CHARACTERIZATION OF *ESCHERICHIA COLI* PATHOTYPES AND FACTORS ASSOCIATED WITH WELL AND WATER CONTAMINATION IN MOMBASA COUNTY

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Characterization of *Escherichia Coli* Pathotypes and Factors Associated with Well and Water Contamination in Mombasa County

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DECLARATION

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

Signature……Date……

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

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DEDICATION

I dedicate this study to my wife, Fatime Hussein and my beloved children, Suleiman, Babie, Sofia, Salma, Suad and Sayana for being so patient with me when I was away from home where they deserve my care, love, help and fatherhood.

This work is also dedicated to all my friends and relatives. Thank you for your support and I wish you blessings from God.

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ACRONYMS AND ABBREVIATIONS

A**MREF**	Africa medical research fund
CDC	center for disease control
DAEC	*Diffusely Adherent E. coli*
DNA	Deoxyribonucleic Acid
EAggEC	*Enteroaggregative E. coli*
EHEC	*Enterohaemorrhagic E. coli*
EIEC	*Enteroinvasive E. coli*
EPEC	*Enteropatho*genic *E. coli*
ETEC	*Enterotoxigenic E. coli*
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
KNBS	Kenya National Bureau of Statistics
LMICS	Low and Middle-Income Countries
MPN	Most Probable Number
PCR	Polymerase Chain Reaction
TCBS	Thiosulphate Citrate Bile Salts Agar
TSI	Triple Sugar Iron Agar

USA	United States of America
VT	verocytotoxins
VT/**Stx**	verocytotoxin/Shiga toxin
VTEC	*Verocytotoxigenic Escherichia coli*
WHO	World Health Organization
WRA	Water Resources Impact Report

ABSTRACT

Delivery of safe water for human consumption is now considered a fundamental right. However, dwindling resources and faulty sanitation in Low and Middle-Income Countries makes the availability of safe water almost unattainable. It is therefore important to continuously evaluate the chemical and biological quality of drinking water. Failure to do so, people will be exposed to numerous water borne enteric diseases. Mombasa and the Coastal Region in general experience perennial water shortages. This cross-sectional study aimed to determine the frequency and characterization of *Escherichia coli* pathotypes from water samples collected from and wells in Mombasa County. One hundred and fifty-seven (157) water samples were collected from all four Sub Counties that is Mvita, Kisauni, Likoni and Changamwe Sub Counties using sterile techniques. Variables such chlorine treatment, distance to pit latrine, borehole or well covers, among others were recorded. The samples were then inoculated to double strength MacConkey broth and incubated at 37^0C for up to 48 hours. Positive results from the double strength MacConkey broth were compared to the 3 tube McCrady Most Probable Number table. The *Escherichia coli* were confirmed by Eijkman's test and antibiotic susceptibility carried out on confirmed isolates. The *Escherichia coli* were then molecularly characterized to determine the pathotypes using polymerase chain reaction. Out of 157 samples collected from around Mombasa County, one hundred and thirty-one (131) samples (83.4%) were contaminated by coliform bacteria. Of the contaminated samples, only 79 (60.3%) were confirmed to have *E. coli*. All the samples with *E. coli* tested (n = 77; 100%) were sensitive to Gentamicin, while all (n = 77; 100%) isolates were resistant to Ampicillin. Molecular characterization indicated that this study's isolates were typed as *Enteroinvasive E. coli*. These findings suggest that *E. coli* are major contaminants of water in wells and boreholes in Mombasa County. The *E. coli* showed a distribution of resistant and sensitivity patterns to commonly used antibiotics. The most dominant pathotype detected was *Enteroinvasive E. coli*.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Water is a chemical substance with the chemical formula H_2O. It is a liquid at room temperature; it occurs on Earth in a solid state (ice), and gaseous state (water vapor or steam) (Pollack, 2013). It covers 70.9% of the earth's surface, and is vital for all known forms of life. On earth, it is found mostly in oceans and other large water bodies at 97%, with 1.6% of water below ground in aquifers, 0.001% in the air as vapor and precipitation and glaciers and polar ice caps at 1.4% (Reddy *et al*., 2012).

Drinking water comes from surface water and ground water. Surface water includes rivers, lakes, and reservoirs while ground water is pumped from wells or that are drilled into aquifers. However due to dwindling resources and faulty sanitation especially in Low and Middle-Income Countries (LMICS), makes the availability of safe water is almost unattainable, this is due to bacterial and chemical contamination (Cappuccino and Sherman, 2002).

Water-borne diseases are one of the major public health problems in Low- and Middle-Income Countries (LMICS). In 2010, contaminated water caused more than 20 million deaths (Gleick, 2002), of which more than 80% were among children under age five (Smith, 2001). Besides the conventional pathogens which are transmitted by water, several emerging water-borne pathogens have become increasingly important during the last decade or so. These include *Vibrio cholerae* O1 and 0139, *Cryptosporidium parvum*, shiga toxin producing *E. coli* especially enterohaemorrhagic *E. coli* (EHEC), *Yersinia enterocolitica*, *Campylobacter jejuni*, Calciviruses, *Microsporidia* and *Aeromonas* species.

More than one billion people in the have no access to safe drinking water, or water for washing their food, hands and utensils before eating, while 2.4 billion also have no

adequate sanitation (AMREF, 2010). This leads to; water-borne diseases (for example cholera, typhoid), water-related diseases (for example malaria, yellow fever, river blindness, sleeping sickness), water-based diseases (for example guinea worm and bilharzias), water-scarce diseases (trachoma and scabies), and diarrhea.

In Kenya, more than 70% of the epidemic emergencies are either water-borne or are water related diseases (AMREF, 2010). Although a substantial amount of work has been carried out on common waterborne pathogens in Kenya, unfortunately only limited information is available on the emerging waterborne pathogens such as *E. coli* pathotypes. Mombasa County and the Coastal region experiences perennial water shortages. There is no sewerage system except within Mombasa Island. Shallow wells are dug near toilets or septic pits. Outbreaks of cholera and dysentery occur during raining seasons or shortly after the rains (Reyburn *et al.*, 2011, Munga *et al.*, 2005). Exchange of microbes between wells and toilets/septic pits has been documented (Luby *et al*., 2006).

A regular surveillance of water source is one of the effective ways of containing pathogens causing water-borne diseases. Therefore, the quality of groundwater supplies is an important public health concern. This study intended to characterize *E. coli* pathotypes and possible factors associated with wells and water contamination in Mombasa County (WRA, 2017)

1. 2 Statement of the Problem

Water being an essential commodity for domestic purposes is also an important vehicle in the transmission of diseases. In 2010, contaminated water caused more than 20 million deaths (Gleick,

2002), of which more than 80% were among children under age five years in the world (Smith, 2001). In Kenya, more than 70% of the epidemic emergencies are either water-borne or are water related (AMREF, 2010). Mombasa and the Coast province

experience perennial water shortages. There is no sewerage system except the Mombasa Island. The shallow wells are dug near toilets or septic pits. Outbreaks of cholera and dysentery occur during raining seasons or shortly after the rains (Munga *et al*., 2005).

1.3 Justification

Mombasa gets most of its water from Mzima springs in Taita Taveta County, Marere in Kwale County and Baricho in Kilifi County water works. However, ground water forms an important source of water for Mombasa and its environs. Contamination of the water sources poses a major threat to human health. Previous disease outbreaks in Mombasa have been linked to contamination of water. The wells are dug near pit latrines, septic tanks and soak pits. The soak pits are dug to the ground water table increasing chances of contamination. Mombasa is also an important tourist destination, so there is a need to determine the safety of the water being consumed by both the locals and tourist populations. The determination of faecal contamination of water is done through the detection of indicator faecal *E. coli* which has been shown to be pathogenic and with a strong association with the presence of definitive pathogens such as cholera (caused by *Vibrio spp*), typhoid (*Salmonella spp*), among others.

1.4 Research Questions

1. Are coliforms associated with well and borehole water contamination in Mombasa County?
2. Are *Escherichia coli* associated with well and borehole water contamination in Mombasa County?
3. Which *E. coli* pathotypes are associated with well and borehole water contamination in Mombasa County?
4. What are the possible factors associated with contamination of well and borehole waters in Mombasa County?

1.5 Objectives

1.5.1 Broad Objective

To characterize coliforms and *E. coli* pathotypes and factors associated with well and water contamination in Mombasa County

1.5.2 Specific Objectives

1. To determine the distribution of coliforms associated with and wells contamination in Mombasa County
2. To determine the distribution of *E. coli* associated with and wells contamination in Mombasa County
3. To characterize *E. coli* pathotypes associated with and wells contamination in Mombasa County
4. To determine factors associated with contamination of well and borehole waters in Mombasa County.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background on Safe drinking water

Drinking or portable water should be safe enough to be consumed by humans. The use of this water (Drinking or portable water) should provide low risk in the short or long term to the consumer. In 2012 for example, about 89% of world population had access to suitable drinking water, with about 1.8 billion people still accessing faecally contaminated water. This could lead to the transmission of diseases to humans resulting to about half a million deaths worldwide. Thus, the importance of providing safe portable drinking water supplies cannot be overemphasized (WHO, 2014).

With increasing industrialization, water sources available for consumption and recreation have been adulterated with industrial as well as animal and human wastes as a result of which water has become an important factor in disease transmission. Polluted water may contain vast amounts of organic matter that serve as excellent nutritional source for the growth and multiplication of microorganisms. The presence of pathogenic organisms responsible for intestinal infections such as bacillary dysentery, typhoid fever, cholera, and paratyphoid fever is important in public health (Cappuccino and Sherman, 2002).

2.2 Sources of Bacteria in Drinking Water

Human and animal wastes are a primary source of bacteria in water. These sources of bacterial contamination include runoff from feedlots, pastures, dog runs, and other land areas where animal and human wastes are deposited. Additional sources include seepage or discharge from septic tanks, sewage treatment facilities, and natural soil/plant bacteria. Bacteria from these sources can enter wells that are either open at the land surface, or do not have water-tight casings or caps (Tumwine *et al*., 2002). Insects,

rodents or animals entering the well are other sources of contamination. Old wells that were dug by hand and lined (cased) with rocks or bricks usually have large openings and casings that often are not well-sealed. This makes it easy for insects, rodents, or animals to enter the well (Pathania, *et al.*,2021).

Another way that bacteria can enter a water supply is through inundation or infiltration by flood waters or by surface runoff. Flood waters commonly contain high levels of bacteria. Small depressions filled with flood water provide an excellent breeding ground for bacteria. Whenever a well is inundated by flood waters or surface runoff, bacterial contamination is likely. Shallow wells and wells that do not have water-tight casings can be contaminated by bacteria infiltrating with the water through the soil near the well, especially in coarse-textured soils (Trojan *et al*., 2003). Older water systems, especially, dug wells, spring-fed systems and cistern-type systems are most vulnerable to bacterial contamination. Any systems with casings or caps that are not watertight are vulnerable. This is particularly true if the well is located so surface runoff might be able to enter the well (Oram, 2007).

2.3 Diarrhea

2.3.1 Background on Diarrhea

Diarrhea is a condition which involves the frequent passing of more than 3 loose and/or watery stools each day. It is the second cause of death among small children. It is caused by viruses, bacteria or parasite which are spread through feacally contaminated water, and later these organisms infect the gut. Water borne diarrhea usually results from the ingestion of viruses and parasites in water contaminated by human or agricultural fecal waste. It causes the loss of fluids from the body which leads to dehydration and electrolyte disturbances such as potassium deficiency or salt imbalance (WHO, 2013). Travelling diarrhea is caused by drinking water or eating food contaminated with fecal material. So, it can be prevented by using boiled or chemically disinfected water and not eating or drinking from unknown sources (Centre for Disease Control (CDC), 2022)

Diarrhea occurs world-wide and causes 4% of all deaths and 5% of health loss to disability. It is most commonly caused by gastrointestinal infections which kill around 2.2 million people globally each year, mostly children in developing countries (Laura *et al*., 2004). Depending on the type of infection, the diarrhea may be watery (for example in cholera) or passed with blood (in dysentery for example) (Roberto *et al*., 2012). Diarrhea due to infection may last a few days, or several weeks, as in persistent diarrhea. Severe diarrhea may be life threatening due to fluid loss in watery diarrhea, particularly in infants and young children, the malnourished and people with impaired immunity (Nataro, 2013). The impact of repeated or persistent diarrhea on nutrition and the effect of malnutrition on susceptibility to infectious diarrhea can be linked in a vicious cycle amongst children, especially in developing countries. Is also associated with other infections such as malaria and measles. Chemical irritation of the gut or non-infectious bowel disease can also result in diarrhea (WHO, 2013).

Diarrhea is more common when there is a shortage of clean water for drinking, cooking and cleaning. Basic hygiene is important in prevention of diarrhea. Water contaminated with human faeces for example from municipal sewage, septic tanks and latrines is of special concern (Robert B spread from person to person, aggravated by poor personal hygiene (Gauthami *et al*., 2017) Food is another major cause of diarrhea when it is prepared or stored in unhygienic conditions (Vinod. 2019). The infectious agents that cause diarrhea are present or are sporadically introduced throughout the world. Diarrhea is a rare occurrence for most people who live in developed countries where sanitation is widely available, access to safe water is high and personal and domestic hygiene is relatively good (Richard *et al.*, 1990). World-wide around 1.1 billion people lack access to improved water sources and 2.4 billion have no basic sanitation (WHO. 2019). Diarrhea due to infection is widespread throughout the world. In Southeast Asia and Africa, diarrhea is responsible for as much as 8.5% and 7.7% of all deaths respectively. Amongst the poor and especially in developing countries, diarrhea is a major killer. In 1998, diarrhea was reported to have killed approximately 2.2 million people, most of

whom were under 5 years of age. Each year there are approximately 4 billion cases of diarrhea worldwide (WHO, 2013).

2.3.2 Gastroenteritis

Gastroenteritis is the intestinal infection marked by diarrhea, cramps, nausea. vomiting and fever due to infections by bacteria parasites and viruses. It is mainly caused by poor hygiene, consuming feacally contaminated food and water. The infected patients will suffer from inflamed stomachs, vomiting, severe abdominal cramps and diarrhea (Healthline, 2015).

At its most basic level, diarrhea caused by infectious pathogens especially bacteria result from an imbalance of absorption and secretion of ions and solute across the gut epithelium. This will be followed by the movement of water in an attempt to restore the appropriate ion concentrations. Often, this imbalance is caused by the presence of bacteria that secrete toxins that disturb the organization of the epithelium. Diarrhea benefits enteric pathogens by facilitating their rapid dissemination into the environment and, consequently, the infection of new hosts. Additionally, host passage increases the virulence of some bacterial pathogens leading to fatality that is associated with the concurrent loss of fluid and electrolytes (Viswanathan, *et al.,* 2009).

2.3.3 Bacterial Causes of Diarrhea

2.3.3.1 Typhoid fever

Typhoid fever is an infection that causes diarrhea and a rash. It is most commonly due to a type of bacterium called *Salmonella typhi* (Wain *et al*., 2015). The bacterium is an aerobic, flagellated, Gram negative bacterium exhibits an acid butt and alkaline slant with weak hydrogen sulphide gas on Triple Sugar Iron Agar (TSI), and is negative for indole, urea and citrate. The pathogen is isolated from blood, bone marrow and or specific anatomical lesions, and is spread through contaminated food, drink, or water (Vittal *et al.*, 2016).

Humans are the natural host of the pathogen. If one eats or drink something that is contaminated with the bacteria, the bacteria enter their body. Then travel into the intestines, and then into the blood stream. The bacteria travel through the blood to your lymph nodes, gallbladder, liver, spleen, and other parts of the body. Some persons become carriers of *S. typhi* and continue to release the bacteria in their stools for years, spreading the disease (Vittal *et al.*, 2016).

Typhoid fever is not common in high income countries. Fewer than 400 cases are reported in the United States of America (USA) each year. Most cases in the U.S.A. are brought in from other countries where typhoid fever is common. Early symptoms include fever, general ill-feeling and abdominal pain. High fever (103°F, or 39.5°C) or higher and severe diarrhea occur as the disease gets worse. Some people with typhoid fever develop a rash called "rose spots," which are small red spots on the abdomen and chest. Other symptoms that occur include; Abdominal tenderness, agitation, bloody stools, chills, confusion, difficulty paying attention (attention deficit), delirium, fluctuating mood, hallucinations, nosebleeds, severe fatigue, slow, sluggish, lethargic feeling, weakness (Wain, *et al.*, 2015).

2.2.3.2 Cholera

Cholera is an infection in the small intestine caused by the bacteria '*Vibrio cholerae'.* It is a Gram negative, comma-shaped bacterium, that is a facultative anaerobic organism having a flagellum at one cell pole Center for Disease Control (CDC, 2013). Its symptoms are diarrhea and vomiting. It is caused by drinking water or eating food that has been contaminated by faeces of an infected persons. When their untreated diarrheal discharge gets into the water system such as groundwater or drinking water, it affects the other people. Poorly cleaned vegetables irrigated by contaminated water sources are another source of contamination. In places like refugee camps and villages with limited water resources and poor sanitation conditions, a single affected victim can contaminate water for the entire population. So, if water source is not cleaned properly, the mortality rate can rise from 1% to 50% - 60%. The bacterium is isolated using selective media

such as Thiosulphate–Citrate–Bile Salts Agar (TCBS), where colonies are oxidase positive, and serological tests are carried out to determine which biotypes (Classical or El Tor) or serotypes (Inaba, Ogawa, Hikojima) the isolates are (CDC, 2013).

2.2.3.3 Dysentery

Dysentery is the disorder of intestine, that results in severe diarrhea, containing blood and mucus in the feces with fever and abdominal pain. It is caused by either an amoeba or bacteria. Bacillary dysentery is mainly caused by the bacteria of the genus *Shigella.* These are Gram negative bacteria, which are facultative anaerobic, non-spore forming, non-motile, rod-shaped bacteria. It mainly and naturally affects humans and apes. This bacterium exhibits an acid butt and alkaline slant with no bubbles of gas in the agar on triple sugar iron agar (TSI), and is negative for indole, urea and citrate biochemical tests (Raquel, *et al.*, 2019)

Generally, dysentery is the mild illness causing symptoms consisting of mild stomach pains and frequent passage of stools. The urge to defecate, the volume of feces passed and the presence of mucus, pus and blood depend on the pathogen that is causing the disease. These pathogens enter the large intestine through oral contact, or from infected and dirty food or water. The person may have elevated body temperature, fever, nausea and vomiting. It is caused mainly by the poor hygiene and it spreads with tainted food and water contaminated with the bacteria. It can be prevented by taking measures to reduce the risk of infection by regularly washing hands, drinking clean water and maintaining good hygiene (yet al., 2021).

2.4 Determination of bacterial contamination of water systems

Bacterial contamination of water supply can be known by testing the water. World Health Organization (WHO) and the Public Health Departments require that all public water suppliers be regularly tested for coliform bacteria and deliver drinking water that meets the WHO Standards requirement (WHO, 2006). Bacterial contamination falls

under the category of pathogens. The WHO standard for coliform bacteria in drinking water is zero (or no) total coliform per 100 ml of water. Testing for all individual pathogens is impractical and expensive. Instead, the WHO has designated total coliform bacteria as a standard to determine bacterial safety of water (WHO, 2006). Coliform bacteria may not cause disease but can be indicators of pathogenic organisms that cause diseases. The pathogenic organisms could cause intestinal infections such as; dysentery, hepatitis, typhoid fever, cholera among other illnesses. However, these illnesses are not limited to disease-causing organisms in drinking water. Other factors such as contamination of water supplies by sewer lines, use of manure (that uses animal or human faces), landfills could lead to seepage of these organisms leading to contamination of drinking water (Borchardt *et al.*, 2004). Wells located in sand and gravel aquifers, are more likely to be contaminated, because the pore sizes in the sand and gravel are too large to impede pathogen transport to wells (Borchardt *et al.*, 2004). Intestinal infections and dysentery are generally considered minor health problems. They can, however, prove fatal to infants, the elderly, and those who are ill. Today typhoid, hepatitis and cholera are mostly encountered during and after rainy seasons (Luby *et al.,* 2006). Other bacteria also may be present in water. No specific sanitary significance or health standards have been indicated for nonpathogenic non-coliform bacteria.

Coliform bacteria originate in the intestinal tract of warm-blooded animals and can be found in their wastes. They can also be found in soil and on vegetation. They are relatively simple to identify and are present in much larger numbers than more dangerous pathogens. They react to the natural environment and treatment processes in a manner and degree similar to pathogens. By monitoring coliform bacteria, the increase or decrease of many pathogenic bacteria can be estimated (Mendez *et al*., 2004). Due to this association, bacterial safety of drinking water is monitored by testing for coliform bacteria and fecal *E. coli* for recent fecal contamination and *Clostridium perfringens* for previous contamination (WHO, 2006).

2.5 *Escherichia coli* pathotypes

The bacterium *Escherichia coli* belong to the family Enterobacteriaceae and are facultatively anaerobic; Gram negative bacilli that live in the intestinal tracts of animals in health and disease (Kaper *et al*., 2004). They are the predominant facultative organism in the human gastrointestinal tract. Pathogenic forms of *E. coli* can cause a variety of diarrheal diseases in hosts due to the presence of specific colonization factors, virulence factors and pathogenicity associated genes which are generally not present in other *E. coli*. Of the strains that cause diarrheal diseases, six pathotypes are now recognized (Regua‐Mangia *et al*., 2010). They include: *Verocytotoxigenic E. coli* (VTEC), *enterotoxigenic E. coli* (ETEC), *enteroinvasive E. coli* (EIEC), *enteropathogenic E. coli* (EPEC), *enteroaggregative E. coli* (EAggEC) and *diffusely adherent E. coli* (DAEC) (Nutan *et al*., 2018)

2.5.1 *Verocytotoxigenic* Escherichia coli (VTEC)

Escherichia coli that produces verocytotoxin/Shiga toxin (VT/Stx) (VTEC/Shiga toxin producing *E. coli*, STEC) are characterized by the production of cytotoxins that disrupt protein synthesis within host cells. These toxins are synonymously either called verocytotoxins (VT), because of their activity on Vero cells, or Shiga toxins (Stx) because of their similarity with the toxin produced by *Shigella dysenteriae*. Shiga toxins exists two major groups, Stx1 and Stx2, whose genes are considered to be part of the genome of lambdoid prophage (Friedman and Court, 2001). *Enterohaemorrhagic E. coli* (EHEC) are a subset of VTEC that are considered to be human pathogens.

The most important EHEC (and VTEC) serotype in public health terms is *E. coli* O157 H7 because it causes food borne illness (Karch 2005). VTEC infection occurs via the fecal-oral route and results in symptoms ranging from mild uncomplicated diarrhea to severe bloody diarrhea. Complications including hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) can occur in some cases, both of which can result in death. The infectious dose of VTEC has been calculated to be as low as 10–100

cells (O'Sullivan, 2007). The disease-causing ability of VTEC in humans is associated with its ability to express VT. The types of VT produced are classified as VT1 and VT2, along with VT2 subtypes; namely: subtypes vtx_2 and vtx_{2c} (Jenkins *et al*., 2003). Waterborne transmission occurs through swimming in contaminated lakes, pools, or drinking untreated water. Direct contact with animal fecal material through recreational activities and person to person contact are also sources of infection. (Dafni *et al*., 2011)

2.5.2 *Enteropathogenic* E. coli (EPEC)

There are two types; Typical and atypical enteropathogenic *E. coli* (EPEC) strains which differ in several characteristics. Typical EPEC is a leading cause of infantile diarrhea in developing countries but rarely in industrialized countries, whereas atypical EPEC seems to be more important cause of diarrhea. For typical EPEC, the reservoir is only humans; while atypical EPEC, comprise both animals and humans as reservoirs (Trabulsi *et al*., 2002). Following the ingestion of EPEC, the organisms adhere to the epithelial cells of the intestine, causing either watery or bloody diarrhea. Enteropathogenic *E. coli* is associated with the attachment to, and physical alteration of, the integrity of the intestine. Bloody diarrhea is associated with attachment and an acute tissue destructive process. Low grade fever and vomiting are also associated with infection. Enteropathogenic *E. coli*, unlike VTEC, do not produce any classic toxins. Water and food contaminated with EPEC have been linked to EPEC infection, with the most common foods implicated in outbreaks being raw beef and chicken.

2.5.3 *Enterotoxigenic* E. coli (ETEC)

Enterotoxigenic *E. coli* are the major cause of traveler's diarrhea worldwide. Infection with ETEC leads to watery diarrhea which may last up to a week (WHO, 2009), which can be with protracted abdominal cramps, sometimes with nausea and headache, fever is usually absent. On infection, ETEC first establishes itself by adhering to the epithelium of the small intestine via one or more colonization factor antigens (CFA). This is followed by the expression of one or more heat-stable (ST) or heat labile (LT)

enterotoxins. These enterotoxins cause inhibition of sodium absorption and stimulation of chloride secretion, which leads to watery diarrhea. Distinct groups of the enterotoxins exist: for the heat stable, STa (STI) and STb (STII) – encoded for on plasmids – and for the heat labile, LTI and LTII – encoded for on the chromosome. Infection occurs when a person ingests food or water contaminated with ETEC bacteria. ETEC are known to cause clinical disease similar to cholera (David *et al*., 2019)

2.5.4 *Enteroinvasive* E. coli (EIEC)

These bacteria typically produce shiga like toxins and are transmitted through the fecal-oral route. Even minimal contact is adequate for transmission. Following the ingestion of EIEC the organisms invade the epithelial cells of the intestine resulting in a mild form of dysentery often mistaken for dysentery caused by *Shigella* species. The illness is characterized by the appearance of blood and mucus in the stools of infected individuals. Characteristic features of EIEC strains are their ability to induce their entry into epithelial cells and disseminate from cell to cell. The EIEC infection can occur through contaminated food or water, or through mechanical vectors such as flies (Gordillo *et al*., 1992). Outbreaks have been associated with hamburger meat and unpasteurized milk (Gordillo *et al*., 1992).

2.5.5 Enteroaggregative E. coli ((EAggEC)

*Enteroaggregative Escherichia coli (*EAggEC) also abbreviated as EAEC are associated with acute or persistent diarrhea, especially in developing countries (Schultsz *et al*., 2000, Weintraub, 2007). Infection is typically followed by a watery, mucoid, diarrheal illness with little to no fever and an absence of vomiting. The precise mechanisms by which EAggEC cause diarrhea and the role of the various pathogenicity factors are poorly understood. EAggEC strains are characterized by their ability to aggregatively adhere to tissue culture cells in a distinctive "stacked, brick-like" manner. EAggEC also produce an enteroaggregative heat-stable toxin (EAST1). EAST1 is similar to ST, and may be responsible for the symptoms of infection (Yatsuyanagi *et al*., 1996). Infant

foodstuffs and formulae, milk and water have all been implicated in EAggEC outbreaks (Nataro *et al*., 1998).

2.5.6 Diffusely Adherent E. coli (DAEC)

Diffusely adherent *Escherichia coli* are a major cause of urinary tract infections worldwide, but its role as a causative agent of diarrhea is controversial. DAEC are comprised of heterogeneous groups of organisms with variable virulence. They are identified by their adherence to Hep-2 cells in a diffuse pattern. DAEC are divided into two classes, those which harbor afimbrial adhesins Afa/Drori antigen (Dr) adhesions and those that express an adhesin involved in diffuse adherence, which is a potential cause of infantile diarrhea. DAEC infection is characterized by the growth of long finger-like cellular projections that wrap around the adherent bacteria. Sources implicated in outbreaks of DAEC include contaminated food, especially undercooked ground beef, contaminated water and contact with livestock and other animals (WHO, 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was conducted in Mombasa County in the coastal region of Kenya. Mombasa County is one of the six Counties in Coastal Region. It is situated along East Coast of Kenya.It lies at a latitude o -4.043740, and the longitude is 39.658871. It has a tropical type of climate with average rainfall of about 100mm annually. It covers an area of about 285 squares kilometres of which 67 squares kilometres is under permanent waters (Indian Ocean) with 218 square kilometres landmass. Administratively the County is divided into 4 Sub Counties: Mainland North (Kisauni), Mainland South (Likoni), Island (Mvita) and Mainland West (Changamwe). The County population is 1,208,333 people as per 2019 population census (KNBS, 2019). The County relies on piped water from Mzima springs in Taita Taveta County, Sabaki River in Kilifi County and Marere springs in Kwale County. Despite all these water resources, the County stills experience acute water shortages. This has led to the residents relying heavily on wells and boreholes which are run by institutions, hotels, private homes and community groups; which sometimes are a source of health hazards due to bacterial contamination.

3.1.1 Map of Mombasa County

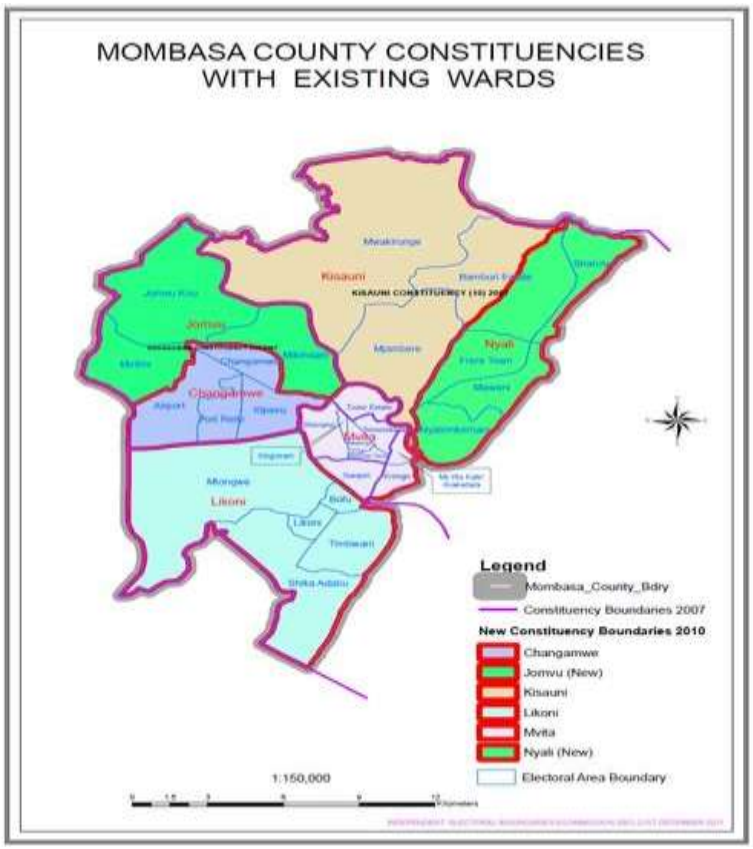

Figure 3.1: Mombasa County Map

source: (Munga *et al.*, 2004)

This was a cross sectional survey that was carried out in Mombasa County, where samples were collected using random sampling from wells or boreholes across the divisions that encompass the county. The samples were collected only once from the targeted sites as explained below.

3.2 Sample Size Determination

The prevalence of bacterial contamination in Mombasa has previously been estimated at 90% (Mwaguni, 2002). The sample size determination for the study used the Cochran formula (Cochran, 1977). Thus:

$$n = (Z^2 pq)/ d^2$$

Where, n = Desired sample size (if target population is greater than 10,000)

Z = Standard normal deviation at the required confidence interval (which =1.96 at 95% confidence interval)

p = proportion in the target population estimated to have measured character

$$q = 1 – p$$

d = level of statistical significance at 95% confidence level = 0.05

Therefore, sample size calculation was;

$= (1.96)^2$ X 0.90 X $0.10/.05^2$

= 138.2976, therefore **138** samples were required for this study.

3.3 Sampling Method

A probability proportional to size cluster sampling method was used to provide the best estimate of the number of samples to be collected from each Sub County in Mombasa County. A cluster in this proposal meant a Sub County within Mombasa County.

A list of all the boreholes and water wells in the four division was obtained from the Public Health Office at Mombasa County Public Health Department. The number of wells and in each division was determined and divided amongst the four (4) Sub Counties using the following formulae;

n = (required samples size) X (total number of well per division)/ (Total number of well and bore well in the county).

Thus, at the time of the study, the number of boreholes and wells in Mombasa County was 3064, where a total number per division was Likoni Sub County was 1094, Mvita Sub County was 679, Changamwe Sub County was 711 and Kisauni Sub County was 580. Using the above formulae, the minimum sample size for each Sub County was as follows; Likoni 44, Mvita 33, Changamwe 35 and Kisauni 26. After getting the number of well/borehole to be sampled per division, all the wells and borehole were assigned numbers and simple random sampling was used to identify the well and to be sampled.

3.4 Methods of Analysis

3.4.1 Collection of Water

Water samples were collected from boreholes and wells aseptically; that is water from boreholes and closed wells, the tap was sterilized by a swab soaked in alcohol and lighted, then 200ml of water was collected in a sterile bottle and labelled. For the open wells a sterile bottle was lowered into the well with rope and water was collected. If free chlorine was present in the water source, 5% sodium thiosulfate was added into the sterile bottles to neutralize the chlorine. The bottle was placed in a chilled cooler for transportation to the Public Health Food Laboratory at Mombasa Public Health Department.

3.4.2 Laboratory Analysis

3.4.2.1 Total Coliforms Count – Most Probable Number (MPN)

MacConkey broth media was prepared in universal bottles to which Durham tubes are added. Using the 200 ml water sample, 3 bottles each with 10ml of double strength MacConkey broth was inoculated with 10ml of water, 3 bottles each with 5ml of single strength MacConkey broth was inoculated with 1ml of water and another 3 bottles each with 5ml of single strength MacConkey broth was inoculated with 0.1ml of water. The bottles were incubated at 37^0 C for 48 hours. (Suton, 2010)

The bottles were checked for lactose fermentation (yellow coloration) and gas production (air space in Durham tube). Number positive was compared with 3- tube MPN McCradys' Table (appendix 4) to determine the most probable number of coliforms.

3.4.2.2 Eijkman's Test to Detect Faecal *E. coli*

All positive bottles were sub-cultured into fresh MacConkey broth and peptone water. The bottles were incubated at 44.5^0 C for 48 hours. The MacConkey bottles were checked for lactose fermentation (yellow coloration) and Gas production (air space in Durham tube).

For all positive MacConkey bottles, Kovacks reagent was added in the corresponding peptone water to detect indole production (red coloured ring). Those found to be positive were noted as positive for fecal *E. coli.* (Suton, 2010).

3.4.2.3 Isolation of *E. coli*

All positive bottles were sub-cultured into fresh MacConkey agar and incubated at 37°C for 18 to 24 hours. The colonial morphology was determined using standard microbiological techniques. Confirmation of *E. coli* was done by Gram stain and

biochemical test (Indole test) *E. Coli* is a Gram-negative bacterium that produces indole from tryptophan (Amaya, *et al*., 2012; Bahiru, *et al.,* 2013; Cernat, *et al.,* 2002).

3.4.2.4 Antibiotic Sensitivity Testing

The antibiotic sensitivity pattern of the isolated pathogens was determined using commonly used antibiotics in Mombasa County by disc diffusion method. The antibiotics used were Ampicillin (30µg/ml), Cotrimoxazole (25µg/ml), Nitrofurantoin (300µg/ml), Sulfamethoxazole (25µg/ml), Nalidixic acid (30µg/ml), Tetracycline (30µg/ml), Gentamicin (120µg/ml) and Streptomycin (10µg/ml).

In this case a pure colony of the isolated organism was spread onto the Muller Hinton agar and the antibiotic disc was placed on the media using disc diffusion method by Kirby-Bauer Disk Diffusion Susceptibility test. The plates were incubated at 37^0C for 18-24 hours and the zones of inhibition measured (Reller, *et al.,* 2009).

3.4.3 Molecular Typing of *E. coli* isolates

3.4.3.1 Deoxyribonucleic Acid (DNA) Extraction

The isolated organisms were further characterized by Polymerase Chain Reaction (PCR) method; a single colony of the isolate was inoculated in 3 ml of standard Trypticase soy broth and incubated at 37°C for 16 to 18 hours for further growth. The bacterial suspension was then centrifuged and resuspended in 200 µl of phosphate buffered saline (PBS) and transferred to a 1.5ml Eppendorf tube. The QIAGEN DNA kit was used to extract the bacterial DNA using the kit manufacturer's instructions, where briefly, twenty microliters (20µl) of QIAGEN proteinase K were then added to the suspension, plus 200µl buffer AL, shaken for 15 seconds and centrifuged at 3000rpm. This was then incubated at 56^0C for 10 minutes. After the incubation, the caps were opened carefully, and 200µl of ethanol (96-100%) was added. This was followed by vigorous shaking for 15 seconds and a short 3000rpm spin. The cell suspension was then transferred to a spin column, and spun at 6000rpm for 4 minutes. The lysate was discarded, and 500µl of

wash buffer 1 (AW1) was added. This again was spun at 6000rpm for 2 minutes and the filtrate discarded. 500µl of wash buffer 2 (AW2) was then added to the spin column and was spun at 6000rpm for 15 minutes and the filtrate discarded. The now clean DNA was eluted from the spin column using 200µl buffer AE and spun 4000rpm for 4minutes.

3.4.3.2 Polymerase Chain Reaction (PCR) to subtype *Escherichia coli*

This was carried out to confirm the results obtained from culture and to subtype the *Escherichia coli* into one of three major pathotypes causing diarrhea in developing countries, including EPEC, ETEC and EAEC. The primers for subtyping them were used (appendix 3) (Nguyen, *et al.,* 2005). The polymerase chain reaction (PCR) mix had the following 2.5µl of 10× PCR buffer (Invitrogen, USA), 2.5µl of magnesium chloride ($MgCl_2$) (Invitrogen, USA), 2µl of 0.4mM dNTPs (Invitrogen, USA), 0.4µl of each of the primers (forward and reverse primers) 7.75µl nuclease free water and 0.25µl of *Taq* polymerase (Qiagen, USA). A 3µl aliquot of DNA was added to give a final volume of 20µl. The cycling conditions for the PCRs were: an incubation at 96^0C for 4 minutes to activate the *Taq* polymerase. This was followed by 35 cycles involving denaturation at 95^0C for 30 seconds, annealing at 57^0C for 30 seconds and strand extension at 72^0C for 1 minute. Finally, a final incubation at 72^0C for 10 minutes followed to fill in the recessed ends of the amplification products. This was carried out on a Gene Amp 9700 (Applied Biosystems, USA). The PCR products were visualized under UV light after gel electrophoresis using Tris-borate EDTA (TBE) buffer on 2% agarose gels stained with ethidium bromide at 100V for 60 minutes.

3.4.3.4 Real Time Polymerase Chain Reaction (PCR) to subtype *E. coli*

This was carried out to subtype the *Escherichia coli* into one of six major pathotypes, including EHEC, ETEC, EPEC, EAEC, EIEC and DAEC. The polymerase chain reaction (PCR) mix had the following, 10µl of QuantiTect PCR Probe (Qiagen, USA) Master mix buffer, 0.5µl of each of the primers (forward and reverse primers) and probe, 5.5µl nuclease free water and 3µl of DNA template was added to give a final

volume of 20µl. The cycling conditions for the PCRs were: an incubation at 95^0C for 15 minutes to activate the *Taq* polymerase. This was followed by 45 cycles involving denaturation at 95^0C for 15 seconds, annealing and extension at 55^0C for 60 seconds. This was carried out on a Rotor Gene Q (Qiagen, USA).

3.5 Risk Factors Determination

The condition of the wells and was assessed using observation and a questionnaire was filled (Appendix 1), to determine whether the well/borehole was covered or not, or whether the source has been chlorinated or not and when it was chlorinated. The distance between wells or boreholes and pit latrines, soak pits or septic tanks and the depth of the well and boreholes was also measured.

3.6 Data Management

3.6.1 Data Quality Control

Quality of data was ensured by; use of standardized questionnaire (see appendix 1), pretesting data collection tools and use of approved laboratory methods for testing. Accuracy and consistency were ensured by entering and cleaning of data.

Statistical analysis was done using Epi- info 3.5.1 software and SPSS version 12. Descriptive analysis was done where proportions were calculated; the association between different variables was done using Chi squire (χ^2).

3.7 Ethical Consideration

Clearance and approval were sought from; Kenya Medical Research Institute (KEMRI) Scientific and Ethical Committees (SSC number 2130), Jomo Kenyatta University of Agriculture and Technology (JKUAT) board of Post Graduate Studies.

Confidentiality of all information obtained from sampled wells and was maintained by not allowing information to be accessible to non-research team, use of password

protected computers, lockable drawers and use of numbers instead of names. The research findings were submitted to the well owners and public health authorities for further actions**.** Consent from owners of water sources was given verbally.

CHAPTER FOUR

RESULTS

4.1 Description of Samples collected from Sampling Sites

A total of 157 samples were collected around Mombasa County from the following areas of Likoni Sub County (n= 51; 32.5%), Mvita Sub County (n= 39; 24.8%), Changamwe Sub County (n= 37; 23.6%) and Kisauni Sub County (n= 30; 19.1%) as shown in the figure below.

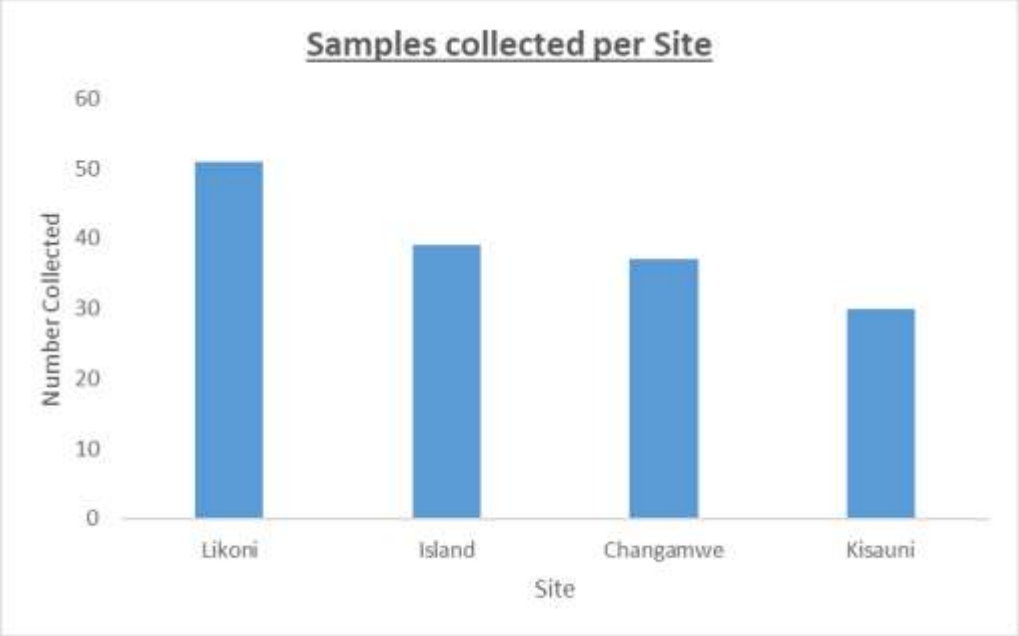

Figure 4.1: Sample distribution per Site.

4.1.1 Water Sources from the Samples Sites

During the study, majority of the samples were collected from (n= 98; 62.4%), while the other samples were collected from wells (n= 59; 37.6%).

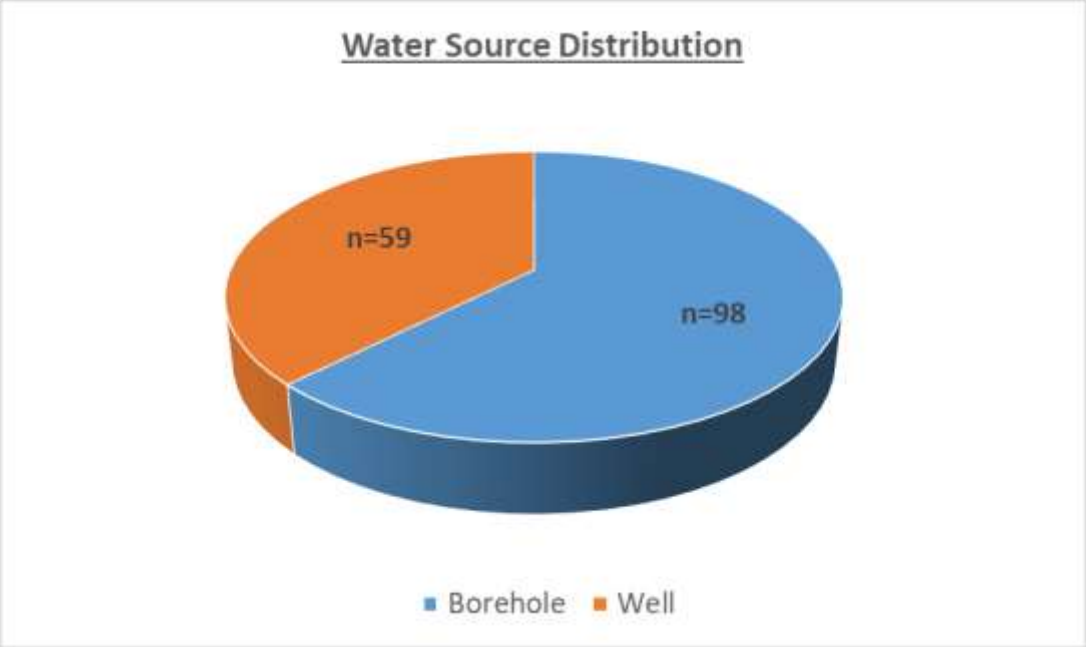

Figure 4.2: Water Source Distribution

4.1.2 Samples Site Description

Majority of the water sources samples were protected (n= 144; 91.7%), while a few of the sampled water sources were not protected (n= 13; 8.3%). Many of water sources sampled had pumps available (n= 145; 92.4%) at the water source compared to a few that had no water pump (n= 12; 7.6%) available at the site. Majority of water sources samples that had pumps available at the water source had not recently over hauled/repaired (n= 137; 87.3%) compared to a few that had the water pump repaired/overhauled (n= 20; 12.7%). Of the 157 samples collected from water sources

around Mombasa County, only 91 representing 58% were treated by the use of chlorine. The remaining 66 representing 42% were not treated with chlorine. This is shown in table 4.1 below.

Table 4.1: Description of Sampling Sites

Sample	Number	Percentage
	98	62%
Wells	59	38%
Protected	144	92%
Chlorine Treated	91	58%
Pumps Available	145	92%
Not over Hauled/Repaired	137	87%

4.2 Contamination

4.2.1 Coliform contamination

Most of the presumptive coliform's tests showed positive coliform tests as shown by universal bottle with yellow color (acid fermentation) and gas in Durham tubes (air space in Durham tubes) while negative test the media remain unchanged (purple color). Out of the 157 samples collected from water sources around Mombasa County and inoculated to MacConkey Broth, 83.4% (n=131) samples were contaminated by coliform bacteria. Of which 60.3% (n=79) of the samples were from , while 39.7% (n=52) were from wells. According to McCrady's MPN tables, the samples that had been contaminated by coliforms 6.9% (n=9) ranged from a value of 3-10, 9.9% (n=13)11-100, 8.4% (n=11) 101-1000 and 78% (n=98) equal to or more than 1000 coliform forming units as shown by the figure below.

Percentage Distribution of MPN Values among positive coliform Samples

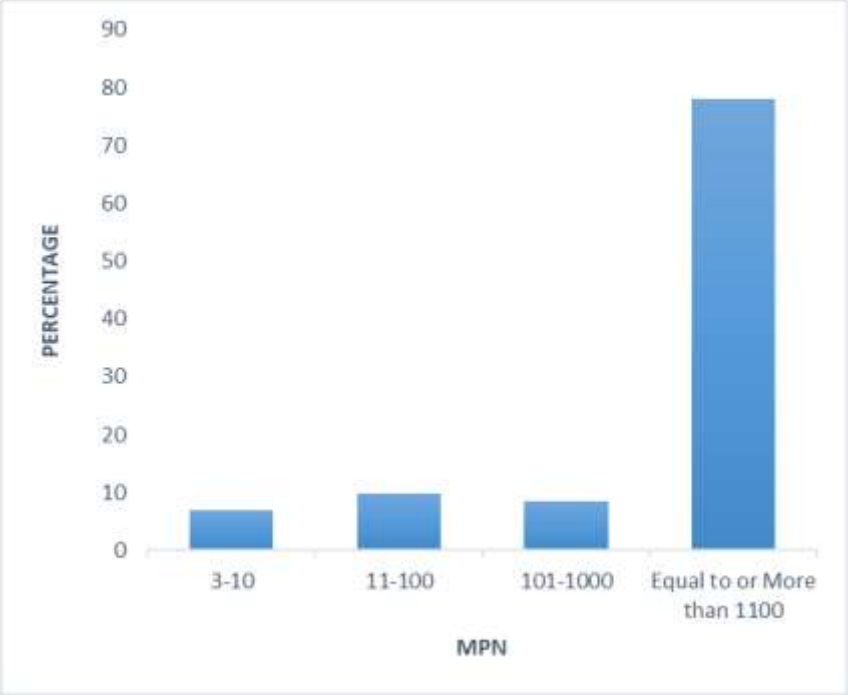

Figure 4.3: Bar chart showing percentage distribution of MPN values of coliform contaminated samples

4.2. 2 *E. coli* contamination

From the total coliform bacteria detected (n= 131), only 79 samples were confirmed to be having *Escherichia coli* after performing the Eijkman Test. This represents 60.3% compared to 52 samples which were not having *Escherichia coli* but had other coliform bacteria. This group without *E. coli* represented 39.7%.

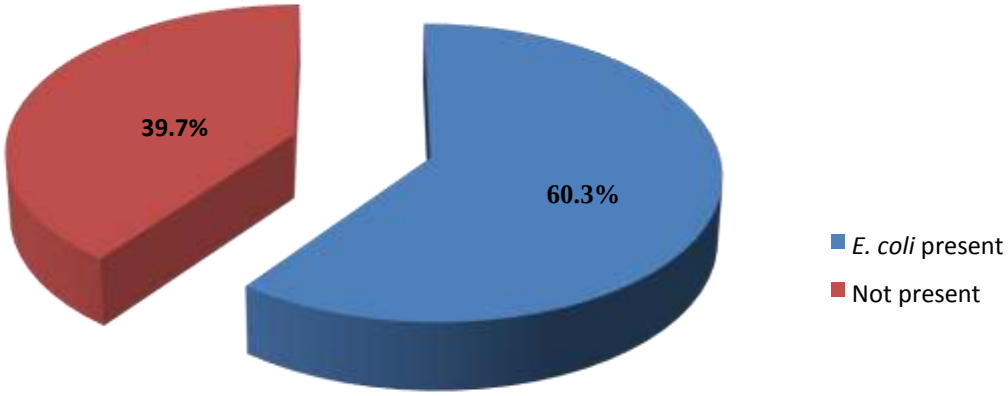

Figure 4.4: Pie chart showing percentage distribution of coliform samples contaminated with *E. coli*.

Of the contaminated water samples that had *E. coli* detected in them (n= 79; 83.4%), 52 representing 65.8% of the samples were from , while 27 representing 34.2% were from wells. This is shown in the figure 12 below.

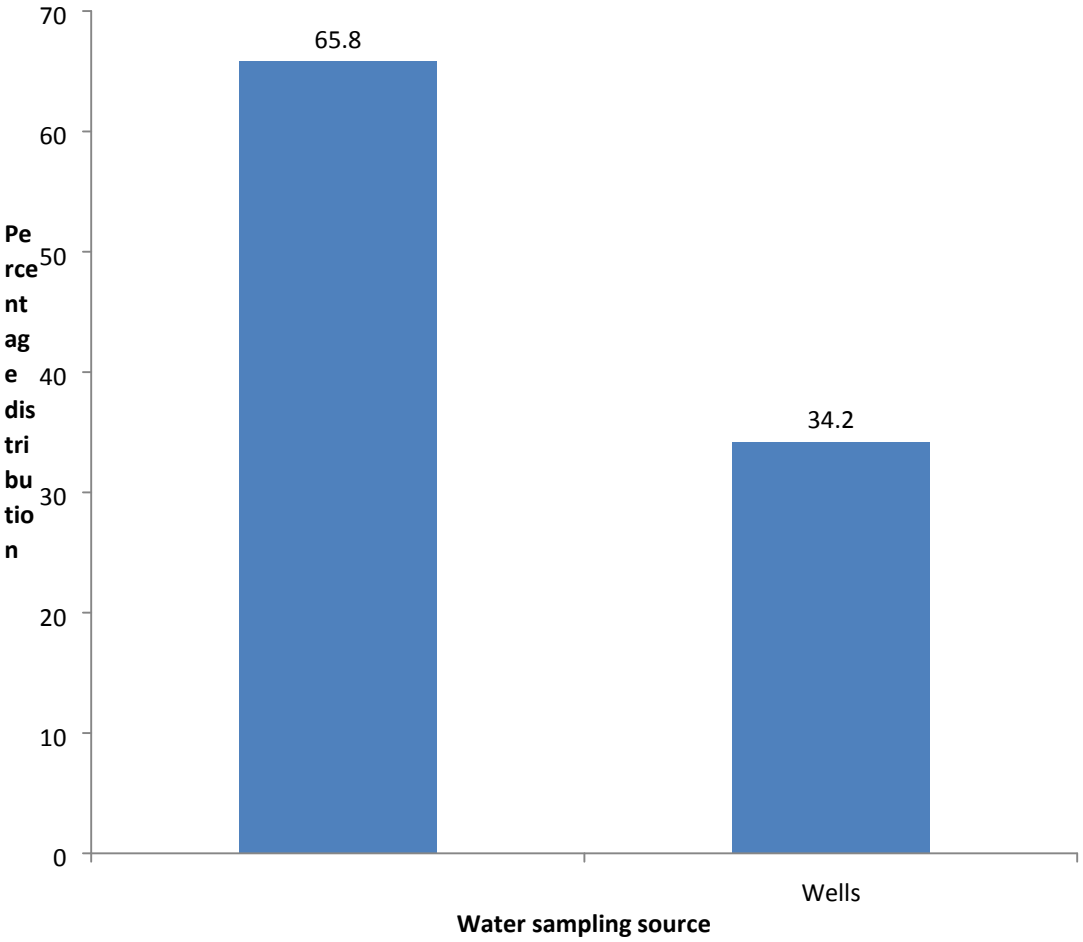

Figure 4.5: Chart showing percentage distribution of water sample source contaminated by *E. coli* bacteria

4.2.3 Antibiotic susceptibility test patterns of the isolated *E. coli*

The figure 4.6 below shows the antibiotic profile of 77 *E.* coli tested against 8 commonly used antibiotics. Of the antibiotics tested, gentamicin (n = 77; 100%) was most sensitive, followed by streptomycin (n = 70; 90.9%). All the *E. coli* isolated were resistant to ampicillin (n = 77; 100%), followed by sulphamethoxazole (n = 33; 42.9%) and cotrimoxazole (n = 33; 42.9%).

Distribution of antibiotic susceptibility

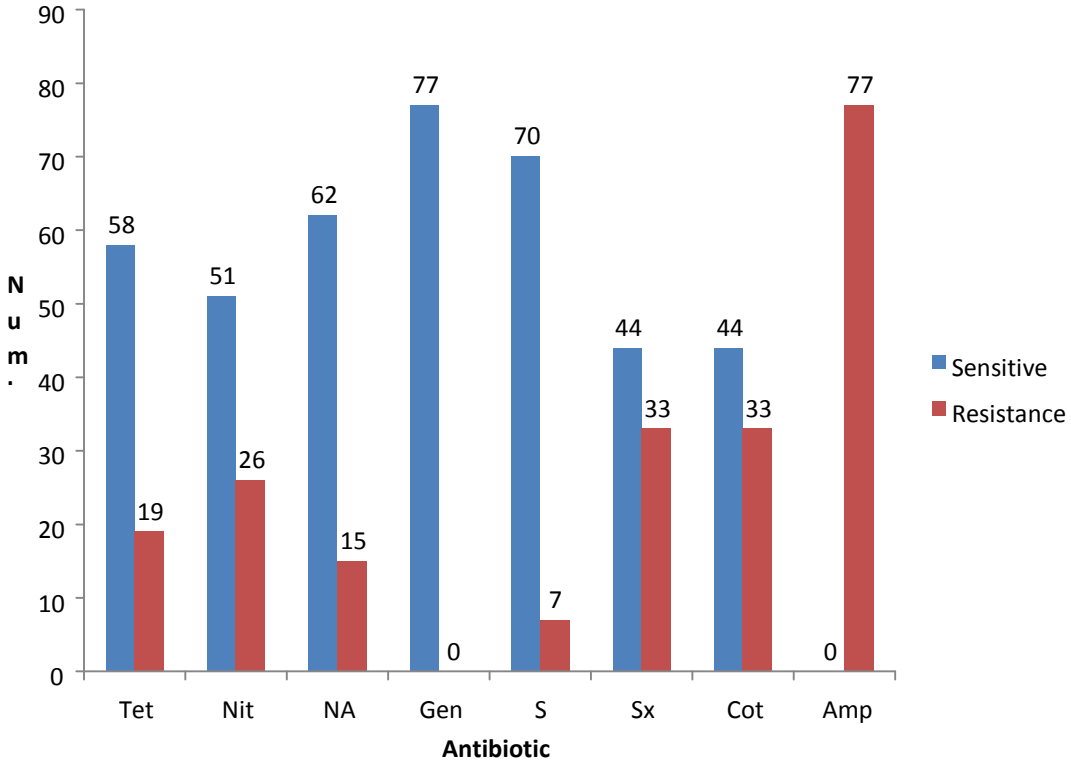

Figure 4.6: Bar graph indicating antibiotic susceptibility profile and distribution of isolated *E. coli*.

Keys: Tet – Tetracycline (30µg); Nit – Nitrofurantoin (300 µg); NA – Nalidixic Acid (30 µg); Gen – Gentamicin (120 µg), S – Streptomycin (10 µg0; Sx – Sulphamethoxazole (25 µg); Cot – Cotrimoxazole (25 µg) and Amp – Ampicillin (30 µg).

4.7.4 Antibiotic Sensitivity and Resistance Patterns

The Table 4.2 below shows the different resistance and susceptibility patterns detected from this study's isolated *Escherichia coli*.

Table 4.2: Antibiotic sensitivity and resistance patterns

Number of Drugs Susceptible	Sensitivity Patterns	Number	Total Number of susceptible isolates to drugs used
1	Gen	2	2
2	Gen & NA Gen & S	4 3	7
3	Gen, S, Sx Gen, NA, S Gen, Cot, Sx	2 4 1	7
4	Gen, NA, S, Tet Gen, Nit, S, Tet Gen, NA, S, Sx Gen, Cot, NA, S	3 4 2 1	10
5	Gen, NA, Nit, S, Tet Gen, Cot, S, Sx, Tet Gen, Cot, Nit, S, Tet	2 2 1	5
6	Gen, Cot, NA, Nit, S, Tet Gen, Cot, NA, S, Sx, Tet Gen, NA, Nit, S, Sx, Tet	7 2 7	16
7	Gen, Cot, NA, Nit, S, Sx, Tet	30	30
Number of Drugs Resistance	**Resistance Patterns**	**Number**	**Total Number of resistance isolates to drugs used**
1	Amp	30	30
2	Amp & Cot Amp & Nit Amp & Sx	7 2 7	16
3	Amp, Cot, Sx Amp, Nit, NA Amp, NA, Sx	2 2 1	5
4	Amp, Cot, Sx, Nit Amp, Cot, NA, Sx Amp, Cot, Nit, Tet Amp, Nit, Sx, Tet	3 4 2 1	10
5	Amp, Cot, NA, Nit, Tet	2	

	Amp, Cot, Nit, Sx, Tet	4	7
	Amp, NA, Nit, S, Tet	1	
6	Amp, Cot, Nit, S, Sx,	4	
	Tet Amp, Cot, NA, Nit, Sx, Tet	3	7
7	Amp, Cot, NA, Nit, S, Sx, Tet	30	30

Keys: Tet – Tetracycline; Nit – Nitrofurantoin; NA – Nalidixic Acid; Gen – Gentamicin, S – Streptomycin; Sx – Sulphamethoxazole; Cot – Cotrimoxazole and Amp – Ampicillin.

The table above shows the number of antibiotics the *E. coli* isolated are sensitive or resistance when was exposed to the commonly used antibiotics used in Mombasa County.

4.2.6 Molecular Characterization of *E. coli*

To further characterize the *E. coli* samples isolated in this study from the contaminated water samples, two molecular assays were carried out using type specific primers were used. The first test was a multiplex PCR to detect three common pathotypes of *E. coli* including ETEC, EPEC and EAEC. The isolated *E. coli* were shown not to be of the three pathotypes as shown in the figure below.

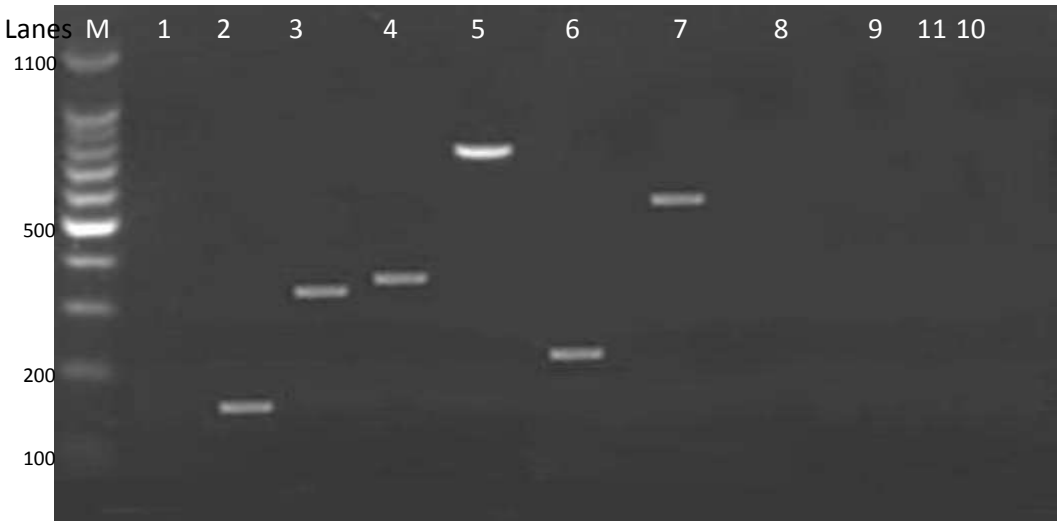

Figure 4.7: Molecular subtyping of isolated *E. coli*.

Lane M = 100bp DNA marker (Invitogen Cat74602-250). Lanes 1 represents an internal negative control. Lane 2 and 3 represents a positive control for ETEC (Strain ESCCO 22) with ST (Lane 2 – 147bp) and LT (Lane 3 – 322bp) indicated. Lanes 4 and 5 represent a positive control for EPEC (Strain S-ESCCO 16 Pl) with bfp (Lane 4 – 367bp) and eae (Lane 5 – 881bp) indicated. Lanes 6 and 7 represent a positive control for EAEC (Strain ESCCO 14) with aaic (Lane 6 – 215bp) and pCVD (Lane 7 – 630bp) indicated. Lanes 8 - 11 are representatives of this study's' *E. coli* isolates tested.

The second molecular test was a real time PCR that was carried out in order to detect all the six *E. coli* pathotypes including ETEC, EPEC, EAEC, EHEC, EIEC and DAEC. The isolated *E. coli* from this study were EIEC had the following results as shown in the figure and table 4 below.

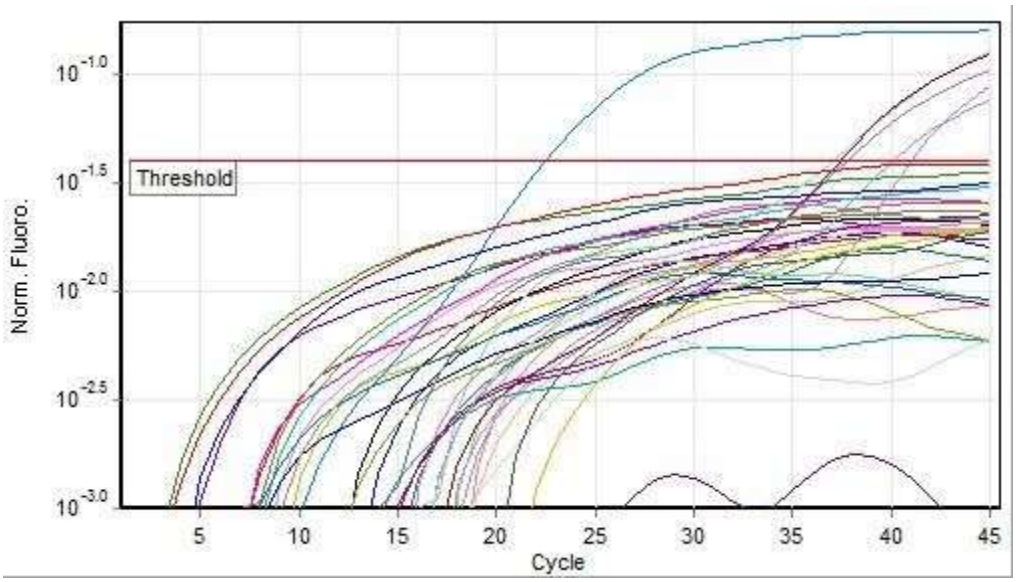

Figure 4.8: A representative Real time PCR results of molecular subtyping of isolated *E. coli*.

Table 4.3: Indicating representative CT values of this study's Real time PCR Results.

Color	Name	Type	CT Value
	EIEC- 65	Unknown	37.15
	EIEC- 70	Unknown	40.09
	EIEC- 72	Unknown	37.78
	EIEC- 97	Unknown	41.01
	EIEC- Positive Control	Unknown	37.42
	EIEC- Negative Control	Unknown	

The Table 3 below compared the association between different variables and compared them with coliform contamination. The results from the location (χ^2 value = 13.308, three degrees of freedom (df=3), and a p value of 0.004), recent overhaul/repair (χ^2 value of 13.308, df=1, and p value of 0.003) and distance to water source (χ^2 value of 9.113, df=1, and p value of 0.021) were significant.

Table 4.4: Comparing Different Variables against Coliform Contamination

Variables	Coliform contamination		Total	P. Value* at 95%
Location	**Yes**	**No**		
Island	31	8	39	
Kisauni	26	4	30	**0.004**
Likoni	49	2	51	
Changamwe	25	12	37	
Sample Source				
Borehole	79	19	98	0.219
Wells	52	7	59	
Protected Water Sources				
Protected	119	25	144	0.369
Unprotected	12	1	13	
Type of cover				
Complete	119	25	144	
Partial	11	1	12	0.653
Open	1	0	1	
Presence of Pump				
Yes	120	25	145	0.425
No	11	1	12	
Recent Overhaul				
Yes	12	8	20	**0.003**
No	119	18	137	
Distance to water Source				
Between 1-10Metres	57	5	62	**0.021**
Equal to & Above 20 Metres	74	21	95	
Chlorine Treatment				
Yes	75	16	91	0.686
No	56	10		

Pearsons' Chi Square Test, CI-Confidence Interval

The Table 4.5 below compared the correlation between different variables and *Escherichia coli* contamination. The results showed no significant differences when compared to the variables.

Table: 4.5: Comparing different variables against *E. coli* contamination

Variables	*Escherichia coli*		Total	P-Value at 95 CI
Location	**Yes**	**No**		
Island	20	19	39	
Kisauni	14	16	30	0.081
Likoni	32	19	51	
Changamwe	13	24	37	
Sample Source				
Borehole	52	46	98	0.376
Wells	52	7	59	
Protected Water Sources				
Protected	74	70	144	0.372
Unprotected	5	8	13	
Type of cover				
Complete	74	70	144	
Partial	4	8	12	0.295
Open	1	0	1	
Presence of Pump				
Yes	75	70	145	0.221
No	4	8	12	
Recent Overhaul				
Yes	6	14	20	0.052
No	73	64	137	
Distance to water Source				
Between 1-10Metres	37	25	62	0.058
Equal to & Above 20 Metres	42	53	95	
Chlorine Treatment				
Yes	45	46	91	0.798
No	34	32		

* Pearsons' Chi Square Test, CI – Confidence Interval

CHAPTER FIVE

DISCUSSION

5.1 Bacteria Contamination of water sources

Water is an important resource that is prone to bacterial contamination from a variety of hosts including mammals and avian species (Ishii *et al*., 2006, Ishii *et al*., 2007, WHO, 2011). The rapid expansion of this county has led to the residents relying on groundwater to supply them with portable water. This has also been observed in other expanding cities around Africa that include Zimbabwe (Dzwairo, 2006), Nigeria (Egwari and Aboaba, 2002), South Africa (Kinge *et al*., 2010), and Ghana (Obiri-Danso *et al*., 2009). Similar observations have also been observed from studies carried out in Kenya at Kitui, Eldoret, Mombasa and Kisumu (Abila *et al*., 2012, KimaniMurage and Ngindu, 2007, Kiptum and Ndambuki, 2012, Munga *et al*., 2005, Mwaguni, 2002, Opisa *et al*., 2012).

5.2 Bacterial Contamination of Water Samples

5.2.1 Prevalence of coliforms and *E. coli*

The presence of coliform bacteria and *E. coli* in water is an indicator of recent fecal contamination indicating the possible presence of disease-causing pathogens, such as bacteria, viruses, and parasites (Egwari and Aboaba, 2002, WHO, 2011). The World Health Organization (WHO) recommends that no coliforms be detected in 100ml of drinking water (WHO, 2011). According to WHO standards, majority of samples collected from this study were contaminated by coliforms. The coliforms in this study were mostly detected in water samples collected from compared to wells. This is different to what was observed in Nigeria where contamination was mostly detected in samples from wells compared to (Egwari and Aboaba, 2002). The prevalence/incidence of coliforms in this study was slightly less than previous studies carried out in the same county in 2002 that determined it at slightly more than 90% (Mwaguni, 2002) and the

study carried out in 2005 (Munga *et al*., 2005) who had a prevalence/incidence of 85.1%. The main difference between this study and the study in 2005 (Munga *et al*., 2005) was that the 2005 study mainly focused with Kisauni Sub County, while this study sampled the whole County thus giving a fairly comprehensive report. A higher prevalence (95%) was also observed in a study using membrane filtration systems to determine bacterial contamination of water samples collected in Kisumu's informal settlements (Opisa *et al*., 2012). This higher prevalence (91.9%) was also noted in another study carried out in a slum in Eldoret (Kimani-Murage and Ngindu, 2007). This study though had a higher prevalence/incidence compared to a study carried out in Zimbabwe (Dzwairo *et al*., 2006), even though it used the membrane filtration technique to determine bacterial contamination. Majority of the Zimbabwe's samples from shallow wells were contaminated due to hygienic practices used by people collecting the water (Dzwairo *et al*., 2006). This study's prevalence was higher when compared to an environmental surveillance study carried out in Nairobi's Kibera slums which had a prevalence of 76.1% contamination by coliforms (Christabel *et al*., 2012).

The bacteria, *E. coli* has been an important indicator of recent contamination of water for many years (Florea, 2011, Wright *et al*., 2004). This is because the bacteria are found in the intestines of warm-blooded animals and released to the environment via deposition of fecal matter (Kaper *et al*., 2004, Ibekwe *et al*., 2011).

5.2.2 Antibiotic Susceptibility and Resistance Patterns

The increase of antibiotic resistance in bacterial isolates is of great concern to health officials locally and worldwide because of the diminishing number of new antibiotics that could be used in the control of bacterial pathogens (Florea, 2011; Magiorakos *et al*., 2012). Therefore, this study's *E. coli* isolates were tested against 8 commonly used antibiotics to determine their antibiotic susceptibility profiles. Of the antibiotics tested, all the isolates from this study were resistant to ampicillin. This observation where high resistance ampicillin was noted, had been reported in other studies (Cernat *et al*., 2002). This is different to a study carried out in Nairobi's Kibera slum, where ampicillin

resistance was also observed at 6.3% (Christabel *et al*., 2012). This was also different from a study carried out in South Africa, where the bacteria had a resistance to ampicillin ranging from between 10 – 80% from the different samples collected (Kinge *et al*., 2010). After resistance to ampicillin, this was followed by resistance to cotrimoxazole and sulphamethoxazole. All of this study's *E. coli* isolates were sensitive to gentamicin. This drug is rarely abused because it is intramuscularly administered compared to majority of the other drugs which are taken orally (Christabel *et al*., 2012). This was followed by sensitivity to streptomycin. This drug is also rarely abused because again, just like gentamicin, it is not orally administered, but intramuscularly or intravenously delivered to patients (Zhu *et al*., 2001). The sensitivity of this study's *E. coli* isolates is in contrast to similar isolates from Ethiopia which were 100% resistant to streptomycin (Bahiru, 2013).

Multidrug resistance which is resistance to more than one antibiotic (Magiorakos *et al*., 2012) was noted in this study's isolates where resistance to 7 antibiotics was most commonly observed. This was followed by resistance to 2 antibiotics. Multidrug resistance has been observed in *E. coli* from studies in Kenya (Christabel *et al*., 2012), South Africa (Kinge *et al*., 2010), Ethiopia (Bahiru, 2013), Nicaragua (Amaya *et al*., 2012) and Romania (Florea, 2011). A study in South Africa, indicated that water bodies could be reservoirs of bacterial antibiotic resistance genes which could be horizontally or vertically transmitted (Biyela *et al*., 2004).

5.2.3 Molecular Sub typing of *E. coli*

In Africa, studies in molecular characterization of *E.* coli isolates from the environment have majorly been carried out in South Africa (Samie *et al*., 2012); (Heine, 2008); (Omar, 2008). Similar studies have also been carried out in Brazil (Oliveira *et al*., 2012). This study's isolates were molecularly characterized as EIEC. These bacteria in Kenya, has been isolated from stool samples (Sang, et al., 2011), but have never been isolated from water sources before. This the first time to characterize *E. coli* pathotypes from water samples in Kenya. This is very different from the South African and Brazilian

studies which detected other *E. coli* pathotypes apart from the EIEC which were rare or were never detected in the water samples. The *EIEC* are causative agents of diarrhea with similarities to *Shigella species*, since they invade colonic epithelium and producing enterotoxin associated with diarrhea (Nataro and Kaper, 1998).

5.2.4 Association of coliform detection with variables tested

From this study, there was association detected between the variables location or sampled site. All the sites sampled in the study had samples contaminated by coliforms. The site at Likoni had most contaminants. This was followed by the Mombasa Island site and then by Kisauni and Changamwe. A similar study carried out in the same county also observed similar results where microbiological contaminations occurred in wells and within the county with more peaks observed during the rainy seasons compared to the wet season (Munga *et al*., 2005). This seasonality was not determined by this study. Many of the residents rely on site sewage management systems including septic tanks and soak pits (Munga *et al*., 2005). Thus, during rains, flooding would occur leading to contamination of these wells and as was noted by the study in Eldoret (Kiptum and Ndambuki, 2012).

The WHO recommends that wells and should located 30 meters away from the soak pits and pit latrines (WHO, 2011). This was not the case as was observed in a study in the same county where pit latrines, waste disposal were very close to wells and (Munga *et al*., 2005, Kimani-Murage and Ngindu, 2007, Kiptum and Ndambuki, 2012). Studies in Zimbabwe (Dzwairo *et al*., 2006), Ghana (Obiri-Danso *et al*., 2009) had water sources (and pit latrines) collected less than 10 meters from pit latrines and in this study, water samples were collected at distances between 1-10 meters and equal to or more than 20meters from or to the pit latrine and the water source. The indiscriminate defecation instead of in pit latrines could be a cause of contamination (Kimani-Murage and Ngindu, 2007).

This study's treated and untreated samples had coliforms detected in them as was observed in a Brazilian study (Nogueira *et al*., 2003). This though was different when compared to the study in Nigeria by Egwari and Aboaba (2002) which sampled water from both treated (piped) and untreated (wells) supplies. The Nigerian study observed no coliforms in treated supplies, while untreated supplies had bacterial contaminants. The presence of these coliforms in treated or untreated water could be due to factors such as inadequate treatment, increased temperatures above 20^0C, and rainfall which may introduce contaminants and nutrients that may increase growth of contaminants to water supplies (LeChevallier *et al*., 1996; Prest, *et al.*, 2016). These factors though were not looked at by the current study.

The covering of wells and has been observed to be an important factor in the reduction of contamination of water sources (LeChevallier *et al*., 1996, WHO, 2011). In this study though, even though majority of the water sources were protected, there was still the presence of coliforms in them. This could have been due to seepage of coliforms from septic tanks or soak pits that were near or close to the wells or and flooding of septic tanks which could then contaminate the wells and with pathogens (Abila *et al*., 2012, Munga *et al*., 2005). In a study in Ghana, all the water samples from and wells were contaminated because the investigators observed that all the water sources were not covered with receptacles that were used for other purposes including bathing and laundering (Obiri-Danso *et al*., 2009). In Kenya, studies in Eldoret (Kimani-Murage and Ngindu, 2007) and Kisumu (Opisa *et al*., 2012), had also noted that samples from uncovered water sources had contamination. In another study in Eldoret, Kenya, water source covers that had been used were mainly made of timber because concrete covers are expensive (Kiptum and Ndambuki, 2012).

In this study, majority of the samples were collected from wells and that had been treated with chlorine but were unfortunately contaminated by coliforms. The WHO recommends further treatment of water using coagulation, flocculation, and filtration processes to remove any other pathogens that could be present in the drinking water (WHO, 2011). In a study in a slum in Eldoret, Kenya, about 42% of the residents boiled

water after collecting it from wells/ (Kimani-Murage and Ngindu, 2007). This study did not look at further treatment procedures carried out by collectors of these water samples from within the county.

From this study, there was no association detected between the variables tested and the detection of *E. coli*. This is similar to a study carried out in Kitui town in Kenya, where no significance when the detection of *E. coli* was compared to distance to the sampling site (Abila *et al*., 2012). The same was also observed when distance from water source was compared to distance to pit latrine in Kisumu (Opisa *et al*., 2012).

5.3 Conclusions

The Findings in this study suggest that coliforms and E. coli and especially of the EIEC subtype are major contaminants of wells and in Mombasa County, showing that water sanitation is limited due to lack of sewage system leaving the majority of people to rely on pit latrines with contaminate the water table.

Majority of the *Escherichia coli* isolated were had multidrug resistance, The isolates have a variety of resistant and sensitivity patterns to commonly used antibiotics. All the isolates were sensitive to Gentamicin and resistant to Ampicillin among the drug used.

5.4 Recommendations

There should be effective control of pit latrine development in the unplanned settlements by the physical planning and public health departments. The pit latrines development should be done according to the WHO guidelines of a distance of more than 20 meters from source of water.

Continuous surveillance of water sources for contamination should be carried out. The boreholes and wells should be frequently monitored and chlorinated to avoid contaminations. The public health department should ensure the boreholes and wells are

chlorinated after every 3 months as stipulated by the guidelines, failure to which legal measures should be taken against the owners.

Further studies could be conducted in the future to determine the effectiveness of chlorination of boreholes and wells as well as determining other pathogens which are associated with water contamination like aeromonads species,

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APPENDICES

Appendix I: Bacteriological Analysis of Water Questionnaire

Sample ID: _____Date of collection: _____

Description and Location of water source (well or Borehole)

Intensity of usage_____

GPS Coordinates: _____

Is it protected? _____ If so, how _____

Depth of the well or Borehole _____ is there a pump _____

Has it been overhauled recently _____

Exact site sample taken: _____

Are there any latrines or other source of pollution? _____
If yes, how far from the water source_____
Time sampling started: _____ Sample collected by:

Residual Chlorine test result: _____ Sodium thiosulfate added to filter? [ ] Yes [ ] No

Back flush Volume: _____

Presumptive coliform test incubation start date/time: _____

Coliform result _____ CFU/100mL. Eijikman's test E. coli result: _____CFU/100mL

MacConkey culture start date/time: _____culture Results-[ ] positive [ ] negative Ryan agar culture start date/time: _____ culture Results-[ ] positive [ ] negative

Appendix II: McCradys Table (3-tube MPN Table)

No. of Tubes Positive in:			MPN in the inoculum of the middle set of tubes
First Set	Middle Set	Last Set	
0	0	0	<0.03
0	0	1	0.03
0	0	2	0.06
0	0	3	0.09
0	1	0	0.03
0	1	1	0.061
0	1	2	0.092
0	1	3	0.12
0	2	0	0.062
0	2	1	0.093
0	2	2	0.12
0	2	3	0.16
0	3	0	0.094
0	3	1	0.13
0	3	2	0.16
0	3	3	0.19
1	0	0	0.036
1	0	1	0.072
1	0	2	0.11
1	0	3	0.15
1	1	0	0.073
1	1	1	0.11
1	1	2	0.15
1	1	3	0.19
1	2	0	0.11

No. of Tubes Positive in:			MPN in the inoculum of the middle set of tubes
First Set	Middle Set	Last Set	
2	0	0	0.091
2	0	1	0.14
2	0	2	0.2
2	0	3	0.26
2	1	0	0.15
2	1	1	0.2
2	1	2	0.27
2	1	3	0.34
2	2	0	0.21
2	2	1	0.28
2	2	2	0.35
2	2	3	0.42
2	3	0	0.29
2	3	1	0.36
2	3	2	0.44
2	3	3	0.53
3	0	0	0.23
3	0	1	0.39
3	0	2	0.64
3	0	3	0.95
3	1	0	0.43
3	1	1	0.75
3	1	2	1.2
3	1	3	1.6
3	2	0	0.93

1	2	1	0.15
1	2	2	0.2
1	2	3	0.24
1	3	0	0.16
1	3	1	0.2
1	3	2	0.24
1	3	3	0.29

3	2	1	1.5
3	2	2	2.1
3	2	3	2.9
3	3	0	2.4
3	3	1	4.6
3	3	2	11
3	3	3	>24

Appendix III: PCR Program and Primers for subtyping *Escherichia coli*

Primer sequence	Primer Name	Amplicon size (bp)
LT-F cacacggagctcctcagtc **LT-R** cccccagcctagcttagttt	LT	508
ST-F gctaaaccagtarggtcttcaaaa **ST-R** cccggtacargcaggattacaaca	ST	147
bfpA-F ggaagtcaaattcatggggg **bfpA-R** ggaatcagacgcagactggt	bfpA	300
CVD-F ctggcgaaagactgtatcat **CVD-R** caatgtatagaaatccgctgtt	CVD432	650
aaiC-F attgtcctcaggcatttcac **aaiC-R** acgacacccctgataaacaa	aaiC	215
eae-F cccgaattcggcacaagcataagc **eae-R** cccggatccgtctcgccagtattcg	eae	881

PCR Master Mix and Programme:

For each reaction, the reagents below were added to make a reaction mix of 20µl per sample (17µl Master mix and 3µl DNA template).

10X PCR Buffer - 2.5µl

MgCl -2.5µl

DNAse Free water - 7.75µl

dNTPs - 2µl

Each of the above listed forward and reverse primers - 4µl (Each primer 0.4µl)

Taq polymerase - 0.25µl

DNA template - 3µl

The above was then preheated at 96^0C for 4 minutes. 35 cycles of denaturation at 95^0C for 30 seconds, annealing at 57^0C for 30 seconds, and an elongation at 72^0 for 1 minute. A final extension was carried out at 72^0C for 7 minutes. The product was viewed in 2% agarose gel using a 1KB ladder.

Appendix IV: Scientific Approval

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
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ESACIPAC/SSC/9999 22nd December, 2011

Thani Suleiman Thani

Thro'
Director, CMR
NAIROBI

forwarded 4/1/2011

REF: SSC No. 2130 (Revised) –Isolation and characterization of aeromonas species and escherichia coli pathotypes and factors associated with well and boreholes water contamination in Mombasa County.

Thank you for your letter dated 22nd November, 2011 responding to the comments raised by the KEMRI SSC.

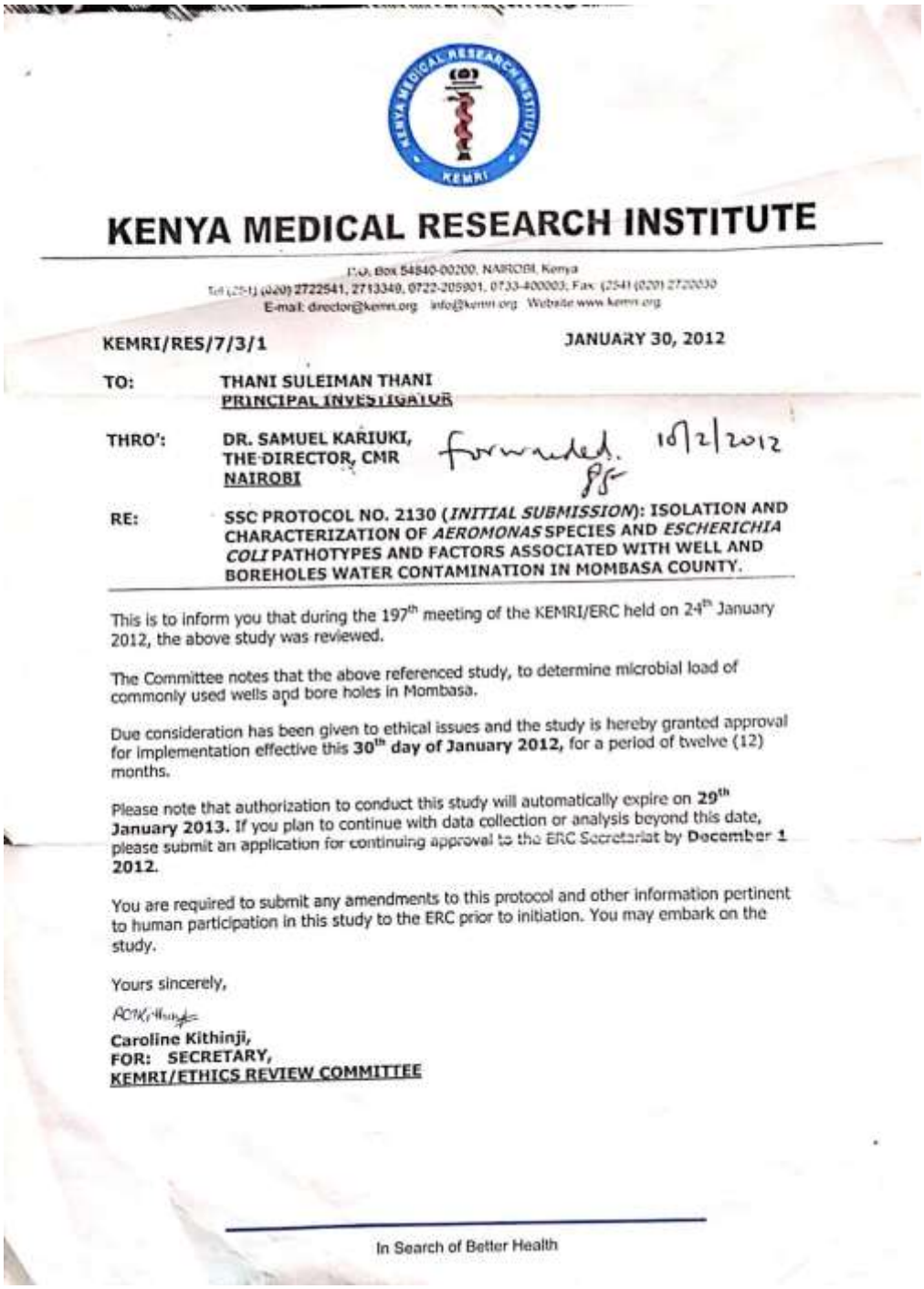
I am pleased to inform you that your protocol now has formal scientific approval from SSC.

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

Sammy Njenga, PhD
SECRETARY, SSC

In Search of Better Health

Appendix V: Ethical Approval



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KEMRI/RES/7/3/1 **JANUARY 30, 2012**

TO: **THANI SULEIMAN THANI**
PRINCIPAL INVESTIGATOR

THRO': **DR. SAMUEL KARIUKI,**
THE DIRECTOR, CMR
NAIROBI

forwarded. 10/2/2012

RE: **SSC PROTOCOL NO. 2130 (*INITIAL SUBMISSION*): ISOLATION AND CHARACTERIZATION OF *AEROMONAS* SPECIES AND *ESCHERICHIA COLI* PATHOTYPES AND FACTORS ASSOCIATED WITH WELL AND BOREHOLES WATER CONTAMINATION IN MOMBASA COUNTY.**

This is to inform you that during the 197th meeting of the KEMRI/ERC held on 24th January 2012, the above study was reviewed.

The Committee notes that the above referenced study, to determine microbial load of commonly used wells and bore holes in Mombasa.

Due consideration has been given to ethical issues and the study is hereby granted approval for implementation effective this **30th day of January 2012,** for a period of twelve (12) months.

Please note that authorization to conduct this study will automatically expire on **29th January 2013.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **December 1 2012.**

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

Yours sincerely,

Caroline Kithinji,
FOR: SECRETARY,
KEMRI/ETHICS REVIEW COMMITTEE

In Search of Better Health

Appendix VI: Publication

Pan Afr Med J. 2016 Jan 22;23:12. doi: 10.11604/pamj.2016.23.12.7755. eCollection 2016.

Isolation and characterization of Escherichia coli pathotypes and factors associated with well and water contamination in Mombasa County

Thani Suleiman Thani [1], Samwel Morris Lifumo Symekher [2], Hamadi Boga [3], Joseph Oundo [4]

Abstract

Introduction: Safe water for human consumption is important, but there is a limited supply. Mombasa County has water shortages making residences rely on other sources of water including and wells. Microbiological evaluation of drinking water is important to reduce exposure to water borne enteric diseases. This cross sectional study aimed at determining the frequency and characterization of Escherichia coli (E. coli) pathotypes from water samples collected from wells and in Mombasa County.

Methods: One hundred and fifty seven (157) water samples were collected from four divisions of the county and a questionnaire administered. The samples were inoculated to double strength MacConkey broth and incubated at 370C for up to 48 hours. Positive results were compared to the 3 tube McCrady MPN table. The E. coli were confirmed by Eijkman's test and antibiotic susceptibility carried out. Using polymerase chain reaction (PCR), the E. coli were characterized to establish pathotypes.

Results: One hundred and thirty one (n = 131; 83.4%) samples had coliform bacteria with only 79 (60.3%) samples having E. coli. Significant values (<0.05) were noted when coliforms were compared to variables with E. Coli showing no significance when compared to similar variables. E. coli (n = 77; 100%) tested were sensitive to Gentamicin, while all (n = 77; 100%) isolates were resistant to Ampicillin. PCR typed isolates as enteroinvasive E. Coli (EIEC).

Conclusion: Findings suggest that coliforms and E. coli are major contaminants of wells and in Mombasa County. The isolates have a variety of resistant and sensitivity patterns to commonly used antibiotics.

Keywords: Boreholes; Coliforms; Escherichia coli; Wells; antibiotic susceptibility.

Figures

Figure 1

Representativeconventional PCR results of isolatedsamples

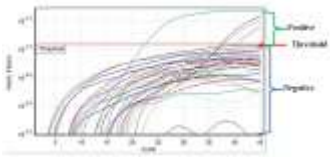

Figure 2

A representative Real time PCR…

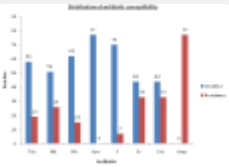

Figure 3

Bargraph indicating antibiotic susceptibility profile…

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