologists to identify pathogens to the level of genus and species fall into three categories; phenotypic, genotypic and immunological. Phenotypic characteristic includes morphology, physiology and biochemistry. Microscopy was used to differentiate the various isolated pathogens according to their gram stain reaction and morphology characteristics.

2.5.2 Biochemical Tests

Biochemical identification relies on determining the presence of specific enzymes and to assess nutritional and metabolic activities of micro- organisms. Examples include tests for fermentation of sugars capacity to metabolize complex polymers

such as proteins, polysaccharides, production of gas, presence of enzymes such as catalase, oxidases, decarboxylases and sensitivity to antimicrobial drugs. Enzymes formed by some organisms either deaminate, dihydrolyze or decarboxylate amino acids and the products (ammonia) are alkaline and other color indicator changes to its original color: bromocresol purple turns purple and phenol red changes to red (Baron *et al.,* 1994).

2.6 Molecular Characterization of Bacteria

2.6.1 PCR Analysis

The PCR technique is based on the annealing of oligonucleotide to homologous sequences in temple DNA, followed by DNA polymerase catalyzed DNA synthesis primed by these oligonucleotide using dNTPs as substrate (Black, 1993). DNA amplification is brought about by repeated temperature cycling through melting of double stranded DNA, annealing of primers to single stranded DNA target sequences and extension of primers using target DNA as the template (Black, 1993). The application of PCR based techniques has a revolutionary impact on the diagnosis of infectious diseases. Many infectious agents that are missed by routine cultures, serological assays, DNA probes and southern blot hybridization can be detected by PCR.

PCR is fast, sensitive and capable of copying a single DNA sequence of viable and non-viable cell over a billion's times within 3-5 h (Forbes *et al*., 1998; Palmer *et al*., 1993). PCR can also be used to detect previously unknown organisms directly in environment or clinical specimens by using broad range primers. PCR primers have successfully developed for all categories of diarrhoeagenic *E. coli.* PCR can also be used both in diagnosing and typing *E. coli* strains. It has been determined that PCR detects significantly more ETEC infections than does the standard probe-based hybridization method (Caeiro *et al*., 1999). In diagnostic, PCR is commonly used for detecting different virulence associated genes of *E. coli*, such as toxin and adherence associated genes. PCR is also widely used in subtyping by doing virulence gene profiles for different diarrheagenic *E. coli* strain (Caeiro *et al*., 1999).

2.7 Antimicrobial Resistance

Many bacterial and parasitic diseases could until recently be treated with inexpensive antimicrobial agents, but treatment has recently been made expensive and less successful by the emergence and spread of drug resistant organisms. Although most diarrhea diseases are self – resolving and should not be treated with antimicrobial agents, invasive or protracted infections require chemotherapy and are typically managed empirically (CDC, 2007). Resistance, however equally compromises the management of acute respiratory infections, HIV, tuberculosis, malaria sexually transmitted diseases and diseases spread by the fecal - oral route, such as typhoid fever, cholera, dysentery and other diarrheal diseases, which are the focus of this perspective (Yeo and Livermore, 1994).

Conventional antimicrobial agents such as ampicillin, chloramphenicol, tetracycline and trimethoprim – sulfamethoxazole have been the drugs of choice in treatment of salmonellosis before 1980 (Cheng *et al.* 2004). However, multidrug resistance with rates of resistance to these antimicrobial agents of more than (50%) has been reported in many areas of world (Figure 1). Extended – spectrum cephalosporins and fluroquinolones have been suggested as alternative agents in the treatment of infections caused by Salmonella serotypes. However, since 1991, cases of infections caused by Salmonella serotypes resistant to extended – spectrum cephalosporins and fluoroquinolones have been increasingly reported (Cheng *et al.,* 2004; Mafu *et al.*, 2009).

According to Aibinu *et al.* (2004), *E. coli* is highly resistant to ampicillin, Amoxillin, tetracycline and trimethoprim – sulphathiazole. The widespread occurrence of drug resistance *E. coli* and other pathogens has necessitated the need for regular monitoring of antimicrobial susceptibility trends to provide the basis for developing rational prescription programs. Data from Gabon, Kenya, Nigeria, Senegal and Tanzania suggest that resistance among causative organisms of diarrhea, such as enterotoxigenic, enteropathogenic and enteroaggregative *E. coli* is high and appears to be rising (Presterl *et al.,* 2003). Unfortunately, most of these studies have not been highly published as opposed to studies in other countries (Figure 2.7).
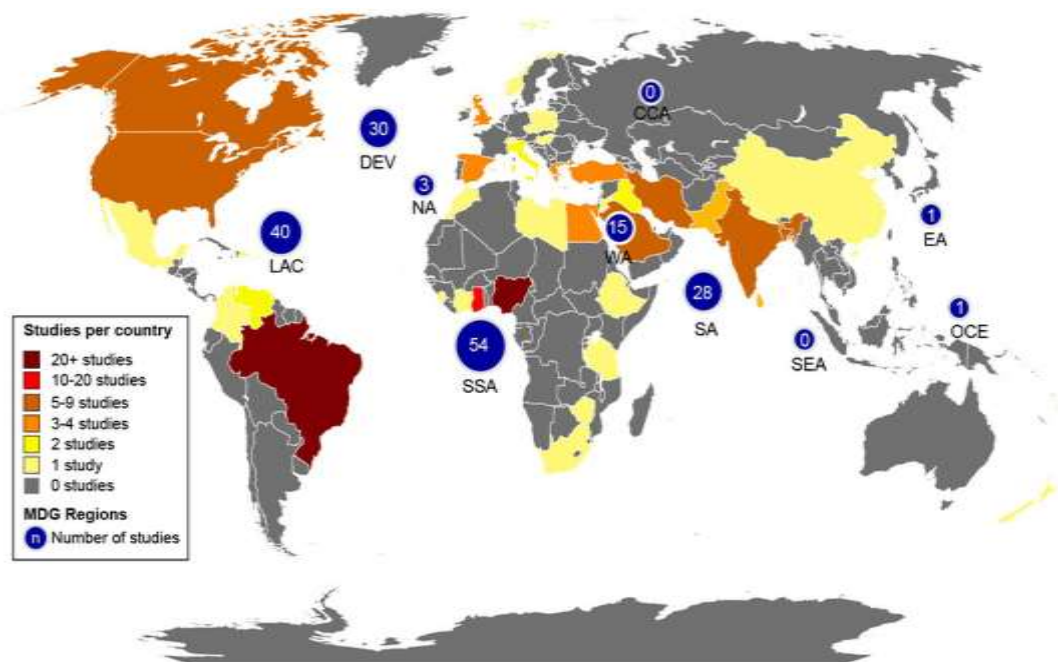
Figure 2.2: Global studies showing the TTC MDR global regions (Adopted from Williams et al., 2015)

2.8 Factors Associated with Ttc Contamination Drinking Water

The use of water sources such as shallow groundwaters (rives, springs, surface waters, wells and boreholes) for drinking and other domestic purposes is common among both low-income urban and rural communities in developing countries (Bojarczuk *et al*., 2018). The communities relying on these water sources are often poor and live in polluted environments with associated high health risks (Stoffel *et al*., 2016). Groundwater sources found in urban areas often show pronounced seasonal variations in microbiological quality, with significant deterioration during the onset of the wet season (Shah *et al*., 2012; Khan *et al*., 2018). The factors leading to contamination of groundwaters are often diverse, but are frequently ascribed to pollution by on-site sanitation facilities, such as pit latrines, as these represent an obvious source of fecal contamination (Bojarczuk *et al*., 2017). Sharing of these sources with animals whether domestic or wild is another source of microbial contamination of these water sources. Poor drainage, and burse sewage systems also contribute significantly to the microbiological contamination of groundwater sources (Ilyas *et al*., 2019). Similar studies have suggested that factors such as the presence

of uncapped wells and poor sanitary completion were as important as subsurface leaching of microbiological contaminants (Ramos *et al*., 2022). In most low-income urban communities, there are numerous sources of feces in the environment, particularly as sanitation coverage is often low (Ramos *et al*., 2022).

Household hygiene, and practices such as hand washing, household water storage type, cleanliness, water treatment, and human waste disposal were important for TTC household drinking water contamination (Too *et al*., 2016). A study conducted in India, showed that fecal contamination occurs principally during storage due to poor water handling (Eshcol *et al*., 2009). Households that have replaced the traditional drinking water containers with covered, narrow-mouthed pots with a tap outlet have significantly less contamination (Mazengia *et al*., 2002). A combination of special storage vessels with point-of-use treatment has been shown to be very effective (Rose *et al*., 2006). Hand washing initiatives and the introduction of point-of-use disinfection can reduce diarrheal incidence (Ejemot *et al*., 2008).

2.9 Research gaps

At the time of this study the following were significant gaps which were important to improve on the microbiological quality of both source and household drinking water. From literature review it was evident that both source and household drinking waters are often contaminated by microbiological pathogen often beyond the WHO recommended guidelines (Shah *et al*., 2012; Khan *et al*., 2018). This information is often vital for authorities to formulate policies to mitigate the consequence of these contamination such as diarrhea outbreak due to cholera and other bacterial pathogens. By determining the contamination levels of thermotolerant *E. coli* of source and household drinking water in Kericho district was significant by informing authorities on potential fecal contamination and usability of these waters with an intention of providing preventive strategies.

Studies have also shown that some of these bacteria contaminating waters are often pathogenic and resistant to serval important antibiotics (Vila *et al*., 2000). Such information was lacking at the study site. By determining the pathotypes, virulence and antibiotics profiles of the thermotolerant *E. coli* isolated from source and

household drinking water in the district aimed at showing the magnitude of the problem. Whether the isolated thermotolerant *E. coli* were pathogenic or part of normal flora. If pathogenic then the study aimed at informing the public and authorities on ways to prioritize water treatment strategies and formulating strategies of investigating the prevalence of household populations (if any) infected with these strains. This will help provide treatment and management strategies for those already infected.

From literature sanitary and hygiene are key to contamination of both source and household drinking water (Mazengia *et al*., 2002; Eshcol *et al*., 2009). Analyzing predisposing factors associated with thermotolerant *E. coli* contamination of water utilized by the study participants in Kericho district, was key in providing a preventative strategy.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Kericho District is one of the Districts in Rift-valley province. The larger District is composed of Kericho east and Kericho west. It lies between longitude 35^0 02' East and 35^0 40' West and between the equator and latitude 0^0 23' South. Kericho District occupies a total area of 1050.6 km^2. The population for the district based on the 2009 population and housing census is approximately 384,100 with a population growth rate of (3.6%) at the beginning of the plan period. The average density is 238.5 people per km^2. Ainamoi division is the most densely populated with 430.2 people per km^2. Soin division is the least densely populated with only 96.2 persons per km^2 (Kenya National Bureau of Statistics-KNBS, 2009).

The district is well drained with rivers, some of the main rivers include River Kipchorian whose source is from Western Mau Forest and it flows through Londiani, Kipkelion and Chilchila divisions to join river Nyando on the Kericho/Nyando District borders. Majority of the household within the township location have piped water supply while the other location relies on shallow wells, river, springs and rain water harvesting (Kenya National Bureau of Statistics-KNBS, 2019). The climate of the district is of highland equatorial type, which enables it to receive high and reliable rainfall that is distributed throughout the year. The district has two main rainy seasons, the long rains occur in March to June, and the main cash crop grown in the area is tea.

3.2 Study Population

The participants in this study were household heads or key responsible persons selected from Kericho East District, between December 2012 and February 2013. The household heads interviewed were the mother/guardian because of their responsibility in the management of drinking-water in the household.

3.2.1 Inclusion Criteria

1. Those who were willing and ready to consent to the study.
2. Residence of the selected areas in the district during the study period.
3. Collect or obtain their water from source (tap, rivers or wells) and stores in different household vessels for drinking.
4. Willing to allow part of their water samples be collected for the purposes of this study.
5. Willing to allocate at least 20 min for the face-to-face interviews.

3.2.2 Exclusion Criteria

1. Those unwilling to consent to the study.
2. Non-residence of the selected areas in the district during the study period.
3. Do not obtain or collect their water from source (tap, rivers or wells) and stores in different household vessels for drinking.
4. Unwilling to allow part of their water samples be collected for the purposes of this study.
5. Unwilling to allocate at least 20 min for the face-to-face interviews.

3.3 Study Design

This was a descriptive cross-sectional study that apart from collecting the water samples, participants were also underwent a face-to-face interview to identified factors associated with TTC contamination of drinking water.

3.4 Sample Size Determination

Sample size was determined using Cochran's formula of 1977,

Equation 1: Cochran's formula (1977)

$$n = \left(\frac{z}{m}\right)^2 p(1-p)$$

Where,

- z is the critical value based on the desired confidence level (e.g., z = 1.96 for (95%) confidence level);

- m is the margin of error or precision of the estimate in this case m=0.05.

- p is the estimated value of the proportion. In this study, p is the average of thermotolerant *E. coli* water contamination rates of sources (13%) (Tole, 1997) and in household vessels (0%) (Kimani-Murage and Ngindu, 2007) giving an average of (13%).

Thus $= 1.96^2 * 0.13 * 0.87 / 0.05^2 = 174$. For this study an additional 32 samples were included giving a total of 206 water samples that were collected. Half 103 water samples were from the sources and 103 from the household drinking vessels.

3.5 Sampling Technique

A two-stage sampling method was be used as follows; sampling of locations; a complete list of all the locations and their population was used (Kenya National Bureau of Statistics-KNBS, 2010). Kericho East has two divisions (Soin and Ainamoi) with 17 locations. Simple random sampling based on probability proportionate to size (PPS) was used to select the number of locations (12) and villages (17) in which sampling was done (appendix Va).

Sampling of households; Simple random sampling was used to pick the first household, for subsequent households, every fifth household was systematically selected until a total of 6 households were sampled per selected village (6 x 17=103 households) (appendix Vb). In urban areas, participating households were selected by the random-route method. Specifically, streets within neighborhoods were randomly selected, and every sixth house was enrolled in the study. For every household sampled, source water was also sampled.

3.6 Data Collection Tools

Water samples were collected aseptically into a clean container. Structured questionnaire was used to collect factors including socio-demographic, hygienic and environmental characteristics associated with contamination of these waters. The questionnaires were administered by trained local interviewers in both local language and English (appendix I). The questions were related to water-extraction patterns, type of water transport, water-treatment methods, hygiene and sanitation related issues. The person interviewed, were the mother/guardian in most cases, as they were responsible for the management of drinking-water in the household.

3.7 Water Sampling Procedures

3.7.1 Sample Collection and Transport

Water samples were collected aseptically as described by WHO, (1998). Water sample was collected from the source and household using sterile water sampling container. About 100mls of the water sample was collected and immediately analyzed for bacteriological qualities, and physical chemical properties (PH, temperature, turbidity, free chlorine) on site using delagua water testing kit. The cultured plates were then transported to Micro Hub Kericho- Walter Reed Project (WRP) for further bacteriological analysis.

3.7.2 Sampling of Drinking Water from the Source

Sampling from rivers, streams, or other surface waters, in areas where residents draw water from rivers, involved drawing water 30cm deep (1ft) below the surface. Sampling from wells and boreholes involved drawing water using a bucket and taking 100ml into sterile container. This was considered a more representative of what is actually being consumed by the household. Sampling from a tap was done directly into collecting containers (WHO, 1998). Different water sources encountered are shown in figure 3.1.

Figure 3.1: Water sources sampling points: A from borehole, B River and C vendors' domain

3.7.3 Sampling of Drinking Water in the Household

The head of household was requested to draw water from storage container using the container used for drawing water he/she normally uses to collect drinking water. About 100ml was put into the sterile container for further tests. Different household water storage containers encountered are shown in figure 3.2.

Figure 3.2: Household drinking water storage found at the field: A Tapped pot, B Open plastic container, C Modern water storage and D conventional pot

3.8 Laboratory Procedures

Water enteropathogens was determined using membrane filtration technique and cultured on heterotrophic plate count media (HPC) for their isolation using conventional methods. PH, chlorine and turbidity were also be determined on site based on figure 3.3.

```
┌──────────────────────┐                              ┌──────────────────────┐
│  Source (river, wells│                              │   Household water     │
│   springs tap &      │                              │ (stored for drinking) │
│     borehole)        │                              │                       │
└──────────────────────┘          ┌───────────────┐  └──────────────────────┘
               \                   │ Water samples │         /
                \                  └───────────────┘        /
                          ┌───────────────┘    └───────────┐
              ┌────────────────────┐      ┌──────────────────────────┐
              │ Physical properties│      │ Membrane lauryl sulphate  │
              │                    │      │ broth –TTC (44ºc)         │
              └────────────────────┘      └──────────────────────────┘
                        │                              │
              ┌────────────────────┐      ┌──────────────────────────┐
              │ PH, temperature    │      │   Polymerase              │
              │ and turbidity      │      │   chain reaction          │
              └────────────────────┘      └──────────────────────────┘
                                                       │
                                          ┌──────────────────────┐
                                          │  Toxigenic strains    │
                                          └──────────────────────┘
                                            │               │
                             ┌──────────────────┐  ┌──────────────────┐
                             │ Antimicrobial    │  │ Non toxigenic    │
                             │ susceptibility   │  │ strains          │
                             │ test             │  │                  │
                             └──────────────────┘  └──────────────────┘
```
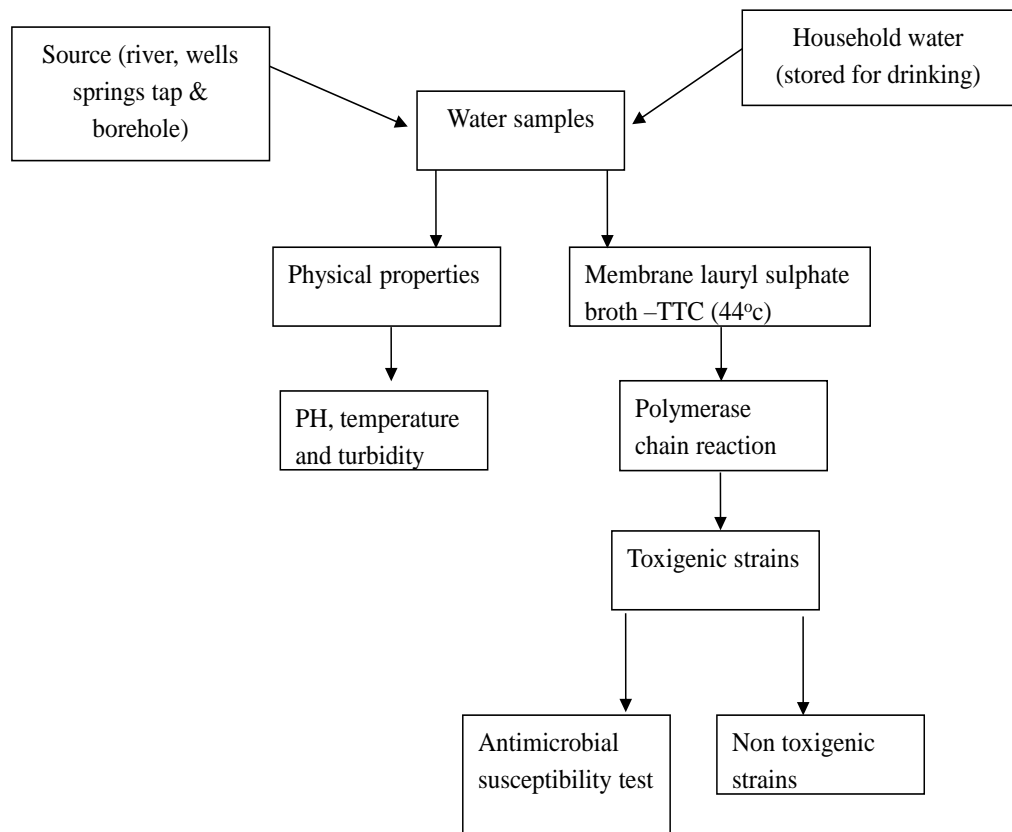
Figure 3.3: Water sample collection and laboratory testing chart (Cheesbrough, 2004)

3.8.1 Total Coliforms and Thermotolerant Coliform Isolation

A water sample was filtered through a 0.45μm pore size membrane filter, which was then incubated on Laurylsulphate agar (LSA) for 18-24 hours at $35 \pm 0.5°C$ or $37 \pm 0.5°C$ for total coliforms and 18-24 hours at $44 \pm 0.25°C$ or $44.5 \pm 0.25°C$ for

thermotolerant coliforms. To confirm thermotolerant coliforms and *E. coli* on membranes, whether incubated at 35, 37 or 44 °C, each colony (or a representative number of colonies) was sub cultured to a tube of lactose peptone water and a tube of tryptone water. Tubes were incubated at 44 °C for 24hs. Growth with the production of gas in the lactose peptone water confirmed the presence of thermotolerant coliforms. Confirmation of *E. coli* was done by the addition of 0.2-0.3 ml of Kovac's reagent to each tryptone water culture. Production of a red color indicated the synthesis of indole from tryptophan which confirmed the presence of *E. coli.* Figure 3.4 shows isolation and identification of TTC using culture and API strips.

Bacteriological water quality was determined by enumeration of the thermotolerant coliforms (TTC) using the membrane filtration technique. *E. coli* was the subjected to multiplex PCR for determination of virulent factors (appendix IV).

Figure 3.4: Culture and API results for the TTC detection

3.8.2 Identification Of *E. Coli* Pathotype

3.8.2.1 DNA Extraction

Unless otherwise specified, standard methods were used for plasmid isolation, genomic DNA isolation, and agarose electrophoresis DNA separation (Sambrook & Russell, 2001).

3.8.2.2 Multiplex PCR identification

E. coli clinical isolates were processed for isolation of genomic DNA as previously described (Gomez-Duarte *et al*., 2009). In brief, overnight liquid cultures were centrifuged, and the pellet was resuspended in water, boiled for 10 min, and centrifuged again. The supernatant containing a crude DNA extract was used as a DNA template on a multiplex PCR for identification of *E. coli* pathotypes, namely, EPEC, STEC, EAEC, ETEC, DAEC, and EIEC. The *E. coli* pathotype two-sample multiplex PCR was carried out using plasmid DNA with cloned targets as positive controls and plasmid DNA vectors as negative clones, as previously described (Gomez-Duarte *et al*., 2009). In brief, there was one plasmid clone for each gene target, while plasmid vectors pCR2.1 and pSC-A and *E. coli* flora genomic DNA were used as negative controls. The PCR 1 contained M1 primers for amplification of *eae*, *bfpA*, VT, and *aggR* genes for identification of STEC, EPEC, and EAEC pathotypes. The PCR 2 contained M2 primers for amplification of LT, ST, *daaE*, *ipaH*, and *virF* gene targets for identification of ETEC, DAEC, and EIEC pathotypes. One microliter of genomic DNA was mixed with 24μL of a premade mix containing primers at a 0.2μM final concentration and Platinum Blue PCR SuperMix polymerase (Invitrogen, Carlsbad, CA). The PCR program used for amplification consisted of 2min at 94°C denaturing temperature, followed by 40 cycles of 30sec at 92°C denaturing temperature, 30sec at 59°C annealing temperature, and 30sec at 72°C extension temperature. At the end of 40 cycles and a 5-min extension at 72°C, samples were separated onto a (2%) agarose ethidium bromide-stained gel, and DNA bands were visualized and recorded under ultraviolet light for further analysis.

Those *E. coli* isolates identified as STEC were further analyzed for determination of the type of verotoxin they carry. This was done by a standard single PCR using specific Shiga-like toxin 1 (VT1) (Vidal *et al*., 2005) and Shiga-like toxin 2 (VT2) (Nguyen *et al*., 2005) oligonucleotide primers) (Appendix IV). Figure 3.5 shows gel electrophoresis identification of pathogenic E. coli strains
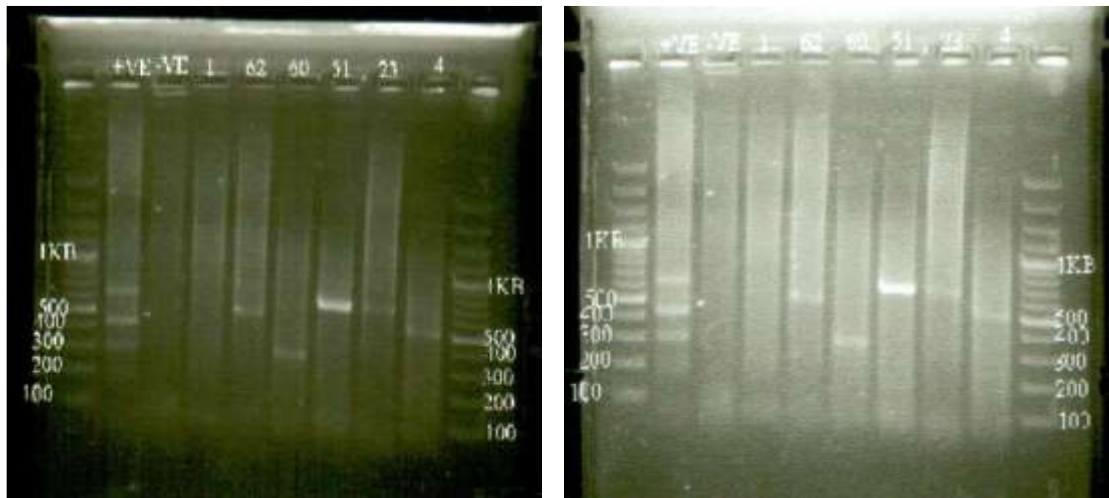
Figure 3.5: Gel electrophoresis identification of pathogenic strains of thermotolerant E. coli strains

3.8.3 antimicrobial susceptibility testing

The disc diffusion technique of Kirby-Bauer (Bauer *et al.,* 1966) was used to test the efficacy of antibiotics available at clinical laboratory settings. The *E. coli* colonies isolated on Mueller Hinton Agar were picked with sterile wire loop and transferred into sterile normal saline to obtain turbidity visually comparable to that of MacFarland 0.5 standard. The mixture was diluted ten times to a density of 10^5 cfu/ml. A sterile cotton swab was dipped into the inoculum suspension while rotating the swab firmly on the inside wall of the tube to remove excess fluid. The dried of Mueller-Hinton Agar plate that had been brought to room temperature was inoculated by streaking the three times over the entire agar surface, rotating the plate at an approximate angle of 60 degrees to ensure an even distribution of the inoculum. The surface of the agar was allowed to dry before placing antibiotic discs gently and pressing to stick to agar surface by use of sterile forceps. The inverted plates were then incubated at 37^0C overnight and the result interpreted according to National Committee for Clinical Laboratory Standards (NCCLS 2003). The panel of antimicrobials were chosen because of importance in the treatment of Gram-negative bacterial infection, widespread availability and use for treatment of enteric fever in Kenya. The antibiotics included Ampicillin, Nalidixic acid, Chloramphenicol, Tetracycline, Cefotaxime, Cotrimoxazole, Ceftazidime and Ciprofloxacin. The zones

inhibition was then interpreted as being either R (resistant), I (intermediate) or S (sensitive) in accordance with the MIC interpretation scheme provided by the manufacturer and performance standards for antimicrobial susceptibility testing (CLSI, 2010).

3.8.4 Quality Assurance

Standard operating procedures of KEMRI/ Micro Hub Kericho- Walter Reed Project (WRP) was adhered to, especially those pertaining to labelling of containers, specimen collection, transportation, analysis and posting of results. Sample collection was done aseptically in sterile tubes and packed in sterile cool boxes and transported to KEMRI/WRP bacteriology laboratory for processing and analysis. All reagents were prepared in accordance with standard operating procedures (SOPs) used at KEMRI/WRP cytology laboratory. Equipment operation were done according to manufacturer's instructions. Tested micro-organisms were confirmed by the supervising microbiologist before results were signed out to the participant's records. All positive samples and every fifth normal waters were re-screened by an independent microbiologist.

3.9 Data Management

All subjects were assigned a subject identification number (SID). All data entered into the study databases were de-identified and only associated with a SID in password protected files. We maintained a double entry system for the data. All paper research records were kept in a password protected; locked filing cabinet located in a restricted-access room at the research center in KEMRI Kericho. The biological samples were stored in a freezer with restricted access.

3.10 Data Analysis

Data were presented in frequencies and percentages using tables and charts. Chi-square was used to test for significance among qualitative data such as distribution of contamination in drinking water quality (*E. coli* counts >10cfu/100mls) between source and household vessels. The overall and type-specific pathogenic

thermotolerant *E. coli* prevalence was determined from the source and household drinking water. In bivariate analyses, odds ratios (OR) and (95%) confidence intervals (CI) for the association between pathogenic thermotolerant *E. coli* contaminating water and socio-demographic, hygienic and environmental characteristics were calculated using Poisson regression. In multivariate analyses, a manual backward elimination approach was used to reach the most parsimonious model including factors that were associated with contamination with pathogenic thermotolerant *E. coli* at the significance level of $p \leq 0.05$. All statistical analyses were performed using Epi info software version 5.3.1.

3.11 Ethical Considerations

The study referred to as SSC No. 2579 was conducted according to the Declaration of Helsinki and International Conference on Harmonization Guideline on Good Clinical Practice (ICH-GCP). The protocol and informed consent form were reviewed and approved by the KEMRI CSC, SSC and ERC prior to any protocol-related procedures (e.g., advertising or recruitment efforts) being conducted. The investigator informed the ERC as to the progress of the study on a regular basis per the ERC requirements, but at minimum once a year. Written informed consent were obtained from each participant/participant's legally authorized representative prior to any protocol-specified procedures being conducted. To maintain confidentiality, initials and coded numbers were used to identify the participants' laboratory specimens, source documents, CRFs, and study reports. All study records were maintained in a secured location. Participant information were not obtained or released without written permission from the participant/participant's legally authorized representative except as necessary for monitoring of the study.

Participation in this study was completely voluntary and the participants were free to withdraw from the study even after accepting to participate. The interviewees were also informed that the study did not have a direct benefit to an individual but it was meant to help in assessing bacteriological quality of drinking water from source to household and factors associated with hygiene-sanitation practices of the consumers

in order to initiate prevention strategies and ensure reduction in morbidity and mortality from diarrheal diseases.

CHAPTER FOUR

RESULTS

4.1 Characteristics of study population

A total of 103 households consented and were recruited in this study seeking to determine total and fecal TTC contamination of source and household drinking water in Kericho District. Majority (30.1%) of the households were located within the Kericho Township. This was followed by (11.7%) in Chepkoinik and the least (4.9%) were situated in Kapcherop locality (Figure 4.1.1). The proportion of household located in township was significantly more than the rest of the locations ($\chi2 = 67.913$; df = 11; P = 0.001).
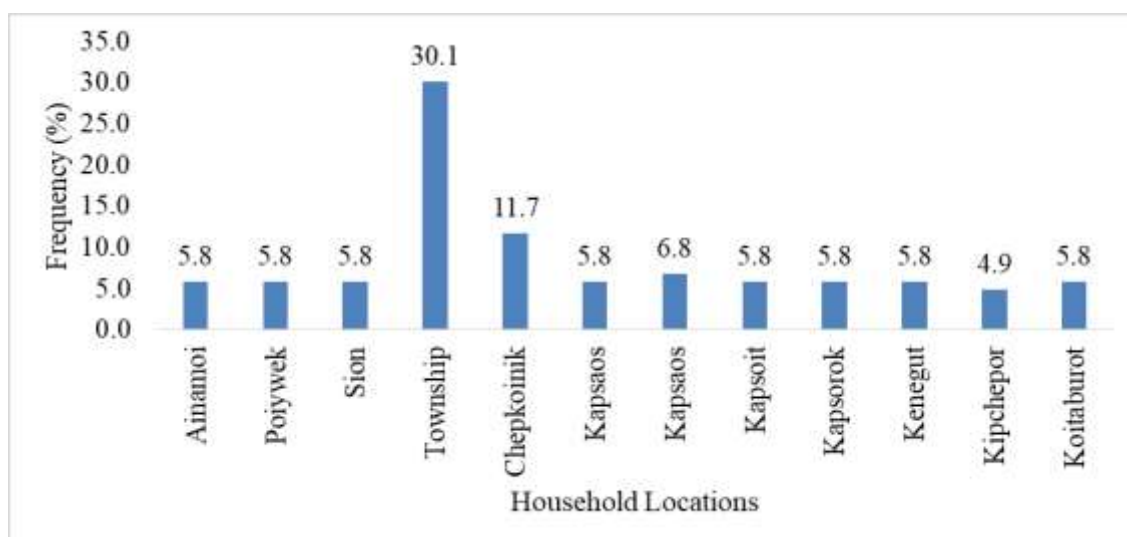
Figure 4.1: Location of the study household

Table 4.1 shows additional socio-demographic characteristics of study population. Majority 68.9% of the households were in the rural locality ($\chi2 = 14.77$; df = 1; P = 0.001), almost all of the interviewed respondents (95.1%) were female ($\chi2 = 83.971$; df = 1; P = 0.001).

The mean age of the 103 respondents was 21.59 years (range 18–29 years) and the majority (42.7%) were aged 21 to 30 years followed by (25%) aged 31 to 40 years. There were about 7.8% of the respondents aged less than 20 years ($\chi 2 = 41.223$; df = 4; P = 0.001). Most of them 57.3% had primary level education followed by (24.3%) and (10.7%) with secondary and tertiary level education respectively. There about 7.8% of them who had no formal education ($\chi 2 = 63.641$; df = 3; P = 0.001). There were two major occupational peaks of the respondents (27.2%) farmers and (24.3%) housewives. Others (17.5%) were in business, (16.5%) employed in various sectors and (14.6%) had no formal occupation or were students. The distribution of respondent's occupation was not significant ($\chi 2 = 6.08$; df = 4; P = 0.193). There was almost equal distribution between households which had children below five years (54.4%) verses those households which did not have children this age (45.6%) ($\chi 2 = 0.79$; df = 1; P = 0.375).

Table 4.1: Study population Socio-demographic characteristics

Social demographic characteristics	Sample size		Pearson X^2	Df	P-Value
	No	%			
Locality					
Rural	71	68.9	14.77	1	0.001
Urban	32	31.1			
Gender					
Female	98	95.1	83.971	1	0.001
Male	5	4.9			
Age group					
<20	8	7.8			
21-30	44	42.7			
31-40	25	24.3	41.223	4	0.001
41-50	10	9.7			
>51	16	15.5			
Education level					
Primary	59	57.3			
Secondary	25	24.3			
Tertiary	11	10.7	63.641	3.0	0.001
Non formal	8	7.8			
Occupation					
Business	18	17.5			
Employee/labourer	17	16.5	6.08	4	0.193
Famer	28	27.2			
Housewife	25	24.3			
Student/Unemployed	15	14.6			
Stay with child below 5 Years					
Yes	56	54.4	0.79	1	0.375
No	47	45.6			

No-Number, %- Percentage, X2- Chi Square, P-Value- Level of significance

The main source for drinking water for the respondents was river (36.9%) followed by 33% who had piped or municipal supply. Others included (18.4%) spring and (11.7%) from rain water or roof catchment (Figure 4.2) ($\chi2 = 17.58$; df = 3; P = 0.001).
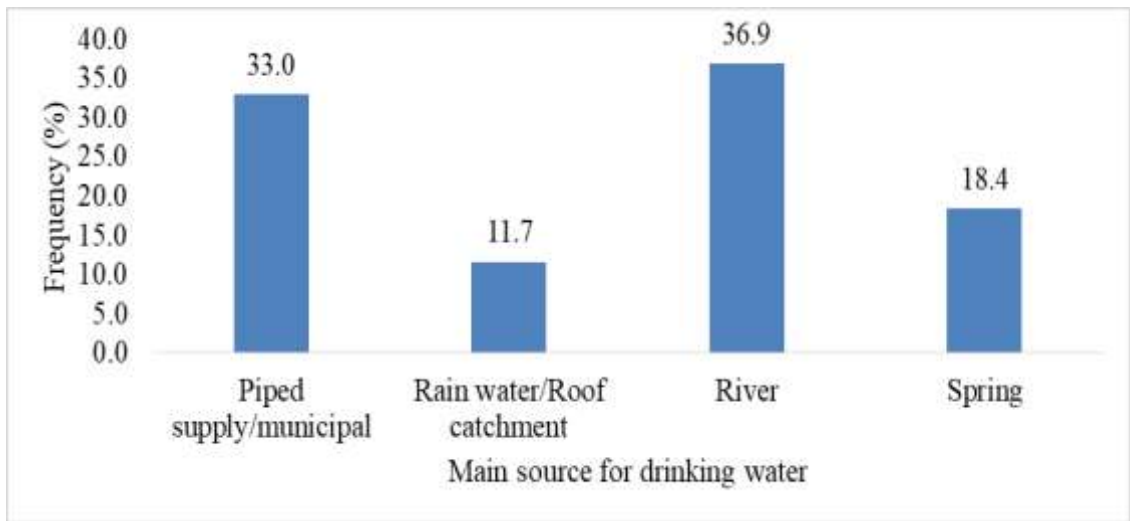
Figure 4.2: The respondent's main source of drinking water

Majority of the households (43.7%) had drinking water source within their premises. About 24.3% water source was less than 15min walk away. Others included (18.4%) who walked for 15 to 30 min while (13.6%) had to walk for 30min to one hour to water source as shown in figure 4.3. The time taken to water source was statistically significant ($\chi2 = 21.544$; df = 3; P = 0.001).
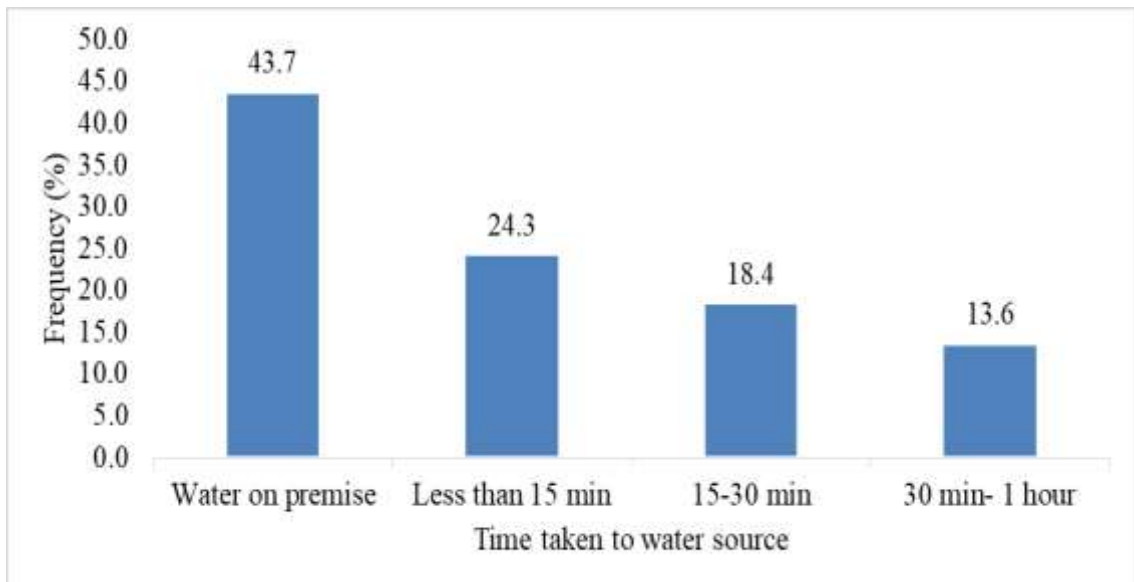
Figure 4.3: Time taken to drinking water source

Table 4.2 shows selected water treatment and storage practices of the study population. Majority (92.2%) considered their drinking water safe ($\chi 2 = 161.32$; df = 2; P = 0.001). Nearly all of them (91.3%) did not treat their drinking water; for about 10% of those who treated drinking water, (2.9%) used filtration method and (2.9%) boiled the water ($\chi 2 = 241.272$; df = 3; P = 0.001). For those who did not treat their water, majority of them (81.6%) stated that their water was clean while 5.8% lacked knowledge and (1.9%) said it was time consuming ($\chi 2 = 177.272$; df = 2; P = 0.001).

Most of the households (64.1%) stored the drinking water for more than two days, about 20.4% stored water for one day while (15.5%) stored water for two days ($\chi 2 = 44.175$; df = 2; P = 0.001). The majority of them (65%) covered their stored water ($\chi 2 = 9.33$; df = 1; P = 0.002).

Most of the households (83.5%) drew drinking water by dipping the cup into the water storage container ($\chi 2 = 46.223$; df = 1; P = 0.001). Nearly half of them had contact with drinking water during drawing ($\chi 2 = 2.184$; df = 1; P = 0.139). The commonest place (50.5%) where the water drawing cup was stored was on the actual water storage cover. Other places included (22.3%) tables or shelves and only (2.9%) stored this cup inside the container or on the floor or wall ($\chi 2 = 47.175$; df = 3; P = 0.001).

Majority 60.2 used clay pot for water storage while other (39.8%) used plastic containers ($\chi 2 = 4.282$; df = 1; P = 0.001). Majority of whom (64.1%) never washed this container and only 2.9% washing the container regularly and (33%) washed the container irregularly ($\chi 2 = 8.165$; df = 1; P = 0.004).

Table 4.2: Water treatment and storage characteristics

Water storage and treatment	Sample size No	%	Pearson X^2	df	P-Value
Consider drinking water source safe					
Yes	95	92.2			
No	1	1.0	161.32	2	0.001
Don't know	7	6.8			
How do you treat drinkig water					
Boil	3	2.9			
Filtration	4	3.9	241.272	3	0.00
Water guard	2	1.9			
Do not treat	94	91.3			
Reasons for not traeting water					
Lack of knowledge	6	5.8			
Water is clean	84	81.6	177.272	2	0.001
Time consuming and costly	2	1.9			
Not applicable	11	10.7			
Water storage period					
One day	21	20.4			
Two days	16	15.5	44.175	2	0.001
More than two days	66	64.1			
Cover water storage container					
Yes	67	65.0	9.33	1	0.002
No	36	35.0			
Drinking water drawing from container					
Dip into container	86	83.5	46.223	1	0.001
Pour directly from container/use tap	17	16.5			
Hand contact with drinking water					
Yes	44	42.7	2.184	1	0.139
No	59	57.3			
Storage of water drawing container					
Table and shelves	23	22.3			
Water storage cover	52	50.5	47.175	3	0.001
Inside container/ Floor/wall	3	2.9			
Not stated	25	24.3			
Type of water container					
Clay pot	62	60.2	4.282	1	0.039
Plastic	41	39.8			
Washing of water storage container					
Regularly	3	2.9			
Iregularly	34	33.0	8.165	1	0.004
Never	66	64.1			

No-Number, %- Percentage, X2- Chi Square, P-Value- Level of significance

4.2 Human waste disposal practices

Nearly half of them (49.5%) used pit latrine without slab (open pit) for their toilet facility. Others included (24.3%) who used shared toilet facility, (14.6%) who went to the filed or bush for long call and only (5.8%) had either piped sewer system/septic tank/pit latrine or ventilated improved pit latrine each as shown in Figure 4.4. The household type of toilet facility was statistically significant ($\chi 2$ = 68.019; df = 4; P = 0.001).
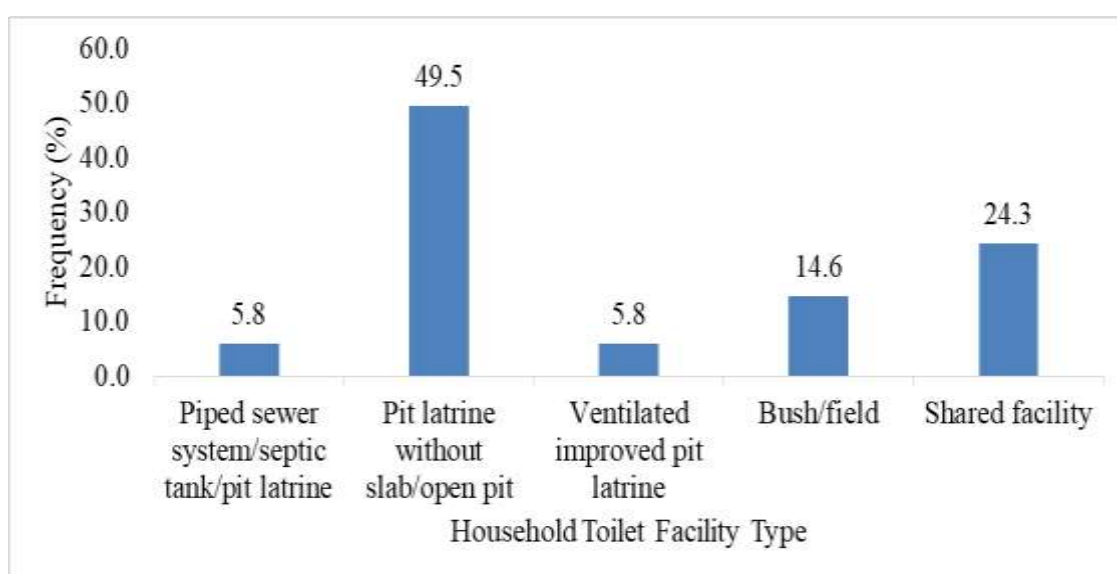
Figure 4.4: Types of Household Toilet Facilities

Evaluating the cleanliness of the household toilet facility for those who had showed most of them (32%) were moderately clean with flies but no visible fecal matter. About (15.5%) had clean toilets (no flies and no visible human waste) while (13.6%) had dirty toilets with flies and visible human waste (Figure 4.5) ($\chi 2$ = 18.981; df = 3; P = 0.001).
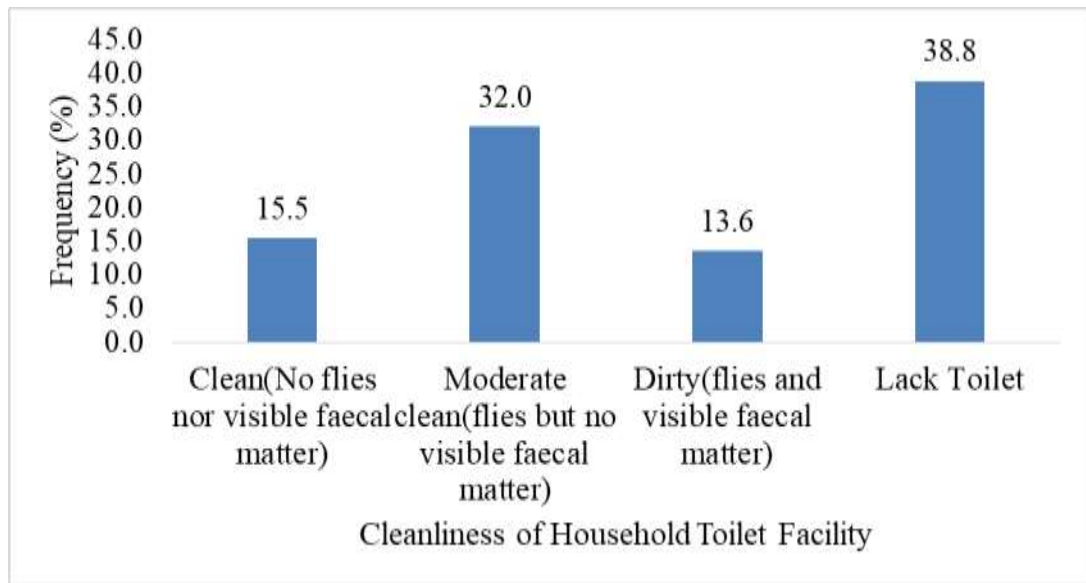
Figure 4.5: Cleanliness of Household Toilet Facility

For the (53.4%) households in the study with children, most of them (31.1%) disposed the child's waste in the pit latrine, (18.4%) rinsed the waste into drain yet other (3.9%) disposed the child's waste into garbage as shown in Figure 4.6. This child waste habit was statistically significant ($\chi2 = 40.883$; df = 3; P = 0.001).
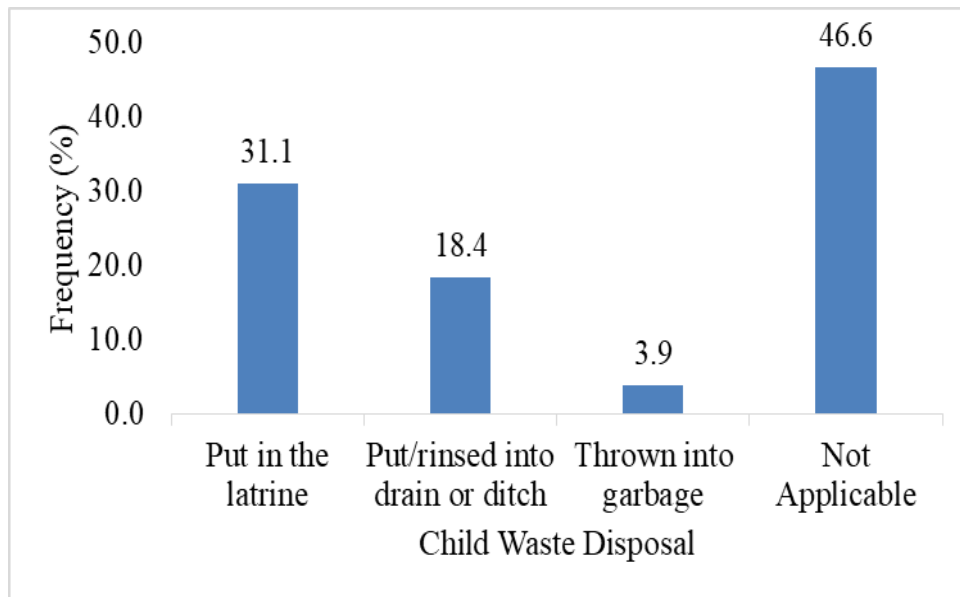
Figure 4.6: Household Child's waste disposal habit

Table 4.3 shows the household hygiene and hand washing practices. Nearly half of them (59.2%) did not wash their hands before drawing water ($\chi2 = 3.505$; df = 1; P = 0.061). Majority (96.1%) of them rinsed the water drawing utensils ($\chi2 = 87.621$; df = 1; P = 0.001), 88.3% were aware of waterborne diseases ($\chi2 = 60.592$; df = 1; P = 0.001), (70.9%) had not suffered from diarrhea, vomiting and fever in the past 3 months ($\chi2 = 17.951$; df = 1; P = 0.001) while (22.3%) of those who had diarrhea, vomiting or fever was not due to water related infection ($\chi2 = 76.971$; df = 2; P = 0.001).

Table 4.3: The Household hygiene and hand washing practices

Hygiene and hand washing practices	Sample size		Pearson X^2	Df	P- value
	No	**%**			
Wash hands before drawing water					
Yes	42	40.8	3.505	1	0.061
No	61	59.2			
Rinse water drawing (drinking) utensils					
Yes	99	96.1	87.621	1	0.001
No	4	3.9			
Aware of waterborne disease					
Yes	91	88.3	60.592	1	0.001
No	12	11.7			
Suffered from diarreaho, vomiting and fever					
Yes	30	29.1	17.951	1	0.001
No	73	70.9			
Water related infection					
Yes	5	4.9			
No	23	22.3	76.971	1	0.001
Not applicable	75	72.8			

No-Number, %- Percentage, X2- Chi Square, P-Value- Level of significance

4.3 Source and household water coliform contaminations

Table 4.4 shows the source and household both total and thermotolerant coliforms levels. The mean and standard deviation (SD) of total coliform counts in the water sources for the study population in Kericho District was 529.85 (SD 1237.2 cfu/100ml) and range 5675 (0 – 5675 cfu/100ml). About (48.5%) of the household had their water sources total coliforms < 10cfu/100ml and (51.5%) had their water source with >10cfu/100ml and was considered contaminated. This distribution was not statistically significant ($\chi2 = 0.087$; df = 1; P = 0.769).

The mean (SD) of total coliform counts in the drinking water within households of the study population in Kericho District was 441.7 (SD 807.3 cfu/100ml) with a

range of 5560 (0 – 5560 cfu/100ml). Majority of the household drinking water 83.5 had total coliforms >10cfu/100ml levels indicating contamination. This distribution was statistically significant ($\chi2$ = 31.544; df = 1; P = 0.001). The mean (SD) of the thermotolerant coliform (TTC) counts in the water sources for the study population in Kericho District was 263.63 (SD 831.45 cfu/100ml) and range 3540 (0 – 3540 cfu/100ml). Majority (77.7%) of the household had their water sources total coliforms < 10cfu/100ml and only (22.3%) had contamination with TTC (>10cfu/100ml) ($\chi2$ = 31.544; df = 1; P = 0.001).

The mean (SD) of thermotolerant coliform counts in households drinking water of the study population in Kericho District was 159.05 (SD 557.05 cfu/100ml) with a range of 3800 (0 – 3800 cfu/100ml). Slightly over (53.4%) of the household drinking water TTC count <10cfu/100ml levels indicating no contamination and only (46.6%) had contaminated TTC levels of >10cfu/100ml ($\chi2$ = 0.476; df = 1; P = 0.49).

Table 4.4: Total and Thermotolerant coliforms in source and household drinking water

Laboratory parametors		Sample size		X2	Df	P-Value
		No	%			
Water source to coliforms						
Mean (=SD)	529.85(1237.2					
Median	98					
Range	5675(0-5675					
≤ 10cfu/100ml		50	48.5	0.087	1	0.768
>10cfu/100ml		53	51.5			
Water source thermotolerant coliforms						
Mean (±SD)	263.63(831.45					
Median	0					
Range	3540(0-3540)					
≤10cfu/100ml		80	77.7	31.544	1	0.001
>10cfu/100ml		23	22.3			
Household total coliforms						
Mean(±SD)	441.74(807.3)					
	266					
	5560(0-5560)					
≤10cfu/100ml		17	16.5	46.233	1	0.001
>10cfu/100ml		86	83.5			
Household thermotolerant coliforms						
Mean (±SD)	159.05(557.05)(807.3)					
Median	0					
Range	3800(0-3800)					
≤10cfu/100ml		55	53.4	0.476	1	0.49
>10cfu/100ml		48	46.6			

No-Number, %- Percentage, X2- Chi Square, P-Value- Level of significance

4.4 Types of coliforms isolated in household drinking water

In all 48 (46.6%) households with TTC, each had thermotolerant *E. coli* as well. Among the 48 thermotolerant *E. coli*, 5 (10.4%) were pathogenic *E. coli*: 2/5 (40%) EAEC, 2/5 (40%) ETEC, and 1/5 (20%) EPEC. There were 35 other types of TTCs isolated from household drinking water. There were other nine different types of thermotolerant coliforms isolated from household drinking water. These included 8/35 (22.8%) *Serratia*, 7/35 (20%) *Enterobacter*, 5/35 (14.3%) *Klebsiella*, 5/35 (14.3%) *Moraxella*, 4/35 (11.4%) *Pseudomonas*, 2/35 (5.7%) *Shigella*, 2/35 (5.7%) *Acinetobacter*, 1/35 (2.9%) *Aeromonas*, and 1/35 (2.9%) Yersinia (Figure 4.7).
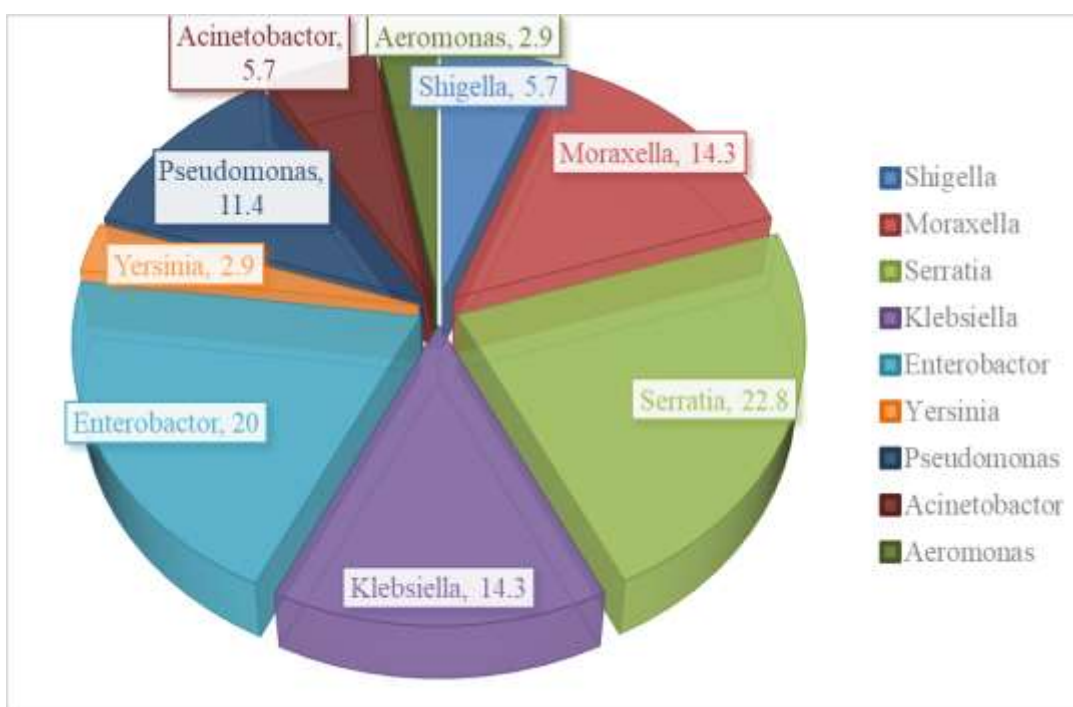
Figure 4.7: Types of Thermotolerant coliforms isolated from household drinking water

4.5 Antibiotics resistant profile for pathogenic E. Coli

Among the 48 TTC isolated from household drinking water, five (10.4%) of these 48 TTCs were toxigenic *E. coli* including 2/5 (40%) Enteroaggregative *E. coli* (EAEC), 2/5 (40%) Enterotoxigenic *E. coli* (ETEC) and 1/5 (20%) Enteropathogenic *E. coli* (EPEC). These toxigenic *E. coli* were subjected to antimicrobial susceptibility patterns and were found susceptible to six antibiotics (Cefotetan, Ciprofloxacin, Levofloxacin, Moxifloxacin, Piperacillin/tazobactam, and Ticarcillin/clavulanic acid). One of the Enteroaggregative *E. coli* (EAEC) was resistant to 15 single antibiotics including (Amikacin, Azitreonam, Cefacolin, Cefepime, Cefotaxime, Cefoxitin, Ceftazidime, CeftRiaxone, CefuRoxime, Ertapenem, Gentamicin, Imipenem, Meropenem, Tobramycin and Amoxicillin/clavulanic acid). Two Enteroaggregative *E. coli* (EAEC) and one Enterotoxigenic *E. coli* (ETEC) isolates were resistant to Cephalothin. On the other hand, all the toxigenic E. coli were multi-drug resistant to the following 4 different antibiotics (Sulfamethoxazole/ trimethoprim, Ampicillin, Tetracycline and Ampicillin/sulbactam) shown in Table 4.5.

Table 4.5: Drug susceptibility patterns for the toxigenic E. coli

| DRUG TYPE | TOXIGENIC E. COLI STRAIN | | | | | |
	EAEC	ETEC	ETEC	EPEC	EAEC	Resistant(%)
Cofotetan	S	S	S	S	S	S
Ciprofloxacin	S	S	S	S	S	S
Moxifloxacin	S	S	S	S	S	S
Piperacillin/tazobactam	S	S	S	S	S	S
Ticarcillin/clavulanic acid	S	S	S	S	S	S
Amikacin	S	S	S	S	R	1(20)
Azitreonam	S	S	S	S	R	1(20)
Cefcolin	S	S	S	S	R	1(20)
Cafepime	S	S	S	S	R	1(20)
Cefatixime	S	S	S	S	R	1(20)
Cefoxitin	S	S	S	S	R	1(20)
Ceftazidime	S	S	S	S	R	1(20)
CeftRiaxone	S	S	S	S	R	1(20)
CefuRixime	S	S	S	S	R	1(20)
Ertapenem	S	S	S	S	R	1(20)
Gentamicin	S	S	S	S	R	1(20)
Imipenem	S	S	S	S	R	1(20)
Meropenem	S	S	S	S	R	1(20)
Tobramycin	S	S	S	S	R	1(20)
Amocicillin/Clavulanic acid	S	S	R	S	R	1(20)
Cephalothin	R	S	R	R	R	3(60)
Sulfamethoxazole/trimethoprim	R	R	R	R	R	5(100)
Ampicilin	R	R	R	R	R	5(100)
Tetracycline	R	R	R	R	R	5(100)
Ampicillin/sulbactam	R	R	R	R	R	5(100)

R-Resistant, S- Susceptible, ETEC- Enterotoxigenic; EPEC-Enteropathogenic and EAEC-enteroaggregative E.coli

4.6 Socio-demographic factors associated with TTC drinking water contamination

In this section we evaluated what household factors were associated with the water contamination with TTC (> 10cfu/100ml). In this case 48/103 (46.6%) of the household drinking water had TTC levels of >10cfu/100ml indicating contamination. In the bivariate analyses, households which were located in the rural set up were more likely to have household drinking water contaminated with Thermo-tolerant coliforms than those households located in the urban areas (OR 2.01, 95% CI 1.09 to 4.12). Further, households whose main source of drinking water was from piped supply or from municipal were less likely to have household drinking water

contaminated with TTC than those households whose source of water was from river/spring (OR 0.38, 95% CI 0.16 to 0.91). In multivariate analyses none of the socio-demographic characteristics (household locality, gender, age, education level, occupation, type of mains source of drinking water and the distance to the main water source) was found to influence TTC water contamination (Table 4.6).

Table 4.6: Socio-demographic factors associated with Household TTC water contamination

Social demographic characteristics	Sample size	Drinking water TTC Contamination No	%	P-Value	Bivariate OR(95%CI)	Multivate OR(95%CI)
Locality						
Rural	71	39	54.9	0.06	2.01(1.09-4.12)	1.13-(0.12-10.37)
Urban	32	32	31.1	Referent	Referent	Referent
Gender						
Female	98	44	44.9	0.528	0.81(0.41-1.57)	0.85(0.36-1.99)
Male	5	5	4.9	Referent	Referent	Referent
Age group						
<20	8	5	62.5	0.816	1.08(0.55-2.12)	1.13(0.47-2.73)
21-30	44	20	45.5	0.898	0.96(0.61-1.55)	1.08(0.54-2.13)
31-40	25	11	44	0.877	0.96(0.57-1.61)	1.01(0.51-1.96)
41-50	10	4	40	0.837	0.933(0.48-1.81)	0.95(0.45-2.02)
>51	16	16	15.5	Referent	Referent	Referent
Education level						
Primary	59	29	49.2	0.985	0.99(0.544-1.82)	1.23(0.52-2.91)
Secondary	25	11	44	0.903	0.96(0.49-1.84)	1.13(0.44-2.91)
Tertiary	11	4	36.4	0.806	0.91(0.42-1.94)	1.33(0.44-4.06)
Non formal	8	8	7.8	Referent	Referent	Referent
Occupation						
Business	18	6	33.3	1.00	1(0.55-1.81)	1.1(0.52-2.39)
Employee/labourer	17	11	64.7	0.470	1.23(0.69-2.19)	1.3(0.59-2.87)
Famer	28	9	32.1	0.974	0.99(0.57-1.71)	1.1(0.47-2.59)
Housewife	25	17	68	0.395	1.26(0.74-2.14)	1.29(0.59-2.82)
Student/Unemployed	15	15	14.6	Referent	Referent	Referent
Stay with child below 5 Years						
Yes	56	20	35.7	0.320	0.85(0.62-1.17)	NS
No	47	28	59.5	Referent	Referent	Referent
Main drinking water source						
Piped supply/ Municipal	34	9	26.5	0.03	0.38(0,16-0.91)	0.78(0.33-1.87)
Rain water/Roof catchment	12	6	50.0	0.525	0.73(0.52-1.92)	0.89(0.39-2.02)
River	38	21	55.3	0.545	0.81(0.41-1.61)	0.98(0.58-1.63)
Spring	19	12	63.2	Referent	Referent	Referent
Time to water source						
Water on premise	45	15	33.3	0.510	0.84(0.52-1.38)	1.03(0.52-2.02)
Less than 15 min	25	15	60.0	0.946	1.01(0.61-1.71)	1.09(0.61-1.96)
15- 30min	19	10	52.6	0.918	0.97(0.55-1.69)	0.92(0.51-1.65)
30min -1 hour	14	8	57.1	Referent	Referent	Referent

No- number, %-percentage, TTC-thermotolerant coliform; OR-Odds ratio, CI-confidence interval, NS-Not significant

46

Table 4.7 shows water storage and treatment characteristics associated with household TTC water contamination. In the bivariate analyses, households which had hand contact with drinking water during water withdrawal were more likely to have household drinking water contaminated with TTC than those households which had no water contact during water withdrawal (OR 1.11, 95% CI 1.11 to 3.39). However, households that washed the water storage containers were less likely to have household drinking water contaminated with TTC than those households which did not wash the water storage container (OR 0.58, 95% CI 0.31 to 0.99). In multivariate analyses none of the storage and treatment practices were found associated with TTC water contamination.

Table 4.7: Water storage and treatment factors associated with Household TTC water contamination

Water storage and treatment	Sample size	Drnking water TTC Contamination		P -value	Bivariate OR (95% CI)	Multivate OR (95%CI)
		No	%			
Consider drinking water source safe						
Yes	95	46	48.4	0.472	1.29(0.64-2.64)	
No	1	1	100.0	0.479	1.75(0.37-8.24)	1.09(0.13-8.58)
Don't know	7	1	14.3	Referent	Referent	Referent
How do you treat drinkig water						
Boil	3	2	66.7	0.943	1.04(0.34-3.18)	0.95(0.22-4.1)
Filtration	4	2	66.7	0.658	0.62(0.07-4.99)	0.61(0.07-5.09)
Water guard	2	0	0	0.798	0.91(0.44-1.85)	1.11(0.32-3.92)
Do not treat	94	43	45.7	Referent	Referent	Referent
Reasons for not traeting water						
Lack of knowledge	6	3	50	0.759	0.88(0.41-1.94)	0.93(0.22-4.1)
Water is clean	84	39	46.4	0.830	0.94(0.54-1.63)	1.11(0.31-3.92)
Time consuming and costly	2	1	50	0.954	0.96(0.27-3.36)	NS
Not applicable	11	5	45.4	Referent	Referent	
Water storage period						
One day	21	8	38.1	0.764	0.92(0.54-1.58)	0.88(0.48-1.61)
Two days	16	8	50.0	0.964	1.01(0.65-1.57)	0.99(0.59-1.67)
More than two days	66	32	48.4	Referent	Referent	Referent
Cover water storage container						
Yes	67	28	41.8	0.582	0.91(0.65-1.27)	0.89(0.57-1.34)
No	36	20	55.5	Referent	Referent	Referent
Drinking water drawing from container						
Dip into container	86	42	48.8	0.674	1.1(0.71-1.71)	1.1990.57-2.51)
Pour directly from container/use tap	17	6	35.3	Referent	Referent	Referent
Hand contact with drinking water						
Yes	36	24	48.8	0.021	1,11(1.11-3.39)	1.19(0.57-1.34)
No	67	24	35.8	Referent	Referent	Referent
Storage of water drawing container						
Table and shelves	23	8	34.7	0.701	0.91(0.56-1.46)	NS
Water storage cover	52	26	50	0.946	1.10(0.68-1.49)	3
Inside container/ Floor/wall	3	2	66.7	0.803	1.12(0.44-2.86)	
Not stated	25	12	48	Referent	Referent	
Type of water container						
Clay pot	62	31	50	0.726	1.06(0.76-1.47)	NS
Plastic	41	17	41.6	Referent	Referent	
Washing of water storage container						
Yes	37	12	32.4	0.049	0.58(0.31-0.99)	NS
No	66	36	54.6	Referent	Referent	

No- number, %-percentage, TTC-thermotolerant coliform; OR-Odds ratio, CI-confidence interval, NS-Not significant

Table 4.8 shows human waste disposal and influence on the on the household TTC water contamination. Both in bivariate and in multivariate analyses none of the human waste disposal practices (type of toilet facility, cleanliness of the latrine and disposable practices of the child waste) were found associated with TTC water contamination.

Table 4.8: Human waste disposal associate with TTC water contamination

Human waste disposal	Sample size	Drinking Water TTC Contamination		P-Value	Bivariate OR(95%CI)	Multivariate OR(95% CI)
		No	%			
House hold kind of toilet facility						
Piped sewer system/septic pit latrine	6	2	33.3	0.844	0.92(0.43-1.99)	
Pit latrine without slab /open pit	51	22	43.1	0.976	0.99(0.66-1.48)	
Ventilated improved pit latrine	6	2	33.3	0.844	0.92(0.43-1.99)	NS
Bush/field	15	11	73.3	0.471	1.2(0.72-1.99)	
Shared facility	25	11	44	Referent	Referent	
Cleanliness of latrine						
Clean(No files nor visible faecal matter)	16	8	50	0.892	0.96(0.1-1.55)	
Moderate clean (flies but no visible faecal matter)	33	12	36.4	0.513	0.87(0.59-1.29)	NS
Dirty (files and visible faecal matter)	14	6	422.8	0.751	0.92(0.55-1.52)	
N/A	40	22	55	Referent	Referent	
Disposal of children faeces						
Put in latrine	32	15	46.8	0.970	1.0(0.69-1.45)	
Put/rinsed into drain or ditch	19	10	52.6	0.897	1.2(0.67-1.57)	NS
Thrown into garbage	4	1	25	0.862	0.91(0.33-2.51)	
Not applicable	48	22	45.8	Referent	Referent	

No- number, %-percentage, TTC-thermotolerant coliform; OR-Odds ratio, CI-confidence interval, NS-Not significant

Table 4.9 shows household hygiene and laboratory parameters and influence on the on the household TTC water contamination. In the bivariate analyses, households which practiced washing hands before drawing drinking water were less likely to have household drinking water contaminated with TTC than those households which did not wash hands (OR 0.33, 95% CI 0.15 to 0.67). Similarly, households whose total coliforms count was less than 10 cfu/100ml were less likely to have household drinking water contaminated with TTC than those households with more than 10 cfu/100ml (OR 0.45, 95% CI 0.26 to 0.81). Further, households whose main water source temperatures was between 15 to 20ºC were less likely to have household drinking water contaminated with TTC than those households with water source temperatures greater than 25.1ºC (OR 0.39, 95% CI 0.16 to 0.96). In multivariate analyses none of the household hygiene and laboratory parameters (hand washing practices, water drawing rinsing practices, water source total coliform, household total coliform, water source TTC, source water temp, Ph and turbidity) were found associated with TTC water contamination.

Table 4.9: Hygiene, hand washing practice and laboratory parameters and Household TTC contamination

Hygiene and laboratory paremeters	Sample size	Drinking water TTC Contamination		P-Value	Bivariate OR (95%CI)	Multivariate OR (95%CI)
		No	%			
Wash hands before drawing water						
Yes	42	9	21.4	0.002	0.33(0.15-0.67)	NS
No	61	39	63.9	Referent	Referent	
Rinse water drawing (drinking) utensils						
Yes	99	47	47.7	0.716	1.17(0.48-2.87)	NS
No	4	1	25	Referent	Referent	
Aware of waterborne diseases						
Yes	91	43	47.2	0.881	1.03(0.71-1.42)	NS
No	12	5	41.6	Referent	Referent	
Suffered from diarreaho vomiting and fever						
Yes	30	14	46.7	0.997	1.1(0.71-1.42)	NS
No	73	34	46.5	Referent	Referent	
Water related infection						
Yes	5	5	100	0.334	1.37(0.72-2.62)	NS
No	23	9	39.1	0.828	0.95(0.64-1.41)	
Not applicable	75	34	45.3	Referent	referent	
Water source thermotolerant coliforms						
$\leq$10cfu/100ml	80	29	36.3	0.095	0.75(0.54-1.04)	NS
>10cfu/100ml	23	19	82.6	Referent	Referent	
Household total coliforms						
$\leq$10cfu/100ml	17	0	0	0.007	0.45(0.26-0.81)	NS
>10cfu/100ml	86	48.0	55.8	Referent	Referent	
Water source total coliforms						
$\leq$10cfu/100ml	50	13	26	0.107	0.74(0.52-1.06)	NS
>10cfu/100ml	53	35	66	Referent	Referent	
Water source temperature						
15-20	47	12	25.5	0.041	0.39(0.16-0.96)	NS
20.1-25	41	20	48.8	0.340	0.67(0.29-1.52)	
>25.1	9	9	100	Referent	Referent	
Water source PH						
5-7	24	14	58.3	0.588	1.1(0.76-1.59)	NS
>7.1	79	34	43	Referent	Referent	

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Introduction

Globally, an estimated 1.7 million people die annually, mainly due to waterborne diseases caused by poor water quality and lack of basic sanitation and hygiene (Ashbolt 2004; WHO 2012). The most affected are children under five years, particularly in developing countries, who often succumb to the ravages of diarrheal diseases (Kosek *et al.* 2003). Notwithstanding the above, WHO estimates that over (90%) of diarrhea cases can be prevented by enhancing the availability of clean water and improving hygiene and sanitation measures. The burden of these diseases is felt mostly in tropical African countries including Kenya (Kung *et al*. 2002). Although these diseases have been reported in most of the tropical African countries, the lack of water sanitation as a hazard has not been exhaustively studied, particularly the total and fecal (*E. coli*) thermotolerant coliforms in the source and household drinking water (Kosek *et al.* 2005; Özdemir *et al.* 2011). For instance, Kenya has experienced recurrent cases of waterborne diseases like cholera (TDN, 2007; Wambua, 2008), with morbidity patterns over the last ten years ranking diarrhea as the fourth priority disease (HSSR 2005). In this Kenyan case, among the most affected regions is the Western and part of Rift Valley Province, notably the districts of Bungoma, Busia, Kakamega and Kericho (Onyango & Angienda, 2010). This study therefore examined Kericho District exploring the water situation in terms of total and *E. coli* thermotolerant coliforms contamination of source and household drinking water as well as identified factors linked to water handling (e.g. water collection, treatment and storage) and sanitation (e.g. waste disposal and pollution alongside rivers). Invariably, the extent of safe water handling was determined by the local people's knowledge and attitudes towards water safety and sanitation (Özdemir *et al.* 2011).

5.2 Study characteristics

This study was among the very first to evaluate both the total and thermotolerant coliforms contamination of water source and the overall impact on the quality of drinking water. In this study a total of 103 households consented and their household and source waters collected. Majority (30.1%) of the households were located within the Kericho Township, (68.9%) were in the rural locality, (95.1%) were female and (42.7%) were aged 21 to 30 years. The respondents mean age was 21.59 years (range 18–29 years) and the majority. The main water source was river (36.9%) and only (33%) had piped or municipal supply. Most (43.7%) of the households had drinking water source within their premises while (13.6%) had to walk for 30 to 60 minutes to water source. Majority (60.2%) of the household used clay pot for water storage with almost all of them (91.3%) not treating their drinking water. The majority (83.5%) drew drinking water by dipping the cup into the water storage container. About (59.2%) did not wash their hands before drawing water while (96.1%) of them rinsed the water drawing utensils.

5.3 Thermotolerant pathogens isolated

The presence of thermotolerant coliforms in water indicates the actual contamination with feces (human and non-human) and potential contamination by disease causing pathogens of all kinds. Bacterial pathogens isolated from the source and household drinking water in this study included Serratia (22.8%), Enterobactor (20%), Klebsiella (14.3%), Moraxella (14.3%), Pseudomonas (11.4%), Shigella (5.7%), Acinetobacter (5.7%), Aeromonas (2.9%) and Yersinia (2.9%). This is worrying given that the isolated bacteria are among those identified as human bacterial pathogens potentially transmitted in drinking water including strains of Esche*richia coli, Shigella ssps, Vibrio cholerae, Yersinia enterocolitica* and *Campylobacter jejune* (WHO, 1996). Similar coliforms have been isolated by others from water sources; Kämpfer *et al*., (2008) isolated *Enterobacter* spp. (species of the *Enterobacter cloacae* complex), *Serratia* spp., *Citrobacter* spp and *Klebsiella* spp.; but species identification remained vague in several cases.

52

5.4 Thermotolerant contamination levels

The World Health Organization (WHO) guidelines for bacteriological quality of drinking water require that all waters intended for drinking must contain no *E. coli* or thermotolerant coliforms in any 100 ml sample. Thermotolerant coliforms in the current study were detected in 23/103 (22.3%) at the water source and 48/103 (46.6%) of the sampled household drinking water points indicating lack of safety for consumption. This bacteriological contamination of water at the source with a further deterioration between the collection points and homes was observed has been observed in other studies. In Kibera Nairobi Chemuliti *et al*. (2002) observed a higher contamination level of fecal coliforms isolated from 57 (95%) in-house sources and 7 (35%) out-house sources. In Western Kenya Muruka *et al*., 2012 observed a lower (2%) source water fecal coliform contamination. In Masaba, Kisii, Nyagwencha *et al*. (2012) observed that (16%) of the households consumed water unsuited for human consumption. In a review conducted by Bain *et al*. (2014) in studies conducted by the Chinese, English, French, Portuguese, and Spanish showed that over a quarter of samples from improved sources contained fecal contamination in 38% of 191 studies. In Peru, Gil *et al*. (2014) found thermotolerant coliforms in (48%) of all water samples. In India Boisson *et al*. (2013) found that (20%) of the total household visits, children's drinking water was assayed for thermotolerant coliforms (TTC), an indicator of fecal contamination. Our study and these others provide strong evidence that the number of people with access to a safe water source has been greatly overstated, and suggests that a large number and proportion in Kericho, Kenya and of the world's population use unsafe water according to the WHO guidelines.

In this study, the thermotolerant coliforms were found to be resistant to various important antibiotics including ampicillin, sulphamethoxazol/trimethoprim, and tetracycline respectively. In Nigeria, resistance pattern of (80.9%) ampicillin, (95.4%) tetracycline and (46.5%) chloramphenicol was observed 41 while in Tanzania resistance rates of (83.1%) to ampicillin, (57%) chloramphenicol, (87.7%) tetracycline and (90.8%) co-trimoxazole were found (Vila *et al*., 2000). Of major concern now is that while all drugs which were more resistant are cheap, inexpensive

and available and could have been abused, drugs such as ciprofloxacin and cefotaxime which are reserve antibiotics in Kenya, showed considerable increasing resistance. This indicates misuse of these and other classes of antibiotics which would have major implications in the treatment of *Escherichia coli* causing diarrhea and drug policies in Kenya and other developing and developed countries

5.5 Socio-demographic factors associated with TTC drinking water contamination

In this study, rural household locality (OR 2.01, 95% CI 1.09 to 4.12) were more likely to have TTC contaminated water while households whose main source of drinking water was from piped supply or from municipal were less likely to have household drinking water contaminated with TTC (OR 0.38, 95% CI 0.16 to 0.91). These findings mirror those of Bain *et al*., (2014) who showed that water sources in low-income countries and rural areas as being more likely to be contaminated. As expected, households located in the rural areas are pronged to poor waste disposal, poor household hygiene, lack of resources and knowledge for water treatment. These factors were likely to contribute to the higher TTC drinking water contamination. This argument is supported by the observation of Bain *et al*. (2014) who observed that a defective water delivery system and inadequate environmental sanitation are shown to be a potential source of contamination for household drinking water. In our study other socio-demographic factors that were found not to be associated with TTC contamination of drinking water included gender, age, education level, occupation, type of mains source of drinking water and the distance to the main water source. Muruka *et al*. (2012) observed a significant association between pit latrine distance and level of fecal contamination of drinking water. This could be interpreted as decreasing distance increases the chances/risk for dug-well contamination to occur. The poor levels of environmental hygiene coupled with a dilapidated water delivery system are major contributors for TTC water contamination (Chemuliti *et al*., 2002).

5.6 Water Treatment, Storage and Waste Management as Factors Household Ttc Water Contamination

Households which had hand contact with drinking water during water withdrawal was associated with TTC contaminated (OR 1.11, 95% CI 1.11 to 3.39). Those households which washed the water storage containers were less likely to have household drinking water contaminated with TTC (OR 0.58, 95% CI 0.31 to 0.99). Households which practiced washing hands before drawing drinking water were less likely to have household drinking water contaminated with TTC (OR 0.33, 95% CI 0.15 to 0.67). Importantly none of the human waste disposal practices (type of toilet facility, cleanliness of the latrine and disposable practices of the child waste) were found associated with TTC water contamination. Eshcol *et al*., (2009) in India showed that fecal contamination occurs principally during storage due to poor water handling. Due to poor hygiene, they observed that the dramatic increase in contamination after collection indicates that until an uninterrupted water supply is possible, the point at which the biggest health impact can be made is at the household level. Household water handling and sanitation practices are key factors in the prevalence and risk of water borne illness outbreaks. An intervention study in Zimbabwe found that homes where traditional drinking water containers are replaced with covered, narrow mouthed urns with a tap outlet have significantly less contamination than the control group (Mazengia *et al*. 2002). A combination of special storage vessels with point of use treatment has been shown to be very effective. Mintz *et al*. (1995) found fecal contamination in households using a specially designed safe water storage container alone, but not in households using both the container and a (5%) calcium hypochlorite solution. Solar disinfection at the point of use was found to reduce childhood morbidity due to diarrhea in southern India (Rose *et al*., 2006). In Calcutta, India, the introduction of a narrow-mouthed and covered container from which water was poured significantly reduced cholera contamination (Deb *et al*., 1986). Luby *et al*. have shown using randomized control trials in Pakistan that handwashing initiatives and the introduction of point-of-use disinfection can reduce diarrheal incidence (Luby *et al*., 2006). A Cochrane review of the efficacy of hand washing interventions concluded that diarrheal episodes may be reduced by about (30%) (Ejemot *et al*., 2008) Other factors such as number of

residents in a household and presence of sewage in streets have been associated with feco-orally transmitted parasitic diseases (Teixeira & Heller, 2006).

5.7 Conclusion and Recommendation

5.7.1 Conclusion

Based on the findings of this study, the following conclusions can be drawn;

1. Majority of the household in Kericho District located within the Kericho Township, are female headed with most of them aged 21 to 30 years but still have rivers as the main water source with a few of them being privileged to access piped or municipal water supply.
2. Majority of the household still uses clay pot for water storage and sadly almost all of them do not treat their drinking water.
3. Most of the household still practice poor water hygiene such as drawing drinking water by dipping the cup into the water storage container, lack of hand wash before drawing water or rinsing the water drawing utensils.
4. Majority of these household drinking water are contaminated by Thermotolerant coliform (TTC) levels of >10cfu/100ml.
5. Most commonly used antibiotics were ineffective against the isolated TTC
6. Household rural household locality, poor hygiene such as (hand contact with drinking water during water withdrawal increased the chances for TTC water contamination
7. Having water supply from piped supply or from municipal and improved hygiene practices such as (hand washing drinking water storage containers, hand washing before drawing drinking water were vital to reduce TTC water contamination

5.7.2 Recommendations

Several recommendations can be drawn from the result of this study. They are necessary in improving the quality of water available for human consumption and reducing the incidences of waterborne disease outbreaks.

1. Awareness creation on personal and environmental hygiene including maintenance of high sanitation standards in water distribution points as well as in water storage and distribution containers.

2. In addition to the inadequate water, resulting in intermittent supply, other systemic weaknesses include the lack of consistent, reliable chlorination (wide fluctuations in chlorination levels throughout the distribution system, with household chlorine levels ranging from nil to over 2mg/L (recommended WHO household level is, 0.5mg/L) and poor responsiveness to complaints, particularly in slum areas with less political clout should be evaluated and addressed. There-fore it is pertinent to ask whether the prevalence of water borne illness can be primarily attributed to the systemic deficiencies or the water handling and storage practices of households.

3. Need to put in place proper sewage disposal and treatment measures to reduce the amount of raw sewage that finds its way into the water sources.

4. Need to supply the communities with clean piped water at their households to keep the population away from the surface sources (rivers and lake). This can assist in protecting these water sources from further quality degradation and pollution.

5. An urgent need to focus on the means and ways of controlling pollution of these community water sources by other organic and inorganic materials through proper solid and liquid waste disposal and regular water monitoring programmes to be established for all water sources in Kericho District.

6. Availing and emphasizing the use of affordable, cheap and locally available and environmentally friendly point of use/household water treatment approaches. These include solar radiation disinfection using water pasteurization kits. This is required in rural and informal urban settlement (slams) as it is the case in Rural Kericho where water is obtained from public standpoints, rivers, lake or from resellers (vendors).

7. Conduct regular water quality assessment specifically to determine the antibiotics profile of TTC with an intention to inform policy about their use

REFERENCES

Aibinu, I, Adenipekun E, & Odugbemi, T. (2004). Emergence of quinolone resistance among *Escherichia coli* strains isolated from clinical infections in some Lagos state hospital in Nigeria. *A Nigeria journal of Health and Biomedical Sciences. 3,* 73-78.

Ashbolt N. (2004). Microbial contamination of drinking water and disease outcomes in developing regions. *Toxicology. 198*, 229–238.

Bain, R, Bartram, J, Elliott M, Matthews R, McMahan L, Tung R, Chuang P, & Gundry, S. (2012). A summary catalogue of microbial drinking water tests for low and medium resource settings. *Int J Environ Res Public Health. 9,* 1609–1625.

Bain, R, Cronk, R, Wright J, Yang H, Slaymaker T, & Bartram, J. (2014). Fecal Contamination of Drinking-Water in Low- and Middle-Income Countries: A Systematic Review and Meta-Analysis. *PLoS Medicine. 11*(5), e1001644.

Baron, JE, Peterson, L, & Finegold, S. (1994). *Diagnostic microbiology. (9th* Edition), Bailey and Scott's.: Mosby.

Barrell P, Hunter G, Nichols. Microbiological standards for water and their relationship to health risk; communicable disease and public health vol 3 no 1

Berendes, M, Fagerli, K, Kim, S, Nasrin D, Powell H, Kasumba I, Ochieng J, Verani J, Doh S, Mintz E, & Kotloff, K. (2023). Survey-Based Assessment of Water, Sanitation, and Animal-Associated Risk Factors for Moderate-to-Severe Diarrhea in the Vaccine Impact on Diarrhea in Africa (VIDA) Study: The Gambia, Mali, and Kenya, 2015–2018, *Clinical Infectious Diseases. 76,* S132–S139.

BGVV. (2001). *Trends and sources of zoonotic agents in animals, feeding stuff, food and man in the European Union and Norway in 1999*. 2001. BGVV's community reference lab for epidemiology of zoonosis.

Black, R, Fontaine, O, & Lamberti, L. (2019). Drivers of the reduction in childhood diarrhea mortality 1980–2015 and interventions to eliminate preventable diarrhea deaths by 2030. *J Glob Health, 9*, 020801

Black, WC. (1993). PCR with arbitrary primers: approach with care. *Insect Molecular Biology. 2*(1), 1-6.

Boisson, S, Stevenson M, Shapiro, L, Kumar, V, & Singh, LP. (2018). Effect of Household-Based Drinking Water Chlorination on Diarrhea among Children under Five in Orissa, India: A Double-Blind Randomised Placebo-Controlled Trial. *PLoS Medicine. 10*(8), e1001497.

Bojarczuk, A, Jelonkiewicz Ł, & Lenart-Boroń, A. (2018). The effect of anthropogenic and natural factors on the prevalence of physicochemical parameters of water and bacterial water quality indicators along the river Białka, southern Poland. Environmental Science *and* Pollution Research. *25,* 10102–10114.

Caeiro, JP, Estrada-Garcia, MT, Jiang, ZD, Mathewson, JJ, Adachi JA, Steffen R, & DuPont, HL. Improved detection of enterotoxigenic Escherichia coli among patients with travelers' diarrhea, by use of the polymerase chain reaction technique. *J Infect Dis. 180*(6), 2053-5.

Callaway, E, & Ledford, H. (2021). How bad is Omicron? What scientists know so far, *Nature*, *600,* 197–199.

Center for Disease Control (CDC) (2010). *Microbiological Indicators Testing in Developing Countries:* A Fact Sheet for the Field Practitioner Version 1.

Center for Disease Control (CDC), (2007). Disease information. *In salmonellosis.*

Chan, EYY, Tong, KHY, Dubois, C, Mc Donnell K, Kim JH, Hung KKC, & Kwok KO. (2021). Narrative Review of Primary Preventive Interventions against Water-Borne Diseases: Scientific Evidence of Health-EDRM in Contexts with Inadequate Safe Drinking Water. *Int J Environ Res Public Health. 18*(23), 12268.

Cheesbrough, M. (2004). *District laboratory practice in tropical countries.* Part 2, Cambridge: Cambridge University press.

Chemuliti, JK, Gathura PB, Kyule MM & Njeruh, F. (2002). Bacteriological qualities of indoor and out-door drinking water in Kibera Sub-location of Nairobi, Kenya. *East African Medical Journal. 79*, 271-274.

Chen, S, Zhao, S, White, DG, Schroeder CM, Lu R, Yang H, McDermott PF, Ayers S, & Meng, J. (2004). Characterization of multiple-antimicrobial-resistant salmonella serovars isolated from retail meats. *Appl Environ Microbiol. 70*(1), 1-7.

Committee on Economic Social and Community Rights. (2002). General comment no. 15, *The right to water.* UN Doc. E/C.12/2010. New York: United Nations.

Deb, BC, Sircar, BK, Sengupta PG, De SP, Mondal SK, Gupta DN, Saha NC, Ghosh S, Mitra U, & Pal SC. (1986). Studies on interventions to prevent eltor cholera transmission in urban slums. *Bull World Health Organ. 64*(1), 127-31.

Ejemot, RI, Ehiri, JE, Meremikwu, MM, & Critchley JA. (2008). Hand washing for preventing diarrhoea. *Cochrane Database Syst Rev. 23*(1), CD004265.

Eshcol, J, Mahapatra P, & Keshapagu S. (2009). Is fecal contamination of drinking water after collection associated with household water handling and hygiene practices? A study of urban slum households in Hyderabad, India. *J Water Health. 7*(1), 145-54.

Forbes, B. A., Sahm, D. F., Weissfeld, A.S., & Bara\on, E. J. (1998). *Dignostic Microbiology. 10th ed.* St. Louis: Mosby.

Giannoulis, N., Maipa, V., Konstantinou, I., Albanis, T. and Dimoliatis, I. (2005). Microbiological risk assesment of Agios Georgios source suplies in North Western Greece based on fecal coliform determination and sanitary inspection survey. *Chemosphere, 58*, 1269 – 1276.

Gil A, Lanata CF, Hartinger SM, Mäusezahl D, Padilla B, Ochoa TJ, Lozada M, Pineda I, Verastegui H. 2014. Fecal contamination of food, water, hands, and kitchen utensils at the household level in rural areas of peru. *J environ health. 76*(6), 102-6.

Gomez-Duarte, OG. (2009). Rapid diagnostics for diarrheal disease surveillance in less developed countries. *Clin Lab Int. 33*, 7–10.

Guillaume, DA, Justus OOS, & Ephantus, KW. (2020). Factors influencing diarrheal prevalence among children under five years in Mathare Informal Settlement, Nairobi, Kenya. *J Public Health Afr. 11*(1), 1312.

Gundry, S, Wright, A, Conroy, R, Du PM, Genthe B, & Moyo S. (2006). Contamination of drinking water between source and point-of-use in rural households of South Africa and Zimbabwe: implications for monitoring the Millennium Development Goal for water. *Water Practical Technology. 1,* 1-9.

Hamzah, L, Boehm, A, Davis, J, Pickering A, Wolfe W, & Mureithi, M. (2020). Ruminant fecal contamination of drinking water introduced post-collection in rural Kenyan households, *Int. J. Environ. Res. Public Health. 2020:* 17 https://doi.org/10.3390/ijerph17020608.

Ilyas, M, Ahmad, W, Khan, H, Yousaf, S, Yasir, M, & Khan, A. (2019). Environmental and health impacts of industrial wastewater effluents in Pakistan: a review. *Rev Environ Health. 34*(2), 171-186.

Kalle, K, (2019). Cholera Forcing" and the Urban Water Infrastructure: Lessons from Historical Berlin, EHES Working Paper. 167, European Historical Economics Society (EHES), s.l.

Kämpfer, P, Nienhüser, A, Packroff, G, Wernicke F, Mehling A, Nixdorf K, Fiedler S, Kolauch C, & Esser M. (2008). Molecular identification of coliform bacteria isolated from drinking water reservoirs with traditional methods and the colilert-18 system. *Int j hyg environ health. 211*(3-4), 374-84.

Kannan, S, Shaik Syed Ali, P, & Sheeza A. (2021). Omicron (B.1.1.529) - variant of concern - molecular profile and epidemiology: a mini review. *Eur Rev Med Pharmacol Sci. 25*(24), 8019-8022.

Kariuki, S, Revathi, G, Kariuki N, Kiiru J, Mwituria, J, & Hart, CA. (2006). Characterization of community acquired non – typhoidal *Salmonella* from bacteraemia and diarrheal infections in children admitted to hospital in nairobi, Kenya. *BMC Microbiology. 6,* 101 – 105.

Kenya National Bureau of Statistics-KNBS (2010). *The 2009 Kenya Population and Housing Census, population distribution by administrative units volume 1 A,* (247-248). Nairobi: KNBS.

Kenya National Bureau of Statistics-KNBS. (2019). 2019 Kenya Population and Housing Census. Distribution of population by Administrative Units. Retrieved from https://housingfinanceafrica.org/app/uploads/VOLUME-II-KPHC-2019.pdf

Kericho District health information office (KDHIO). (2008). *District morbidity report*. Kericho: KDHIO.

Khan, I, Khan, A, Khan, MS, Zafa, r S, Hameed, A, Badshah, S, Rehman, SU, Ullah H, & Yasmeen, G. (2018). Impact of city effluents on water quality of Indus River: assessment of temporal and spatial variations in the southern region of Khyber Pakhtunkhwa, Pakistan. *Environ Monit Assess. 190*(5), 267.

Kimani-Murage, EM & Ngindu, A. (2007). Quality of Water the Slum Dwellers Use: The Case of a Kenyan Slum. *Journal of Urban Health: Bulletin of the New York Academy of Medicine. 84* (6), 829-838.

Kirby, B. W. (1966). Antimicrobial susceptibility testing by the Kirby Bauer disc diffusion.

Kosek, M, Bern, C, & Guerrant, RL. (2003). The global burden of diarrheal disease, as estimated from studies published between 1992 and 2000. *Bull World Health Organ. 81*(3), 197-204.

Kosek, M, Black, R, & Keusch, G. (2005). Nutrition and Micronutrients in Tropical Infectious Diseases', in L. Richard, Guerrant, D.H. Walker & Weller P.F., (eds.), *Tropical Infectious Diseases: Principles, Pathogens, and Practice*, (2nd ed.), New York: Elsevier Health Sciences.

Kosek, M, Yori, P, Pan W, Olortegui S, Gilman H, Perez J, Chavez C, & Hall, E. (2008). Epidemiology of highly endemic multiply antibiotic-resistant shigellosis in children in the Peruvian Amazon, *Pediatrics. 122*, e541–e549.

Kung, WN, Musau, P, Ochieng, A, Wachira, E, Omol, RT, Rakwar, J, Muthwii, S, …. & Osaki, Y. (2002). Diarrhea prevalence and risk factors in slums. *Journal of National Institute for Public Health. 51*, 73-76.

Larson, A, Hartinger SM, Riveros M, Salmon-Mulanovich G, Hattendorf J, Verastegui H, Huaylinos ML, & Mäusezahl D. (2019). Antibiotic-Resistant *Escherichia coli* in Drinking Water Samples from Rural Andean Households in Cajamarca, Peru. *Am J Trop Med Hyg, 100*(6), 1363-1368.

Larson, A, Haver S, Hattendorf J, Salmon-Mulanovich G, Riveros M, Verastegui H, Mäusezahl D, Hartinger S. (2023). Household-level risk factors for water contamination and antimicrobial resistance in drinking water among

households with children under 5 in rural San Marcos, Cajamarca, Peru. *One Health. 16*(2023), 100482.

Lin, I, Van T, & Smooker, M. (2015). Live-Attenuated Bacterial Vectors: Tools for Vaccine and Therapeutic Agent Delivery. *Vaccines.3*, 940-972.

Luby, SP, Agboatwalla, M, Painter, J, Altaf A, Billhimer W, Keswick B, & Hoekstra, R. (2006). Combining drinking water treatment and hand washing for diarrhea prevention, a cluster randomised controlled trial. *Trop. Med. Int. Health. 11*(4), 479–489

Mafu, NC, Pironcheva, G, & Okoh, A. (2009). Genetic diversity and in *vitro* antibiotic susceptibility profile of *Salmonella* species isolated from domestic water and wastewater sources in the Eastern Cape Province of South *Africa. African Journal of Biotechnology. 8* (7), 1263 – 1269.

Mazengia, E, Chidavaenzi, M, Bradley, M, Jere M, Nhandara C, Chigunduru D, & Murahwa, C. (2002). Effective and culturally acceptable water storage in Zimbabwe: maintaining the quality of water abstracted from upgraded family wells. J. Environ. Health. 2002: 64(8), 15–18, 25.

Mintz, ED, Reiff, FM, & Tauxe R. (1995). Safe water treatment and storage in the home. A practical new strategy to prevent waterborne disease. *JAMA. 273*(12), 948–953.

Murray, CJ, Aravkin AY, & Zheng, P. (2020). Global burden of 87 risk factors in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet. 396,* 1223–49.

Muruka, C, Fagbamigbe, FA, Muruka, A, Njuguna, J, Otieno, DO, Onyando, J, … & Wamalwa D. (2012). The Relationship between Bacteriological quality of Dug-Wells & Pit Latrine Siting in an Unplanned Peri-Urban Settlement: A Case Study of Langas – Eldoret Municipality, Western Kenya. *Public Health Research. 2*(2), 32-36

Nguyen, TV, Le Van, P, Le Huy C, Gia KN, & Weintraub A. (2005). Detection and characterization of diarrheagenic Escherichia coli from young children in Hanoi, Vietnam. *J Clin Microbiol. 43*, 755–760.

Nyagwencha, JM, Kaluli, JW, Home, PG, & Hunja M. (2012). Water and water-borne diseases in North Masaba District, Kenya *JAGST. 14*(1), 116.

Nyberg, T, Ferguson, NM, Nash, S, Webster, H, Flaxman, S, Andrews, N, … & Bhatt, S. (2022). Comparative analysis of the risks of hospitalisation and death associated with SARS-CoV-2 Omicron (B. 1.1. 529) and Delta (B. 1.617. 2) variants in England: a cohort study, *Lancet. 399,* 1303–1312.

Olofin, I, McDonald CM, & Ezzati, M. (2013). Associations of suboptimal growth with all-cause and cause-specific mortality in children under five years: a pooled analysis of ten prospective studies. *PLoS One, 8,* e64636.

Onyango, D, & Angienda, P. (2010). Epidemiology of Waterborne Diarrhoeal Diseases among Children Aged 6-36 Months Old in Busia - Western Kenya. World Academy of Science, Engineering and Technology, *International Journal of Environmental, Chemical, Ecological, Geological and Geophysical Engineering. 4*, 38-45.

Opisa, S, Odiere, MR, Jura, WO, Karanja, MS, & Mwinzi, PMW. (2012). Faecal contamination of public water sources in informal settlements of Kisumu City, western Kenya. *Water Sci Technol. 66*(12), 2674-81.

Osiemo, M, Ogendi, G, & M'Erimba, C. (2019). Microbial Quality of Drinking Water and Prevalence of Water-Related Diseases in Marigat Urban Centre, Kenya. *Environ Health Insights. 13*, 1178630219836988.

Oswald, W, Lescano, Bern M, Calderon M, Cabrera L, & Gilman, R. (2007). Fecal contamination of drinking water within peri-urban households, Lima, Peru, *Am. J. Trop. Med. Hyg. 77*, 699–704.

Özdemir, S, Elliott, M, Brown J, Nam P, Hien V, & Sobsey, M. (2021). Rainwater harvesting practices and attitudes in the Mekong Delta of Vietnam. *Journal of Water, Sanitation and Hygiene for Development. 1*, 171–177.

Palmer C, Tsai YL, Pasko – Kolva C, Mayer C, & Sangermano LR. (1993). Detection of *Legionella* species in sewage and ocean water by polymerase chain reaction, direct fluorescent antibody and plate culture methods. *Applied Enviromental Microbiology. 59,* 3618 - 3624.

Paulson, KR, Kamath, AM, & Alam, T. (2021). Global, regional, and national progress towards Sustainable Development Goal 3.2 for neonatal and child health: all-cause and cause-specific mortality findings from the Global Burden of Disease Study 2019. *Lancet. 398,* 870–905.

Presterl Ewick, H, Reichman, S, Aichelburg, A, Winkler, S, Kremsner, G, & Graninger W. (2003). Frequency and virulence properties of diarrhoegenic *Escherichia coli* in children with diarrhea in Gabon. *American Journal of Tropical Medicine and Hygien. 69*, 406 – 410.

Ramos, MDN, Rangel, AS, Azevedo, KS, Melo, MGB, Oliveira, MC, Watanabe, CMU, Pereira, FF, Silva, CM, & Aguiar, A. (2022). Characteristics and treatment of Brazilian pulp and paper mill effluents: a review. *Environ Monit Assess. 194*(9), 651.

Republic of Kenya (2014). Kericho District Strategic Plan 2005-2010 for Implementation of the National Population Policy for Sustainable Development. National Coordination Agency for Population and Development Ministry of Planning and National Development. Retrieved from http://www.ncapd-ke.org/images/stories/districts/Kericho

Rose, A, Roy, S, Abraham, V, Holmgren, G, George, K, Balraj, V, … & Kang, G. (2006). Solar disinfection of water for diarrheal prevention in southern India. *Arch. Dis. Child. 91*(2), 139.

Sambrook, J. & Russell, DW. (2001). *Molecular Cloning: A Laboratory Manual. Cold Spring Harbor,* NY: Cold Spring Harbor Laboratory Press.

Shah, M, Kathiiko C, & Wada, A. (2016). Prevalence, seasonal variation, and antibiotic resistance pattern of enteric bacterial pathogens among hospitalized diarrheic children in suburban regions of central Kenya. *Trop Med Health. 44*, 39.

Shah, MT, Alizai, A, & Khan, D. (2012). Environmental geochemistry of surface and subsurface water from Dera Ismail Khan Division, Khyber Pakhtunkhwa, Pakistan. *Journal of Chemical Society of Pakistan. 34*, 243–250.

Snow, J. (1855). *On the mode of communication of cholera. (*2nd Edition), London, J Churchill. reprinted in 'Snow on cholera, New York: Hafner.

Stoffel, M, Wyżga, B, & Marston, RA. (2016). Floods in mountain environments: a synthesis. *Geomorphology. 272,* 1–9.

Talaro KP. (2008). *Foundations in Microbiology.* (Sixth edition). New York: McGraw – Hill International edition.

Teixeira, C, & Heller, L. (2006). Impact of water supply, domiciliary water reservoirs and sewage on faeco-orally transmitted parasitic diseases in children residing in poor areas in Juiz de Fora, Brazil. *Epidemiol. Infect. 134*(4), 694–698.

Tole, MP. (1997). Pollution of groundwater in the coastal Kwale District, Kenya. Sustainability of Water Resources under Increasing Uncertainty (Proceedings of the Rabat Symposium S1. IAHS Publ. no. 240, 1997. 287

Trajano Gomes da Silva, D, Ebdon, J, Okotto-Okotto, J, Ade, F, Mito, A, Wanza, P, Yu J. & Wright, J. (2020). A longitudinal study of the association between domestic contact with livestock and contamination of household

point-ofuse stored drinking water in rural Siaya County (Kenya), *Int. J. Hyg. Environ. Health. 2020*, 230.

Tropical Disease News (TDN), (2007). Seven People Killed by Cholera in Western Kenya, More Affected, Retrieved from www.medindia.net/news/Seven-People-Killed-by-Cholera-in-Western-Kenya-More-Affected-30819-1.htm

United Nations, (2021). WHO: 110 Countries have Found Omicron Mutants. Retrieved from https://news. un.org/zh/story/2021/12/1096642, 2021.

United Nations. (2010). *Resolution on Human Right to Water and Sanitation*. United Nations General Assembly. A/64/292. New York: United Nations.

United Nations. (2013). UN Goal 7: Ensure Environmental Sustainability. Retrieved from http://www.un.org/millenniumgoals/environ.shtml.

UN-Water (2014). A Post-2015 Global Goal for Water. Synthesis of key findings and recommendations from UN-Water. Retrieved from http://bit.ly/Prg2lt

Vidal, M, Kruger, E, Duran, C, Lagos, R, Levine, M, Prado, V, Toro, C, & Vidal, R. (2005). Single multiplex PCR assay to identify simultaneously the six categories of diarrheagenic Escherichia coli associated with enteric infections. J Clin Microbiol. 2005. 43:5362–5365.

Vila, J, Vargas, M, & Henderson, IR. (2000). Enteroaggregative Escherichia coli virulence factors in travellers' diarrhea strains. *J Infect Dis. 182*, 1780e3.

Vogel, L, Muller, A, Odingo, S, Onyango, Z, & De Geus, A. (1982). *Health and Disease in Kenya.* Joint production of Medical Research Centre, Nairobi and Department of the Royal Tropical Institute, Amsterdam, Netherlands. *Kenya Literature Bureau.* 180 -187.

Wambua J, (2008). Cholera in Kenyan situation a worry, Retrieved from http://keyscientist.org/talk/viewtopic.php?f=4&t=3

WHO. (1997). *Guidelines for Drinking-Water Quality*. Geneva: World Health Organization.

WHO. (2011). *Guidelines for drinking-water quality*. Geneva: World Health Organization.

WHO/UNICEF (2010). JMP Technical Task Force Meeting on Monitoring Drinking-water Quality. Geneva and New York: WHO/UNICEF Joint Monitoring Programme. Retrieved from: http://www.wssinfo.org/ fileadmin/user_upload/resources/JMP-Task-Force-Meeting-on-Monitoring-Drinking-water-Quality.pdf.

World Health Organisation (WHO), (2012). Burden of disease and cost-effectiveness estimates, Retrieved from http://www.who.int/water_sanitation_health/ diseases/burden/en/index.html

World Health Organization (WHO). (1993). *Guidelines for drinking water quality*. Geneva: World Health Organization.

World Health Organization (WHO). (1994). *Guidelines for drinking-water quality*, (2nd edition); Volume 1: Recommendations. Geneva: WHO.

World Health Organization (WHO). (1998). *Guidelines for drinking-water quality. V. 3. Surveillance and control of community supplies*.(2nd ed.). Geneva: WHO.

World Health Organization (WHO). (1999). *Environmental health indicators*: Frame work and methodologies. Geneva: WHO.

World Health Organization (WHO). (2000). *Global Water Supply and Sanitation Assessment 2000 Report.* Geneva: WHO.

World Health Organization (WHO). (2002). *The World Health Report 2002*. Geneva: WHO.

World Health Organization (WHO). (2009). *Diarrhea Diseases Update*; Geneva: World Health Organization.

World Health Organization and United Nations Children's Fund (WHO/UNICEF) (2010). *Joint Monitoring Programme for Water Supply and Sanitation: Progress on Sanitation and Drinking-water*: 2010 Update, Geneva: WHO/UNICEF.

World Health Organization, (2018). *Antimicrobial Resistance*. Retrieved from http://www.who.int/topics /antimicrobial_resistance/en/,

World Health Organization. (1996). Guidelines for drinking water quality. (2nd edition), Retrieved from https://www.who.int/publications/ i/item/ 9241 544805

World Water Assessment Programme (WWAP). (2006). *Kenya National Water Development Report. Case Study*. In UN – water / WWAP / 2006/12.

Wright, J, Gundry, S, & Conroy, R. (2004). Household drinking water in developing countries: a systematic review of microbiological contamination between source and point-of-use, Tropical Med. *Int. Health, 9*(2004), 106–117.

Yeo, S, & Livermore, DM. (1994). Effect of inoculum size on the in – vitro susceptibility to beta – lactam antibiotics of *M. Catarrhalis* isolates of different beta lactamase types. *Journal of Medical Microbiology. 40*(40), 252 – 255.

APPENDICES

Appendix I: Study Questionnaire

SECTION A: SOCIO-DEMOGRAPHIC INFORMATION

1. **Questionnaire Number………… Date of interview…………………………**
2. **Residence**: a. Location: _____ b. Village _____ c. Household no. _____
3. **Locality**: a. Rural (*Resop*)   b. Urban *(Taon)*
4. **Age of respondent (Years) (***Kenyisiek.***)** _____

5. **Respondent gender**   a**.** Male (*Murenik)*   b. Female *(Chepyoset)*

6. **Religion** (*Kaniset)* a. Christian   b. Muslim   c. Hindu

7. **Education level of the respondent** *(Kisoman koit ano.)*

a. Non   b. Primary   c.   Secondary   d. Tertiary

8. **Occupation of the respondent.** *(Yae kasit ainon.)*

 a. Farmer   b. Laborer   c. Business   d. Government officer   e. Employee
f. Housewife   g. Student   h. Unemployed

9. Any child in the household under the age of 5 years. *(Mi lakwet nemasire kenyisiek muut?)*

a. Yes   b. No

SECTION B: STORAGE AND TREATMENT OF DRINKING WATER

10. What is the <u>main</u> source of drinking water for members of your household?
(record name of source where possible) (Oe beek achon?)

a. Traditional/ open well   b. Well with hand pump   c. Borehole

d. River   e. Pond/ dam   f. Rain water /

roof catchment   g. Spring   h. Piped supply/municipal

11. How long on <u>average</u> does it takes to reach the water source *(one trip go and come back, estimate) (Ibe saisiek ata kesor beek en ole oibunen?)*

    a. Less than 15 min   b. 15-30 min   c. 30 min-1 hour

    d. More than 1 hour   e. Water on premise   f. don't know

12. **Who usually goes to this source to fetch the water for your household?** *(Ng'o ne ibu beek?)*

Probe: Is this person under the age of 15 years? What sex? Circle the code that best describes this person.

    a. Adult woman   b. Adult man   c. Female child

    (under 15 years)   d. Male child (under 15

    years)   e. Don't know

13. **Do you consider water from the current source safe for drinking**? *(Kororon beek en ole oibunen?)*

    a. Yes   b. No   c. Don't Know

14. **Do you treat your water before drinking?** *(Inyoi beek kotomo iye?)*

    a. Yes   b. No

15. If you treat your water, which method do you use? *(Ngot inyoi beek, iyoitoi ano?)*

    a. Boil   b. Use chlorine   c. Filtration   d. Water guard

16. If you do not treat your water, why? *(Ngot imenyoi beek ko amunei?)*

   a. The water is clean   b. Don´t know how to treat   c. Too time
consuming

   d. Too costly   e. Limited access to firewood   f. Lack of
knowledge

17. How long do you store your drinking water? *(O konori beek kasarta netian?)*

   a. One day   b. Two days   c. More than two days

18. Is drinking water containers covered in this household (*Observe***).** *(Tugotin beek en koiton?)*

         a. Yes   b. No

19. (i) Now I would like to see how you draw water from the container. (*Observe demonstration***).** *(Amache ager eleromdoi beek)*

   a. Dip into container   b. Pour directly from container / use tap

   (ii). While drawing water was there contact of the hands to water. (Observe**)** *(Ker ngo katiny   eut ngorome beek)*

      a. Yes   b. No

   (iii) Where do you place your water drawing (drinking**) utensils?** *(O ndai ano ki neoramen beek?)*

     a. Tables and/or shelves   b. Storage cover   c. Inside the
container   d. Hang on wall   e. Floor

 20. (i) Type of water storage container (*observe***)** *(Ne neindo beek.)*

   a. Clay pot   b. Plastic   c. Metal

(ii) Do you wash the water storage container? *(Kiune kit nekindo beek)* a. Yes   b. No

(iii) If Yes, how often? *(Ngot kiune ko ou?)* a. Regular   b. Irregularly   c. Never

SECTION C: FECAL MATTER DISPOSAL PRACTICES

21. (i) Do you have a toilet facility? *(Otinye ye kitoreten nge?)* a. Yes   b. No (**Skip to 22**).

(ii) If Yes, What kind of toilet facility do members of your household usually use? *If "flush" or "pour flush" probe:* **Where does it flush to?** *(Ngot komiten ko ainon?)*

a. Piped sewer system/septic tank /pit latrine   b. Unknown place/

c. Ventilated improved pit latrine   d. Pit latrine with slab

e. Pit latrine without slab/open pit   f. No facilities

(iii) Is the latrine clean *(Tililindo nebo kapchoo.)*

a. Clean (*no flies nor visible faecal matter*)   b. Moderate clean (*flies but no visible faecal matter*)   c. Dirty (*flies and visible faecal matter)*

22. If No, where do you usually go for your long call? *(Ngot ko mami toilet, otoreten ge ano?)*

a. Bush/field   b. indiscriminate disposal   c. Shared facility

23. If Q9 was Yes, how do you dispose of the feces of your child/children under 5 years? *(Ngot nambait 9 ko Eeh, ondoi ano chebo lagok?)*

- a. Leave it in the yard and do-nothing
- b. Bury it
- c. Put/rinsed into drain or ditch
- d. Put in the latrine
- e. Thrown into garbage

SECTION D: HYGIENE AND HAND WASHING PRACTICES

24. Do you normally wash your hands before drawing water? *(Ouni eunek kotomoram beek?)*

a. Yes   b. No

25. What do you <u>usually</u> use to wash your hands? (*Obaisien ne ouni eunek?*)

a. Water Only **b.** Water and Soap **c.** Water and ash or sand

26. Do you rinse your water drawing (drinking) utensils? *(Ouni kit neoramen beek?)*

a. Yes b. No

27. Where do you dispose your household garbage? *(Owirchini ano tagatagek?)*

a. Anywhere **b.** In garbage pit **c.** Behind house **d.** In the gutter **e.** Bury **f.** Burn **g.** In the River

28. (i) Do you know some diseases associated with drinking contaminated water? *(Tos ingen mianwogik che namege ak beek che kiye chesomis.)*

a. Yes b. No (If No, go to SECTION F)

(ii) If yes, name them; *(Ngot ko Eeh, mwa.)*

a. Typhoid b. Cholera c. Intestinal worms d. Diarrhea e. Round worms f. Eye diseases g. Amoebiasis

(iii) How can one prevent these diseases? *(Ki ter to ano mianwogik chu?)*

a. Treat water before drinking   b. Safe disposal of fecal

matter   c. Washing hands at appropriate times

d. Hygienic handling of food

SECTION E: INCIDENCE OF SANITATION AND HYGIENE RELATED DISEASES

29. (i) Has any of the members in your household suffered from any of the following ailments: Diarrhea, vomiting, fever (associated with diarrhea and vomiting) In the last three months**?** *(Mi chi nekikomian en mianwogik chu?)*

     a. Yes   b. No   c. Specify_____

(ii) If Yes, what do you think was the cause of the ailment? *(Ngot ko Eeh, tos ko nee ne kitau miondo*
noto?)_____

Section F: RESEARCH CO-ORDINATORS OBSERVATION OF SITE.

30. General hygiene of the of the house hold environment. *(Tililindo ab bomanito.)*

   a. Open litter   b. Stagnant water on the ground   c. Litter ground

   d. Dusty ground near   e. Presence of rubbish pit   f. Clean

31. The distance of water point(*ground water***) and the latrines.** *(Loindab ye kitachen beek ak kapchoo.)*

   a. Less than 10 m   b. Between 10-50 M   c. Between 50-100M   d. Over 100m

32. Is there any impermeable platform preventing any surface water into the well   especially during the rains? *(Mi ki ne tere beek che mi barak matkochut keringet ab beek?)*

       a. Yes   b. No

33. What activities are present around the water point   *(surface water) (Ne netesetai en yenegit ak ye kiramen beek?)*

a. Washing clothes   b. Watering of animals   c. Bathing

d. Farming   e. None

34. Is water for drinking stored in a separate container from water intended for other purposes? *(Kikonori beek che kiye ter ak che kiboisien en ko?)*

       a. Yes   b. No

35. Major complaints by the consumer *(Magutik ab biik.)*

_____

Appendix II: Informed Consent Form

Title of study: Characterization and factors associated with thermotolerant *Escherichia coli* contamination of source and household drinking water in Kericho District, Kenya

FOR QUESTIONS ABOUT THE STUDY, CONTACT: Johana K. Too (0721-273157) or William Sang (0720950385) both of the ITROMID and KEMRI.

Description: You are asked to participate in a research study to test whether the water that you use both stored at homes or from the sources that you collect from are contaminated with bacteria known as thermotolerant *Escherichia coli* which are known to cause diarrheal diseases. We would also like to find out what are the factors contributing to contamination of drinking waters in the two sources. This information would help us to provide interventions that will contribute in lowering and preventing sources of water contamination not only in Kericho District but the whole of Kenya. If you agree to participate, we will take water samples at your home as well as ask you to direct us where you routinely collect or obtain your drinking water. The water will be transported to KEMRI in Kericho for storage and testing.

Risks and benefits: One potential risk of being in the study is the loss of privacy. However, we will do our best to make sure that the personal information gathered during this study is kept private. Further some of the questions might make you uncomfortable. Other than this we do not see any other potential risk of this study to you. There is no monetary benefit for your participation in this study. The benefit which may reasonably be expected to result from this study includes your contributions to efforts to provide quality water to the Kericho community as well as knowing the factors contributing to water contamination. If your waters is found contaminated with this bacteria, every efforts will be made to inform you and advice you on how to decontaminate your water before consumption. Your decision whether or not to participate in this study will not affect your current benefits (if any) you get from KEMRI programs in the district.

Time involvement: This study will take about 30 minutes of your time.

Subject's rights: If you have read this form and have decided to participate in this project, please understand your participation is voluntary and you have the right to withdraw your consent or discontinue participating at any time without penalty. You have the right to refuse to answer particular questions. Your individual privacy will be maintained in all published and written data resulting from the study.

If you have questions about your rights as a study participant, or are dissatisfied at any time with any aspect of this study, you may contact - anonymously, if you wish – if you wish – The secretary, KEMRI Ethical Review Committee, PO Box 54840 – 00200 Nairobi, Kenya; Tel: 020-2722541, 0722205901, 0733400003; Email address: erc@kemri.org.

I have read this form or had it read to me in a language that I understand. I have discussed the information with study staff. My questions have been answered. My decision whether or not to take part in the study is voluntary. If I decide to join the study I may withdraw at any time. By signing this form I do not give up any rights that I have as a research participant.

_____   _____   _____

Participant Name   **Participant Signature/ Thumb print**   **Date**

_____   _____   _____

Study Staff Conducting   **Study Staff Signature**   **Date**

Appendix III: Laboratory Request Form

Location: ……………………………. Village Name: …………………………

Household No: …………………………… Questionnaire No: ………………………

Date Collected: …………………………..

Laboratory test requested

	HOUSEHOLD SAMPLES	SOURCE SAMPLES
1. Water Specimen		
2. Time Collected		
3. Temperature (ºC)		
4. PH Level		
5. Turbidity (T.U)		
6. Free Chlorine (mg/l)		

☐   Thermotoleran*t* coliform counts/*E coli*

Method used	sample type	results (cfu)
Membrane Lauryl Sulphate Broth – TTC	Household	
	Source	

☐   Enteropathogens

Method used	sample type	results
HPC media	Household	
	Source	

☐   Toxigenic *E. coli (t*ypical *E. coli)*

Appendix IV: Primers used in amplification of specific genes fragment in E. coli pathotypes

TARGET	FORWARD	REVERSE	BAND	REFERENCE
ETEC – LT	CACACGGAGCTCCTCAGTC	CCCCCAGCCTAGCTTAGTTT	508	Pass *et al.*, 2000
ETEC-ST	GCTAAACCAGTARGGTCT	CCCGGTACARGCAGGATTACAACA	147	Nguyen *et al.*, 2005
EHEC-Stx1	CAGTTAATGTGGTGGCGAAGG	CACCAGACAATGTAACCGCTG	348	MHM Nazmul, 2008
EHEC-Stx2	ATCCTATTCCCGGGAGTTTACG	GCGTCATCGTATACACAGGAGC	584	MHM Nazmul, 2008
EPEC-eae	CCCGAATTCGGCACAAGCATAAGC	CCCGGATCCGTCTCGCCAGTATTCG	881	Pons *et al.*, 2011
EPEC-bfpA	GGAAGTCCAATTCATGGGGGTAT	GGAATCAGACGCAGACTGGTAGT	300	Pons *et al.*, 2011
EIEC-IpaH	TGGAAAAACTCAGTGCCTCT	CCAGTCCGTAAATTCATTCT	423	Martha *et al.*, 2000
EAEC-aatA	CTGGCGAAAGACTGTATCAT	CAATGTATAGAAATCCGCTGTT	650	Schmidt *et al.*, 1995
EAEC-aaiC	ATTGTCCTCAGGCATTTCAC	ACGACACCCCTGATAAACAA	215	Pons *et al.*, 2011

key

LT –heat labile toxin

ST –heat stable toxin

Stx1- Shiga like toxin 1

Stx2 -Shiga like toxin 2

eae- enteropatognic attachment and effacement

bfpA –bundle forming pilus

IpaH-invasion plasmid antigen H

Appendix V: Sampling Procedures –Locations

A two-stage sampling method will be used as follows

A complete list of all the locations and the population was used (Kenya National Bureau of Statistics-KNBS, 2009). Simple random sampling based on probability proportionate to size (PPS) was used to select the number of locations to be sampled, there are 17 locations, and 12 locations were selected, with their respective number of villages to be sampled. A total of 17 villages were selected.

No	LOCATION	POPULATION	CUMULATIVE	SELECTED NO	NO OF VILLAGE
1	TOWNSHIP	55,801	55,801	16997, 27674, 38351, 49028, 59705	5
2	POIYWEK	7504	63305	70382	1
3	TENDWET	9265	72570		0
4	KAPSOIT	4148	76718	81059, 91736	2
5	AINMOI	16533	93251	102413	1
6	TELANET	9520	102771		0
7	KAPCHEBOR	7509	110280	113090	1
8	SITOTWET	3755	114035		0
9	KENEGUT	8022	122057	123767	1
10	CHEPKOINIK	2400	124457	134444	1
11	KAPSAOS	19856	144313	145121	1
12	KAPSEGUT	3098	147411		0
13	KAITUI	4620	152031	155798	1
14	KOITABUROT	10008	162039	166475	1
15	SOIN	11064	173103	177152	1
16	SOLIAT	5613	178716		0
17	KAPSOROK	2793	181509	**187829**	1
				TOTAL	**17**
Radom number 6,320 **Interval 10,677**					

SAMPLING OF HOUSEHOLDS

No	Location	No of villages	Village selected	No of household (6)	
5	Township	215	Chepyos Koita Chepnabe Kipmugen Mitikubwa	30	
1	Poiywek	19	Chepkoyo	6	
1	Kapsoit	12	Kapboswa	6	
1	Ainamoi	21	Cheplanget	6	
1	Kapchebor	14	Kooma	6	
1	Kenegut	15	Torsagek	6	
2	Chepkoinik	9	Chepsoo kapsisiywo	12	
1	Kapsaos	43	Mureret	6	
1	Kaitui	14	Kipboywo	6	
1	Kiotaburot	29	Kiotaburot	6	
1	Soin	24	Simbi	6	
1	kapsorok	9	Siswet	6	
17			**TOTAL**	**103**	

A list of all selected villages names was used; Simple random sampling was used to select villages to be sampled per location. In each village selected 6 households will be sampled. Simple random sampling was used to pick the first household, and then every fifth household will be systematically selected for sampling till 6th household is sampled (6x17=103 households)