ISOLATION AND MOLECULAR CHARACTERIZATION OF *Escherichia coli* FROM SELECTED DRINKING WATER SOURCES IN NJORO SUBCOUNTY, KENYA

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Egerton University

APRIL, 2017

DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been submitted or presented in any institution.

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This thesis has been submitted with our approval as supervisors for examination according to Egerton University regulations.

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DEDICATION

This research thesis is dedicated to my Mother Charity Karianyoni and Late father Joseph Ruciaka. Esteemed regards to my brothers and sisters for being perfect role models. I wish to recognize in a special way, Maria Teresa Gaetti and Francesca Moiana for their steadfast support, love, and encouragement throughout my studies.

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ABSTRACT

Contamination of drinking water sources remains a big challenge and many people lack access to safe potable water. In Njoro Sub-county the main water sources are rivers, man-made wells (protected and unprotected), boreholes, rainwater and springs (protected and unprotected). Although *Escherichia coli* (*E. coli*) is an indicator organism for fecal contamination of water, some *E. coli* strains have acquired the ability to cause intestinal (gastroenteritis) and extraintestinal diseases in humans. This study aimed at determining the Physico-chemical parameters and microbiological quality (using *E. coli* as indicator organism) in water used for drinking in Njoro Sub-county. Water samples were collected from drinking water sources and household storage containers. *E. coli* was isolated and quantified using the Compartmental Bag Test kit (CBT). The Physico-chemical properties were measured to determine water quality at points of collection and use, respectively. Characterization of pathogenic *E. coli* strains was done using a published multiplex-PCR protocol (mPCR). All data was imported into SAS 9.1 statistical software package for analysis. Numerical variables were summarized using arithmetic means and frequencies. The means were subjected to One way ANOVA and compared using Least Significant difference (LSD) at p= 0.05. Turbidity and electrical conductivity were above the WHO recommended levels in the water samples. *E. coli* was detected in 62.36% (n=111) of all the sampled drinking water in the sources and household storage containers. In total, 53.15% (n=59) of the *E. coli* positive samples were positive for pathogenic strains. However, only 38.98% (n=23) of the samples had different combinations of virulence strains. This indicated that the majority of drinking water were contaminated with the organic and inorganic matter at sources, during storage and handling and hence the need for frequent water quality monitoring and treatment to minimize contamination.

Keywords: Physico-chemical parameters, *E. coli*, Njoro, drinking water.

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LIST OF ABBREVIATIONS AND ACRONYMS

AAF	Aggregative Adherence Fimbriae
ARGs	Antibiotic Resistance Genes
BFP	Bundle-Forming Pili
BOD	Biological oxygen Demand
CBT	Compartmental Bag Test
CFU	Colony Forming units
CNF	Cytotoxic Necrotizing Factor
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
E. coli	*Escherichia coli*
EAEC	Enteroaggregative *Escherichia coli*
EC	Electrical Conductivity
EHEC	Enterohemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EMB	Eosin Methylene Blue
EPA	Environmental Protection Agency
EPEC	Enteropathogenic *Escherichia coli*
FC	Fecal coliforms
LEE	Locus of Enterocyte Effacement
LT	Heat Labile
mL	Milliliters
MPN	Most Probable Numbers
Nm	Nanometers
NTU	Nephelometric Turbidity Unit
PCA	Plate Count Agar
PCR	Polymerase Chain Reaction
SDWA	Safe Drinking Water Act
ST	Heat Stable
ST	Heat Stable
TC	Total coliforms
TCU	True Color Units

TDS	Total Dissolved Solids
TVCC	Total viable cell counts
UNEP	United Nations Environmental programme
UPEC	Uropathogenic *Escherichia coli*
USEPA	United States Environmental Protection Agency
UTI	Urinary Tract Infection
UV	Ultra Violet
VFs	Virulence Factors
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Water an important component to our physiology and anatomy because all cells and organs functions depend on it for their proper functioning (Soetan *et al.,* 2010). The quality of drinking water should be just as important as the quantity and thus should be clean and free of contaminants to ensure proper health (Hillie and Hlophe, 2007). However, population growth, urbanization, and the desire to have a better living impact fresh drinking water supplies resulting in increased health and environmental issues (Pruss-Ustun and Corvalan, 2007). It is estimated that by 2025, 40% of the world population will live in water scarce regions (Arnell, 2004). Already approximately 783 million people worldwide have no access to safe drinking water while an estimated 2.5 billion people lack access to sanitation, placing them at risk for waterborne illnesses like diarrhea (Bharti and Jalota, 2015).

One of the major causes of diarrheal diseases is consumption of microbe-contaminated drinking water. These diarrheal diseases weaken the immune system leading to higher risk of other diseases which present themselves as opportunistic infections (Agholi *et al.,* 2013). Since the majority of the available water systems used are not reliable, the communities are left without water for weeks and even months (WHO, 2011a). In Africa, most of the people in rural areas draw water from unsafe sources using containers and then transports it back to the households for use and even storage (WHO, 2006). While there are many etiological agents responsible for diarrhea, pathogenic *E. coli* is a major contributor. As a facultative anaerobe, *E. coli* is commonly used as a fecal indicator for the possible presence of other bacterial pathogens in water sources (Croxen *et al.,* 2013).

During rainfalls, snow melts, or other types of precipitation, *E. coli* may be washed into creeks, rivers, streams, lakes, or groundwater which is the major water sources for most communities (Vignesh *et al.,* 2013). Direct water fetching and use from water sources by local residents impact the water quality and increases microbial contamination. If water from these sources is not properly and frequently treated, it ends up causing illnesses to the consumers (Fick *et al.,* 2009). *E. coli* is a fecal coliform found in the gastrointestinal tract of healthy warm-blooded humans and animals hence present in fecal matter (Ishii and Sadowsky, 2008). Most *E. coli* strains are harmless and serve a useful function in the body by stopping the growth of harmful bacteria species and synthesizing necessary vitamins (Ho and

Hsu, 2003). Some strains, however, can be opportunistic pathogens and cause gastrointestinal illnesses in healthy humans when ingested (Rolfe, 2000).

Pathogenic *E. coli* strains cause a broad range of human diseases that span from the intestinal tract to extra-intestinal sites such as the urinary tract and central nervous system and are serologically classified into various pathogenic groups according to their virulence determinants (Kozub-Witkowski *et al.,* 2008). These determinants confer each group with the ability to present clinical syndromes with distinct pathologic characteristics and subsequent diarrhea. Enterotoxigenic *E. coli* (ETEC) for example, may cause watery diarrhea in children in developing countries (Qadri *et al.,* 2005). Enterohemorrhagic *E. coli* (EHEC) may cause hemorrhagic colitis and hemolytic uremic syndrome because of the production of Shiga toxins, while Enteropathogenic *E. coli* (EPEC) causes nonspecific gastroenteritis, especially in children (Nguyen *et al.,* 2006). Fecal contaminants and specifically *E. coli* are responsible for acute diarrhea in Kenya and other developing countries and account high proportion of enteritis in children <5 years with significant morbidity and mortality. High frequency of diarrheal episodes in children leads to decreased ability of the intestine to absorb nutrients (malnutrition) which have even more implications such as stunting and decreased intelligence (Mondal *et al.,* 2012). In many developing countries, it is a common practice to dump untreated sewage into lakes, rivers, and streams that people use for drinking. In addition, these sources are further contaminated by people washing and bathing in the same water used as a drinking source.

Studies to determine the prevalence of persistent diarrhea in children aged 3 to 36 months at the Kenyatta National Hospital in Kenya found that there was a fatality rate of 13.6 % (Mbori-Ngacha *et al.,* 1995). Similarly, the high prevalence of undiagnosed diarrhea at the Njoro Health Centre and Nakuru Provincial General Hospital (NPGH) in Kenya was closely linked to consumption of pathogen-polluted waters (NPGH, 2009). Generally, having access to safe drinking water is a major factor in preventing deaths and improving the quality of life for low-income households around the world. Water quality is thus not frequently tested because the tests that are available either do not give quantitative results, are not designed for populations to self-test, and/or are priced beyond what people can afford (Heitzinger *et al.,* 2015). As a result, the majority of the people drink water which is fecally contaminated as millions continue to get sick and die from unsafe water. On the other hand, in-vitro assays such as bacterial cell culture and cytotoxicity for the identification of virulent strains are expensive, time-consuming and require expertise which may be lacking in developing countries.

The ability to provide a reliable, simple and inexpensive test in limited resource settings may reduce the consumption of unsafe drinking water, thereby reducing the risk of waterborne disease. The Compartmental Bag Test (CBT) is a new test made available to the water testing world and has several advantages that make it the ideal water quality test kit available in the market today (Stauber *et al.*, 2014). The CBT kit is portable since it is compact and light weight, it is convenient to use since it operates at variable ambient temperatures from 25°C and above, simple to use by anyone with little training and does not require a laboratory. The test kit has not been extensively tested in the field yet and therefore this study used this test in Kenya to assess the drinking water quality in water sources and household storage containers.

1.2 Statement of the problem

The commonly used microbial water quality tests include the multiple tube fermentation, Colilert Quantitray test, and the membrane filtration techniques. However, these tests are all expensive, time-consuming, employ specialized laboratory equipment and require technical expertise. The solution needed is to have a reliable and fast, simple to use and inexpensive test available which can be used by any person to determine drinking water quality without the need of expensive equipment. The CBT kit had been used in other countries in the USA as well as Tanzania but it has not yet been tested in Kenya. Previous studies have been done on the microbial contamination in River Njoro, others on the fluoride levels of stored water in Njoro division and another one on the microbial quality of water in households of various income levels in Njoro town (which is located in Njoro location). However, no study has done an extensive analysis of drinking water from the other areas in the Njoro Sub-County or used CBT as an assessment tool to target the presence of *E. coli* genes.

Thus this study aimed at assessing the use of the CBT to determine the presence of *E. coli* in water that is used for household consumption. Molecular characterization of the *E. coli* strains was done on positive CBT samples in order to determine the presence of pathogenic strain(s) circulating in the communities. The data can be used to assess the potential health risk in the fight against diarrhea.

1.3 Objectives

1.3.1 Main objective

The main objective of the study was to isolate and characterize *Escherichia coli* strains from drinking water sources in the Njoro Sub-County, Kenya.

1.3.2 Specific objectives

i. To determine the physical-chemical quality of drinking water from drinking water sources and water in household storage containers in Njoro Sub-County, Kenya.
ii. To identify and quantify *E. coli* from drinking water sources and water in household storage containers using the commercially available Compartmental Bag Test (CBT) kit in Njoro Sub-County, Kenya.
iii. To characterize pathogenic *E. coli* strains from drinking water sources and water in household storage containers in Njoro Sub-County, Kenya using a published mPCR protocol.

1.4 Hypotheses

i. There are no variations in the physical and chemical determinants from drinking water in Njoro Sub-County, Kenya.
ii. There are no significant levels of *E. coli* in drinking water in Njoro Sub-County, Kenya.
iii. There are no various *E. coli* pathotypes present in drinking water in Njoro Sub-County, Kenya, which could be considered a health risk to vulnerable community members.

1.5 Justification of the study

The WHO states that drinking water should either be free of fecal bacterial contamination or if it is there, it should be within the set tolerated levels. Most drinking water sources in Njoro Sub-County are harvested rainwater, boreholes, springs (protected and unprotected), wells (protected and unprotected), dams and rivers. These sources are subject to fecal contamination from sewage leakages, agricultural run-offs, and livestock wastes. This is due to unavailability of a basic water service and poor maintenance and operation in areas where the service is available. WHO guidelines for drinking water quality encourage water testing on a regular basis to verify its quality. In contrast, in developing countries authorities do not perform frequent water analysis at water points due to lack of affordable microbial tests. The CBT kit is portable and can be used anywhere and by anybody including non-

educated people at their homes and it does not require electricity or any special equipment. In most developing countries, differentiation of pathogenic *E. coli* from non-pathogenic normal flora is achieved by identification of the surface O-antigen and molecular typing is mostly done on fecal samples to determine the bacterial strains. However, the O-antigen method is often inconclusive as many strains do not belong to known O-serotypes and are non-reactive with O-antisera. In vitro assays such as cell culture and cytotoxicity for the identification of virulent strains are expensive, time-consuming, require expertise and expensive equipment which may be lacking in developing countries such as Kenya. The presence of any *E. coli* strain in a drinking water sources is in itself a concern because it indicates fecal pollution or poor hygiene practices. For health risk assessment it is necessary to determine the type of *E. coli* strain present in the water sources. Several mPCR protocols have been published and when access is available to a laboratory where molecular characterization is done, and then it is of utmost importance to determine the strain specificity (Abbasi *et al.,* 2015). The results from this study will provide information needed in diarrheal treatment in Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 General introduction

The major pathogens of significance to untreated water are bacteria, viruses, fungi, and protozoa. Bacteria, which are the most common pathogens in water, gain entrance into water commonly through fecal contamination (Cabral, 2010). Pathogenic bacteria are normally absent from a healthy intestine but in the case of an infection, large numbers of them are passed in the feces (Ramaswamy *et al.,* 2007). Some of the bacteria pathogens found in polluted water include Salmonella spp, Shigella spp, pathogenic strains of *E. coli*, Yersinia spp, Campylobacter spp and Vibrio spp (Harnisz, 2013). Cholera is caused by Vibrio cholera and transmitted through consumption of contaminated water. It is characterized by an acute and very intense diarrhea that can exceed one liter per hour. Salmonella spp causes Salmonellosis which in turn causes typhoid and paratyphoid fever, which are associated with ingestion of fecal-contaminated water (Ramirez-Castillo *et al.*, 2015). Shigella spp cause shigellosis in human and transmission through water is favored by their high surviving nature. In the epithelial cells of the intestinal tract of humans, Shigella spp produces a high level of cytotoxic Shiga toxin resulting in fever, anorexia, fatigue and malaise (Emch and Yunus, 2008). Pathogenic *E. coli* strains cause intestinal and extra-intestinal diseases in humans especially in children below 5 years of age in developing countries (Munshi *et al.,* 2012).

Globally, more than 5,000 children die daily as a result of diarrheal diseases and 78% of the deaths occur in the African and South-East Asian regions (Farthing *et al.,* 2013). Only fifteen countries, including Kenya, contribute to almost three-quarters of the total diarrhea cases worldwide (Walker *et al.,* 2013). Mortality in children is common in those under 5 years of age, immunosuppressed and the elderly people because they have a weak immune system (Walker *et al.,* 2012). The Millennium Development Goal (MDG) Section 7c call for sustainable access to safe water and basic sanitation throughout the world was targeted to reduce by half, the number of people without access to safe drinking water by the year 2015 (WHO, 2012). Previous reports indicate that the proportion of the Kenyan population who had access to safe drinking water sources and sanitation was 59% and 31% respectively (WHO, 2011b). On the other hand, Kenya has been ranked among the top ten countries without enough and reliably improved drinking water sources (United Nations Children's Emergency Fund and WHO, 2012).

Reports by the United Nations Environmental Programme (UNEP) predicted that Kenya was unlikely to attain the MDG goals. This is because the country's population with access to improved water source and sanitation was 41% and 39% during the 1990s and was targeted to be 82% and 78% respectively in the 2000s. However, the country only managed to attain just 57% and 42% in the year 2008 (UNEP, 2009). Upon the realization of the inability to achieve the MDG targets, the government of Kenya shifted its goals further to the vision 2030 program.

2.2 *E. coli* is a waterborne pathogenic bacteria

E. coli is a rod-shaped Gram-negative facultative anaerobe bacteria in the family Enterobacteriaceae and moves via peritrichous flagella (Sinha and Pandey, 2011). It has a circular genome with 4.6 kilobase pair sequence and 4,288 protein-coding genes annotated, 38% of which have no attributed functions (Blattner *et al.,* 1997). Its biochemical characteristics are: lactose fermentation, possession of lysine decarboxylase, Vogues-Proskauer negative, production of indole, doesn't grow on nitrate, and doesn't produce hydrogen sulfide (Paul *et al.,* 2010). *E. coli* typically colonizes the gastrointestinal tract of human infants within a few hours after birth and its human host coexists in good health and with mutual benefit. The commensal *E. coli* strains rarely cause disease except in immunocompromised hosts or where the normal gastrointestinal barriers are breached for example in peritonitis (Vaishnavi, 2013). The niche of commensal *E. coli* is the mucous layer of the mammalian colon (Tenaillon *et al.,* 2010). Several highly adapted *E. coli* clones have acquired specific virulence attributes, which confers an increased ability to adapt to new niches and cause a broad spectrum of diseases in humans (Wiedenbeck and Cohan, 2011). Therefore, the presence of genes encoding for virulence attributes can be used to distinguish these pathogens from the nonpathogenic or commensal *E. coli*.

Virulence factors (VFs) are frequently encoded on genetic elements that can be mobilized into different strains to create novel combinations of VFs (Reisner *et al.,* 2006). Only the most successful combinations of VFs have persisted to become specific pathotypes of *E. coli* that are capable of causing disease in healthy individuals (Donnenberg and Whittam, 2001). Three general clinical syndromes can result from infection with pathogenic *E. coli* strains: enteric/diarrhoeal disease, urinary tract infection and sepsis/meningitis (Nataro and Kaper, 1998). Enteropathogenic *E. coli* (EPEC) does not produce toxins but have in common the locus of enterocyte effacement (LEE), a pathogenicity island that promotes the development of attaching and effacing lesions (Kaper *et al.,* 2004). The LEE Island encompasses the *eae* gene that encodes intimin, an outer membrane adhesin fundamental to

the establishment of attaching, and *esp* which codes for secreted proteins and effacing lesions (Lara-Ochoa *et al.,* 2010). These genes are characterized by intimate adherence of bacteria to the intestinal epithelium causing a disruption of the cell surface leading to the effacement of microvilli (Chen and Frankel, 2005). Low-grade fever, vomiting, and bloody diarrhea are associated with attachment and an acute tissue destructive process (Navaneethan and Giannella, 2008).

EPEC adherence factor *bfp* (codes for bundle-forming pili) is responsible for the localized virulence and auto-aggression mechanisms upon infection (Bakhshi *et al.,* 2013). EPEC strains that form *bfp* are referred to as typical EPEC and are coded on *bfp* plasmids while those that do not are termed atypical EPEC and are coded by *eae* genes (Hernandes *et al.,* 2009). Enterotoxigenic *E. coli* (ETEC) is a diverse pathotype which is a major cause of traveler's diarrhea (three or more unformed stools passed by a traveler within a 24-hour period) and endemic in most underdeveloped countries (Okoh and Osode, 2008). ETEC is highest during the warm season, suggesting that travelers are more vulnerable to the diarrheal illnesses at these times (Hill and Beeching, 2010). Infection with ETEC leads to watery diarrhea which lasts up to a week, abdominal cramps, sometimes with nausea and headache (Fleckenstein *et al.,* 2010). ETEC adheres to the epithelium of the small intestines via one or more colonization factor antigens and expresses one or more heat-stable (ST) or heat labile (LT) enterotoxins (Johnson *et al.,* 2009). These enterotoxins cause inhibition of sodium absorption and stimulation of chloride secretion, which leads to watery diarrhea and loss of electrolytes (Dubreuil, 2012). Distinct groups of ST enterotoxins are the STa and STb encoded on plasmids, and LTI and LTII for LT encoded on the chromosome.

Enterohaemorrhagic *E. coli* (EHEC) excretes potent toxins called verotoxins or Shiga-like toxins which cause hemolytic-uremic syndrome (Proulx *et al.,* 2001). Diarrhea may range from mild and non-bloody to bloody stools with no leukocytes (Tarr *et al.,* 2005). Children have a higher risk for serious infection from non-O157 strains and screening for Shiga toxin rather than the O157 antigen is the most preferred (Pennington, 2010). Transmission may also occur by ingestion of contaminated food or water and treatment involves intravenous fluid replacement and supportive care. Enteroinvasive *E. coli* (EIEC) infection results in shigellosis-like symptoms in both adults and children and its characterized by the appearance of blood and mucus in the stools of infected individuals (Pereira and Giugliano, 2013). They induce their entry into epithelial cells of the intestine and disseminate from cell to cell causing a mild form of dysentery. Infection commences in the colon, where the bacteria passes through microfold cells by transcytosis to reach the underlying submucosa

(Croxen *et al.,* 2013). Genes required for entry of EIEC into host cells are clustered on a 220kb virulence-associated plasmid which is also found in Shigella spp (Abbasi *et al.,* 2015). Expression of several plasmid-encoded proteins is required for the complete virulence phenotype of EIEC. These invasion plasmid antigens (*Ipa*) proteins are encoded in the *ipa* operon (Gibotti *et al.,* 2004). Thus, all tests applied for the determination of the virulence of Shigella species are as well suitable for testing EIEC virulence.

Enteroaggregative *E. coli* (EAEC) are associated with acute watery mucoid diarrhea, intestinal inflammation (presence of inflammatory markers in the stool) and low-grade to no fever (Kaur *et al.,* 2010). Participation of EAEC as the causative agent of diarrheal diseases in human immunodeficiency virus-infected adults in the developed world has also been described (Medina *et al.,* 2010). EAEC possess hemagglutinating activity, indicative of their adhesive properties to the intestinal mucosa surfaces in a "stacked, brick-like" manner. Aggregative adherence in EAEC is mediated by either aggregative adherence fimbriae I or II, which are encoded for by *aggR* genes (Bernier *et al.,* 2002). EAEC produce an enteroaggregative heat-stable toxin encoded on a plasmid by *astA* genes. Neonatal meningitis causing *E. coli* (NMCEC) type K1 is the most common cause of meningitis in premature infants and is the second common agent in full-term neonates. This results in inflammation of the meninges of the brain and spinal cord in children (Bonacorsi and Bingen, 2005). The prognosis of meningitis is difficult until the bacteria reaches the cerebrospinal fluid, by which proinflammatory cytokines are circulating in the blood and the progression of brain damage has begun (Dubois *et al.,* 2009).

Uropathogenic *E. coli* (UPEC) are a major cause of urinary tract infection (UTI) and accounts for very many of the community-acquired cases as well as hospital-acquired infections (Lau *et al.,* 2008). UTI due to *E. coli* can progress to bacteremia, which is associated with significant mortality. Fecal bacteria colonize the urethra and spread up to the urinary tract and finally to the urinary bladder. UPEC possess several VFs necessary for persistence and colonization of the bacteria in the urinary tract, overcome host defenses, and cause extra intestinal disease (Johnson and Russo, 2005). The VFs are fimbrial adhesions, (P, type 1, S, and F1C fimbriae), afimbrial adhesin, toxins (hemolysin and cytotoxic necrotizing factor), siderophores (aerobactin system), and capsular polysaccharides (Tiba *et al.,* 2008). For UTI initiation, colonization followed by expression of the P-fimbriae in patients with acute pyelonephritis, cystitis and a normal immune system is crucial (Hannan *et al.,* 2012). Then, G adhesin, located at the tip of P-fimbriae and encoded by the pap operon, contributes to the progress of the disease by binding to specific receptors in the urinary tract.

Uropathogenic *E. coli* produce alpha and beta-hemolysins, which cause lysis of urinary tract cells (Giray *et al.,* 2012).

2.3 Water quality in Kenya

Kenya is generally a dry country since 80% of it is arid and semi-arid. The average rainfall is 630 mm with less than 200 mm in Northern Kenya and over 1,800 mm on the slopes of Mt. Kenya (Monteiro *et al.,* 2010). Access to clean and safe drinking water is critical to the economic development in Kenya. Many people in the urban and rural parts of Kenya lack access to potable water mostly due to recurrent droughts, poor management of water sources, misuse of water and the arid and semi-arid climate of some regions (Kalungu *et al.,* 2014). In other cases, water shortage occurs due to low investment in management of water sources in rural areas by the government. In 2006 Kenya's rural population had a much lower access rate to clean water than the urban population, with 49 % and 85 % respectively. However, there has been an overall progress made in the percentage of people having access to clean water with an increase from 41 % of the total population in 1990 to 57 % of the total population in 2006 (Marshall, 2011).

Forest degradation is the leading causes of drought and hence the shortage of rain, surface and underground water (Beresford *et al.,* 2013). The largest forest in Kenya, the Mau forest, which distributes water to six lakes as well as eight wildlife reserves, while about ten million people depend on its rivers for a living, has had about 400 000 (a quarter) hectares destroyed (Baldyga *et al.,* 2008). This has resulted in increased runoff, which has negative implications in both the rainy as well as the subsequent dry seasons and hence insufficient water sources. Analysis of water from the Nairobi and Athi Rivers which traverses along Nairobi city with a population of more than 3 million people and across most informal settlements was done by Musyoki *et al*., (2013). Although these rivers receive effluents from Nairobi water treatment, this water is mainly used for irrigation and drinking purposes by downstream communities. Results found that the water was contaminated with *E. coli* (1.0 x104 CFU/100mL) and Shigella flexneri (1.2x101 CFU/100mL) respectively.

Due to rapid urbanization, Njoro Sub-County has experienced rapid population growth with many residential houses being constructed at a high rate. This has led to a generation of more industrial, household, fecal and agricultural wastes which find their way into water bodies especially during rains (Mainuri and Owino, 2013). As a result, the Kenyan government has adopted various strategies such as rehabilitation and protection of indigenous

forest covers, increased water harvesting and storage as well as the adoption of water resources and management systems.

2.4 Water quality standards

There are guidelines provided for a wide array of physical-chemical and microbial contaminants commonly found in drinking water. These guidelines provide a framework for achieving safe drinking water by the implementation of health-based targets, creation of water safety plan, and maintenance of water surveillance (WHO, 2006). In Kenya, the quality of drinking water and wastewater management is controlled by the Kenya Bureau of Standards (KEBS, 2010). Table 2.1 provides an overview of the criteria that must be used to evaluate drinking water quality based on the two regulatory bodies. The pH is important in the effectiveness of disinfection and impacts corrosion of pipes. A pH of less than 8.0 is most effective for chlorine disinfection, but pH less than 7 has a higher likelihood of being corrosive, although alkalinity and calcium content also influence corrosivity. The total dissolved solids (TDS) constitute the dissolved inorganic anions and cations in water and include magnesium, calcium, sodium, potassium, sulfates and bicarbonates among others. They come from sewage, runoffs, chemicals used in water treatment and nature of the materials used in the piping systems of drinking water (Ahmad and Chand, 2015). In other cases, TDS can originate from carbonate deposits, mineral springs and leakage of salty water from the lakes and ocean and into fresh water sources.

Water temperature is the degree of coldness or hotness which in turn affects the rate at which chemicals such as metals dissolve in water. More turbid water may have a higher temperature because the suspended and dissolved materials can absorb heat from the sun (Perlman, 2014). Higher water temperatures are less pleasing to consumers and warm water encourages microorganism growth. Dissolved oxygen also forms an important aspect of the water bodies like fish for respiration. When this dissolved oxygen is depleted to levels below 5 mg/L (Table 2.1), most of the water bodies will die (Zhang *et al.,* 2015). This drop occurs as a result of sewerage leakage, runoff from fertilizers and inorganic wastes from industrial and domestic activities. A survey carried out on the quality of surface water in Githurai are in Nairobi city revealed that Biological Oxygen Demand (BOD) and TDS were above the recommended values and about 30-40 % of the interviewed patients visiting the hospitals were suffering from of diarrhea (Kaluli *et al.,* 2012). BOD refers to the amount of oxygen that is required to break down organic matter in water through aerobic processes by microorganisms. This way, the bioconvertability of a proportion of organic matter in a water

sample via oxidation can be determined (Razif and Persada, 2015). The water is, therefore, able to perform a self-cleaning by converting organic matter into carbon dioxide and inorganic ions which are released in water or are recycled. Therefore, a high BOD indicates that the water is highly polluted while a low BOD indicates that water is of good quality (Bhuiyan *et al.,* 2010).

Nitrates get into the soil through the application of nitrogen-containing fertilizers. Above the recommended limits, nitrates are very hazardous to expectant mothers and infants especially when nitrates are converted to nitrites in the stomach leading to the development of blue babies (Knobeloch *et al.,* 2000). Fluoride is a mineral found in the earth crust and can find its way into underground drinking water sources (Maheshwari, 2006). In low concentrations of 1 mg/L, fluoride is added to drinking water via the process of fluoridation which in turn strengthens the enamel and prevents it from corrosion by microbial acids. However, in levels above 1.5 mg/L, fluoride causes dental fluorosis characterized by brown teeth whereby fluoride replaces the hydroxyl group on the enamel with the hydroxyapatite (Munoz *et al.,* 2013). Dental fluorosis is a major concern in Njoro Sub-County which falls under the Great East African Rift valley where underground fluoride is in high quantities (Moturi, 2002). Dissolved iron is in the earth crust and exists as both soluble (ferrous) and insoluble (ferric) forms. When water containing ferrous iron is exposed to air, it forms an insoluble precipitate of ferric iron (Wendt *et al.,* 2016). As such, the water appears brownish in color thus affecting its aesthetic properties as well as staining linens. Iron can also harbor some bacteria which form slime in the water piping systems leading illnesses to the users. Dissolved manganese just like iron occurs naturally in the ground rocks and can come into contact with drinking water. Water contaminated with manganese stains the fabrics and alters its aesthetic properties. Since manganese dissolves slowly in water, it requires a longer period of time to remove than iron. In very high concentrations, manganese affects the nervous system and causes diseases with similar symptoms to Parkinson's disease (Aschner *et al.,* 2009).

Turbidity is a measure of light transmission through water which is influenced by the organic and inorganic particles suspended in the water (Juntunen *et al.,* 2013). It is measured is the Nephelometric Turbidity Unit (NTU). Turbidity indicates microbial contamination as microorganisms prefer to attach to these particles. During the treatment process, turbidity is lowered through coagulation, sedimentation and, filtration prior to disinfection makes the disinfection much more efficient as the pathogens attached to particles are removed (Pichler *et al.,* 2012). Increase in turbidity during distribution is indicative of biofilms inside pipes or

of outside contamination entering pipes. Electrical conductivity is a measure of the capacity of water to carry an electric charge and indicates the amount of TDS. EC values higher than 0.05 µS/cm (Table 2.1) makes drinking water significantly and increasingly unpalatable. Since there is a wide range of pathogens present in untreated water, microbial analysis of water depends on detection of the presence of indicator organisms (Girones *et al.*, 2010). Bacteria indicators are the most important indicators of fecal contamination and they include members of the Enterobacteriaceae family i.e. total coliforms (TC) and fecal coliforms (FC). FC are widely used as indicators due to their continuous association in fecal wastes of both humans and animals (Arthurson, 2008). TC includes a wide range of bacteria such as *E. coli*, Citrobacter, Klebsiella, and Enterobacter. Of the FC group, *E. coli* is the most numerous in mammalian feces, hence is considered the most specific indicator of fecal pollution (National Health and Medical Research Council, 2003).

The *E. coli* test confirms presumptive fecal coliforms by testing for the presence of the β-D-glucuronidase enzyme which is selective for the *E. coli* organism. This separates them from non-fecal thermo-tolerant coliforms although both classes are positive for the enzyme β- D-galactosidase (Odonkor and Ampofo, 2013). The features for these non-pathogen fecal indicators are: universal presence in human and animal feces, does not multiply in water, behaves and responds to treatment similarly to fecal pathogens, and can be easily measured (Gruber *et al.*, 2014). Although they do not meet all the criteria for being fecal indicators, TC can also be measured to monitor the cleanliness of the distribution system and to indicate a level of disinfection. The guideline value presented by the WHO for *E. coli* is that it "must not be detectable in any 100mL sample." The total viable cells counts (TVCC) include the mold, fungi, and bacteria which require carbon for their growth. Once obtained through culture methods, these microorganisms consist of the natural microbiota of water as well as those derived from diverse sources of pollutants.

Table 2. 1: Drinking water guidelines by WHO (2011) and KEBS (2010).

Parameter	WHO	KEBS
pH	6.5-8.5	6.5 – 8.5
Total dissolved solids	600 mg/L	1200 mg/L
Temperature	Not specified	Not specified
Nitrate-NO_3	50 mg/L	10 mg/L
Fluoride	1.5 mg/L	1.5 mg/L
Biochemical Oxygen Demand	1-6 mg/L	30 mg/L
Iron	0.3 mg/L	0.3 mg/L
Manganese	0.4 mg/L	0.1mg/L
Turbidity	5 NTU	5 NTU
Dissolved oxygen	4 mg/L	8 mg/L
Electrical conductivity	0.05 µS/cm	0.05 µS/cm
Total coliforms	Not specified	30 CFU/100mL
Fecal coliforms	0 CFU/100mL	0 CFU/100mL
E. coli	0 CFU/100 mL	0 CFU/100 mL
Total viable cell counts	-	500 CFU/1mL

2.5 Microbial water quality tests currently used

2.5.1 The membrane filtration (MF) method

The (MF) (MF) test (Figure 2.1) enumerates *E. coli* colonies on agar medium within 24 hours as required by Environmental Protection Agency (US EPA) (US EPA, 2002). It involves filtering 100mL of sample water through a 0.45µm pore size membrane filter using a filter funnel and a vacuum pressure pump. The vacuum suction pulls the sample water through the membrane, leaving *E. coli* bacteria evenly distributed across the membrane filter surface. The membrane filter is then transferred from the filter funnel to an agar medium using sterile forceps (Watkins and Sartory, 2015). The agar media used are differential and selective for the growth of indicator and other coliform bacteria. The agar medium plate containing the membrane filter is then incubated at 44.5˚C for 24 hours (Hannan *et al.*, 2010). The advantages of this method are: the indicator organisms are trapped on or within the filter paper and the ease of enumeration of distinct colonies of bacteria on the grids of the filter paper. The disadvantages of this method are that it requires expensive laboratory equipment

such as the filter assembly, a source of vacuum and electricity in order to carry out the analysis. Moreover, highly turbid waters can block the membrane filters while the filters themselves are expensive to buy.

Figure 2. 1: Membrane filtration unit.

2.5.2 The multiple tube fermentation (MTF)

The MTF technique gives the most probable number (MPN) estimation of *E. coli* concentration in water samples (Kimani-Murage and Ngindu, 2007). It utilizes differential and selective liquid broth media and multiple sample volumes that are scored as positive or negative. The scores are distinctive of *E. coli* growth such as the appearance of fluorescence under long ultraviolet (UV) light using fluorogenic 4-methylumbelliferyl-ß-D-glucuronide substrate. This method uses several culture tubes (Figure 2.2) requiring precise measurements using pipets, racks to hold the culture tubes, and other sterile laboratory equipment (Wohlsen *et al.,* 2006). Positive results take 48 hours and have lower precision than methods based on enumerating colonies, such as membrane filtration (Maheux *et al.,* 2008).

Advantages of MTF are its higher sensitivity than MF and it is ideal for turbid water samples. Disadvantages of this method are that it requires preparation of sterile broth culture media usually by autoclaving, trained personnel, electricity, and expensive laboratory equipment, and time consuming.

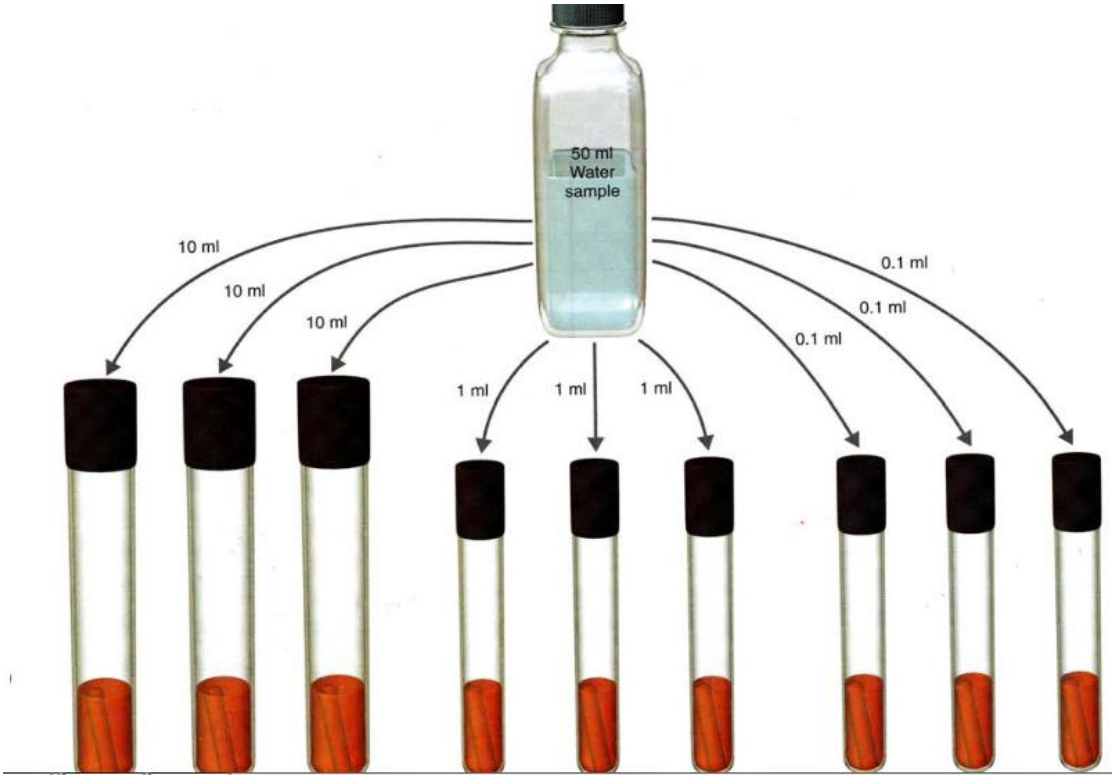

Figure 2. 2: Multiple tube fermentation.

2.5.3 Colilert-Quantitray method

The IDEXX Colilert Quanti-Tray 2000 method is widely used for quantifying *E. coli* in water samples as MPN (Chao *et al.*, 2004). It uses multi-well trays (IDEXX Quanti-trays), 100 mL bottles, a chromogenic and fluorogenic medium (Colilert), and an IDEXX Quanti-tray sealer, all of which are expensive and require an electrical power source. Water samples are poured into specific bottles in 100mL volumes and the Colilert defined substrate reagent medium is added and shaken until dissolved. The sample is then poured into wells, sealed and incubated at 44.5 ˚C for 24 hours. The results for *E. coli* are read as fluorescence in the wells of the tray using UV light. *E. coli* concentration is expressed as an MPN value per 100mL sample based on the number of positive and negative wells. This method is more efficient than the traditional culture methods in terms of time consumption. However, there can be

interferences due to presence of other bacteria in the sample and possibility of false positives in case the enzyme is present in other organisms.

Figure 2. 3: Colilert Quantitray method.

2.5.4 Compartment Bag Test (CBT)

The CBT test detects and quantifies *E. coli* based on the MPN (Stauber *et al.,* 2014). The components of the compartment bag test are: a clear, sterile, disposable plastic bag, dry chromogenic substrate culture media, a reusable plastic clip, chlorine tablets and a water collection container. The test involves mixing the chromogenic medium with 100mL of the water sample, pouring it into the compartment bag, and gently squeezing the bag to distribute the correct volumes into the designated compartments (Weiss *et al.,* 2016). The bag is then sealed with a reusable plastic clip and incubated at 35˚C for approximately 24 hours. *E. coli* is detected by production of a blue-green color change in the water sample due to the utilization of the specific substrate in the medium (Heitzinger *et al.,* 2015). Unlike many other drinking water quality tests, the compartment bag test does not require the use of electricity. Advantages of CBT are its portability, operation at variable ambient temperatures from 25°C, simplicity of use by anyone and it does not need laboratories, expensive incubators.

Figure 2. 4: A compartmental bag test kit.

2.6 Summary of Literature Review

Since the 1970s, the United States Environmental Protection Agency (USEPA) has been apprehensive about the quality of drinking water in all countries. This can be confirmed by the introduction of the Clean Water Act, which necessitates both regulatory and non-regulatory means of action for point and nonpoint source of pollution to surface waters. Safe Drinking Water Act amendments oblige relevant authorities to keep watch of the quality of the country's waters. Moreover, people are put at risk through drinking contaminated water by eating food prepared with utensils washed with contaminated water, bathing and washing in unhygienic water. Improving access to clean water and sanitation is an effective way of improving public health and saving lives. Previous studies have been done on bacterial contamination in the River Njoro (Kiruki *et al.,* 2011) and another on microbial contamination of water in households of Njoro division (Macharia *et al.,* 2015). However, no information exists on the quality of drinking water from the other four locations, at sources and households in Njoro Sub-County using *E. coli* as an indicator organism. Moreover, no studies have yet isolated and characterized *E. coli* strains to use as a measure of health risk. As such, this study determined the safety of drinking water from sources and household

storage containers in Njoro Sub-County by isolating and identifying pathogenic *E. coli* strains in drinking water.

CHAPTER THREE

STUDY DESIGN AND SAMPLE COLLECTION IN NJORO SUB-COUNTY, KENYA

3.1 Study Area

This study was carried out in Njoro Sub-County, Kenya. Njoro is located at 0°19'60" S and 35°55'60" E, at an elevation of from 1 600 to 2 000 meters above sea level and 20 km Southwest of Nakuru town, in the Kenyan Rift Valley Province, Kenya National Bureau of Statistics (KNBS, 2013). The total annual rainfall ranges from 500 mm in the lowlands to 1800 mm in the highlands falling during two seasons; the long rains from March to April, and the short rains from October to December and it is classified as semi-arid area. River Njoro is the major source of water but its volume reduces during dry seasons (Figure 3.1). Lake Nakuru which is the nearest lake from Njoro Sub-County is a salty lake leaving the residents to rely on ground water as their main source of water. Njoro Sub-County is divided into five administrative locations namely: Njoro, Lare, Kihingo, Maunarok and Mauche (Figure 3.1) and the total population in Njoro Sub-County is 188,124 (KNBS, 2009). The mainstay of the economy in this area are agri-based industries including vegetable and milk processing, large-scale maize, wheat and barley farming, light manufacturing industries such as timber milling, canning, and quarrying (Kiruki *et al.,* 2011).

3.2 Study design and sample collection

Simple random sampling design was used to select the participating villages and water was collected at intervals of ten homesteads. There was no definite criterion used to determine the number of each type of source or containers. Therefore any water source as well as domestic storage container (having drinking) water found within the villages and the visited homesteads respectively were sampled. The sample size of this study was calculated using the formula n=z2p.q/d2, where n=desired sample size; z=standard normal deviation at 1.96 (obtained from a 2 tailed normal table); q=1-p; d2 =0.052 and p=prevalence of the condition under study (SD prevalence of fecal coliforms contamination of drinking water in the Shabab area of Nakuru district was 0.12) (Kiruki *et al.,* 2013). Each of the samples was replicated three times during sample collection. Water samples from five locations of Njoro Sub-County were collected from the river Njoro, springs, water vendor kiosks, household storage containers, taps, wells and boreholes. Sterile 500 mL plastic bottles were used for sample collection. Wells were purged for at least 3 minutes to flush out any standing water

from the bottom while taps were allowed to run continuously for two minutes before sampling. The sample bottle was then rinsed with the water sample thrice before taking the sample. Samples were collected directly into the sample container to minimize contamination and transported immediately on ice to Egerton University Limnology laboratories for further analysis within six hours of sample collection.

3.3 Data analysis

All the data generated in triplicates was coded and entered into Ms Excel 2010 for cleaning, editing, and determination of averages for each parameter. This data was then imported into SAS version 9.1 for analysis. The means were subjected to analysis of variance and the means compared using the Least Significant difference (LSD) at P<=0.05. The LSD method separates means by use of small letters (a,b,c,d, e, f……..e.t.c) whereby in this study if the means within a column are followed by the same letter, they are not statistically different. On the other hand, the means followed by different letters indicates that the said variable level significantly (say the type of water source) affects the parameter (s) being analyzed for instance dissolved oxygen.

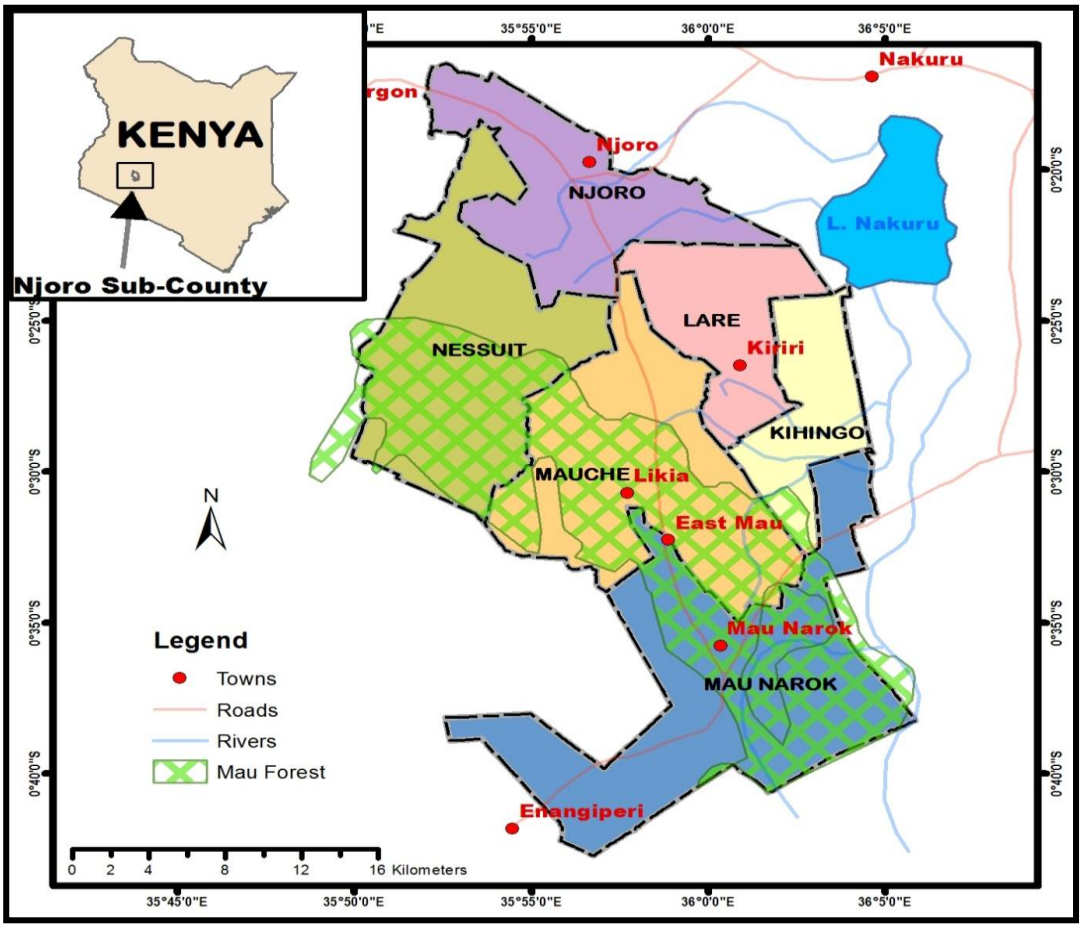

Figure 3. 1**:** Map of Njoro Sub- County (Philip and John, 2016)

3.4 Water sources assessed in this study

Water sources assessed in the study area were categorized as improved and unimproved sources according to the WHO criteria (WHO, 2011a). Improved drinking water sources are sources that by nature of construction are protected from contaminations from outside for instance taps. The water sources and storage containers were sampled randomly depending on the type that was being used at the exact time of sample collection. On the other hand, unimproved drinking water sources are not protected in any way from contamination for instance a river. Improved drinking water sources were the primary sources of drinking water for the population constituting 89.52 % (n=111) while the unimproved sources formed 10.48 % (n=13). The proportions of improved drinking sources assessed in this study included: taps/piped water (25.23 %), tanks (40.54 %), boreholes (21.62 %), protected wells (7.21 %) and protected springs (5.41 %) as shown in figure 3.2.

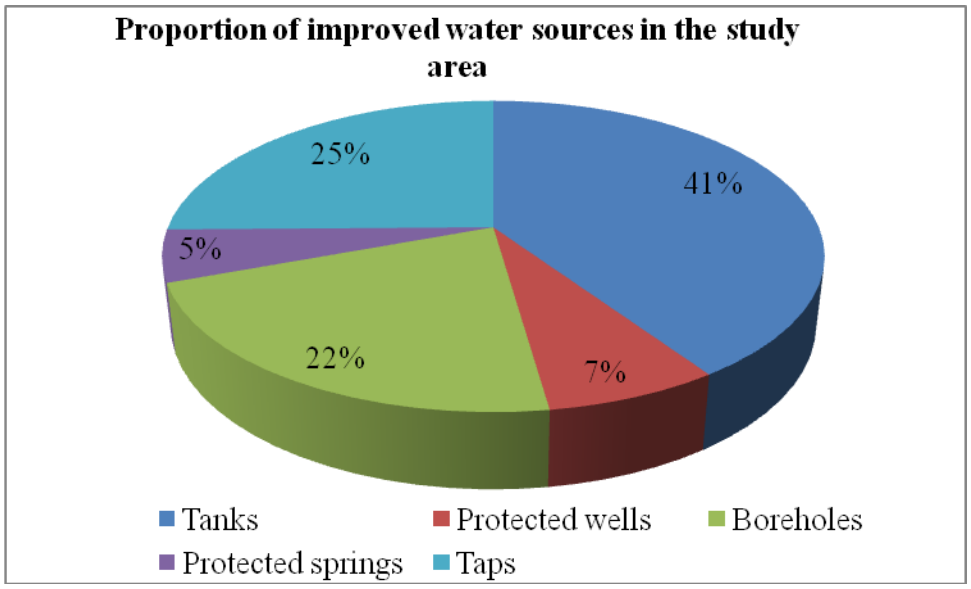

Figure 3. 2: Proportion of improved water sources in the study area.

The proportion of unimproved water sources assessed in this study included: rivers (38.46 %), unprotected wells (38.46 %), dams (15.38 %) and unprotected springs (7.69 %) (Figure 3.3). This indicates that the majority of the unprotected water sources in the study area were the unprotected wells and River Njoro.

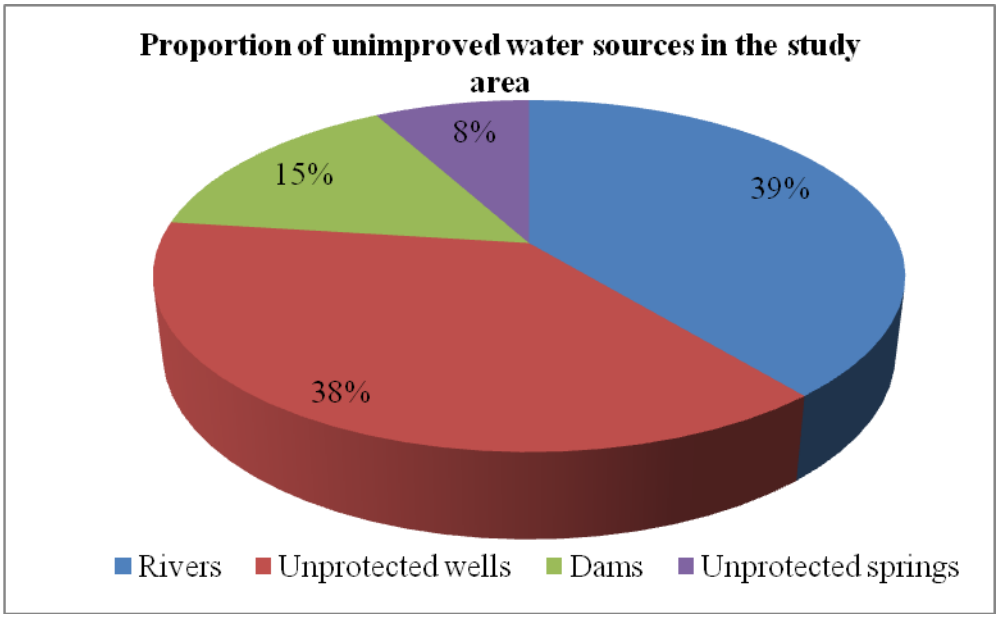

Figure 3. 3: Proportion of unimproved water sources in the study area.

Tables 3.1 and 3.2 provide a summary of the water sources assessed in Njoro Sub-County and in the specific administrative units. Figure 3.4 shows the different sources assessed in this study.

Table 3. 1: Water sources assessed in Njoro Sub-County.

Water source type		**Number assessed in this study**	**Prevalence of the sampled water sources**
Unimproved sources	Rivers	5	38.46 %
	Springs (unprotected)	1	7.69 %
	Wells (unprotected)	5	38.46 %
	Dams	2	15.38 %
Improved sources	Springs (protected)	6	5.41 %
	Taps/piped water	28	25.23 %
	Tanks	45	40.54 %
	Boreholes	24	21.62 %
	Wells (protected)	8	7.21 %

Table 3. 2: Water sources assessed in each of the five locations in Njoro Sub-County.

Water source type		Kihingo	Njoro	Lare	Mauche	Maunarok
Unimproved sources	Rivers	-	4	-	1	-
	Springs (unprotected)	-	1	-	-	-
	Wells (unprotected)	3	1	-	-	1
	Dams	-	-	-	1	1
Improved sources	Springs (protected)	-	1	-	2	3
	Taps/piped water	2	11	5	9	1
	Tanks	14	12	2	6	11
	Boreholes	2	17	2	2	1
	Wells (protected)	5	-	-	-	3

-no sample of that type was found in the respective locations during sampling

3.5 Water storage containers assessed in this study

The household water storage containers assessed in this study are summarized in Tables 3.3 and 3.4. Moreover, some of the water sources sampled in this study are shown in figure 3.4. The containers included jugs (3.70 %), cups (3.70 %), gallons (5.41 %), skyplasts (25.68 %), plastic containers (74.07 %), ceramic pots (3.70 %) and buckets (3.70 %).

Figure 3. 4: Some common water sources in Njoro Sub-County: A (unprotected well) B (borehole water at communal taps).

Table 3. 3: Specific water containers assessed in Njoro Sub-County.

Serial No.	Water storage container	Number assessed in this study	Prevalence of each type of storage containers sampled
i.	Sufuria	2	3.70 %
ii.	Cups	2	3.70 %
iii.	Jugs	2	3.70 %
iv.	Pots	2	3.70 %
v.	Jerrycans	40	74.07 %
vi.	Buckets	2	3.70 %
vii.	Gallons	4	7.41 %

Table 3. 4: Water containers assessed in each of the 5 locations in Njoro Sub-County.

Serial No.	Water storage container	Kihingo	Njoro	Lare	Mauche	Maunarok
i.	Sufuria	-	1	-	1	-
ii.	Cups	-	-	1	1	-
iii.	Jugs	-	1	-	1	-
iv.	Clay pots	-	1	-	1	-
v.	Jerry cans	9	10	4	7	10
vi.	Buckets	-	1	1	-	-
vii.	Gallons	3	-	1	-	-

-no sample of that type was found in the respective locations during sampling a

The fecal matter gets into the household storage containers during water collection as well as handling by use of dirty hands and small fetching containers like cups and tins. Some of the domestic storage containers from which drinking water was collected for analysis are shown in figure 3.5.

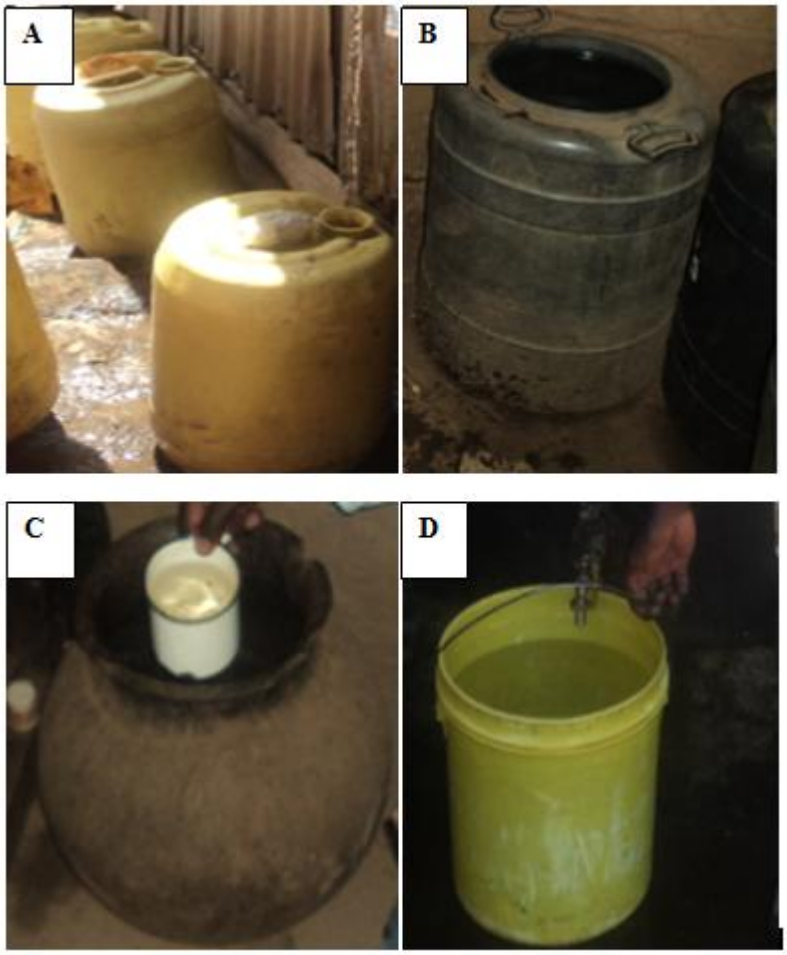

Figure 3. 5: Water containers used by study population.

Earthen clay pots were used only by a few households who believed that it offers self-cleansing of the water and their volumes depend on the size of the pot. The most common encountered volumes were 10 liters and 20 liters clay pots. Sufuria were silver in color and made of aluminum. They are commonly used for cooking although a few other people use them for storage of water. They can carry different volumes depending on its size ranging from 5, 10, 20, 40, 60, 100 liters. Plastic buckets were used to store water especially when the drums had been filled up and could store at least more than 20 liters of water, especially for storage. The plastic jerry can containers had volumes of 10 liters and above and were the most common domestic containers used to convey water from short as well as long distances to the homesteads. Cups/mugs and jugs were used to store water especially in areas that received water periodically so as to store as much as possible.

CHAPTER FOUR

PHYSICAL AND CHEMICAL PROPERTIES OF DRINKING WATER FROM SELECTED SOURCES IN NJORO SUB-COUNTY, KENYA

Abstract

The quality of drinking water is affected by the concentration of physical as well as chemical parameters. These could be in form of dissolved, suspended matter or organic and inorganic materials. This study was carried out to determine the concentration of some physical and chemical contaminants in water sources and water stored in domestic containers in five locations in Njoro Sub-County. During sample collection, some parameters like DO, EC, turbidity, TDS, pH and temperatures were taken on site. In the laboratory, the BOD, nitrates, iron, manganese and fluoride were analyzed. All the readings were pooled for the locations, their means calculated and then compared against WHO and KEBS standards. High concentrations of EC, turbidity, fluoride and iron were observed in water sampled from various sources and domestic storage containers in various locations. The highest turbidity readings were recorded in Mauche 273.85±52.85NTU (protected springs). The highest mean pH were recorded in Njoro (8.73±0.28). The mean EC in water sources was high in all the locations ranging from 0.06±0.02 (Maunarok) to 3.36±0.64 µS/cm (Njoro). High levels of fluoride in water sources were observed in Njoro location (4.01±1.29mg/L). In the stored water, the highest fluoride concentration was 3.39±1.14mg/L. The water sources and stored water sampled in this study. The increased levels of these contaminants could be due to pollution and infiltration of organic and inorganic materials from the ground surfaces.These findings indicated that the water sources as well as domestic containers are contaminated and hence frequent water monitoring and treatment at homes should be adopted.

Key words: Chemical contaminants, Physical contaminants, Pollution.

4.1 Introduction

The quality of drinking water is affected by its smell, taste, concentrations of organic and inorganic materials as well as colour. If they are consumed, water contaminants could lead to negative health effects. The inorganic chemicals may find their way into the nervous system and other organs thus interfering with their normal physiological functions (Khan *et al.,* 2013). The rising anthropogenic activities have resulted in detrimental effects to the water sources which are otherwise meant for consumption. As a result it is necessary to

perform a frequent physical-chemical assessment of water sources to determine their suitability for use (Ubomba-Jaswa *et al.,* 2009). The temperature of water is important for aquatic life such as fish while in terms of consumption, it has an effect on its taste (Blokker and Pieterse-Quirijns, 2013).

Turbidity affects the aspects of light absorption, scattering and appearance. If there is an increase in the concentration of scattered light there is an increase in turbidity. The pH of water is determined by establishment of a balance between carbon dioxide and carbonate ions although during the day, pH tends to increase due to high rates of photosynthesis associated with usage of carbon dioxide and decreases during the night due to respiratory activity (Sofi *et al.,* 2015). EC is affected by motion, total concentration, mobility, temperature and valence of the solution of ions (Henquin *et al.,* 2013). Therefore, conductance of water is expressed as the reciprocal of the resistivity involved and it's expressed as micro Siemens per centimetre (µS/cm). Dissolved oxygen is vital for the water bodies such as fish in order to carry out respiration. It enters water by photosynthesis or diffusion from air (Kumar and Puri, 2012). Biological oxygen demand (BOD) is used to determine the levels of pollution in drinking and waste water and the effectiveness of water treatment methods (Shan *et al.,* 2015). High nitrates are from inorganic fertilizers in farms, drainage from livestock feeds, domestic and industrial wastes (Crandall *et al.,* 2013).

The reports of fluoride mapping in Kenya shows that Nakuru (where Njoro Sub-County is located) is among several other regions (including Baringo, Kajiado, Nairobi, Narok, Thika, Kericho and Laikipia) with at least half of boreholes containing fluorides levels above 1.5mg/L (KEBS, 2010). Specifically Njoro Sub-County suffer from high levels of fluoride and majority of the people have mottled teeth as a result of dental fluorosis (Mavura *et al.,* 2003). This is because this area is found in the East African Rift valley which has fluoride bearing rocks closer to the earth surface and the major sources of water are from ground sources; indicating high leaching volcanic soils in water (Shen & Schafer, 2015). Low fluoride levels below 0.8mg/L causes dental carries while higher levels above 1.5mg/L causes dental and skeletal fluorosis and thus the recommended levels are 1.5mg/L (Jagtap *et al.,* 2012). It is therefore against the stated background that the water sources and water stored in household containers in Njoro Sub-County were analyzed for physical and chemical parameters to determine its safety for consumption.

4.2 Materials and methods

Physical parameters used in this assessment included temperature, TDS, EC and pH and were measured at the point of sample collection using a multimeter (Combo, Hanna Instruments, USA). All measurements were based on standard procedures, (APHA, 2008). All water sample readings were taken in triplicate and averages recorded using MS Excel. The bulb end of the multi-meter was rinsed in sterile distilled water and carefully placed into the water sample, allowed to stabilize and each of the readings taken after two minutes. The electrode of the pH meter was first calibrated against a pH buffer 7, 9 and 12 respectively to adjust to the response of the glass electrode. Turbidity was measured in the laboratory using a turbidity meter (Hach 2100Q, USA). A total of 25 ml of each of each water sample was gently agitated until all air bubbles disappeared. The sample was added to a sample cell and the turbidity read directly from the instrument display screen and recorded in NTU. DO was measured at the point of sample collection using a portable DO meter (Hach HQ 40d, USA). The probe was rinsed in distilled water and immersed into the water sample. The reading displayed on the screen was read and recorded in mg/L. Five days BOD was determined using standard procedures whereby the samples were put in the aluminum foil-covered BOD bottles and incubated in the dark for five days at 20ºC and the readings recorded.

Chemical parameters included metal ions nitrate, manganese, fluoride and iron which were determined spectrophotometrically using a spectrophotometer (Hach DR-3900). Fluoride was assayed using the USEPA SPADNS reagent. Approximately 10mL of the sample was pipetted into a dry sample cell while 10mL of deionized water (control) was pipetted into another sample cell. Then 2mL of SPADNS 2 reagent was added to each cell and swirled to mix the contents. The control followed by the sample was read at 580nm and results recorded. The SPADNS 2 method involves the reaction between fluoride and a red zirconium dye solution. Fluoride combines with part of the zicornium to form a colourless complex that bleaches the red color in an amount proportional to the fluoride concentration. Nitrate was assayed using cadmium reduction method whereby the sample cell was filled with 10mL of the sample followed by the addition of nitraVer 5 nitrate reagent powder (cadmium) and the contents swirled for one minute to obtain a uniform mixture. An amber color read at 500nm was indicative of presence of nitrate. Cadmium metal reduces nitrate in the water sample to nitrite which in turn reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt couples with gentisic acid to form an amber colored solution.

Manganese was analyzed by periodate oxidation method whereby 10 mL of the water sample and distilled water were added into separate sample cells and the citrate powder pillow followed by sodium periodate powder pillow poured into each of the cells. If manganese is present in the water sample, it is oxidized to the purple permanganate compound by the sodium periodate upon buffering with citrate. The intensity of the purple color is directly proportional to the manganese concentration in the respective water sample. The sample reading was taken after taking the reading of the control at a wavelength of wavelength of 525 nm. Iron was analyzed using the ferrover method and read spectrophotometrically at a wavelength of 510 nm. Briefly, 10 mL of the sample was put in a sample cell followed by addition of the contents of the FerroVer Iron Reagent pillows. In case iron is present in the water sample, the ferroVer reagent converts all the iron into soluble ferrous iron. The 1-10 phenanthroline indicator contained in the powder forms an orange color in proportion to the amount of iron present. All the metal ions readings were recorded in mg/L. The GPS co-ordinates of each sampling point were taken for reference using a Global Positioning System (Etrox 10, Garmin inc, Taiwan).

The guideline levels shown in Chapter 2 (Table 2.1) was used as the reference guideline measurements for each of the physical and chemical assessment in water sources and water tested inside household storage containers.

4.3 Results

4.3.1 Physical parameters

4.3.1.1 Physical parameters for water sources

The physical parameters for the water sources used by communities in the five locations in Njoro Sub-County are shown in Tables 4.1 to 4.5. In Kihingo location, the physical parameters for the sampled sources in this location are shown in Table 4.1. There was no water sample collected for river, unprotected wells, dams and unprotected springs because none of these water sources was found in this location during sampling. The mean pH, BOD and TDS were within the KEBS recommended levels (KEBS, 2008) across the sources. Turbidity in protected wells (13.26±1.14NTU) and electrical conductivity in all sources

were above the KEBS recommended levels. The mean DO was low in all sources in Njoro location ranging from 2.67±0.46 (protected wells) to 6.31±0.29mg/L (tanks).

In Lare location, the mean pH, BOD and TDS were within the KEBS recommended levels as shown in Table 4.2. There were no samples taken from river, unprotected springs, unprotected wells, dams and protected springs because none of these water sources was found in this location during sampling. The highest mean temperature of water was recorded in boreholes (27.55±0.55°C). The mean EC concentrations were high in all the sources ranging from 0.17±0.10 µS/cm (taps) to 0.47±0.03 µS/cm (boreholes) while DO was low in tap water (4.48±0.89 mg/L), although the differences were not statistically significant. The physical parameters from drinking water sources in Mauche are shown in Table 4.3. There were no samples taken from unprotected springs and unprotected wells because none of these water sources was found in this location during sampling. The mean pH BOD and TDS levels were within the standard limits as recommended by the KEBS. All the water sources had high levels of EC which ranged from 0.18±0.02µS/cm to 0.35±0.03µS/cm but the differences were not statistically significant. Turbidity in protected springs was higher than all other sources and measured 229.15NTU.

In Maunarok, the means of physical parameters of water sources are presented in Table 4.4. There were no water samples taken from river and unprotected springs because none of these water sources was found in this location during sampling. The mean BOD and TDS were within the recommended KEBS levels in all water sources. The highest pH levels were recorded in taps (8.73±0.28) while the lowest levels were in protected spring (7.10±0.32) respectively. Mean turbidity level was highest in dams (59.70±3.62NTU). The mean temperature and EC were highest in protected wells recording 22.97±1.10°C and 0.220.01µS/cm. In Njoro location the physical parameters for drinking water in various sources are shown in table 4.5. There were no water samples taken from dams and protected wells because none of these water sources was found in this location during sampling. The mean temperature, pH, BOD and TDS concentrations were within the recommended levels as set by the WHO (WHO, 2011). Although DO in all the water sources sampled in Njoro location were lower than the KEBS recommended levels with the lowest reading being in unprotected wells (2.59±0.06mg/L). On the other hand, the mean turbidity concentrations in water samples were higher than the recommended WHO limits except in taps (4.96±2.93 NTU). The mean EC levels were high in all the sampled sources and ranged from 0.10±0.04 µS/cm (in tanks) to 3.36±3.21 µS/cm (in river samples).

Table 4. 1: **Physical parameters for various water sources types in Kihingo location in Njoro Sub-County.**

WATER SOURCE		PHYSICAL PARAMETERS (mean ± standard error)						
		TEMP (°C)	**pH**	**EC (µS/cm)**	**DO (mg/L)**	**TURB (NTU)**	**BOD (mg/L)**	**TDS (mg/L)**
Unimproved sources	Rivers (n=0)	-	-	-	-	-	-	-
	Springs (n=0)	-	-	-	-	-	-	-
	Wells (n=9)	22.23±0.75^{a}	7.56±0.11^{a}	0.76±0.19^{a}	3.23±0.23^{b}	1.80±0.21^{a}	0.82±0.07^{b}	0.37±0.11^{a}
	Dams (n=0)	-	-	-	-	-	-	-
Improved sources	Springs (n=0)	-	-	-	-	-	-	-
	Taps/piped water (n=6)	21.55±0.25^{a}	7.91±0.66^{a}	0.68±0.07^{a}	5.41±1.56^{a}	0.55±0.05^{a}	2.16±0.23^{a}	0.34±0.04^{a}
	Tanks (n=42)	20.73±0.59^{a}	8.05±0.17^{a}	0.15±0.05^{b}	6.31±0.29^{a}	4.99±1.90^{a}	0.94±0.16^{b}	0.08±0.03^{b}
	Boreholes (n=6)	23.85±0.05^{a}	8.09±0.06^{a}	0.51±0.04^{ab}	6.05±0.30^{a}	0.40±0.08^{a}	0.35±0.10^{b}	0.25±0.02^{ab}
	Wells (n=15)	20.86±0.49^{a}	7.42±0.15^{a}	0.86±0.13^{a}	2.67±0.46^{b}	13.26±1.14^{a}	0.76±0.23^{b}	0.39±0.04^{a}

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

Table 4. 2: Physical parameters for various water sources types in Lare location in Njoro Sub-County.

WATER SOURCE		PHYSICAL PARAMETERS (mean ± standard error)						
		TEMP (°C)	**pH**	**EC (µS/cm)**	**DO (mg/L)**	**TURB (NTU)**	**BOD (mg/L)**	**TDS (mg/L)**
Unimproved sources	Rivers (n=0)	-	-	-	-	-		
	Spring (n=0)	-	-	-	-	-	-	-
	Wells (n=0)	-	-	-	-	-	-	-
	Dams (n=0)	-	-	-	-	-	-	-
Improved sources	Spring (n=0)	-	-	-	-	-	-	-
	Taps/piped water (n=15)	21.88±0.75[a]	7.85±0.24[a]	0.17±0.06[a]	4.48±0.89[a]	21.92±4.14[a]	1.01±0.37[a]	0.08±0.01[a]
	Tanks (n=6)	23.35±3.55[a]	7.83±0.27[a]	0.25±0.05[a]	5.93±0.74[a]	1.50±0.20[a]	0.81±0.06[a]	0.13±0.02[a]
	Boreholes (n=6)	27.55±0.55[a]	8.42±0.06[a]	0.47±0.03[a]	6.21±0.30[a]	6.70±1.28[a]	1.12±0.07[a]	0.24±0.02[a]
	Wells (n=0)	-	-	-	-	-	-	-

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

Table 4. 3: Physical parameters for various water sources types in Mauche location in Njoro Sub-County.

WATER SOURCE		PHYSICAL PARAMETERS (mean ± standard error)						
		TEMP (°C)	**pH**	**EC (µS/cm)**	**DO (mg/L)**	**TURB (NTU)**	**BOD (mg/L)**	**TDS (mg/L)**
Unimproved sources	Rivers (n=3)	24.70±1.87[a]	7.51±0.34[a]	0.31±0.06[a]	5.83±1.46[a]	44.70±2.87[b]	0.30±0.06[a]	0.15±0.02[a]
	Springs (n=0)	-	-	-	-	-	-	-
	Wells (n=0)	-	-	-	-	-	-	-
	Dams (n=3)	19.00±2.83[a]	7.89±0.32[a]	0.27±0.04[a]	6.93±1.83[a]	20.30±2.81[b]	1.49±0.07[a]	0.14±0.03[a]
Improved sources	Springs (n=6)	25.20±7.50[a]	7.34±0.52[a]	0.35±0.03[a]	6.27±0.66[a]	273.85±52.85[a]	1.94±0.21[a]	0.18±0.08[a]
	Taps/piped water (n=27)	21.21±0.70[a]	7.78±0.14[a]	0.25±0.05[a]	5.65±0.35[a]	5.79±1.12[b]	1.02±0.20[a]	0.12±0.03[a]
	Tanks (n=18)	20.82±0.60[a]	7.60±0.08[a]	0.35±0.21[a]	6.16±0.18[a]	4.38±1.25[b]	1.61±0.34[a]	0.18±0.02[a]
	Boreholes (n=6)	23.70±0.90[a]	7.53±0.02[a]	0.18±0.02[a]	6.85±0.01[a]	7.90±0.60[b]	0.61±0.08[a]	0.09±0.01[a]
	Wells (n=0)	-	-	-	-	-	-	-

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

Table 4. 4: Physical parameters for various water sources types in Maunarok location in Njoro Sub-County.

WATER SOURCE		PHYSICAL PARAMETERS (mean ± standard error)						
		TEMP (°C)	**pH**	**EC (µS/cm)**	**DO (mg/L)**	**TURB (NTU)**	**BOD (mg/L)**	**TDS (mg/L)**
Unimproved sources	Rivers (n= 0)	-	-	-	-	-	-	-
	Springs (n= 0)	-	-	-	-	-	-	-
	Wells (n= 3)	22.00±0.31^{a}	7.67±1.84^{a}	0.18±0.06^{ab}	6.71±1.56^{a}	9.50±2.31^{b}	0.63±0.01^{ab}	0.09±0.03^{ab}
	Dams (n= 3)	22.50±1.73^{a}	7.81±0.41^{a}	0.21±0.04^{a}	6.25±1.24^{a}	59.70±3.62^{a}	0.17±0.02^{b}	0.10±0.03^{a}
Improved sources	Springs (n= 9)	19.43±0.34^{a}	7.10±0.32^{a}	0.16±0.01^{b}	6.47±0.18^{a}	11.70±1.14^{b}	0.26±0.04^{b}	0.07±0.01^{b}
	Taps/piped water (n= 3)	21.20±0.37^{a}	8.73±0.28^{a}	0.19±0.01^{a}	7.04±1.39^{a}	0.30±0.08^{b}	0.91±0.06^{a}	0.10±0.01^{a}
	Tanks (n= 33)	19.16±0.92^{a}	7.79±0.13^{a}	0.06±0.02^{b}	5.90±0.26^{a}	3.83±0.81^{b}	0.92±0.07^{a}	0.03±0.01^{b}
	Boreholes (n= 3)	19.80±2.57^{a}	8.24±1.33^{a}	0.19±0.02^{a}	6.41±1.39^{a}	0.20±0.08^{b}	0.17±0.06^{b}	0.10±0.02^{a}
	Wells (n= 9)	22.97±1.10^{a}	7.73±0.32^{a}	0.22±0.01^{a}	6.30±0.18^{a}	11.70±1.14^{b}	0.26±0.04^{a}	0.11±0.01^{a}

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

Table 4. 5: Physical parameters for various water sources types in Njoro location in Njoro Sub-County.

WATER SOURCE		PHYSICAL PARAMETERS (mean ± standard error)						
		TEMP (°C)	**pH**	**EC (µS/cm)**	**DO (mg/L)**	**TURB (NTU)**	**BOD (mg/L)**	**TDS (mg/L)**
Unimproved sources	Rivers (n=12)	16.35±1.09^{b}	8.17±0.09^{ab}	3.36±0.64^{a}	7.27±0.16^{a}	94.53±22.80^{a}	0.87±0.23^{a}	0.07±0.01^{b}
	Springs (n=3)	24.00±1.64^{a}	6.74±0.71^{b}	0.34±0.02^{a}	6.81±0.39^{a}	72.40±10.31^{ab}	0.50±0.19^{a}	0.17±0.04^{b}
	Wells (n=3)	18.00±2.81^{b}	6.65±0.15^{b}	0.21±0.03^{a}	2.59±0.06^{b}	36.70±9.59^{c}	1.82±0.12^{a}	0.10±0.03^{b}
	Dams (n=0)	-	-	-	-	-	-	-
Improved sources	Springs (n=3)	22.10±1.07^{b}	7.12±0.06^{b}	0.88±0.19^{a}	6.84±1.31^{a}	37.90±2.87^{bc}	0.47±0.11^{a}	0.44±0.03^{a}
	Taps/piped water (n=33)	22.28±0.72^{ab}	8.36±0.15^{a}	0.32±0.03^{a}	6.20±0.28^{a}	4.96±0.59^{c}	1.10±0.36^{a}	0.16±0.01^{b}
	Tanks (n=36)	20.51±0.75^{b}	7.60±0.19^{b}	0.10±0.04^{a}	5.27±0.34^{a}	2.90±0.77^{c}	1.02±0.15^{a}	0.05±0.01^{b}
	Boreholes (n=51)	24.22±0.52^{a}	7.67±0.14^{b}	0.37±0.03^{a}	6.39±0.33^{a}	5.99±0.70^{c}	0.84±0.10^{a}	0.19±0.01^{b}
	Wells (n=0)	-	-	-	-	-	-	-

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

4.3.1.2 Physical parameters for water storage containers

The physical parameters for the water storage containers used by communities in the five locations in Njoro Sub-County are shown in Tables 4.6 to 4.10. In Kihingo location (Table 4.6) the mean pH, BOD and TDS of water were within the recommended guideline levels across all the domestic containers as stated by the KEBS (Table 2.1). There were no water samples taken from jugs, cups/mugs, claypots, buckets and sufurias because none of these containers was found having stored water in this location during sampling. The mean temperature was highest in gallons and recorded 23.90±0.75°C. The mean EC and turbidity levels were above the KEBS recommended levels ranging from 0.28±0.09 to 0.53±0.05µS/cm and 6.74±0.67 to 7.87±1.42NTU, but their differences were not statistically significant. DO was lower than the recommended KEBS guidelines i.e. 6.12±0.41 (jerrycans) and 5.13±0.98 mg/L (gallons).

In Lare location, the mean BOD and TDS were within the recommended KEBS guideline levels (Table 4.7). There were no water samples taken from jugs, claypots and sufurias because none of these containers was found having stored water in this location during sampling. The water temperatures were highest in water sampled from buckets (27.20±1.94°C) while turbidity was highest in jerrycans (12.88±2.40NTU). However, the mean differences of temperatures and turbidity across the containers were not statistically significant. The mean pH was above the WHO recommended limits in mugs (8.65±0.12) while EC was high and ranged from 0.03±0.01µS/cm (buckets) to 0.44±0.11µS/cm (buckets). The mean DO in all storage containers in Lare location were lower than KEBS guidelines ranging from 4.59±0.38 to 7.05±1.12mg/L. The mean differences among all parameters in Lare household storage containers were not statistically significant.

In Mauche location, the mean pH, BOD and TDS were within the KEBS recommended guideline levels (Table 4.8). There were no water samples taken from gallons and buckets because none of these containers was found having stored water in this location during sampling. EC was high in all containers except in jugs (0.03±0.01µS/cm) while turbidity was within the recommended levels except in cups (7.10±1.59NTU). The mean DO was lower than the recommended KEBS guideline limits in all storage containers ranging from 5.32±0.13 (jug) to 7.10±0.70mg/L (jerrycan). In Maunarok, only jerryans were used for water storage purposes at the time of sampling. There were no water samples taken from gallons, jugs, cups/mugs, claypots, buckets and sufurias because none of these containers was found having stored water in this location during sampling. All the physical parameters were within the recommended KEBS levels (Table 4.9) except EC which was higher than the

KEBS recommended guideline limits recording a mean of 0.13±0.04µS/cm and DO was low with a mean of 6.24±0.31mg/L.

In Njoro location, there were no gallons and cups found to be storing water in the households that were sampled. The mean concentrations of BOD and TDS were within the acceptable guideline levels as recommended by KEBS (Table 4.10). There were no water samples taken from gallons and cups/mugs because none of these containers was found having stored water in this location during sampling. The pH was within the recommended levels in all containers except in water collected from clay pots which recorded higher levels (8.67±0.49) than the KEBS guidelines. The EC measurements were higher than KEBS guidelines in all storage containers ranging from 0.07±0.02µS/cm (sufuria) to 0.37±0.05µS/cm (jerry cans). Turbidity was higher than the recommended guideline limits in buckets (6.80±1.38NTU) and clay pots (8.80±2.34NTU). DO was low in all storage containers in Njoro location ranging from 5.77±0.48 (buckets) to 6.89±0.79mg/L (sufurias).

Table 4. 6: Physical parameters for water storage container types in Kihingo location in Njoro Sub-County.

WATER CONTAINER	PHYSICAL PARAMETERS (mean ± standard error)						
	TEMP (°C)	pH	EC (µS/cm)	DO (mg/L)	TURB (NTU)	BOD (mg/L)	TDS (mg/L)
Gallon (n=9)	23.90±0.75[a]	8.10±0.08[a]	0.53±0.05[a]	5.13±0.98[a]	7.87±1.42[a]	0.62±0.05[a]	0.26±0.03[a]
Jug (n=0)	-	-	-	-	-	-	-
Cup/mug (n=0)	-	-	-	-	-	-	-
Jerrycan (n=27)	21.14±0.61[b]	7.97±0.16[a]	0.28±0.09[a]	6.12±0.41[a]	6.74±0.67[a]	0.87±0.28[a]	0.14±0.05[a]
Claypot (n=0)	-	-	-	-	-	-	-
Bucket (n=0)	-	-	-	-	-	-	-
Sufuria (n=0)	-	-	-	-	-	-	-

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

Table 4. 7: Physical parameters for water storage container types in Lare location in Njoro Sub-County.

WATER CONTAINER	PHYSICAL PARAMETERS (mean ± standard error)						
	TEMP (°C)	pH	EC (µS/cm)	DO (mg/L)	TURB (NTU)	BOD (mg/L)	TDS (mg/L)
Gallon (n=3)	18.50±1.14[a]	8.09±0.08[a]	0.11±0.03[a]	7.05±1.12[a]	2.70±0.10[a]	0.73±0.21[a]	0.05±0.01[a]
Jugs (n=0)	-	-	-	-	-	-	-
Cup/mug (n=3)	20.90±1.19[a]	8.65±0.12[a]	0.44±0.11[a]	6.64±1.81[a]	0.70±0.02[a]	2.31±0.31[a]	0.22±0.02[a]
Jerrycan (n=12)	23.78±1.00[a]	7.90±0.18[a]	0.40±0.04[a]	4.59±0.38[a]	12.88±2.40[a]	1.47±0.42[a]	0.20±0.02[a]
Claypot (n=0)	-	-	-	-	-	-	-
Bucket (n=3)	27.20±1.94[a]	7.18±0.33[a]	0.03±0.01[a]	6.16±0.86[a]	3.00±0.39[a]	2.00±0.16[a]	0.04±0.01[a]
Sufuria (n=0)	-	-	-	-	-	-	-

Means followed by the same small letter in a column are not significantly different at 5% LSD. - Not tested since no sample of that type was found for collection during sampling

Table 4. 8: Physical parameters for water storage container types in Mauche location in Njoro Sub-County.

WATER CONTAINER	PHYSICAL PARAMETERS (mean ± standard error)						
	TEMP (°C)	pH	EC (µS/cm)	DO (mg/L)	TURB (NTU)	BOD (mg/L)	TDS (mg/L)
Gallon (n=0)	-	-	-	-	-	-	-
Jug (n=3)	19.00±1.18[a]	7.13±0.42[a]	0.03±0.01[a]	5.32±0.13[a]	3.80±0.10[a]	1.08±0.02[a]	0.04±0.01[a]
Cup/mug (n=3)	21.00±0.63[a]	8.35±0.27[a]	0.18±0.04[a]	6.83±0.76[a]	7.10±1.59[a]	3.08±0.04[a]	0.09±0.03[a]
Jerrycan (n=21)	21.14±1.08[a]	7.87±0.10[a]	0.16±0.02[a]	7.10±0.70[a]	4.74±0.65[a]	2.59±0.58[a]	0.08±0.01[a]
Claypot (n=3)	14.10±0.30[a]	7.76±0.82[a]	0.19±0.03[a]	5.66±0.34[a]	4.20±1.39[a]	2.19±0.11[a]	0.09±0.03[a]
Bucket (n=0)	-	-	-	-	-	-	-
Sufuria (n=3)	16.10±2.32[a]	8.04±0.12[a]	0.19±0.09[a]	6.22±0.11[a]	3.70±1.41[a]	3.45±0.14[a]	0.10±0.03[a]

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

Table 4. 9: Physical parameters for water storage container types in Maunarok location in Njoro Sub-County.

WATER CONTAINER	PHYSICAL PARAMETERS (mean ± standard error)						
	TEMP (°C)	pH	EC (µS/cm)	DO (mg/L)	TURB (NTU)	BOD (mg/L)	TDS (mg/L)
Gallon (n=0)	-	-	-	-	-	-	-
Jugs (n=0)	-	-	-	-	-	-	-
Cup/mug (n=0)	-	-	-	-	-	-	-
Jerrycan (n=30)	19.01±1.06	7.84±0.14	0.13±0.04	6.24±0.31	3.62±0.95	0.63±0.08	0.07±0.02
Claypot (n=0)	-	-	-	-	-	-	-
Bucket (n=0)	-	-	-	-	-	-	-
Sufuria (n=0)	-	-	-	-	-	-	-

Means followed by the same small letter in a column are not significantly different at 5% LSD, - Not tested since no sample of that type was found for collection during sampling

Table 4. 10: Physical parameters for water storage container types in Njoro location in Njoro Sub-County.

WATER CONTAINER	PHYSICAL PARAMETERS (mean ± standard error)						
	TEMP (°C)	pH	EC (µS/cm)	DO (mg/L)	TURB (NTU)	BOD (mg/L)	TDS (mg/L)
Gallon (n=0)	-	-	-	-	-	-	-
Jug (n=3)	24.20±0.43^{a}	8.21±0.89^{a}	0.32±0.11^{a}	6.03±0.34^{a}	1.50±0.08^{a}	0.58±0.15^{b}	0.16±0.01^{a}
Cup/mug (n=0)	-	-	-	-	-	-	-
Jerrycan (n=30)	21.51±0.81^{a}	7.97±0.14^{a}	0.37±0.05^{a}	5.86±0.28^{a}	2.33±0.94^{a}	0.85±0.26^{b}	0.18±0.03^{a}
Claypot (n=3)	21.70±0.83^{a}	8.67±0.49^{a}	0.35±0.13^{a}	5.91±0.89^{a}	8.80±2.34^{a}	1.06±0.02^{b}	0.18±0.07^{a}
Bucket (n=3)	20.60±1.21^{a}	8.04±0.54^{a}	0.09±0.03^{a}	5.77±0.48^{a}	6.80±1.38^{a}	5.62±0.11^{a}	0.05±0.01^{a}
Sufuria (n=3)	17.30±0.92^{a}	8.12±0.59^{a}	0.07±0.02^{a}	6.89±0.79^{a}	2.00±0.41^{a}	0.73±0.28^{b}	0.03±0.01^{a}

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

4.3.2 Chemical parameters

4.3.2.1 Chemical parameters for water sources

The chemical parameters for the water sources used in the five locations in Njoro Sub-County are shown in Tables 4.11 to 4.15. The water samples were not filtered and so the measurements were carried out for total chemical contaminants. In Kihingo location, there were no water samples taken from the river, unprotected springs, dams and protected springs because none of these water sources was found in this location during sampling. The mean nitrate concentrations were within the WHO recommended levels of less than 50 mg/L (short term exposure). However fluoride levels were higher than the recommended WHO limits in all sampled water sources apart from protected and unprotected wells which recorded a mean of 0.84±0.25 and 1.05±0.47mg/L respectively (Table 4.11). Generally iron levels were above the KEBS maximum permissible levels (0.3 mg/L) in all water sources apart from tap water (0.25±0.21 mg/L).

In Lare location, there were no water samples taken from the river, unprotected springs, unprotected wells, dams and protected springs because none of these water sources was found in this location during sampling. The mean nitrates concentrations were within the acceptable guideline levels for drinking water by the WHO (Table 4.12). Iron and manganese was higher than the KEBS limits in all the sources while fluoride was higher than WHO limits in all sources except in boreholes (1.18±0.23mg/L). In the water sources in Mauche location (Table 4.13), the mean nitrate levels were in the range of 1.45±0.15mg/L (boreholes) to 85.00±5.00mg/L in protected springs, while manganese was within the KEBS recommended guidelines. There were no samples taken from unprotected springs, unprotected wells and protected wells because none of these water sources was found in this location during sampling. The mean iron levels were higher than the KEBS acceptable limits in drinking water and ranged from 0.53±0.23mg/L (dams) to 1.12±0.02mg/L (protected springs). The mean Fluoride was higher than the WHO recommended levels in all water sources in Mauche location and was in the range of 2.11±0.49mg/L (taps) to 2.99±0.17mg/L (protected springs).

In Maunarok, there were no water samples taken from the river and unprotected springs because none of these water sources was found in this location during sampling. The mean concentrations of nitrates were within the WHO guidelines for short term exposure in all sampled water sources (Table 4.14). While manganese was within the accepted KEBS guideline levels, the iron levels were within the acceptable KEBS limits in all sources except dams (0.31±0.13mg/L) and protected springs (0.34±0.15mg/L). The mean fluoride levels

were in the range of 0.15±0.04mg/L in boreholes to 2.28±0.32mg/L in protected springs. In Njoro location, there was no water samples collected from the dams and protected wells because none of these water sources was found in this location during sampling. The mean nitrate was within the WHO recommended limits. The mean manganese levels were within the acceptable limits except in water sampled from rive, taps and tanks. Iron was higher than the KEBS recommended guideline levels in all water sources except the unprotected wells (0.16±0.007mg/L). The mean fluoride concentrations in unprotected wells were in the range of 0.88±0.30mg/L (river samples) to 4.01±1.29mg/L (unprotected wells).

Table 4. 11: Chemical parameters for water sources types in Kihingo location in Njoro Sub-County.

WATER SOURCE		CHEMICAL PARAMETERS (mean ± standard error)			
		NITRATE (mg/L)	MANGANESE (mg/L)	IRON (mg/L)	FLUORIDE (mg/L)
Unimproved Sources	River (n=0)	-	-	-	-
	Spring (n=0)	-	-	-	-
	Wells (n=9)	5.13±0.70[a]	0.08±0.03[a]	0.77±0.32[a]	0.84±0.25[a]
	Dams (n=0)	-	-	-	-
Improved Sources	Spring (n=0)	-	-	-	-
	Taps/piped water (n=6)	1.09±0.11[a]	0.05±0.01[a]	0.25±0.04[a]	1.71±0.32[a]
	Tanks (n=42)	3.09±1.29[a]	0.24±0.05[a]	0.53±0.09[a]	1.73±0.27[a]
	Boreholes (n=6)	6.40±0.78[a]	0.36±0.07[a]	0.67±0.22[a]	1.62±0.49[a]
	Wells (n=15)	6.86±0.70[a]	0.37±0.14[a]	0.52±0.13[a]	1.05±0.47[a]

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

Table 4. 12: Chemical parameters for water sources types in Lare location in Njoro Sub-County.

WATER SOURCE		CHEMICAL PARAMETERS (mean ± standard error)			
		NITRATE (mg/L)	MANGANESE (mg/L)	IRON (mg/L)	FLUORIDE (mg/L)
Unimproved Sources	River (n=0)	-	-	-	-
	Spring (n=0)	-	-	-	-
	Wells (n=0)	-	-	-	-
	Dams (n=0)	-	-	-	-
Improved Sources	Spring (n=0)	-	-	-	-
	Taps/piped water (n=15)	4.77±0.58[a]	0.20±0.05[a]	0.55±0.05[a]	2.37±0.57[a]
	Tanks (n=6)	1.23±0.44[a]	0.19±0.03[a]	0.87±0.34[a]	2.80±0.10[a]
	Boreholes (n=6)	6.12±0.84[a]	0.19±0.03[a]	0.58±0.09[a]	1.18±0.23[a]
	Wells (n=0)	-	-	-	-

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

Table 4. 13: Chemical parameters for water sources types in Mauche location in Njoro Sub-County.

WATER SOURCE		CHEMICAL PARAMETERS (mean ± standard error)			
		NITRATE (mg/L)	MANGANESE (mg/L)	IRON (mg/L)	FLUORIDE (mg/L)
Unimproved Sources	Rivers (n=3)	27.90±0.29[b]	0.03±0.01[a]	0.87±0.23[a]	2.42±0.64[a]
	Springs (n=0)	-	-	-	-
	Wells (n=0)	-	-	-	-
	Dams (n=3)	8.20±0.21[c]	0.03±0.01[a]	0.53±0.23[a]	2.54±0.17[a]
Improved Sources	Springs (n=6)	85.00±5.00[a]	0.31±0.05[a]	1.12±0.02[a]	2.99±0.17[a]
	Taps/piped water (n=27)	3.90±1.86[c]	0.11±0.05[a]	0.54±0.16[a]	2.11±0.49[a]
	Tanks (n=18)	3.42±1.40[c]	0.29±0.13[a]	0.64±0.17[a]	2.20±0.59[a]
	Boreholes (n=6)	1.45±0.15[c]	0.03±0.01[a]	0.65±0.08[a]	2.61±0.31[a]
	Wells (n=0)	-	-	-	-

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

Table 4. 14: Chemical parameters for water sources types in Maunarok location in Njoro Sub-County.

WATER SOURCE		CHEMICAL PARAMETERS (mean ± standard error)			
		NITRATE (mg/L)	MANGANESE (mg/L)	IRON (mg/L)	FLUORIDE (mg/L)
Unimproved Sources	Rivers (n=0)	-	-	-	-
	Springs (n=0)	-	-	-	-
	Wells (n=3)	0.09±0.02 [b]	0.08±0.01[a]	0.07±0.02[a]	1.25±0.10[a]
	Dams (n=3)	25.80±3.89[a]	0.08±0.03[a]	0.31±0.13[a]	1.44±0.11[a]
Improved Sources	Springs (n=9)	4.27±0.60[b]	0.04±0.01[a]	0.34±0.15[a]	2.28±0.32[a]
	Taps/piped water (n=3)	1.20±0.03[b]	0.05±0.01[a]	0.25±0.11[a]	1.89±0.04[a]
	Tanks (n=33)	1.70±0.52[b]	0.07±0.02[a]	0.30±0.06[a]	0.80±0.31[a]
	Boreholes (n=3)	0.90±0.21[b]	0.05±0.01[a]	0.18±0.03[a]	0.15±0.04[a]
	Wells (n=9)	2.42±0.21[b]	0.09±0.03[a]	0.25±0.03[a]	1.84±0.59[a]

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

Table 4. 15: Chemical parameters for water sources types in Njoro location in Njoro Sub-County.

WATER SOURCE		CHEMICAL PARAMETERS (mean ± standard error)			
		NITRATE (mg/L)	MANGANESE (mg/L)	IRON (mg/L)	FLUORIDE (mg/L)
Unimproved sources	Rivers (n=12)	9.23±1.38[ab]	0.27±0.04[a]	0.89±0.05[a]	0.88±0.30[b]
	Springs (n=3)	13.30±1.38[a]	0.10±0.02[a]	0.53±0.19[a]	1.59±0.12[b]
	Wells (n=3)	2.00±0.01[b]	0.10±0.03[a]	0.16±0.07[a]	4.01±1.29[a]
	Dams (n=0)	-	-	-	-
Improved Sources	Springs (n=3)	8.60±0.39[b]	0.07±0.02[a]	0.54±0.15[a]	2.75±1.01[ab]
	Taps/piped water (n=33)	2.74±1.00[b]	0.20±0.05[a]	0.64±0.20[a]	1.80±0.37[b]
	Tanks (n=36)	3.58±1.30[b]	0.26±0.06[a]	0.83±0.18[a]	0.99±0.21[b]
	Boreholes (n=51)	2.32±0.50[b]	0.06±0.02[a]	0.40±0.12[a]	1.86±0.28[b]
	Wells (n=0)	-	-	-	-

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

4.3.2.2 Chemical parameters for water storage containers

The chemical parameters for water in various storage containers used by communities in the five locations in Njoro Sub-County are shown in Tables 4.16 to 4.20. In Kihingo, water samples were collected from jerrycans and gallons only because none of the other storage containers was found having stored drinking water during sampling. The mean chemical parameters are indicated in Table 4.16. The mean nitrate levels were within the recommended guidelines by the WHO. The mean iron and manganese were in the range of 0.25±0.03 to 0.55±0.13 and 0.11±0.06 to 0.30±0.10mg/L. The mean fluoride levels ranged between 1.29±0.46mg/L to 2.04±0.37mg/L respectively. In Lare location, the chemical parameters in household storage containers are presented in Table 4.17. There were no water samples collected from the jugs, claypots and sufurias because none of these storage containers was found having stored drinking water during sampling. The mean nitrates and manganese levels were within the acceptable guideline limits set by the WHO. Iron was above the WHO recommended guideline limits in jerrycans (1.61±0.47mg/L) and bucket (1.06±0.01mg/L) while fluoride was high than the WHO limits and was in the range of 1.63±0.19mg/L (buckets) and 2.43±1.01mg/L (gallons).

The mean chemical parameters from drinking water storage containers in Mauche location are presented in Table 4.18. No water samples were collected from the gallons and buckets because none of these storage containers was found having stored drinking water during sampling. The mean nitrate concentrations were within the recommended guideline levels by the WHO. The mean iron levels were higher that the acceptable guideline limits by KEBS in sufuria (0.24±0.06mg/L), while fluoride was in the range of 0.23±0.10mg/L (cups) to 3.39±1.14mg/L (jugs). In Maunarok, all the chemical parameters were within the acceptable guideline levels set by the WHO and KEBS as shown in Table 4.19. Water was collected from jerrycans only because none of the other storage containers was found having stored drinking water during sampling. The mean nitrate, manganese, iron and fluoride levels were 3.82±0.36mg/L, 0.13±0.04mg/L, 0.34±0.09mg/L and 1.20±0.33mg/L respectively.

The mean chemical parameters of drinking water in domestic storage containers in Njoro location are shown in Table 4.20. No water samples were collected from the cups/mugs and gallons because none of these storage containers was found having stored drinking water during sampling. The highest mean nitrate levels were recorded in sufuria (8.12±3.11mg/L) while the highest manganese levels were in jerrycans (0.17±0.04 mg/L) and they were within the WHO and KEBS recommended guideline levels respectively. On the other hand, iron exceeded the KEBS acceptable guideline limits in all storage containers with samples from

sufuria recording the highest levels of 2.10±0.17mg/L. Fluoride levels were higher than the WHO acceptable guideline levels in drinking water of 1.5mg/L in all domestic containers except jugs (0.42±0.16) and jerrycans (1.34±0.06mg/L).

Table 4. 16: Chemical parameters for water storage container types in Kihingo location in Njoro Sub-County.

WATER CONTAINER	CHEMICAL PARAMETERS (mean ± standard error)			
	NITRATE (mg/L)	MANGANESE (mg/L)	IRON (mg/L)	FLUORIDE (mg/L)
Gallon (n=9)	5.31±0.97[a]	0.11±0.01[a]	0.25±0.03[a]	1.29±0.46[a]
Cup (n=0)	-	-	-	-
Jugs (n=0)	-	-	-	-
Jerrycan (n=27)	3.49±1.33[a]	0.30±0.10[a]	0.55±0.13[a]	2.04±0.37[a]
Claypot (n=0)	-	-	-	-
Bucket (n=0)	-	-	-	-
Sufuria (n=0)	-	-	-	-

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

Table 4. 17: Chemical parameters for water storage container types in Lare location in Njoro Sub-County.

WATER CONTAINER	CHEMICAL PARAMETERS (mean ± standard error)			
	NITRATE (mg/L)	MANGANESE (mg/L)	IRON (mg/L)	FLUORIDE (mg/L)
Gallon (n=3)	2.69±0.09[a]	0.04±0.01[a]	0.26±0.05[a]	2.43±1.01[a]
Jug (n=0)	-	-	-	-
Cup/mug (n=3)	4.21±0.91[a]	0.06±0.02[a]	0.04±0.01[a]	1.80±0.33[a]
Jerrycan (n=12)	7.20±1.88[a]	0.17±0.02[a]	1.61±0.47[a]	1.67±0.16[a]
Claypot (n=0)	-	-	-	-
Bucket (n=3)	9.25±1.28[a]	0.04±0.01[a]	1.06±0.01[a]	1.63±0.19[a]
Sufuria (n=0)	-	-	-	-

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

Table 4. 18: Chemical parameters for water storage container types in Mauche location in Njoro Sub-County.

WATER CONTAINER	CHEMICAL PARAMETERS (mean ± standard error)			
	NITRATE (mg/L)	MANGANESE (mg/L)	IRON (mg/L)	FLUORIDE (mg/L)
Gallons (n=0)	-	-	-	-
Jugs (n=3)	1.85±0.38[a]	0.32±0.08[a]	0.53±0.17[a]	3.39±1.84[a]
Cups/mugs (n=3)	0.65±0.19[a]	0.05±0.02[a]	1.05±0.02[a]	0.23±0.10[b]
Jerrycans (n=21)	4.60±0.48[a]	0.20±0.02[a]	0.83±0.16[a]	3.19±0.23[a]
Claypots (n=3)	0.25±0.08[a]	0.15±0.03[a]	0.39±0.17[a]	3.11±0.13[a]
Buckets (n=0)	-	-	-	-
Sufuria (n=3)	0.88±0.21[a]	0.04±0.01[a]	0.24±0.06[a]	0.44±0.17[b]

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

Table 4. 19: Chemical parameters for water storage container types in Maunarok location in Njoro Sub-County.

WATER CONTAINER	CHEMICAL PARAMETERS (mean ± standard error)			
	NITRATE (mg/L)	MANGANESE (mg/L)	IRON (mg/L)	FLUORIDE (mg/L)
Gallons (n=0)	-	-	-	-
Jugs (n=0)	-	-	-	-
Cups/mugs (n=0)	-	-	-	-
Jerrycans (n=30)	3.82±0.36	0.13±0.04	0.34±0.09	1.20±0.33
Claypots (n=0)	-	-	-	-
Buckets (n=0)	-	-	-	-
Sufuria (n=0)	-	-	-	-

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

Table 4. 20: Chemical parameters for water storage container types in Njoro location in Njoro Sub-County.

WATER CONTAINER	CHEMICAL PARAMETERS (mean ± standard error)			
	NITRATE (mg/L)	MANGANESE (mg/L)	IRON (mg/L)	FLUORIDE (mg/L)
Gallons (n=0)	-	-	-	-
Jugs (n=3)	5.63±0.39^{a}	0.03±0.01^{a}	1.56±0.35^{ab}	0.42±0.16^{a}
Cups/mugs (n=0)	-	-	-	-
Jerrycans (n=30)	2.56±0.77^{a}	0.17±0.04^{a}	0.43±0.11^{b}	1.34±0.06^{a}
Claypots (n=3)	0.96±0.22^{a}	0.05±0.02^{a}	1.15±0.02^{b}	3.12±1.05^{a}
Buckets (n=3)	0.33±0.11^{a}	0.04±0.01^{a}	0.89±0.29^{b}	1.87±0.04^{a}
Sufuria (n=3)	8.12±3.11^{a}	0.03±0.01^{a}	2.10±0.17^{a}	1.73±0.08^{a}

Means followed by the same small letter in a column are not significantly different at 5% LSD

- Not tested since no sample of that type was found for collection during sampling

4.4 Discussion

This study aimed at assessing the physical-chemical quality of drinking water in different sources and water stored in various household containers in Njoro Sub-County. Scarcity and inconsistency in water supply prompts individuals to store water in containers for future use. The type and cleanliness of the containers determines the quality of water at the points of use. Generally, the mean temperature, BOD and TDS were within the acceptable levels in the WHO guidelines of drinking water.

It was found that in water sources from all of the five locations, turbidity and EC levels were above the acceptable guideline limits by the WHO. High levels of EC are indicative of contamination with materials with mineral ions such as soil or chemicals such as aluminium sulphate used for water treatment, saline intrusion, nitrate and fecal pollution (Morrison *et al.,* 2001). High levels of turbidity is due to the fact that water carries along it many substances and also receive a lot of materials from run-offs and erosion and finds its way to homes (Shadrack, 2012). The results of this study were similar to Palamuleni and Akoth (2015) investigating borehole water in Mahikeng, South Africa.

Temperature measurements were high in water sampled from boreholes in Lare while the low temperatures of water at households can be attributed to the storage conditions under the roof as well as low intensity of sunlight. Temperature of water affects the concentration of DO and hence a good indication of contamination. An increase in water temperature reduces oxygen solubility and increases the metabolic activity of aquatic organisms (Delpla *et al.,* 2009). pH control is necessary at all stages of water treatment to ensure satisfactory water clarification and disinfection. For effective disinfection with chlorine, the pH should be less than 8, but pH less than 7 is acidic and corrosive. The mean pH at sources and households were within the recommended WHO standards for drinking water although higher values in cups in Mauche and claypots in Njoro could be attributable to either the use of chlorine for disinfection or contamination with acidic or fecal contaminants (Liang and Singer, 2003). Moreover, Das *et al.,* 2014, also reported that the pH of water from Ganges River in India was in the range of 7.06 to 8.35 similar to the results of this study.

The low levels of DO in some drinking water samples could be as a result of increased temperatures of the sample which in turn lowered the rate of oxygen solubility (Munoz *et al.,* 2015). On the other hand, the high BOD in water samples was indicative of the high fecal contamination of the drinking water sources as well as household storage containers. Das *et al.,* 2014 reported that the BOD in drinking water in Dhalai district, India ranged between 0.28 to 14.25 mg/L. BOD analysis was done in Njoro district by Kiruki *et al*

(2011) to determine the extent of organic pollution of River Njoro whereby the five day-BOD was in the range of 2.00 to 44 mg/L. The level of fluoride was higher in most ground water sources like wells and boreholes due to the location of Njoro Sub-County to the East African Great Rift Valley whereby the underground water from acquifiers interacts with the fluoride-bearing rocks. In fact, previous studies have reported high fluoride levels in the Kenyan Rift valley in raw beverages such as raw vegetable fruit juice as well as milk (Njenga *et al.,* 2005). As a result, all the vessels whose water had high fluoride levels were probably from underground water sources while those with low levels were probably rain harvested.

These findings are similar to others reported in a study to determine the levels of fluoride levels in Gilgil area, Nakuru County which reported high fluoride levels in water from taps, tanks, boreholes and Lake Elementaita (Wambu and Muthakia, 2011). In Njoro Sub-County, high fluoride concentrations have been previously documented in Njoro location with a large population suffering from dental fluorosis whereby the mean concentrations in rainwater, dam, wells, springs and boreholes were 0.5, 2.4, 4.1, 5.5, and 6.6 mg/L respectively (Moturi, 2002). This study also indicated that only the water stored in metal containers and clay pots reduced fluoride by up to 8.2% and 34.3% respectively and that 48.3% of children in Njoro location presented with moderate to severe dental fluorosis. In a study to determine the level of fluoride in the Rift valley, the samples collected from 18 sources in Njoro location recorded the mean fluoride levels which ranged from 0.78 mg/L (river samples) to 11.00 mg/L (boreholes) (Moturi, 2002).

Nitrates were low although a few sources like river and protected springs Mauche and dam water samples in Maunarok had high levels. This is due to extensive agricultural activities carried in these regions and hence excessive application of inorganic nitrogenous manure and fertilizers, waste water treatment and oxidation of human excreta (Maghanga *et al.,* 2012). Nitrates are associated with formation of methaemoglobinaemia or blue baby syndrome (Knobeloch *et al.,* 2000). In this case, nitrate is reduced to nitrite in the stomach of infants which in turn oxidizes hemoglobin to methaemoglobin which is unable to transport oxygen in the body. Furthermore, Khazenzi *et al.,* 2014 reported that 11 % of the wells used as sources of ground water in Langas, Kenya had mean nitrate levels above 10 mg/L. The presence of high levels of nitrates in drinking water sources during dry seasons is attributed to the leakage of these ions from the pit latrines through the soil into water bodies (Brender *et al.,* 2013).

The high levels of iron observed in the water sampled sources and household containers across the locations could be as a result of infiltration of iron from soil and pipes

for water supply, corrosion of steel as well as use of iron coagulants in water supply systems. The high iron levels from underground water such as boreholes or improved sources like tanks fed from boreholes could be due to high iron levels in rocks and soil due to its abundance in aquifers thus dissolving into water bodies. The findings of this study are similar to others which reported high iron levels in drinking water and ground water in Ota, Nigeria (Anake *et al.,* 2014). High iron has no clinical impacts on human health but leads to unacceptability of drinking water by the public due to altered color, taste, odor and staining of clothes. Such water should be treated using iron filters or water softeners before distribution to the communities. High manganese levels detected in some underground water sources such as boreholes as well as tanks and taps probably supplied by these sources could be due to prolonged contact of water with rocks bearing manganese (Li and Li, 2014). In the case of river water, contamination with manganese could due to application of inorganic fertilizers which are washed into the river during heavy rains (Kilonzo *et al.,* 2014). Analysis of heavy metals in River Athia-Galana-Sabaka tributaries in Kenya which are source of drinking water found that manganese was in concentrations of 0.187-1.048 mg/L (Muiruri *et al.,* 2013).

4.5 Conclusion

This study showed that various water sources as well as water stored in domestic storage containers in the Njoro Sub-County are vulnerable to physical and chemical contamination. The level of contamination varied across various locations although a common trend of high EC, turbidity and fluoride levels were observed. During the sampling periods, severe drinking water shortages and weak drinking water storage practices in homes were also observed. In some areas, very old tanks with and without covers which may stay for over six months without cleaning were used to store drinking water. This is an indication of pollution hazards in these areas which, in turn, have implications on the health of the people. Due to the toxicity levels imposed by the accumulation of metals like fluoride and iron in living body systems, it is important to constantly monitor their levels. Since most communities used borehole water, they are exposed to detrimental effects of fluorosis. The defluoridation methods as catridge filter (filled with bone char), *Moringa oleiferia* powder, rice husks and clay ion exchange resins can be applied to reduce the levels of fluoride in water. There should be an adequate training of communities on water storage, handling and treatment to ensure improvement in water quality.

4.6 References

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CHAPTER FIVE

ANALYSIS OF MICROBIAL QUALITY OF DRINKING WATER IN NJORO SUB-COUNTY, KENYA

Abstract

Drinking water should be free of microbial pathogens so as to be regarded as potable water and safe for drinking. However, water is prone to fecal contaminants which are the sources of gastrointestinal illnesses. In this study, various water sources and water stored in different containers was analyzed for its microbial quality. The various microbial parameters such as total viable colony counts, total coliforms, fecal coliforms and *E. coli* were evaluated. as total viable colony counts, total coliforms, fecal coliforms were tested by use of the culture methods while *E. coli* was tested using the CBT technique. Most of the water sources were contaminated. TVCC ranged from 0.47 to 1.76CFU/1mL. in water sources and 0.48 to 2.04CFU/1mL in domestic storage containers. TC was in the range of between 0.30 to 1.89CFU/100mL in water source and 0.59 to 2.47CFU/100mL in domestic storage containers. The mean FC in water sources was from 0.10 to 1.68CFU/100mL and from 0.81CFU/100mL in jerry cans. The *E. coli* as a fecal indicator was present in most of the water from sources and domestic storage containers in the range of 0.5 to >100MPN/100mL. Therefore frequent water testing should be performed by various water authorities as recommended by WHO. At households, the people should employ various water treatment methods and practice safe water handling so as to avoid gastrointestinal infections.

Keywords: *E. coli*, CBT, coliforms, water quality, contamination.

5.1 Introduction

Microbiological quality of water is measured by the use of indicator organisms such as Total viable colony counts (TVCC), Total coliform bacteria (TC) and fecal coliform bacteria (FC) specifically *E. coli* bacteria (McFeters, 2013). Total viable colony counts are also known as total plate count, heterotrophic plate count or pour plate method. It is used in the measurements of heterotrophic microorganisms in drinking. Heterotrophs consist of yeasts, molds, and bacteria which needs external sources of organic carbon in order to grow. TVCC method, therefore, accesses the formation of colonies on a culture media and hence it

is a measure of the overall bacteriological quality of drinking water in both public as well as private water systems. TC are bacterial species of fecal origin as well as other non-fecal bacterial groups (An *et al.,* 2002). The TC are indicative of the general hygienic quality of the water and potential risk of infectious diseases from the water. It is not economical and practical to test for each and every microorganism thus the presence of TC indicates the presence of pathogenic bacteria. Coliforms should not be detected in treated water supplies and, if found, suggest inadequate treatment, post-treatment contamination, or excessive nutrients. (Muhammad *et al.,* 2009). FC bacteria are associated with intestinal tract and hence are released to the environment by fecal wastes from animals and humans (Casanovas-Massana and Blanch, 2013). Being the most abundant in the fecal coliforms, *E. coli* is commonly used as an indicator of fecal pollution (Djuikom *et al.,* 2006). The optimum temperature for *E. coli* growth is 37 ºC and it thrives well in the intestines of warm-blooded animals and thus regarded as the best indicator of fecal contamination (Brennan *et al.,* 2013).

In a study to determine the bacteriological quality of the Njoro River by Kiruki et al (2011), the frequency of bacteria isolated in 216 samples collected from the River included species such as Hafnia alvei (29.2 %), Salmonella typhimurium (18 %), Aeromonas hydrophila (52 %) and *E. coli* (19.8 %). In another study that investigated the contamination chain of domestic water in the Njoro Township in Kenya (Macharia *et al.,* 2015), the *E. coli* density was in the range of 0–220 CFU/100 mL (point of collection) and 0–520 CFU/100 mL (low-income households and vendors). This study, therefore, sought to determine the microbial quality of drinking water from various sources and water stored inside various household storage containers within the five locations in Njoro Sub-County.

5.2 Materials and methods

5.2.1 Determination of TVCC in drinking water

TVCC test was done using the standard pour plate method according to the method of Donde *et al.,* (2013) (Figure 5.1b). Briefly, 1 ml of the water sample was aseptically transferred into clearly marked sterile Petri dishes. A volume of 15 ml of sterile molten Plate Count Agar (PCA) at 4 °C was added to each of the Petri dishes and thoroughly swirled to facilitate sample distribution in the media. The plates were then left to solidify at room temperature, inverted upside down and incubated for 24 hours at 37°C. The colonies were recorded as colony forming units per ml (CFU/1 mL).

5.2.2 Total coliform counts (TC)

TC was enumerated by membrane filtration technique method (Stuart, Bibby scientific, UK). A sterile 0.45 µm, 47 mm membrane filter (Sartorius, Germany) was placed on a filter funnel. A volume of 10 mL of each water sample was added to a membrane and the vacuum pump turned on. After the sample was passed through the filter, the funnel was rinsed with 20 mL of distilled water and maintained in the vacuum until all liquid had passed. The filter was then transferred using a sterile forceps to a 50 mm disposable Petri dish containing Eosin Methylene Blue (EMB) agar. Each funnel was rinsed with 20 mL distilled water between each water sample. All Petri dishes were incubated upside down in an incubator for 24 ± 2 hours at 37 °C. All samples were analyzed by counting the blue and pink colonies under a colony counter (Acculite, Fisher, USA) as CFU/100 mL as shown in Figure 5.1.

5.2.3 Isolation and Enumeration of *E. coli*

The commercially available Compartmental Bag Test (CBT) and methodology according to the manufacturer were followed (Aquagenx, Chapel Hill, NC, USA). Briefly: after the water sample was collected, 100 ml was poured into a sterile sample bottle and the chromogenic growth medium X-Gal (5 Bromo 4-Chloro 3-Indolyl β-D-Galactopyranoside) which was supplied by the manufacturer, was added to the sample bottle and swirled until all the substrate had dissolved in water (turn brown in color). The prepared water sample was then poured into the supplied CBT bag and evenly distributed into all compartments by manually gently squeezing to ensure that each sample volume was filled to the set mark (Hi-media laboratories, Mumbai, India). Each bag was sealed using a two-piece plastic clip and incubated at 30-37ºC overnight.

E. coli density was scored in a spreadsheet provided as MPN/100 ml of water through the combination of positive and negative compartments of the bag per sample. Blue/green color was indicative of *E. coli* positive while brown/yellow color indicated negative *E. coli* for the sample. A total of 2mL of each *E. coli* positive compartment samples were collected in sterile cryotubes using sterile syringes and stored at 4°C for further analysis. The remaining water samples in the CBT bag were decontaminated by adding three chlorine tablets and swirling until all the tablets dissolved and then safely discarded according to the manufacturer's specifications.

5.3 Results

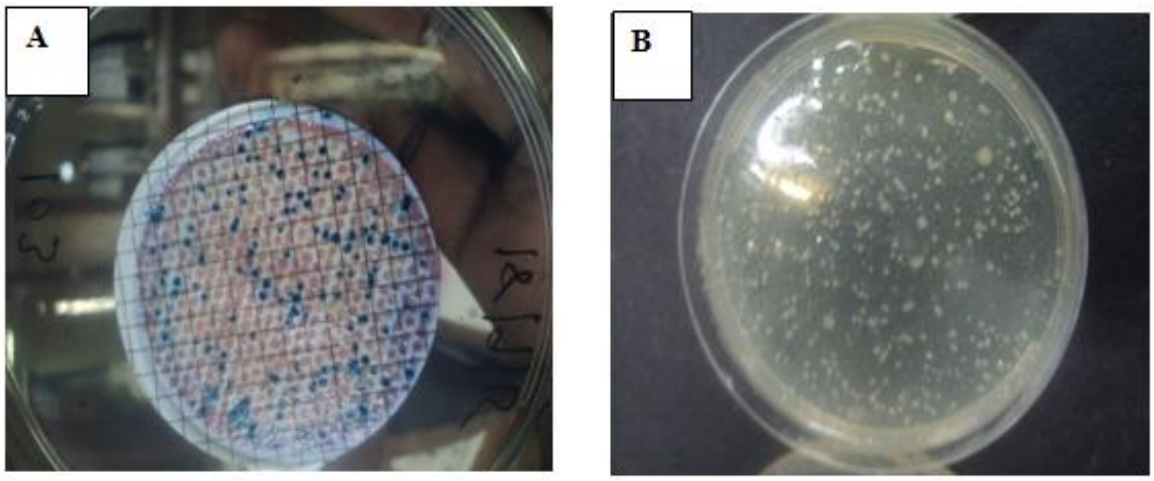

Figure 5. 1: TC (A) and TVCC (B) colonies after incubation in EMB and PCA media respectively.

5.3.1 TVCC in water sources and water in various household storage containers

All the sampled water sources were contaminated with TVCC which was in the range of $\log_{10}$ 0.48±0.12 (unprotected wells in Njoro) to 1.76±0.05 (taps in Maunarok and boreholes in Mauche) CFU/1mL as shown in Table 5.1 In household storage containers, the highest $\log_{10}$ mean of TVCC CFU/1mL were recorded in buckets used to store drinking water in Lare (2.04±0.01CFU/100 mL) while lowest means were in sufuria in Mauche (0.48±0.09CFU/100 mL) as indicated in Table 5.2.

5.3.2 TC counts in water sources and water in various household storage containers

All the water sources in Njoro Sub-County were contaminated with TC as shown in Table 5.3. The mean $\log_{10}$ TC CFU/100 mL in the water sources were highest in tanks in Mauche (2.12±0.27CFU/100 mL) and lowest in taps in Mauche (0.30±0.00 CFU/100 mL) and in unprotected wells in Njoro (0.30±0.07CFU/100mL). All the sampled household containers were contaminated with TC which ranged from $\log_{10}$ 0.59±0.09CFU/100mL (jerry cans in Njoro) to 2.47±0.23CFU/100mL (buckets in Lare) as shown in Table 5.4.

5.3.3 FC counts in water sources and water in various household storage containers

The mean $\log_{10}$ FC CFU/100mL is presented in Table 5.5 whereby the counts ranged from 0.00 to 1.68±0.07CFU/100mL. In household containers, all the containers had mean $\log_{10}$ FC counts of 0.00CFU/100mL apart from jerry cans in Maunarok (0.81±0.25CFU/100mL) respectively (Table 5.6).

Table 5. 1: Mean log_{10}TVCC CFU/1mL for water source types in Njoro Sub-County.

WATER SOURCE		KIHINGO mean±SE (n = number of samples)	LARE mean±SE (n = number of samples)	MAUCHE mean±SE (n = number of samples)	MAUNAROK mean±SE (n = number of samples)	NJORO mean±SE (n = number of samples)
Unimproved sources	River	-	-	-	-	1.18±0.03[a] (n=3)
	Springs	-	-	-	-	-
	Wells	-	-	-	-	0.48±0.12[a] (n=3)
	Dams	-	-	-	-	-
Improved sources	Springs	-	-	-	-	-
	Taps/piped water	-	1.14±0.18[a] (n=9)	0.70±0.14[a] (n=3)	1.76±0.05[a] (n=3)	1.06±0.16[a] (n=12)
	Tanks	1.20±0.18[a] (n=9)	0.63±0.15[a] (n=6)	0.94±0.29[a] (n=9)	1.31±0.14[a] (n=15)	-
	Boreholes	0.69±0.14[a] (n=6)	-	0.48±0.13[a] (n=3)	-	-
	Wells	1.47±0.11[a] (n=6)	-	-	-	-

SE = standard error - Not tested since no sample of that type was found for collection during sampling
Means followed by the same small letter in a column are not significantly different at 5% LSD

Table 5. 2: Mean log_{10} TVCC CFU/1mL for domestic containers in Njoro Sub-County.

WATER CONTAINER	KIHINGO mean±SE (n = number of samples)	LARE mean±SE (n = number of samples)	MAUCHE mean±SE (n = number of samples)	MAUNAROK mean±SE (n = number of samples)	NJORO mean±SE (n = number of samples)
Gallons	1.00±0.02 (n=3)	-	-	-	-
Jugs	-	-	1.08±0.14 (n=3)	-	-
Cups	-	-	-	-	-
Jerry cans	-	1.07±0.17[a] (n=6)	-	1.20±0.30 (n=6)	0.93±0.08 (n=6)
Clay pots	-	-	1.40±0.11 (n=3)	-	-
Buckets	-	2.04±0.01[a] (n=3)	-	-	-
Sufurias	-	-	0.48±0.09 (n=3)	-	-

SE = standard error - Not tested since no sample of that type was found for collection during sampling
Means followed by the same small letter in a column are not significantly different at 5% LSD

Table 5. 3: Mean log_{10} TC counts/100mL for water source types in Njoro Sub-County.

WATER SOURCE		KIHINGO mean±SE (n = number of samples)	LARE mean±SE (n = number of samples)	MAUCHE mean±SE (n = number of samples)	MAUNAROK mean±SE (n = number of samples)	NJORO mean±SE (n = number of samples)
Unimproved sources	River	-	-	-	-	1.40±0.03[a] (n=3)
	Springs	-	-	-	-	-
	Wells	-	-	-	-	0.30±0.02[a] (n=3)
	Dams	-	-	-	-	-
Improved sources	Springs	-	-	-	-	-
	Taps/piped water	-	1.59±0.50[a] (n=9)	0.30±0.07[a] (n=3)	1.64±0.49[a] (n=3)	0.90±0.22[a] (n=12)
	Tanks	1.87±0.03[a] (n=9)	1.56±0.11[a] (n=6)	2.12±0.27[a] (n=9)	1.73±0.13[a] (n=15)	-
	Boreholes	1.11±0.41[a] (n=6)	-	0.60±0.21[a] (n=3)	-	-
	Wells	1.89±0.08[a] (n=6)	-	-	-	-

SE = standard error - Not tested since no sample of that type was found for collection during sampling

Means followed by the same small letter in a column are not significantly different at 5% LSD

Table 5. 4: Mean log_{10} TC CFU/100mL for domestic containers in Njoro Sub-County.

WATER CONTAINER	KIHINGO mean±SE (n = number of samples)	LARE mean±SE (n = number of samples)	MAUCHE mean±SE (n = number of samples)	MAUNAROK mean±SE (n = number of samples)	NJORO mean±SE (n = number of samples)
Gallons	0.95±0.31 (n=3)	-	-	-	-
Jugs	-	-	2.09±0.21 (n=3)	-	-
Cups	-	-	-	-	-
Jerry cans	-	1.42±0.27[a] (n=6)	-	1.76±0.18 (n=6)	0.59±0.09 (n=6)
Clay pots	-	-	2.09±0.39 (n=3)	-	-
Buckets	-	2.47±0.23[a] (n=3)	-	-	-
Sufurias	-	-	1.74±0.08 (n=3)	-	-

SE = standard error - Not tested since no sample of that type was found for collection during sampling

Means followed by the same small letter in a column are not significantly different at 5% LSD

Table 5. 5: Mean log_{10} FC CFU/100mL for water source types in Njoro Sub-County.

WATER SOURCE		KIHINGO mean±SE (n = number of samples)	LARE mean±SE (n = number of samples)	MAUCHE mean±SE (n = number of samples)	MAUNAROK mean±SE (n = number of samples)	NJORO mean±SE (n = number of samples)
Unimproved sources	River	-	-	-	-	0.00±0.00[a] (n=1)
	Springs	-	-	-	-	-
	Wells	-	-	-	-	0.00±0.00[a] (n=3)
	Dams	-	-	-	-	-
Improved sources	Springs	-	-	-	-	-
	Taps/piped water	-	1.08±0.12[a] (n=9)	0.00±0.00[a] (n=3)	1.43±0.05[a] (n=3)	0.00±0.00[a] (n=12)
	Tanks	0.10±0.02[b] (n=9)	0.64±0.11[a] (n=6)	0.00±0.00[a] (n=9)	0.97±0.28[a] (n=15)	-
	Boreholes	0.00±0.00[b] (n=6)	-	0.00±0.00[a] (n=3)	-	-
	Wells	1.68±0.07[a] (n=6)	-	-	-	-

SE = standard error - Not tested since no sample of that type was found for collection during sampling

Means followed by the same small letter in a column are not significantly different at 5% LSD

Table 5. 6: Mean log_{10} FC CFU/100mL for domestic storage containers in Njoro Sub-County.

WATER CONTAINER	KIHINGO mean±SE (n = number of samples)	LARE mean±SE (n = number of samples)	MAUCHE mean±SE (n = number of samples)	MAUNAROK mean±SE (n = number of samples)	NJORO mean±SE (n = number of samples)
Gallons	0.00±0.00 (n=3)	-	-	-	-
Jugs	-	-	0.00±0.00[a] (n=3)	-	-
Cups	-	-	-	-	-
Jerry cans	-	0.00±0.00[a] (n=6)	-	0.81±0.25 (n=6)	0.00±0.00 (n=6)
Clay pots	-	-	0.00±0.00[a] (n=3)	-	-
Buckets	-	0.00±0.00[a] (n=3)	-	-	-
Sufurias	-	-	0.00±0.00[a] (n=3)		-

SE = standard error - Not tested since no sample of that type was found for collection during sampling

Means followed by the same small letter in a column are not significantly different at 5% LSD

5.3.4 CBT analysis of *E. coli* density in water from sources and in various household storage containers

Figure 5.2 shows the *E. coli* counts in MPN/100mL as appearing on the CBT bag after overnight incubation at 37ºC.

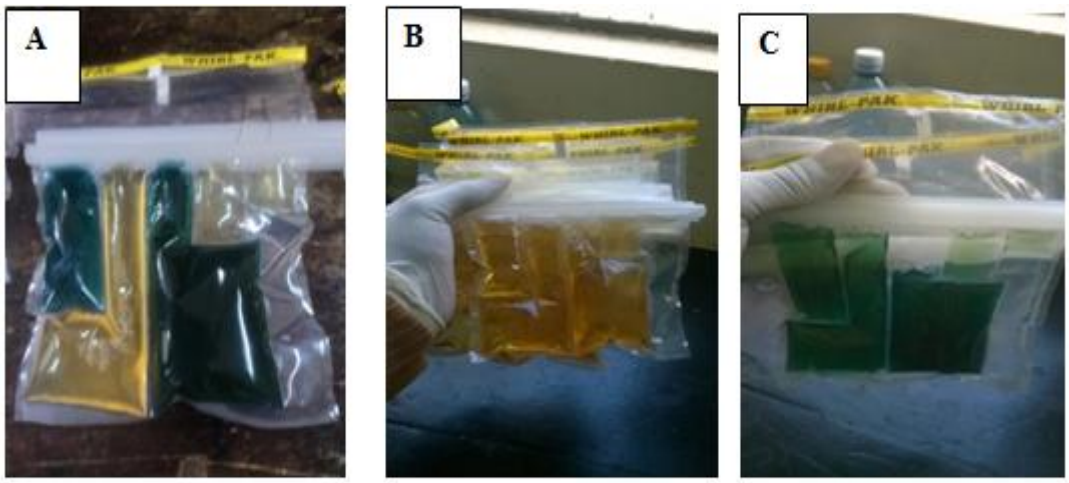

Figure 5. 2: CBT bags showing *E. coli* counts: 3.4 (A), 0 (B) and >100 (C)*E. coli* MPN/ 100 ml respectively.

The classification of water based on the WHO health risk categories system categories (WHO, 2011) is shown in 5.7 to 5.9. The mean *E. coli* concentrations across the sampling locations were above the recommended levels for drinking water as shown in the Tables 5.8 and 5.9. In Kihingo, the mean *E. coli* concentrations were highest in water sampled from the wells and were in the range of 2.10±0.32MPN/100mL (boreholes) to 71.87±29.13 MPN/100mL (unprotected wells). In Lare, the *E. coli* density was in the range of 0.80±0.30 (boreholes) to 24.34±9.94MPN/100mL (tanks) while in Mauche, the protected springs and the River recorded similar highest *E. coli* levels of more than 100.00±22.49MPN/100mL. In Njoro, the samples from the River, unprotected springs, and unprotected springs had a mean density of more than 100MPN/100mL while boreholes had the lowest mean density of 3.68±0.56MPN/100mL. In Maunarok location, the samples from dams recorded means of more than 100.00±15.69MPN/100mL while protected wells and protected springs recorded densities of 49.93±5.80MPN/100mL and 34.00±6.70MPN/100mL respectively (Table 5.8).

In Kihingo, all the water sources fall into the high risk/probably unsafe category except for borehole sources that are in the intermediate/probably safe risk category. In Lare location, boreholes and tanks are a source of intermediate/probably safe risk drinking water while the taps/piped water system is in the high risk/probably unsafe group. In Mauche, the

River and protected springs fall into the very high risk/unsafe category, while dams, taps, boreholes and tank water is in the high risk/probably unsafe categories (Table 5.8). In Maunarok location, the dam water sources are in the very high/unsafe risk while boreholes, protected springs, and protected wells are in the high risk/probably safe categories respectively. On the other hand, unprotected wells, improved tanks and tap water in Maunarok are in the intermediate/probably safe category (Table 5.8). In Njoro location, River, unprotected springs, and unprotected wells are in the very high risk/unsafe category while protected springs, taps, and tank water samples are on the high risk/probably safe categories respectively and the boreholes are all in the intermediate/probably safe risk category (Table 5.8).

Table 5. 7: color coding to indicate health risk categories of drinking water (based on WHO-4th Edition, 2011).

Risk level	**Very high risk/ unsafe**	**High risk/ probably unsafe**	**Intermediate risk/ probably safe**	**Safe**
***E. coli* count**	>100 MPN/100 mL	>10-100 MPN/100mL	1-10 MPN/100mL	<1MPN/ 100mL
Color code				

Table 5. 8: *E. coli* MPN/100mL for water source types in Njoro Sub-County.

WATER SOURCE		KIHINGO mean±SE (n = number of samples)	LARE mean±SE (n = number of samples)	MAUCHE mean±SE (n = number of samples)	MAUNAROK mean±SE (n = number of samples)	NJORO mean±SE (n = number of samples)
Unimproved sources	River	-	-	101.00±12.92^{a} (n=3)	-	101.00±21.05^{a} (n=12)
	Springs	-	-	-	-	101.00±10.38^{a} (n=3)
	Wells	71.87±29.13^{a} (n=9)	-	-	4.70±1.37^{a} (n=3)	101.00±8.24^{a} (n=3)
	Dams	-	-	48.30±9.24^{a} (n=3)	101.00±15.69^{a} (n=3)	-
Improved sources	Springs	-	-	101.00±22.49^{a} (n=6)	34.00±6.70^{a} (n=9)	48.30±7.67^{ab} (n=3)
	Taps/piped water	57.30±8.74^{bc} (n=6)	24.34±0.41^{a} (n=15)	17.56±2.33^{a} (n=27)	1.50±0.02^{a} (n=3)	20.38±2.41^{b} (n=33)
	Tanks	15.51±1.60^{c} (n=42)	4.70±0.18^{a} (n=6)	36.88±4.07^{a} (n=18)	4.51±1.80^{a} (n=33)	24.03±11.04^{b} (n=36)
	Boreholes	2.10±0.32^{c} (n=2)	0.80±0.30^{a} (n=6)	51.05±9.99^{a} (n=6)	13.60±2.72^{a} (n=3)	3.68±0.56^{b} (n=51)
	Wells	69.38±12.91^{ab} (n=15)	-	-	49.93±5.80^{a} (n=9)	-

SE = standard error - Not tested since no sample of that type was found for collection during sampling

Means followed by the same small letter in a column are not significantly different at 5% LSD

The mean *E. coli* density in household storage containers across all the five locations in Njoro Sub-County are presented in table 5.9. Generally, the concentrations were higher than the WHO recommended levels of 0MPN/100mL. All the water samples in gallons and jerry cans in Kihingo storage containers fall into the high risk/probably unsafe category. In Lare, water in gallons and cups is classified as intermediate/probably safe risk while water in buckets and jerry cans is high risk/probably unsafe category. In Mauche, water stored in clay pots is in the very high risk/unsafe category since the *E. coli* density exceeds 100 MPN/100 mL, jerry cans and sufuria water samples are in the high risk/probably unsafe risk category while jug and cup water are in the intermediate/probably safe category. Water from jerry cans

in Maunarok location are in the intermediate/probably safe risk category. In Njoro, samples from jerry cans, clay pots, and buckets are in the high risk/probably unsafe category while samples from sufuria and jugs were in the intermediate/probably safe risk group.

Table 5. 9: *E. coli* MPN/100mL for domestic storage containers in Njoro Sub-County.

WATER CONTAINER	KIHINGO mean±SE (n = number of samples)	LARE mean±SE (n = number of samples)	MAUCHE mean±SE (n = number of samples)	MAUNAROK mean±SE (n = number of samples)	NJORO mean±SE (n = number of samples)
Gallons	34.00±6.70[a] (n=9)	4.70± 078[a] (n=3)	-	-	-
Jugs	-	-	0.50±0.13[a] (n=3)	-	3.40±1.03[a] (n=3)
Cups	-	4.70±1.31[a] (n=3)	9.10±2.19[a] (n=3)	-	-
Jerry cans	17.28±2.34[a] (n=27)	75.88±25.13[a] (n=12)	32.96±3.54[a] (n=21)	9.50±0.88 (n=30)	13.06±1.96[a] (n=30)
Clay pots	-	-	101.00±18.38[a] (n=3)	-	13.60±2.44[a] (n=3)
Buckets	-	13.60±3.48[a] (n=3)	-	-	13.60±2.18[a] (n=3)
Sufurias	-	-	48.30±9.74[a] (n=3)	-	0.50±0.07[a] (n=3)

SE = standard error - Not tested since no sample of that type was found for collection during sampling

Means followed by the same small letter in a column are not significantly different at 5% LSD

5.4 Discussion

The results obtained for microbial quality in Njoro Sub-County indicated that majority of the drinking water sources were contaminated. The high concentrations of TVCC and TC were an indication of a load of contamination in water otherwise meant for drinking purposes. A similar study on microbial analysis of stored and treated drinking water in Nakuru North Sub-County found that 35% (189/540) of all the samples were positive for TC (51.8%), *E. coli* (32.3%) and Salmonella (15.9%) respectively (Nyamache *et al.*, 2014). This indicated that the water from sources and storage household containers in Nakuru North-Sub-County did not meet the microbial quality guidelines by WHO in order to qualify for drinking purposes. High numbers of TVCC bacteria in a distribution system might be a result re-growth of bacteria that resisted treatment. Bacterial re-growth leads to corrosion of pipes and increases the growth of slime (Walter, 2009).

The decrease in TVCC, TC and FC levels in some household storage containers as compared to sources was probably due to employment of treatment methods such as boiling and chlorination. An increase in the bacterial counts at households as compared to the water sources might be linked to further deterioration of drinking water with fecally contaminated hands or objects. A study conducted in Vietnam also found off-premises piped sources to contain more fecal contamination than on-premises piped sources, with evidence of similarly stored water quality for both source types (Brown *et al.,* 2013). Additionally, there is a possibility of contamination of water by vendors or during transportation from off premises to homesteads, during storage and handling (Macharia *et al.,* 2015).

The high levels of *E. coli* in storage containers may refer to the lack of regular cleaning, defects on the pipelines or contamination during distribution. High *E. coli* in gallons and pots are due to their small diameters which make it hard to clean them with ease frequently (Jensen *et al.,* 2002). The trends of storing water for long in households can result in a possibility of fecal contamination of maybe initially good-quality drinking water. Such contamination can arise from dipping of fecally contaminated hands or utensils in the storage containers, especially by children. This form of contamination pathway at the household is independent of pollution at the source because the source might be free from contamination (Eshcol *et al.,* 2009). On the other hand, in case the source is polluted, further pollution at the household can increase the load and hence a greater health risk to the users (Pickering *et al.,* 2010).

The high *E. coli* density in Mauche is due to the fact that the area is largely served by the Njoro River as their source of drinking water. This implies that either they do not treat their water adequately before storage or it can be due to poor hygiene practices within the households. These findings are supported by other studies on the bacteriological quality of drinking water sources in Njoro Division, that indicated that there were higher fecal coliform counts in the Njoro River as compared to the WHO guidelines (Mavura *et al.,* 2006), while another study reported that Njoro River was highly contaminated with indicator bacteria *E. coli* (Kiruki *et al.,* 2011).

5.5 Conclusion

The presence of *E. coli* in drinking water is of great public health significance and may lead to the onset of various diseases. Being a faecal-oral pathogen, there are other vehicles necessary for its transmission for instance contaminated hands, foods and utensils. The untreated water sources and household stored water used for drinking and other domestic

purposes as well as the *E. coli* bacteria from these sources are potential threats to the health of residents. This calls for urgent intervention strategies by the government, the community and other stakeholders to minimize the health risks associated with consumption of contaminated water.

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CHAPTER SIX

MOLECULAR CHARACTERISATION OF *E. COLI* USING MULTIPLEX POLYMERASE CHAIN REACTION

Abstract

The diarrheagenic *E. coli* (DEC) are classified into several pathotypes depending on the nature of the intestinal disease that they cause. However, the commensal (Com *E. coli*) is the normal strain that exists in the human gut but does no cause disease. Therefore m-PCR was used in this study to amplify the DEC and ComEC genes simultaneously with the inclusion of both internal and external controls to monitor inhibition. In this study, the samples which were positive for *E. coli* using the CBT techniques were subjected to multiplex PCR to determine the virulent strains present in drinking water samples and their relative frequencies. A total of 111 CBT positive water samples collected in Njoro Sub-County were analyzed in a PCR reaction which targeted the *stx, stx2, eagg, astA, bfp, eaeA, lt, st* and *ial* virulence genes. The *mdh* and *gapdh* genes were included in the reaction as internal and external controls, respectively. The external control *gapdh* gene in all samples excluded any possible PCR inhibition. The internal control *mdh* gene was used to confirm the classification of the samples as PCR positive samples because it was detected in 100 % of the waters samples. All the pathogenic, as well as commensal *E. coli* pathotypes were detected at varying frequencies per water sample. Moreover, strain combinations were detected in the same water samples. This indicates that some water used for drinking purposes is contaminated and the communities are likely to suffer from diarrhoeal diseases.

Keywords: Diarrhoeagenic *E. coli*, Multiplex PCR, pathotypes, water samples.

6.1 Introduction

Diarrhoeal diseases in Kenya are among the five main causes of mortality in children younger than five years (Berkley *et al.,* 2005). Bacterial diarrhea has been reported to account for up to 30% of all cases of infantile diarrhea and as the most common cause of travelers' diarrhea (Oundo *et al.,* 2008). Apart from protozoans such as Giardia lamblia, Entamoeba histolytica, Cryptosporidium parvum, Isospora belli and viruses such as Rotavirus and Norovirus implicated in cases of diarrhea, frequently isolated bacterial diarrhoeagenic agents include *E. coli*, Campylobacter jejuni/coli, Salmonella, Shigella, and Aeromonas species (Obi *et al.,* 2004). Evidence from studies indicates that diarrhoeagenic *E. coli* (DEC) are potential

public health risks, with EHEC O157: H7 causing life-threatening sequelae, including HUS and thrombocytopenic purpura, which causes kidney failure, hemolytic anemia, and thrombocytopenia (Trachtman *et al.*, 2012).

The spread of different pathogenic *E. coli* is a major concern in developing countries where it is enhanced by factors such as harsh climatic conditions, poor sanitation, malnutrition, immunosuppression related to HIV and AIDS and consumption of contaminated water (Clarke, 2001). DEC constitutes a potential human health risk because it is an indicator of the presence of intestinal pathogens of fecal origin (Kaper *et al.*, 2004). At the moment there are seven groups of pathogenic *E. coli* but for this study, five pathotypes were selected they are very important as far as surface-water pathogenicity is concerned. The DEC pathotypes are classified into entero-haemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), and enteroinvasive *E. coli* (EIEC) (Jafari *et al.*, 2012). The virulence mechanisms which characterize *E. coli* are genetically coded for chromosomal, plasmid and bacteriophage DNAs. They are heat-labile and heat-stable toxins, Verotoxin, cytotoxic necrotizing factor (CNF1 and CNF2), attaching and effacing mechanisms (*eaeA*), enteroaggregative mechanisms (*Eagg*) and enteroinvasive mechanism (*Einv*). Major differences in virulence between pathotypes might be related to the presence of specific pathogenicity islands which increase gene mobility within various mobile elements such as plasmids (Makobe *et al.*, 2012).

Recently, health consequences associated with DEC infection has been worsened by the emergence of multidrug-resistant *E. coli* to antibiotics recommended by WHO as first-line treatment for diarrhea such as ampicillin, tetracycline, sulphamethoxazole–trimethoprim, chloramphenicol, and augmentin (Kariuki *et al.*, 1997). It is therefore recommended that children presenting with persistent diarrhea, epidemic gastroenteritis episodes and immunocompromised patients with severe diarrhea get diagnosed for *E. coli* pathotypes. In Kenya, most of the documented researches involving DEC strains are carried out on clinical fecal samples. From the literature study, it seems like no study has been done in Kenya on molecular characterization of DEC strains found in water. Therefore, this study took the positive *E. coli* samples from the various sources and household storage containers and used a standardized multiplex PCR (m-PCR) protocol to detect the prevalence of virulence factors of *E. coli* in an attempt to elucidate the molecular epidemiology of virulent strains.

6.2 Materials and methods

6.2.1 DNA extraction

From each of the positive *E. coli* CBT samples (Chapter five), a total volume of 2 mL of the positive contents of the CBT bag was collected and preserved in 2 mL cryotubes at -70°C for DNA extractions. Genomic DNA was isolated using a DNA extraction kit according to the manufacturer's instructions (Qiagen, USA). All the preparations were stored at -20°C until used. Briefly, 1.5ml of the CBT positive samples were transferred to a 1.5ml Eppendorf tube and centrifuged at 15,00g for two minutes to pellet the cells. The supernatant was discarded and the pellet was then resuspended in lysis 600µl buffer and incubated at 8°C for five minutes to denature membranes and proteins. Then 3µl of the RNase solution was added to the contents of the tube mixed and incubated at for 30°C minutes to break down the RNA. After adding the precipitation solution, the tube contents were centrifuged at 15,000g for 3minutes. The supernatant containing the genomic DNA was then transferred to a clean 1.2ml microcentrifuge tube containing 600µl of isopropanol and vortexed. The tube contents were centrifuged at 15,000g for two minutes and the supernatant poured. Then 600µl of 70% ethanol vortex and centrifuge at 15,000g for two minutes. The pellet was air-dried for 15minutes and then 100µl of RNA rehydration solution to the tube containing the DNA pellet and incubated at 65°C for 1 hour and stored at 4°C.

6.2.2 Detection of *E. coli* pathotypes by m-PCR

The protocol described by Omar and Barnard (2014) was used to detect the presence of DEC genes in all water samples. All m-PCR reactions were performed in a Biorad MycyclerTM Thermal cycler in a total volume of 20µl. The primers used are shown in Table 6.1. Each reaction consisted of 1X Qiagen® PCR multiplex mix (containing HotstartTaq® DNA polymerase, m-PCR buffer, and dNTP mix) (Qiagen, USA); 2µl of the primer mixture [0.1 lM of *mdh* and *lt* primers, 0.2 lM of *ial*, *eagg*, *astA*, *bfp* and *gapdh* primers (F and R), 0.3 lM of *eaeA* and stx2 primers (F and R), 0.5 lM of *stx1* and *stx2* primers], 2 µl of DNA sample, 1 µl of *gapdh* cDNA, hotstart *Taq* polymerase and 5ul of PCR grade water. The reactions were subjected to denaturing step at 95°C for 15min, 35 cycles that consisted of denaturation at 95°C for 45seconds, annealing at 55°C for 45 seconds, extension at 68°C for 2minutes and final elongation at 72°C for 5minutes (Omar and Barnard, 2014).

The negative control lacking the template DNA was included to rule out the possibility of reagent contamination. A positive *E. coli* control was supplied by the

University of Venda microbiology laboratory. Bacterial DNA was analysed using a 2.5% (w/v) agarose gel in TAE buffer (40 mmol-1 Tris-acetate; 2 mmol-1 EDTA, pH 8.3) with 0.5µgml-1 Ethidium Bromide. DNA was electrophoresed for 1-2 hrs in electric field strength of the 8Vcm-1 gel. DNA was visualized using UV light (Gene Genius Bio Imaging system, Vacutec®) and the relative sizes of the DNA fragments were estimated by comparing their electrophoretic mobility with that of the standards run with the samples on each gel, either 1 kB or 100 bp markers (Fermentas®).

Table 6. 1: Primers used for identification of DEC (Omar and Barnard, 2014).

PATHOGENIC STRAIN	PRIMER	SEQUENCE	REFERENCE
E. coli	Mdh (forward and reverse)	GGTATGGATCGTTCCGACCT GGCAGAATGGTAACACCAGAGT	Tarr et al (2002)
EIEC	Lal (forward and reverse)	GGTATGATGATGATGAGTGGC GGAGGCCAACAATTATTTCC	Paton and Paton (1998)
EHEC/EPEC	EaeA (forward and reverse)	CTGAACGGCGATTACGCGAA GACGATACGATCCAG	Aranda et al (2004)
EAEC	Eagg (forward and reverse)	AGACTCTGGCGAAAGACTGTATC ATGGCTGTCTGTAATAGATGAGAAC	Kong et al (2002)
EHEC	Stx 1 (forward and reverse)	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	Moses et al (2006)
	Stx 2 (forward and reverse)	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCACTTTG	
ETEC	LT (forward and reverse)	GGCGACAGATTATACCGTGC CGGTCTCTATATTCCCTGTT	Pass et al (2000)
	ST (forward and reverse)	TTTCCCCTCTTTTAGTCAGTCAACTG GGCAGGATTACAACAAAGTTCACA	

6.3 Results

Out of the 111 water samples tested, *st* and *lt* genes (ETEC), *eagg* gene (EAEC), *stx1* gene, *stx2* gene (EHEC), *eaeA* gene (Atypical-EPEC), *bfp* gene (Typical-EPEC) and *ial* gene (EIEC) tested positive as shown in tables 5.2 and 5.3 with their relative frequencies. DEC was found in 59 samples (53.15%) while ComEC was detected in 52 samples (46.85%). The

results indicated that 100% of the water samples had the *mdh* gene which confirms that they were positive for *E. coli* (either ComEC or DEC).

Table 6. 2: Summary of the pathogenic *E. coli* combination frequencies.

Pathotype Combination	**Frequency**
aEPEC+EAEC	2
tEPEC+ETEC	6
ETEC+EAEC	2
aEPEC+ETEC	5
EHEC+EAEC	1
EHEC+ETEC	1
aEPEC+EHEC+ETEC	1
tEPEC+EIEC	1
tEPEC+EHEC+ETEC	1
tEPEC+EAEC	1
tEPEC+EHEC+ETEC+EAEC	1
aEPEC+ETEC+EAEC	1

6.3.1 Pathogenic *E. coli* strains in water sources

The results of the pathogenic *E. coli* strains present in the water sources are shown in Tables 6.2. Water samples from the sampled improved water sources indicated a higher variety and frequency of DEC strains as compared to the unimproved water sources.

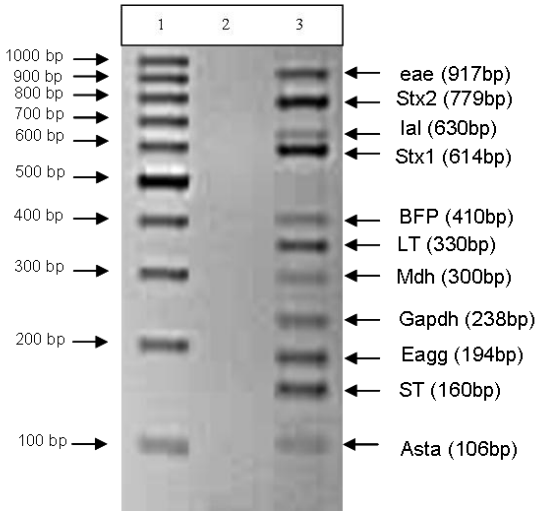

Figure 6. 1: Gel electrophoresis set-up of pathogenic *E. coli.* Line 1 = molecular weight marker; Line 2 = negative control; Line 3 = *E. coli* virulence markers.

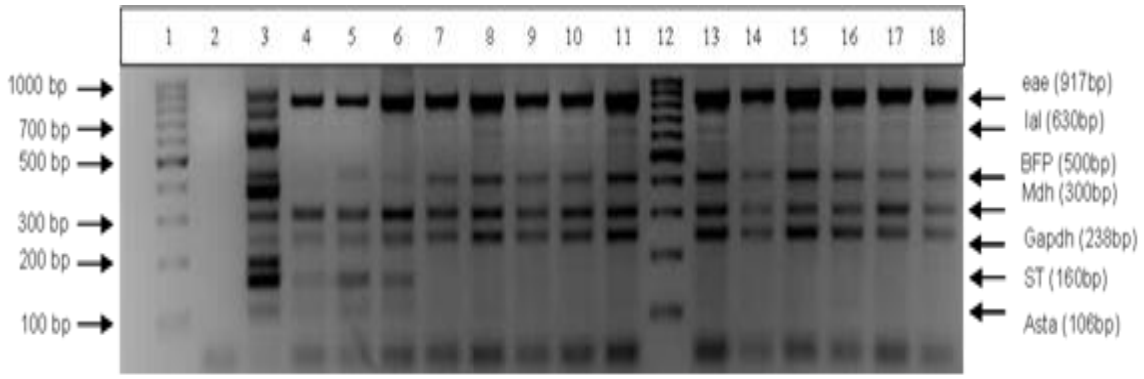

Figure 6. 2: Agarose gel image for water samples: Lane 1 and 12 (molecular weight markers), Lane 2 (No template control included) and Lane 3 (*E. coli positive* reference strains) Lane 4-11, 13-18 (water samples).

In Kihingo, the highest density of commensal *E. coli* was recorded in unprotected wells (66.67%) followed by protected wells (60%), tap water (50%) and tank (7.14%) water. In Lare, the prevalence was 50% in boreholes and tanks while in taps commensal *E. coli* was 60%. In Mauche, highest numbers were in protected springs and boreholes (50%) and the lowest numbers were in taps (11.11 %). In Maunarok, commensal *E. coli* were detected in all boreholes and dams water samples (100%) while the same strain was only detected in 18.18% of the tank water samples. In Njoro location commensal *E. coli* were detected in all

the unprotected springs and wells samples (100%) while they were few in borehole water samples (5.88%).

In Kihingo, approximately half of the water samples collected from taps and boreholes were contaminated with aEPEC. While no aEPEC strains were detected in any of the water sources in Mauche, about half of the water samples from taps in Lare were contaminated with aEPEC strain. On the other hand, in Maunarok location, protected wells and springs were detected in 33.33% of the samples while the lowest numbers were detected in tanks. In Njoro location, aEPEC were detected in tanks and borehole water only. tEPEC was detected in 33.33% of the unprotected wells in Kihingo, absent in all sources in Lare, while in Mauche they were only detected in 11.11% of the tap water. In maunarok, the tEPEC were detected in all unprotected wells and taps (100%) while in Njoro, there was no tEPEC strain detected in all water samples.

EHEC was not detected in water sources across all sources except in Kihingo and Maunarok. In Kihingo, the highest prevalence was in protected wells (20%) while in Maunarok they were in 9.09% of the tank water. EIEC strain was only detected in tap water sampled in Lare and recorded a prevalence of 20%. The ETEC strain was detected across all the locations apart from Lare. However, the highest prevalence of ETEC was in taps in Kihingo (50%), and in protected springs and boreholes in Mauche (50%). In Maunarok location, ETEC was detected in high numbers in dams while in Njoro, the protected springs were highly contaminated with ETEC. The EAEC strains were detected in water sources across all the locations. In Kihingo location, the EAEC was found in 7.14% of the tanks and in 20% of the tap water in Lare. In Mauche and Maunarok locations, the highest prevalence of EAEC 100% was recorded in dams and unprotected wells respectively, while in Njoro, highest EAEC were in about a quarter of the river water samples.

In this study, mixed strains were also seen in many of the sources. In unimproved water sources, the DEC strains combinations were found in river samples in Njoro location (ComEC+ETEC+EAEC) as well as in unprotected wells in Kihingo location (ComEC+tEPEC+ETEC) and Maunarok (tEPEC+EAEC) respectively. In improved water sources, DEC strains combinations in Kihingo location were detected in taps (ComEC+aEPEC+ETEC), tanks (ComEC+tEPEC+aEPEC+EHEC+ETEC+EAEC) and protected wells (ComEC+aEPEC+EHEC+ETEC). In Lare location, the DEC strain combinations were in taps (ComEC+EIEC+EAEC) and tank (ComEC+aEPEC) water samples. In Mauche location, strain combinations were recorded in protected springs (ComEC+ETEC), taps (ComEC+tEPEC+ETEC), tanks (ComEC+ETEC+EAEC) and

boreholes (ComEC+ETEC). In Maunarok location, strain combinations were detected in taps (tEPEC+ETEC), tanks (ComEC+tEPEC+aEPEC+EHEC+ETEC+EAEC) and protected wells (tEPEC+aEPEC+ETEC). In Njoro location, the DEC strain combinations in improved water sources was detected in tanks (ComEC+EPEC+ETEC+EAEC) and boreholes (ComEC+aEPEC + EAEC) water samples.

Table 6. 3: Virulent *E. coli* genes identified in water sources in the Njoro Sub-County.

WATER SOURCE		KIHINGO nr of pos samples/total nr of samples (percentage)	LARE nr of pos samples/total nr of samples (percentage)	MAUCHE nr of pos samples/total nr of samples (percentage)	MAUNAROK nr of samples/total nr of samples (percentage)	NJORO nr of pos samples/total nr of samples (percentage)
Unimproved Sources	River	-	-	EAEC 1/1 (100%)	-	ComEC 1/4 (25%) ETEC 2/4 (50%) EAEC 1/4 (25%)
	Springs	-	-	-	-	ComEC 1/1 (100%)
	Wells	ComEC 2/3 (66.67%) tEPEC 1/3 (33.33%) ETEC 1/3 (33.33%)	-	-	tEPEC 1/1 (100%) EAEC 1/1 (100%)	ComEC 1/1 (100%)
	Dams	-	-	EAEC 1/1 (100%)	ComEC 1/1 (100%)	-
Improved Sources	Springs	-	-	ComEC 1/2 (50%) ETEC 1/2 (50%)	aEPEC 1/3 (33.33%)	ETEC 1/1 (100%)
	Taps/piped water	ComEC 1/2 (50%) aEPEC 1/2 (50%) ETEC 1/2 (50%)	ComEC 3/5 (60%) EIEC 1/5 (20%) EAEC 1/5 (20%)	ComEC 1/9 (11.11%) tEPEC 1/9 (11.11%) ETEC 3/9 (33.33%)	tEPEC 1/1 (100%) ETEC 1/1 (100%)	ComEC 3/11 (27.27%)
	Tanks	ComEC 1/14 (7.14%) tEPEC 1/14 (7.14%) aEPEC 1/14 (7.14%) EHEC 2/14 (14.29%) ETEC 2/14 (14.29%) EAEC 1/14 (7.14%)	ComEC 1/2 (50%) aEPEC 1/2 (50%)	ComEC 2/6 (33.33%) ETEC 1/6 (16.67%) EAEC 1/6 (16.67%)	ComEC 2/11 (18.18%) tEPEC 2/11 (18.18%) aEPEC 1/11 (9.09%) EHEC 1/11 (9.09%) ETEC 1/11 (9.09%) EAEC 2/11 (18.18%)	ComEC 4/12 (33.33%) aEPEC 3/12 (25%) ETEC 3/12 (25%) EAEC 2/12 (16.67%)
	Boreholes	aEPEC 1/2 (50%)	ComEC 1/2 (50%)	ComEC 1/2 (50%) ETEC 1/2 (50%)	ComEC 1/1 (100%)	ComEC 1/17 (5.88%) aEPEC 3/17 (17.65%) EAEC 2/17 (11.76%)
	Wells	ComEC 3/5 (60%) aEPEC 1/5 (20%) EHEC 1/5 (20%) ETEC 1/5 (20%)	-	-	tEPEC 1/3 (33.33%) aEPEC 1/3 (33.33%) ETEC 1/3 (33.33%)	-

tEPEC = typical Enteropathogenic *E. coli* ComEC = commensal *E. coli* EHEC = Enterohaemorragic *E. coli* ETEC = Enterotoxigenic *E. coli* pos-positive

aEPEC = atypical Enteropathogenic *E. coli* EAEC = Enteroaggregrative *E. coli* EIEC = Enteroinvasive *E. coli* nr-number -No sample found for testing

6.3.2 Pathogenic *E. coli* strains in water storage containers

The results of the pathogenic *E. coli* strains present in the water storage containers are shown in Tables 6.3. From the data, it can be seen that Jerry cans and buckets had the most variety of virulent strains. The ComEC strains in Kihingo location were present in 33.33% 0f the gallons and 11.11% of the jerrycans while in Lare, they were prevalent in water stored in cups followed by jerrycans. In Mauche location, the water sampled from all domestic containers was contaminated with ComEC only which ranged from 75 to 100% of the containers, whereas ComEC was absent in all containers in Maunarok. However, in Njoro location, the high prevalence of ComEC was recorded in clay pots (100 %) followed by jerrycans (60%). aEPEC strain in Kihingo location was recorded in jerrycans only while in Lare, it ranged from 75% (jerrycans) to 100% (cups). In Maunarok and Njoro locations, the prevalence of aEPEC was 30% and 10% respectively.

The tEPEC strains in Kihingo, Maunarok, and Njoro were detected in water sampled from jerrycans and had a prevalence of 33.33%, 20% and 10% respectively. EHEC strain in Lare and Njoro were in 100% of the buckets. The EIEC strain was detected in water collected from jerrycans in Kihingo only. The prevalence of ETEC strains sampled from Lare, Maunarok and Njoro locations were 100% (buckets), 50% (jerrycans) and 10% (jerrycans) respectively. The EAEC strain detected only in Maunarok location (jerry cans) while in Njoro they were present in jerrycans, jugs and bucket water samples. In this study, mixed strains were also seen in several of the household storage containers. The most prevalent combinations in jerrycans were ComEC+tEPEC+aEPEC+ETEC+EAEC (Njoro), tEPEC+aEPEC+ETEC+EAEC (Maunarok) and ComEC+tEPEC+aEPEC+EIEC (Kihingo). There were no mixed pathogenic *E. coli* strains which were detected in water samples collected from gallons, cups, jugs, pots and sufuria. The mixed DEC in buckets were aEPEC+EHEC+ETEC (Lare) and EHEC+EAEC (Njoro).

The tEPEC strains in Kihingo, Maunarok and Njoro were detected in water sampled from jerrycans and had a prevalence of 33.33%, 20% and 10% respectively. EHEC strain in Lare and Njoro were in 100% of the buckets. The EIEC strain was detected in water collected from jerrycans in Kihingo only. The prevalence of ETEC starin sampled from Lare, Maunarok and Njoro locations were 100% (buckets), 50% (jerrycans) and 10% (jerrycans) respectively. The EAEC strain detected only in Maunarok location (jerry cans) while in Njoro they were present in jerrycans, jugs and bucket water samples. In this study, mixed strains were also seen in several of the household storage containers. The most prevalent combinations in jerrycans were ComEC+tEPEC+aEPEC+ETEC+EAEC (Njoro),

tEPEC+aEPEC+ETEC+EAEC (Maunarok) and ComEC+tEPEC+aEPEC+EIEC (Kihingo). There were no mixed pathogenic *E. coli* strains which were detected in water samples collected from gallons, cups, jugs, pots and sufuria. The mixed DEC in buckets were aEPEC+EHEC+ETEC (Lare) and EHEC+EAEC (Njoro).

Table 6. 4: Virulent *E. coli* genes identified in domestic storage containers in the Njoro Sub-County.

WATER CONTAINER	**KIHINGO** nr of pos samples/total nr of samples (percentage)	**LARE** nr of pos samples/total nr of samples (percentage)	**MAUCHE** nr of pos samples/total nr of samples (percentage)	**MAUNAROK** nr of pos samples/total nr of samples (percentage)	**NJORO** nr of pos samples/total nr of samples (percentage)
Gallons	ComEC 1/3 (33.33%)	aEPEC 1/1 (100%)	-	-	-
Jugs	-	-	-	-	EAEC 1/1 (100%)
Cups	-	ComEC 1/1 (100%)	ComEC 1/1 (100%)	-	-
Jerry cans	ComEC 1/9 (11.11%) tEPEC 3/9 (33.33%) aEPEC 1/9 (11.11%) EIEC 1/9 (11.11%)	ComEC 3/4 (75%)	ComEC 4/7 (57.14%)	tEPEC 2/10 (20%) aEPEC 3/10 (30%) ETEC 5/10 (50%) EAEC 1/10 (10%)	ComEC 6/10 (60%) tEPEC 1/10 (10%) aEPEC 1/10 (10%) ETEC 1/10 (10%) EAEC 1/10 (10%)
Clay pots	-	-	ComEC 1/1 (100%)	-	ComEC 1/1 (100%)
Buckets	-	aEPEC 1/1 (100%) EHEC 1/1 (100%) ETEC 1/1 (100%)	-	-	EHEC 1/1 (100%) EAEC 1/1 (100%)
Sufurias	-	-	ComEC 1/1 (100%)	-	-

tEPEC = typical Enteropathogenic *E. coli* ComEC = commensal *E. coli* EHEC = Enterohaemorragic *E. coli* ETEC = Enterotoxigenic *E. coli*

pos-positive

aEPEC = atypical Enteropathogenic *E. coli* EAEC = Enteroaggregrative *E. coli* EIEC = Enteroinvasive *E. coli* nr-number -No sample found for testing

6.4 Discussion

The communities in Njoro Sub-County depend on improved and unimproved water for their drinking purposes. In most cases, these sources get contaminated and are not treated before use. Since some of the sources are not protected, they are therefore open to microbial contamination of animal and human origin. Since *E. coli* is among the major microbial contaminants in water, its presence in water sources is a public health concern. *E. coli* exhibits a biphasic life cycle whereby it can comfortably exist in the human host or the environment when released in the fecal matter. The detection of pathogenic *E. coli* in water sources in this study was an indication of the health hazard to the people. The purpose of this study was to identify the presence of pathogenic strains of *E. coli* from drinking water sources. The most frequently isolated pathogenic strains were EPEC (35.53%) and ETEC (24.32%). EPEC has been reported to a major cause of diarrheal diseases especially in young children (Kuhnert *et al.,* 2000).

The results of this study indicated that commensal *E. coli* were the most prevalent in the water samples used for drinking purposes in Njoro Sub-County. However, among the DEC pathotypes, EPEC (aEPEC and tEPEC) was the most prevalent followed by ETEC, EAEC, EHEC and EIEC respectively. These findings on contamination of drinking water with pathogenic *E. coli* were similar to others by Makobe *et al.,* 2012 who reported that the highest *E. coli* pathotypes prevalent in stool samples were EPEC, ETEC, EAEC, STEC and EIEC respectively. This means that if this stool was released into the water bodies, the DEC pathogens would likely be transmitted to humans through the fecal-oral route. Similarly, studies to determine the bacteriological quality of drinking water in river Njoro reported the pathogenic *E. coli* strains were in the following proportions: EAEC 9.2%, Necrotoxigenic *E. coli* 7.4% and EPEC 3.2% respectively (Kiruki *et al.,* 2011). The presence of different combinations of pathotypes in water samples showed the exchange of genes that takes place within the Enterobacteriaceae group which makes the *E. coli* bacteria not only an indicator of fecal contamination but also a strong candidate for diarrhoeal diseases within the population.

6.5 Conclusion

The pathogenic *E. coli* strains especially EPEC and ETEC have been observed to be the leading causes of diarrhea in children, especially in the developing countries. The presence of DEC pathotypes can result in persistence invasion of the intestinal and urinary tract. In favorable conditions, the bacteria can grow and during interaction with other bacteria

exchange their genetic materials which can result in the emergence of more virulence strains with complex virulence factors. A development of drug resistance *E. coli* strains can ensue from the exchange of genetic materials and hence a health concern. This study, therefore, indicated that the virulence markers of *E. coli* were present in some of the sampled drinking water sources as well as water stored in domestic containers for drinking purposes. These will in turn help to detect and identify the pathogens for risk assessment to the vulnerable communities. It is furthermore very important that the social aspects in these communities are investigated to determine how hygiene and sanitation risks can be eliminated and living conditions improved to limit the health risk associated with unsafe water, poor sanitation, and unhygienic practices.

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CHAPTER SEVEN

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

The main objective of this study was to isolate *E. coli* strains using the CBT test and characterize the pathogenic genes of *E. coli* strains from drinking water sources and water in household storage containers in Njoro Sub-County, Kenya. In order to achieve this objective, three specific objectives were formulated.

The first specific objective was to determine the physical-chemical quality of drinking water from water sources and household containers used for storing drinking water in Njoro Sub-County, Kenya. The results are shown in Chapter four and indicated that the physical parameters such as turbidity and electrical conductivity levels were above the WHO recommended guideline levels in various drinking water sources and water in household storage containers. On the other hand, DO was found to be low in sources, indicating the possibility of low self-purification of the water systems and the threat caused to aquatic life forms. Other parameters like pH, total dissolved solids, dissolved oxygen and biochemical oxygen demand were within the required WHO guideline levels for safe drinking water quality. Considering the chemical parameters: fluoride levels were high in most sources especially the underground waters as well as the containers where drinking water had been stored. This indicated the potential risks of dental fluorosis and sclerosis exposed to the children and the elders respectively, a problem that has adversely affected the residents of Njoro Sub-County. Other metals such as iron and were slightly higher than the WHO recommended guideline levels in some water sources as well as water stored in domestic containers indicating the negative effect posed to the aesthetic properties of the water. Manganese levels were found to be within the WHO recommended guideline levels.

The second specific objective was to identify and quantify *E. coli* from drinking water sources and water inside household storage containers in Njoro Sub-County, Kenya using the commercially available Compartmental bag test (CBT) kit. *E. coli* was found in about 62.36% of the total samples from both the water sources and household domestic storage containers. The improved sources formed about 56.76% of the CBT-positive samples (boreholes=10, protected wells=7, protected springs=4, tank=26 and taps=16) while the unimproved sources formed 11.71% of the CBT-positive samples (unprotected wells=5,

river=5, unprotected spring=1 and dams=2). In addition *E. coli* was detected in 31.53% of the household water storage containers (cups=2, jugs=1, jerry cans=25, clay pots=2, sufuria=1, gallons=2 and buckets=2). Using the WHO risk levels, all the water sources were in the range between intermediate/probably safe to the very high/unsafe risk which indicated a strong need for health interventions by the Kenya government authorities.

The third specific objective was to characterize pathogenic strains of *E. coli* from drinking water sources and water inside various household storage containers in Njoro Sub-County, Kenya using mPCR protocol. The presence of the pathogenic genes within the water samples indicated a potential health risk for vulnerable individuals within these communities. The results showed that 46.85% (n=52) of the CBT-positive samples were commensal *E. coli* while 53.15% (n=59) were pathogenic *E. coli.* Furthermore, 61.02% (n=36) of the pathogenic *E. coli* positive samples had only one type of pathogenic strain, while 38.98% (n=23) of the pathogenic *E. coli* positive samples were positive for more than one pathogenic strain, indicating the risks involved to vulnerable individuals within the communities.

Therefore the hypothesis set out in Chapter one was rejected and the alternate hypotheses accepted because the results obtained in this study showed that:

1) There were variations in the physical-chemical determinants from drinking water and water stored inside household water storage containers in Njoro Sub-County;
2) There were significant levels of *E. coli* counts in drinking water sources and household water storage containers in the Njoro Sub-County;
3) There were various *E. coli* pathotypes present in drinking water sources and household water storage containers in the Njoro Sub-County which could be considered a health risk to vulnerable community members.

7.2 Recommendations

The following recommendations were made following the outcome of this study:

1) Source and household water treatment options by relevant authorities and individuals respectively should be adopted.
2) The community should be adequately educated on the available methods of water treatments such as chlorination and boiling as well as adopting safe agricultural practices, protection of drinking water sources and adherence to safe wastes disposal.
3) The water authorities should adopt a frequent water monitoring system on water sources so that early signs of physical-chemical or microbiological contamination can reduce the emergence of waterborne outbreaks early enough.

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APPENDICES

Appendix 1: Summary of pathogenic *E,coli* strains frequencies.

Strain	Frequency
ComEC	46.85% (n=52)
ETEC	24.32% (n=27)
aEPEC	18.92% (n=21)
EAEC	15.32% (n=17)
tEPEC	12.61% (n=14)
EHEC	4.32% (n=6)
EIEC	1.80% (n=2)

Appendix 2: A comprehensive **g**eneralized ANOVA table

Source	df	MS							
		pH	**Temperature**	**DO**	**Turbidity**	**EC**	**BOD**	**TDS**	**MPN**
Location	4	0.219^{ns}	37.940***	5.958**	1673.605***	0.856^{ns}	4.794***	0.078***	2213.769^{ns}
Stype	1	0.812^{ns}	16.662^{ns}	3.161^{ns}	3913.742***	0.382^{ns}	4.484**	0.004^{ns}	1584.931^{ns}
Container type (Location)	39	0.407*	15.396***	3.138***	4400.082***	1.003^{ns}	1.233***	0.026***	2925.564***
Error	133	0.242	6.316	1.394	219.612	0.973	0.550	0.010	1108.976

df= degrees of freedom, MS= Mean Squares, DO= Dissolved Oxygen, EC= Electrical Conductivity, BOD= Biochemical Oxygen Demand, TDS= Total Dissolved Solids, MPN= Most Probable Number