**DETERMINATION OF ANTIMICROBIAL RESISTANCE OF *Escherichia coli* ISOLATED FROM *Rastrineobola argentea* AND WATER SAMPLES FROM LAKE VICTORIA - KENYA**

**BY**

**SOTE, TONY BUSISA**

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**SCHOOL OF BIOLOGICAL AND PHYSICAL SCIENCES**

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# DECLARATION

I hereby declare that this thesis is my original work and has not been presented for the award of a degree in any other university or institution. All sources of information have been duly acknowledged in the references unless by inadvertent omission.

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

**ADM. No. PG/MSc./042/2008**

**SOTE TONY**

Maseno University, School of Biological and Physical Sciences, Department of Zoology

**SUPERVISOR(S)**

This thesis has been submitted for examination with our approval as university supervisor (s):

**Prof. DAVID MIRUKA ONYANGO, PhD**

School of Biological and Physical Sciences, Department of Zoology,

Maseno University, Kenya.

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

**Prof. ELIUD N. WAINDI, PhD**

School of Biological and Physical Sciences, Department of Zoology,

Maseno University, Kenya.

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

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# DEDICATION

This thesis is dedicated to my father, who taught me that the best kind of knowledge to have is that which is learned for its own sake, to my mother, who taught me that even the largest task can be accomplished if it is done one step at a time. It is also dedicated to my brother and sisters who taught me to be resilient.

# ABSTRACT

Many strains of *Escherichia coli* can be pathogenic leading to diarrheal diseases. The occurrence of antimicrobial resistant bacteria is increasing in aquatic environments infecting fish which are later consumed by humans when not well cooked posing a major problem in the management of bacterial infections. *Rastrineobola argentea* is food for many people livingaround Lake Victoria due to its nutritive value and highprotein content. Sun dried *R. argentea* retailed in Kisumu city markets have been shown to be contaminated with antimicrobial resistant *E. coli* which could be as a result of how they are processed including drying them on the sand and dirty mats. Studies done so far with *Salmonella* species have indicated a difference in *Salmonella* species contamination in different types of beaches and different bacteria concentrations at different distances from the shoreline into the lake interior. *Vt1* gene has been shown to be a determinant factor for virulence. This was why this study was undertaken to determine the antimicrobial resistance of *E. coli* isolated from freshly fished *R. argentea* and in lake water in Dunga and Uyoma Naya beaches in Lake Victoria Kenya. A cross sectional study design was used to collect 36 *R. argentea* and 36 water samples from Dunga beach and 36 *R. argentea* and 36 water samples from Uyoma Naya beach in sterilized plastic bags and bottles. Standard microbiological procedures were used to isolate and determine *E. coli* phenotypes from the samples within which 16 *R. argentea* and 9 water samples from Dunga and 8 *R. argentea* and 8 water samples from Uyoma Naya were found to be contaminated with verotoxin - producing *E. coli* (VTEC).The isolates were later screened for antibiotic resistance using the disk – diffusion technique. All 41 isolates were resistant to Ampicillin/Cloxallin, 65.9% were resistant to Tetracycline, 4.9% were resistant to Cefuroxime, and 2.4% were resistant to Nalidixic Acid. There was no resistance to Chloramphenicol, Gentamicin and Co-Trimoxazole. Monoplex PCR was performed on eight of the *E. coli* isolates that were both resistant to Ampicillin/Cloxallin and Tetracycline using a verotoxin gene (*vt1*) as the primer of 5’CGCTGAATGTCATTCGCTCTGC3’ nucleotide base sequence and a 772 – 812 product size in base pairs. Dunga and Uyoma Naya beaches have antimicrobial resistant *E. coli*. There was a significant difference in *E. coli* contamination levels between the two beaches shown by Ts = 36.9928 (P< 0.05). The results give an insight for continuous research on antibiotic susceptibility surveillance in the aquatic environments where *R. argentea* and water are obtained for human consumption since the rate of antibiotic resistance keep on changing from time to time due to the fact that *E. coli* is a hardy environmental isolate and is indicative of fecal contamination in food stuff or water for it is only found as a resident microbe in the intestine.

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# ABBREVIATIONS

1. 0157:H7 - An Enterohaemorrhagic strain of the bacterium *Escherichia coli*

and a cause of food borne illness

1. ANOVA - Analysis of Variance
2. DNA - Deoxyribonucleic Acid
3. *E. coli - Escherichia coli*
4. EHEC - Enterohaemorrhagic *E. coli*
5. EIEC - Enteroinvasive *E. coli*
6. EPEC - Enteropathogenic *E. coli*
7. ETEC - Enterotoxigenic *E. coli*
8. H antigen - A bacterial flagellar antigen important in the serological

classification of enteric bacilli

1. IMViC Test - Indole, Methyl Red, Voges-Proskauer and Citrate Tests
2. K – 12 - A laboratory Strain of *E. coli*
3. K antigen - A bacterial capsular antigen
4. Km2 - Square kilometers
5. KNBS - Kenya National Bureau of Statistics
6. LST - Lauryl Sulphate Tryptose
7. MDR - Multi Drug Resistance
8. MR – VP - Methyl Red – Voges-Proskauer
9. NNIS - National Nosocomial Infections Surveillance
10. O Antigen - One occurring in the lipo-polysaccharide layer of the wall of

gram-negative bacteria

1. O:H - The ratio between the O and H antigens
2. PCR - Polymerase Chain Reaction
3. pH - preferential hydrogen
4. *R. argentea* - *Rastrineobola argentea*
5. TBE - Tris/Borate/Ethylenediaminetetraacetic acid
6. TSB - Tryptone Soy Broth
7. UV - Ultra Violet
8. *vt1* - Verocytotoxin gene
9. VTEC - Verocytotoxin *E. coli*
10. μg - micrograms
11. μl - micro liters
12. μm - micro moles

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**CHAPTER ONE**

**INTRODUCTION**

* 1. **Background**

The emergence of antimicrobial resistance in members of the *Enterobacteriaceae* family is posing a major problem in the management of bacterial infections (Ashok, 2008) and the occurrence of antimicrobial resistant bacteria is also increasing in aquatic environments (Onyango *et al,.* 2009). According to Acha and Szyfres (2003), drug resistant bacteria may occur in aquatic environments and make their way to humans and spread drug resistant genes leading to persistence of ill health in the human population. For example, antimicrobial resistant bacteria have been implicated in cases of diarrhea in Western Kenya (Onyango *et al.,* 2009).

As a member of the *Enterobacteriaceae* family, Escherichia coli is naturally found in the intestines of humans and warm-blooded animals. Within human and animal faeces, *E. coli* is present at a concentration of approximately 109 per gram (Edberg *et al.,* 2000). Unlike other bacteria in this family, E. coli does not usually occur in plants or in soil and water. Most of the *E. coli* are non-pathogenic, meaning they do not cause disease in the intestine. Nevertheless, these non-pathogenic *E.* *coli* can cause disease if they spread outside the intestine. The pathogenic strains of *E. coli* may cause diarrhea by producing and releasing toxins (Todar, 2007).

The survival time of *E. coli* in the environment is dependent on many factors collectively referred to as stress factors, which include temperature, sunlight, preferential hydrogen concentration, the structure of the indigenous microbiota and type of terrestrial and aquatic habitats. In general terms, E. coli survives for about 4-12 weeks in water containing a moderate microflora at a temperature of 15-18°C (Edberg et al., 2000). Re-growth of E. coli in water distribution systems is not a concern, since E. coli rarely grows outside the human or animal gut (Geldreich, 1996). The inability of E. coli to grow in unpolluted water combined with its short survival time in clean water environments means that the detection of E. coli in a water system is a good indicator of recent faecal contamination.

Commensal *E.* *coli* serotypes that colonize the epithelial cells in the large intestinal tract are harmless and are important in maintaining the intestinal physiology. Among the pathogenic strains of *E. coli* are 4 serotypes that are classified according to their individual virulence, clinical symptoms and distinct surface antigens (O antigen which is part of [lipopolysaccharide](http://en.wikipedia.org/wiki/Lipopolysaccharide) layer; H antigen which is part of the [flagellin](http://en.wikipedia.org/wiki/Flagellin) and K antigen which is part of the capsule). The pathogenic serotypes according to Todar (2007) are Enteropathogenic *E*. *coli* (EPEC), Enterotoxigenic *E*. *coli* (ETEC), Enterohaemorrhagic *E*. *coli* (EHEC), verocytotoxin producing *E.* *coli* (VTEC) or *E.* *coli* (0157:H7) and Enteroinvasive *E*. *coli* (EIEC). They are found in faecal polluted water or sewage, and are known to survive and multiply for a long time in the polluted water (Jiménez *et al.,* 1989). These pathogenic *E. coli* infect *R. argentea,* which have a lake-wide pelagic distribution both inshore and offshore and are mostly found in the 0 – 20 m depth range where they feed on zooplanktons which are primarily found in surface and polluted waters (Wanink, 1999). The infection occurs when the pathogenic *E. coli* lounge themselves on the fish gills, injured body surfaces like the scales and the soft tissues found in the eyes, eventually affecting the fish physiology.

*R*. *argentea* is consumed by many people livingaround Lake Victoria due to its nutritive value and highprotein content (Abdullahi *et al.,* 2001). According to Gitonga (2006), fish from Lake Victoria represents 85% of Kenya’s fish supply and constitutes 25% of total catch from Africa’s inland fisheries. In addition, it is documented that fish living in natural environment are known to harbor pathogenic *Enterobacteriaceae* (Pillay, 1990). According to Sifuna *et al.,* (2008), sundried *R. argentea* are contaminated with *E. coli* and the prominence of co-trimoxazole, tetracycline and ampicillin resistance among *E. coli* isolates demonstrates the similarities in the development of resistance among the isolates and the *e. coli* of clinical importance. In the study by Sifuna *et al.,* (2008), it was postulated that the exposure to antibiotics might be of a human origin. This was further supported by the presence of antibiotic resistance to co-trimoxazole, a synthetic antibiotic generally used in humans only in the same study. Differences in levels of resistance and resistance patterns may be due to levels of exposure to the agents or other factors that may have increased or decreased the likelihood of the development of resistant bacteria. The fish, *R. argentea,* is not exposed directly to antibiotics but may be contaminated with antibiotic resistant bacteria through handling, polluted waters or animal droppings (Sifuna *et al.,* 2008).

Bacteria may be intrinsically resistant to one or more than one class of antimicrobial agents, or may acquire resistance by *de novo* mutation or via the acquisition of resistance genes from other organisms (NNIS Report, 2004). Drug resistant bacteria may occur in aquatic environments from the sewage emanating from the surrounding villages, the numerous cottages and the agricultural sector around the Lake Victoria region and natural wildlife (Wandili *et al.,* 2011).

The commonly used antibiotics include ampicillin/cloxacillin, chloramphenical, nalidixic acid, gentamicin, cefuroxime, tetracycline and co-trimoxazole depending on their bacteriostatic or bactericidal nature (Sifuna *et al.,* 2008).

In an earlier study by Onyango *et al.,* (2009), enteric bacteria isolated from *Rastrineobola argentea* and *Oreochromis* that were collected from three fish landing beaches namely Dunga, Luanda Rombo and Sirongo and from three markets: Kisumu Municipality, Luanda and Bondo showed resistance to these commonly used antibiotics.

Pathogenic isolates of *E. coli* possesses a number of virulence factors that are important for the ability to cause disease. These *E. coli* strains have been categorized into pathogenicity groups, based on their virulence properties (Kaper *et al.*, 2004). One of these groups is characterized by the production of potent cytotoxins that inhibit the protein synthesis within eukaryotic cells. Shiga toxins (stxs) or verocytotoxins (*vts*) is considered to be the major virulence factor of VTEC and comprise a family of structurally related cytotoxins with similar biological activity. Human pathogenic Enterohaemorrhagic strains produce *vt1* gene or *vt2* gene, while *vt2e* gene is specific for strains causing edema disease in pigs. O157:H7 produces *vt1* gene (Kaper *et al.,* 2004).

This study aimed at determining the antimicrobial resistance patterns of *E. coli* isolated from *R. argentea* and from water in two beaches; Dunga beach (located near Kisumu city) which has a closed shoreline is prone to various contamination from both inland waters and the surrounding since water circulation is not strong so this makes it a very rich place to do this type of    surveillance study. While Uyoma Naya beach which is an open gulf with the Nile River flowing through has a good clearing time and we do not expect to have a higher rate of contamination (Onyango *et al.,* 2009). So the two sites were chosen so as to compare the possible rates of contamination by the bacteria population.

**1.2 Problem Statement**

*Escherichia coli* is a hardy environmental isolate and is indicative of fecal contamination in food stuff and water since it is only found as a resident microbe in the intestine of both cold and warm blooded animals. Many people livingaround Lake Victoria use the Lake water for their daily chores and also consume *Rastrineobola argentea* due to its nutritive value and highprotein content (Abdullahi *et al.,* 2001). Fish living in natural environment are known to harbor pathogenic *Enterobacteriaceae* (Pillay, 1990). *E. coli* in water can infect the fish in the gills, scales and eventually flesh and this can be transferred back to the general population leading to a vicious cycle (Hussein, 2007). The growth of these pathogenic strains of *E. coli* in the human intestine is known to produce large quantities of enterotoxins, which cause severe damage to the lining of the intestine and other organs of the body (Hussein, 2007). The emergence of antimicrobial resistance in members of the *Enterobacteriaceae* family is posing a major problem in the management of bacterial infections (Ashok, 2008) and the occurrence of antimicrobial resistant bacteria is also increasing in aquatic environments (Onyango *et al.* 2009). Due to over the counter drug administration, so many bacteria are exposed to various antibiotics that they are not intended to and hence they develop antimicrobial resistance genes for survival a condition called phase variation. They may also acquire plasmids through conjugation and transduction mechanisms that enhance antibiotic resistance and if they are passed in the water where fish is found, due to seasonality they may infect the fish and be passed back to the general public population (Onyango *et al.,* 2009) which translates in a form of bacteremic disease prevalence within the neighboring fishing communities (Pillay, 1990). A high percentage of individuals have been reported to suffer from *E. coli* related illnesses among which antibiotic resistant strains of *E. coli* of which co-trimoxazole, tetracycline and ampicillin are the most resistant (Onyango *et al.,* 2009). According to Sifuna *et al.,* (2008) in their study on the microbiological quality and safety of *R. argentea* retailed in Kisumu town markets, *E. coli* was isolated from all the fish products examined and were susceptible to chloramphenicol, nalidixic acid and norfloxacin. The expression of *vt1* gene which is a determinant factor for virulence has been shown to be the main feature of *E. coli* 0157:H7 a bacteria implicated diarrhea outbreaks. This is why the study was undertaken to determine the antimicrobial resistance of *E. coli* isolated from fresh *R. argentea* and Lake Victoria water in Dunga and Uyoma Naya beaches in Kenya.

# 1.3 Justification of the Study

Clinical prevalence of various gastrointestinal infections is high due to antibiotic resistance that keep on changing from time to time as can be seen from the number of individuals attending health centers with faecal oral infections leading to diarrhea (Onyango *et al.,* 2009). Pathogenic strains of *E. coli* are responsible for three types of infections in humans: urinary tract infections, neonatal meningitis, and intestinal diseases (gastroenteritis). The diseases caused depend on distribution and expression of an array of virulence determinants (Vogt and Dippold, 2005). Fish such as *R. argentea* is food that is consumed by many people livingaround Lake Victoria due to their nutritive value, highprotein content and is affordable (Abila, 1998; Abdullahi *et al.,* 2001). According to Sifuna *et al.,* (2008), sun dried *R. argentea* retailed in Kisumu city markets were contaminated with antimicrobial resistant *E. coli* that were most frequently resistant to cotrimoxazole, tetracycline and ampicillin showing resistance to two or more antimicrobial agents which could have been because of how the fish were being handled and processed. According to Wandili *et al.,* (2011), there was a high concentration of *Salmonella* species in water samples from Dunga beach which has a closed shoreline and is in Kisumu town within Winam gulf and the lake water at the beach could be more polluted compared to the *Salmonella* species concentration in lake water at Sirongo which has an open shore line and away from the city. Verocytotoxin producing *E. coli* have been shown to possess *vt1* gene which is a determinant factor for virulence and has been shown to be produced by *E. coli* O157:H7 that has been implicated in disease outbreaks (Kaper *et al.,* 2004). The presence of the gene in the study region is indicative of the presence of *E. coli* O157:H7 in the region. The Kenya’s Ministry of Fisheries Development, (Fisheries department annual report, 2006), reported that the fisheries sector plays an important role in the national economy especially through the sale of fish and other fish products locally and internationally. With the presence of pathogenic serotypes of *E. coli* in fish, water and the environment surrounding the fish, the pathogenic serotypes are hypothesized to infect the fish leading to poor fish health and welfare resulting in low revenue for the fishing community and the country at large. When the infected fish are consumed by humans, the pathogenic [serotypes](http://en.wikipedia.org/wiki/Serotype) can cause serious [food poisoning](http://en.wikipedia.org/wiki/Foodborne_illness) in [humans](http://en.wikipedia.org/wiki/Human), and are occasionally responsible for [product recalls](http://en.wikipedia.org/wiki/Product_recall) (Vogt and Dippold, 2005). Therefore contamination of fish and water sources with antimicrobial resistant bacteria can pose serious public health risk to people living in the Lake Victoria region and beyond. Since antibiotic susceptibility testing contributes directly to patient care, and have great influence on antibiotic usage and hence on the pressures that facilitate the emergence of antimicrobial drug resistance (Sudha *et al.,* 2001), it was therefore important to determine antimicrobial resistance patterns of *E. coli* isolated from freshly fished *R. argentea* and the lake waters in Dunga and Uyoma Naya beaches in Kenya, to determine if these point sources act as reservoirs for infections that would lead to outbreaks and epidemics.

# 1.4 Objectives

**1.4.1 Main Objective**

To determine antimicrobial resistance of *E. coli* isolated from freshly fished *R. argentea* and in lake water samples from Dunga and Uyoma Naya beaches in Lake Victoria Kenya.

# 1.4.2 Specific Objectives

1. To determine the prevalence of *E. coli* from *R. argentea* and water samples from Dunga and Uyoma Naya beaches.
2. To determine antimicrobial resistance of *E. coli* isolates to selected antimicrobials.
3. To determine the presence of *vt1* virulence factor from the *E. coli* isolates.

**CHAPTER TWO**

**LITERATURE REVIEW**

**2.1 *Escherichia coli***

*Escherichia coli* commonly form part of the normal microbial flora of the lower [intestine](http://en.wikipedia.org/wiki/Gastrointestinal_tract) of [warm-blooded](http://en.wikipedia.org/wiki/Warm-blooded) organisms. Most *E. coli* serotypes are harmless, but some [serotypes](http://en.wikipedia.org/wiki/Serotype) can cause serious [food poisoning](http://en.wikipedia.org/wiki/Foodborne_illness) in [humans](http://en.wikipedia.org/wiki/Human), and are occasionally responsible for [product recalls](http://en.wikipedia.org/wiki/Product_recall). The pathogenic strains are important causes of different kind of infections which include; urinary tract infection that encompasses cystitis, pyelitis and pyelonephritis, they cause wound infections, bacteremia, meningitis and diarrheal diseases in humans (Vogt and Dippold, 2005).

Drug resistant *E. coli* causes diarrheal disease in the world and remains a major public health problem (Vogt and Dippold, 2005). The harmless serotypes of *E. coli* are part of the [normal flora](http://en.wikipedia.org/wiki/Human_flora) of the [gut](http://en.wikipedia.org/wiki/Gut_%28zoology%29), and can benefit their hosts by producing [vitamin K](http://en.wikipedia.org/wiki/Vitamin_K)2 (Bentley and Meganathan, 1982) and by preventing the establishment of [pathogenic](http://en.wikipedia.org/wiki/Pathogen) bacteria within the intestine e.g. *Clostridium spp*., and *Bacteroides spp*.. This commensal *E. coli* strains rarely cause disease except in immune - compromised hosts or where the normal gastro intestinal barriers are breached (Hudault *et al.,* 2001). According to Eckburg *et al*., (2005) [fecal-oral transmission](http://en.wikipedia.org/wiki/Fecal-oral_transmission) is the major route through which pathogenic serotypes of the bacterium cause disease. Cells are able to survive outside the body for a short period of time, which makes them ideal [indicator organisms](http://en.wikipedia.org/wiki/Indicator_organism) to test environmental samples for [fecal contamination](http://en.wikipedia.org/wiki/Feces) (Feng *et al*., 2002).

*E. coli* encompasses an enormous population of bacteria that exhibit a very high degree of both genetic and phenotypic diversity. *E. coli* remains one of the most diverse bacterial species with only 20% of the genome being common to all serotypes (Lan and Reeves, 2002). Similarly, other serotypes of *E. coli* (e.g. the K-12 strain commonly used in [recombinant DNA](http://en.wikipedia.org/wiki/Recombinant_DNA) work) are sufficiently different that they would merit reclassification (Darnton *et al.,* 2006).

Enteric *E. coli* (EC) are classified on the basis of serological characteristics and virulence properties (Todar, 2007). Virotypes include:

[Enterotoxigenic *E. coli*](http://en.wikipedia.org/wiki/Enterotoxigenic_Escherichia_coli) (ETEC) which causes diarrhea (without fever) in humans, pigs, sheep, goats, cattle, dogs, and horses uses [fimbrial adhesins](http://en.wikipedia.org/wiki/Fimbria_%28bacteriology%29) (projections from the bacterial cell surface) to bind [enterocyte](http://en.wikipedia.org/wiki/Enterocyte) cells in the [small intestine](http://en.wikipedia.org/wiki/Small_intestine). ETEC can produce two [proteinaceous](http://en.wikipedia.org/wiki/Protein) [enterotoxins](http://en.wikipedia.org/wiki/Enterotoxins): the larger of the two proteins, [heat labile enterotoxin](http://en.wikipedia.org/wiki/LT_enterotoxin), is similar to [cholera toxin](http://en.wikipedia.org/wiki/Cholera_toxin) in structure and function. The smaller protein, the [heat stable enterotoxin](http://en.wikipedia.org/wiki/ST_enterotoxin) causes cyclic guanosine monophosphate accumulation in the target cells and a subsequent secretion of fluid and electrolytes into the intestinal [lumen](http://en.wikipedia.org/wiki/Lumen_%28anatomy%29). ETEC strains are noninvasive, and they do not leave the intestinal lumen. ETEC is the leading bacterial cause of diarrhea in children in the developing world, as well as the most common cause of [traveler's diarrhea](http://en.wikipedia.org/wiki/Traveler%27s_diarrhea). Each year, ETEC causes more than 200 million cases of diarrhea and 380,000 deaths, mostly in children in developing countries (Vogt and Dippold, 2005).

Enteropathogenic *E. coli* (EPEC) is a causative agent of diarrhea in humans, rabbits, dogs, cats and horses Like ETEC, EPEC also causes diarrhea, but the molecular mechanisms of colonization and etiology are different. EPEC lack fimbriae, heat stable and heat labile toxins, but they use an [adhesin](http://en.wikipedia.org/wiki/Adhesin) known as [intimin](http://en.wikipedia.org/wiki/Intimin) to bind host intestinal cells. This virotype has an array of virulence factors that are similar to those found in [*Shigella*](http://en.wikipedia.org/wiki/Shigella), and may possess a [shiga toxin](http://en.wikipedia.org/wiki/Shiga_toxin). Adherence to the intestinal mucosa causes a rearrangement of [actin](http://en.wikipedia.org/wiki/Actin) in the host cell, causing significant deformation. EPEC cells are moderately invasive (i.e. they enter host cells) and elicit an inflammatory response. Changes in intestinal cell ultra-structure due to “attachment and effacement” are likely the prime cause of diarrhea in those afflicted with EPEC (Todar, 2007).

[Enteroinvasive *E. coli*](http://en.wikipedia.org/wiki/Enteroinvasive_Escherichia_coli) (EIEC) is found only in humans. EIEC infection causes a syndrome that is identical to [shigellosis](http://en.wikipedia.org/wiki/Shigellosis), with profuse diarrhea and high fever.

[Enterohaemorrhagic *E. coli*](http://en.wikipedia.org/wiki/Shiga-like_toxin-producing_Escherichia_coli) (EHEC) infects humans, cattle, and goats. The most famous member of this virotype is strain [O157:H7](http://en.wikipedia.org/wiki/O157:H7), which causes bloody diarrhea but elicits no fever. EHEC can cause [hemolytic-uremic syndrome](http://en.wikipedia.org/wiki/Hemolytic-uremic_syndrome) and sudden kidney failure. It uses bacterial fimbriae for attachment, is moderately invasive and possesses a phage-encoded shiga toxin that can elicit an intense inflammatory response (Smaijlovic *et al.,* 2007).

[Enteroaggregative *E. coli*](http://en.wikipedia.org/wiki/Enteroaggregative_Escherichia_coli) (EAEC) which infects only humans have fimbriae which aggregate [tissue culture](http://en.wikipedia.org/wiki/Tissue_culture) cells, EAEC bind to the intestinal mucosa to cause watery diarrhea but without fever. EAEC are noninvasive. They produce a [hemolysin](http://en.wikipedia.org/wiki/Hemolysin) and a heat stable enterotoxin similar to that of ETEC (Darnton *et al.,* 2007).

Antimicrobial drugs have played an indispensable role in decreasing illness and death associated with infectious diseases in animals and humans. However, selective pressure exerted by antimicrobial drug use also has been the major driving force behind the emergence and spread of drug-resistance traits among pathogenic and commensal bacteria. Surveillance data show that resistance in E. coli is consistently highest for antimicrobial agents that have been in use the longest time in human and veterinary medicine (Sirinavin and Garner, 2000). *E. coli* and related bacteria possess the ability to transfer [DNA](http://en.wikipedia.org/wiki/DNA) via [bacterial conjugation](http://en.wikipedia.org/wiki/Bacterial_conjugation), [transduction](http://en.wikipedia.org/wiki/Transduction_%28genetics%29) or [transformation](http://en.wikipedia.org/wiki/Transformation_%28genetics%29), which allows genetic material to [spread horizontally](http://en.wikipedia.org/wiki/Horizontal_gene_transfer) through an existing population a reason for bacterial [antibiotic resistance](http://en.wikipedia.org/wiki/Antibiotic_resistance) and the evolution of bacteria that can degrade novel compounds such as human-created antibiotics. This process has led to the spread of the gene encoding [shiga toxin](http://en.wikipedia.org/wiki/Shiga_toxin) from [*Shigella*](http://en.wikipedia.org/wiki/Shigella) to [*E. coli* O157:H7](http://en.wikipedia.org/wiki/Escherichia_coli_O157:H7) (Brüssow *et al.,* 2004), carried by a [bacteriophage](http://en.wikipedia.org/wiki/Bacteriophage) through a process called [horizontal gene transfer](http://en.wikipedia.org/wiki/Horizontal_gene_transfer).

# 2.1.1 Microscopy

*E. coli* is a [Gram-negative bacterium which stains red or pink with a counter stain usually safranin during gram staining. It is rod shaped and motile](http://en.wikipedia.org/wiki/Gram-negative). Some strains are capsulated (Vogt and Dippold, 2005).

# 2.1.2 Culture

*E. coli* is an aerobe and facultative anaerobe. It produces colonies of 1 – 4 mm in diameter on blood agar after overnight incubation at 35 – 370C. The colonies may appear mucoid and some strains are hemolytic (Fotadar *et al.,* 2005).

In the development of a rapid detection method for *E. coli* 0157:H7 using Idaho Technology’s R.A.P.I.D. ® LT Real – Time PCR system in spinach and ground beef, a wide variety of liquid enrichment media were examined for the *E. coli* O157:H7 LT FSS including TSB, modified TSB, modified TSB with novobiocin, and buffered peptone water. Buffered peptone water was chosen for its attributes in PCR compatibility, its speed and reliability of enrichment and its low relative cost (Vogt and Dipploid, 2005).

On MacConkey agar, most *E. coli* strains produce lactose fermenting colonies. Some enteropathogenic *E.* coli are late or non – lactose fermenters (Fotadar *et al.,* 2005).

Most strains of *E. coli* do not grow or are markedly inhibited on Xylose Lysine Deoxycholate agar, Salmonella – Shigella agar or other selective media used to isolate *Shigella* Spp. and *Salmonella* Spp.

Optimal growth of *E. coli* occurs at 37°C (98.6°F) but some laboratory serotypes can multiply at temperatures of up to 49°C (120.2°F) (Fotadar *et al.,* 2005). Growth can be driven by [aerobic](http://en.wikipedia.org/wiki/Aerobic_respiration) or [anaerobic respiration](http://en.wikipedia.org/wiki/Anaerobic_respiration), using a large variety of [redox pairs](http://en.wikipedia.org/wiki/Redox), including the oxidation of [pyruvic acid](http://en.wikipedia.org/wiki/Pyruvic_acid), [formic acid](http://en.wikipedia.org/wiki/Formic_acid), [hydrogen](http://en.wikipedia.org/wiki/Hydrogen) and [amino acids](http://en.wikipedia.org/wiki/Amino_acid), and the reduction of substrates such as [oxygen](http://en.wikipedia.org/wiki/Oxygen), [nitrate](http://en.wikipedia.org/wiki/Nitrate), [dimethyl sulfoxide](http://en.wikipedia.org/wiki/Dimethyl_sulfoxide) and [trimethylamine N-oxide](http://en.wikipedia.org/wiki/Trimethylamine_N-oxide) (Ingledew and Poole, 1984). Upon exposure to oxygen, E. coli increases the expression of enzymes essential for aerobic respiration, such as components of the citric acid cycle and terminal oxidase complexes. This increase requires the elimination of repression mediated by the Arc regulatory system under anaerobic conditions.Also, the synthesis of enzymes that function in anaerobic processes such as fermentation decreases, partly due to the inactivation of the transcription factor Fnr. E. coli is thus able to adjust the levels of respiratory enzymes to fit its environmental circumstances, and in this case, reduces the production of the less energy efficient fermentation enzymes in favor of the aerobic pathways (Madigan and Martinko, 2006).

# 2.1.3 Biochemical Reactions

The basic biochemical reactions of *E. coli* are breakdown of tryptophan to release indole which reacts with dimethylaminobenzaldehyde, doesn’t produce sufficient acidity in a buffered medium to give a color change of indicator, produces acetoin which is oxidized to diacetyl which reacts with creatine, doesn’t use citrate as its source of carbon, doesn’t decompose sulphur containing amino acids to release hydrogen sulphide, doesn’t have the enzyme urease which hydrolyzes urea producing ammonia, lactose fermenters, Mannital fermenters, Glucose fermenters and they produce a gas in triple sugar iron agar slants.

Analytical Profile Index (API) 20E is the confirmatory test of choice which identifies the species and subspecies of *Enterobacteriaceae* and groups and species identification of non-fermenting organisms. The plastic sterile strip is inoculated with isolated pure culture of microorganism suspension. This process also rehydrates the desiccated medium in the miniature tubes. And some probes (tubes) are overlaid with sterile mineral oil for the anaerobic reactions (Carson *et al.,* 2001).

Some of the *E. coli* strains have been reported to possess a battery of virulence determinants which enable them to overcome the host’s defense mechanisms and produce disease. The well-characterized virulence factors to date are: P-fimbriae, type 1 fimbriae, haemolysin, aerobactin, and serum resistance. The polysaccharides of O and K antigens protect the organism from the bactericidal effects of complement and from phagocytosis in absence of specific antibodies (Darnton *et al.,* 2006). Adhesin has also been shown to be an important virulence factor of *E. coli* (Smaijlovic *et al.,* 2007).

A [strain](http://en.wikipedia.org/wiki/Strain_%28biology%29) is a sub-group within the species that has unique characteristics that distinguish it from other serotypes. These characteristics which include virulence determinants are often detectable only at the molecular level; however, they may result in changes to the physiology or lifecycle of the bacterium. For example, a strain may gain [pathogenic capacity](http://en.wikipedia.org/wiki/Pathogenicity), the ability to use a unique carbon source, the ability to take upon a particular [ecological niche](http://en.wikipedia.org/wiki/Ecological_niche) or the ability to resist antimicrobial agents. Different serotypes of *E. coli* are often host-specific, making it possible to determine the source of faecal contamination in environmental samples (Feng *et al.,* 2002). For example, knowing which *E. coli* serotypes are present in a water sample allows researchers to make assumptions about whether the contamination originated from a human, another [mammal](http://en.wikipedia.org/wiki/Mammal) or a [bird](http://en.wikipedia.org/wiki/Bird).

# 2.1.4 Antimicrobial Drug Susceptibility

Antimicrobial drugs include antibiotics, which are produced by living organisms and chemical antimicrobials, which are synthetically produced compounds. Not all antimicrobials at the concentration required to be effective are completely non – toxic to human cells. Most of the antimicrobials however show sufficient selective toxicity to be of value in the treatment of microbial diseases (Calderon and Sabundayo, 2007).

Antibacterials are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity, most target bacterial functions or growth processes (Calderon and Sabundayo, 2007). They are also classified according to the National Committee for Clinical Laboratory Standards protocol (1999). Those that target the bacterial cell wall ([penicillin’s](http://en.wikipedia.org/wiki/Penicillin) and [cephalosporin’s](http://en.wikipedia.org/wiki/Cephalosporin)) or the cell membrane ([polymixins](http://en.wikipedia.org/wiki/Polymixins)), or interfere with essential bacterial enzymes ([rifamycins](http://en.wikipedia.org/wiki/Rifamycin), [lipiarmycins](http://en.wikipedia.org/wiki/Lipiarmycin), [quinolones](http://en.wikipedia.org/wiki/Quinolone), and [sulfonamides](http://en.wikipedia.org/wiki/Sulfonamides)) have [bactericidal](http://en.wikipedia.org/wiki/Bactericidal) activities. Those that target protein synthesis ([macrolides](http://en.wikipedia.org/wiki/Macrolide), [lincosamides](http://en.wikipedia.org/wiki/Lincosamides) and [tetracyclines](http://en.wikipedia.org/wiki/Tetracycline)) are usually [bacteriostatic](http://en.wikipedia.org/wiki/Bacteriostatic) (with the exception of bactericidal [aminoglycosides](http://en.wikipedia.org/wiki/Aminoglycoside)) (Finberg *et al.,* 2004). Further categorization is based on their target specificity. "Narrow-spectrum" antibacterial antibiotics target specific types of bacteria, such as [Gram-negative](http://en.wikipedia.org/wiki/Gram-negative) or [Gram-positive](http://en.wikipedia.org/wiki/Gram-positive) bacteria, whereas broad-spectrum antibiotics affect a wide range of bacteria (Calderon and Sabundayo 2007). According to the Clinical and Laboratory Standards Institute (2009), the antibiotics of choice for *E. coli* depending on their phenotypic characteristics are Ampicillin/Cloxallin, Tetracycline, Cefuroxime, Nalidixic Acid, Chloramphenicol, Gentamicin and Co-Trimoxazole.

According to the Clinical and Laboratory Standards Institute (2009), combinations of antimicrobials are used to remedy mixed infections, to prevent drug resistance, to manage severe infection or when it is necessary to obtain a greater antimicrobial effect. New drugs that have a greater resistance, have increased activity, have better diffusibility, have a greater toxicity and simpler route of administration are being developed in response to the need for less expensive drugs and antimicrobials.

Antimicrobial resistance is a worldwide problem. This is because the antimicrobials can be obtained outside of recognized treatment centers and taken without medical authorization, lack of staff and facilities to perform sensitivity tests, lack of guidelines regarding selection of drugs and inadequate procedures in hospitals resulting in spread of infectious diseases and resistant strains (Jorgensen and Turnidge, 2007).

Bacteria becomes resistant to antimicrobials by genetic mutations, by producing enzymes that destroy or inactivate antimicrobials, by changing to other metabolic systems not affected by the antimicrobial being used and by the bacteria altering the permeability of their cell membrane (Calderon and Sabundayo 2007).

According to the Clinical and Laboratory Science institute (2007), the treatment and control of infectious diseases, especially when caused by pathogens that are often drug resistant, requires sensitivity test be performed to select effective antimicrobial drugs. Laboratory antimicrobial sensitivity testing can be performed using a diffusion technique in which a disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial and this is placed on a plate of sensitivity testing agar inoculated with the test organism. The second method is dilution sensitivity test which measures minimum inhibitory concentration. Optimal interpretation of minimum inhibitory concentration requires knowledge of the pharmacokinetics of the drug in humans, and information on the likely success of a particular drug in eradicating bacteria at various body sites. This is best accomplished by referring to an expert source, which publishes interpretive criteria for minimum inhibitory concentration of all relevant antibiotics for most bacterial genera (Clinical and Laboratory Science Institute, 2008).

# 2.1.5 Molecular Analysis

Pathogenic isolates of *E. coli* possess a number of virulence factors that are important for the ability to cause disease. These *E. coli* strains have been categorized into pathogenicity groups, based on their virulence properties (Kaper *et al.*, 2004). One of these groups is characterized by the production of potent cytotoxins that inhibit the protein synthesis within eukaryotic cells. These toxins are either termed verocytotoxins (*vts*) because of their activity on vero cells or shiga toxins (*stxs*) because of their similarity with the toxin produced by *Shigella dysenteriae* (Kaper *et al.,* 2004). Enterohaemorrhagic *E.* coli constitute a sub – set of serotypes of verocytotoxin producing *E.* coli that has been firmly associated with bloody diarrhea and hemolytic uremic syndrome (Caprioli *et al.,* 2005). Shiga toxin (stx) is considered to be the major virulence factor of VTEC and comprise a family of structurally related cytotoxins with similar biological activity. Most VTEC included in the EHEC group colonize the intestinal mucosa with a mechanism that subverts the epithelial cell function and induce a characteristic histopathologic lesion defined as attaching and effacing. The lesion is due to the *eae* gene which is essential for the ability of the strains to attach to the host mucosal surface (Kaper *et al.,* 2004). *vt* is divided into four subgroups: *vt1, vt2, vt2c* and *vt2e.* Human pathogenic Enterohaemorrhagic strains produce *vt1* or *vt2*, while *vt2e* is specific for strains causing edema disease in pigs. O157:H7 produces *vt1* (Kaper *et al.,* 2004). Genetic analysis of the complete DNA sequence of VTEC 0157:H7 showed that almost 20% of its chromosome is constituted by foreign DNA not present in the chromosome of *E. coli* K – 12 and has probably been acquired from other bacterial species through horizontal gene transfer (Caprioli *et al.,* 2005). Similarly other regions of the foreign DNA can be considered as putative alternative splicing since they carry virulence associated genes, show lower guanine and cytosine content. VTEC 0157 posses a large virulence plasmid of approximately 90 kilo bases which encodes proteins some of which are presumably involved in the pathogenesis of EHEC infections (Caprioli *et al.,* 2005).

# 2.1.5.1 Polymerase Chain Reaction

Molecular methods have developed to increase the rapidity of analysis. They are able to achieve a high degree of sensitivity and specificity without the need for a complex cultivation and additional confirmation steps. These molecular methods applied to the specific detection of coliforms include immunological methods which are based on the specific recognition between antibodies and antigens and the high affinity that is characteristic of this recognition reaction and the nucleic acid – based methods which use molecular hybridization properties that involve the complementary sequence recognition between a nucleic probe and a nucleic target. The more frequently used nucleic acid based methods are polymerase chain reaction and the *in situ* hybridization methods. The major limitation of immunological methods is linked to the very low number of targeted cells in the samples. A number of nucleic acid based methods have been reported for the detection and characterization of verocytotoxin producing *E. coli*. The most commonly reported methods are based on the use of the polymerase chain reaction (PCR) to amplify a specific gene target in verocytotoxin producing *E. coli* since other techniques for example *in situ* hybridization technique has some limitations which includes its very vulnerable to showing false positives from non-specific binding, because of the relatively low level of mRNA, it can only be used for fairly abundant messages and also, it does not tell about the level of protein translation when applied to the detection of nutrient starved bacterial cells(Posse *et al.,* 2007). The primers used in the PCR may detect a characteristic virulence factor in verocytotoxin producing *E. coli*, i.e. *vt* genes (*vtx1, vtx2* or *vtx2e* subtypes) as well as others such as *eae* (intimin) gene sequences. Closely related strains of *E. coli* have *rfb* genes encoding different O antigens and this can be exploited to differentiate between different sero - groups of verocytotoxin producing *E. coli*. While *vtx1* has a relatively homogeneous nucleotide sequence implying it doesn’t show significant variation in sequence, several variants of *vtx2* (*vt2, vt2c, vt2d, vt2e* and *vt2f*) associated with more severe human diseases and which are found in the genome of lambdoid prophages integrated in the verocytotoxin producing *E. coli’s* chromosome have been described. There are a number of primer pairs available which will detect most *vtx2* variants in a single assay and others which discriminate between the variants. This can give additional characterization of the strains and information regarding their virulence potential with some variants more associated with severe clinical illness (Posse *et al.,* 2007). Conventional PCR relies on amplification of the target gene(s) in a thermo - cycler, separation of PCR products by gel electrophoresis, followed by visualization and analysis of the resultant electrophoretic patterns (Perelle *et al.,* 2006). The development of monoplex PCR which uses one primer sequence to detect the presence/ absence of a particular gene has greatly increased the sensitivity of PCR - based detection methods (Posse *et al.,* 2007). Monoplex PCR assays have been developed for the detection of verocytotoxin producing *E. coli* carrying the major associated virulence genes i.e. *eae*, *vtx1* and *vtx2*. Also, there are monoplex PCR assays which confirm sero - group for example O157, O26, O111, O103 and O145 by targeting genes found in the O – antigen gene cluster in each sero - group. Some of these targets have been combined into multi - plex assays (Perelle *et al.,* 2006).

**2.2 Physical Chemical Properties of Water**

The rapid increase in human population in Lake Victoria catchment area has had significant impacts on the dynamics of the lake water resources in the last few decades (Edberg et al., 2000). Oxygen concentrations in water bodies are not only a measure of environmental health of the aquatic ecosystem, but are also the lifeline of the aquatic life. For example, a slight difference in temperature with depth would make the water column stable thereby reducing re - oxygenation through mixing particularly at shallow waters (Kenyanya, 2000). Decomposition of the organic materials from sewage discharge and dead algae depletes the dissolved oxygen in lakes especially at bottom layers (Edberg et al., 2000). The increased lake water temperature could be attributed to increased suspended matter in the lake. Increased heat in lake water results in increased stability of stratified water column and hence reducing mixing depth (Kenyanya, 2000). According to Kenyanya, (2000), conductivity of waters varied slightly among and within the sampling stations implying that the sampled areas contained similar dissolved nutrients that are responsible for carrying electric current. High degrees of turbidity (corresponding to low transparency) are attributed to the abundance of phytoplankton and stirring of water by regular wind action that leads to re-suspension of bottom silt. It might also be attributed to an increase in human population in the catchment area with subsequent increase in anthropogenic input of materials to the lake (Kenyanya, 2000). Numerous rivers draining into the lake are among the possible reasons causing observed lower secchi depths as recorded by Lyimo and Sekadende (2003). The drastic increase in nitrogen, which is considered as a limiting nutrient in Lake Victoria (Lyimo and Sekadende, 2003), is due to excess input of organic materials into the lake.

Fish perform all their bodily functions in water. Because fish are totally dependent upon water to breathe, feed, grow, excrete wastes, maintain a salt balance, and reproduce, understanding the physical and chemical qualities of water is critical in any fish designed studies (Boyd, 1979). In this waters where fish is found, *E. coli* also finds itself as a result of surface run –offs that eventually contaminate the waters. Also, *E. coli* survives longer in polluted water with the following factors, including temperature (40C - 250C), less exposure to sunlight, presence and types of other microflora, and in turbid water (Edberg et al., 2000).

*E. coli* is used as an indicator of possible sewage contamination because it is commonly found in human and animal feces. Although it is generally not harmful, it indicates the possible presence of pathogenic bacteria, viruses, and protozoans that also live in human and animal digestive systems. Their presence in water suggests that pathogenic microorganisms might also be present and that swimming and eating fish might pose a health risk (Feng *et al*., 2002).

According to Onyango *et al.* (2009), Dunga beach area which is densely populated with a closed shoreline, has poor water circulation in the shore line. Also, the effects of urbanization have serious implications on Lake Victoria along Dunga beach in Kisumu city. Uyoma Naya beach on the other hand has an open shoreline with adequate water circulation.

**CHAPTER THREE**

**MATERIALS AND METHODS**

**3.1 Study Sites**

In this study, lake water and fish (*Rastrineobola argentea*) samples were collected from two fish landing beaches namely; Dunga beach in Kisumu county and Uyoma Naya beach in Siaya county. These beaches are in Winam gulf which lies south of the Equator between 00 6’ S – 00 32’S and 340 13’ - 340 52’E at an altitude of 1134 m above sea level and covers an area of 1,920 sq. km (approximately 6% of whole lake) between 6 km and 30 km (Fig. 1). It has a catchment area of 3,600 km2 (KNBS, 2010).



**Uyoma**

**Legend**

Sampling Sites

…. International

Boundaries

Fig. 1 A map showing the sampling sites in Lake Victoria (Courtesy of K.N.B.S. 2010).

# 3.2 Sample size

Sample size was determined following the method of Kipkemboi *et al.,* (2012) in a study that determined the prevalence and antimicrobial susceptibility patterns of bacterial pathogens, 25% of samples were contaminated with *E. coli.* At 95% confidence level and a precision of 0.05, the resulting sample size was determined as follows:

no = Z2pq

e2

Where no = Sample size

Z = desired confidence level

p = Estimated proportion of an attribute present in a population

q = 1 - p

e = Desired level of precision

Sample Size = (1.96)2(0.25)(0.75)

(0.05)2

= 288

This value was divided by 2 and a value of 144 obtained since with this sample, we were 95% percent confident that the samples contaminated were within the expected range of the true population of contaminated samples.

Sampling was done in two beaches, 72 samples were taken from Dunga beach and 72 samples from Uyoma Naya beach. The 72 samples included 36 *R. argentea* and 36 water samples from each beach. So as to have enough sample size and also to assess if there is seasonality of infection of fish by the pathogen since pathogenicity has got seasonality when it rains and when it is dry, basing on the resources available, the time required to analyze the samples and the objectives, four visits were made to each beach and during each visit, 9 water samples and 9 *R. argentea* samples were collected for analysis.

# 3.3 Sampling Procedure

A cross-sectional study design which involves data collection from a population, or a representative subset at one specific point in time was used to collect the lake water and *R. argentea* samples. This study was done during the long rain season (March - June). A volume of 250ml of lake water per sample was collected in sterilized bottles. The samples were taken from three different locations in each fish landing beach. At each location, three samples were taken, meaning that nine water samples were collected from each fish landing beach per visit. The water samples’ collection points were separated by at least 50 meters inshore (0m, 50m, and 100m) since microbial concentrations tend to decrease towards the lake interior this is as a result of eroded sediments, debris, and other pollutants being washed from watersheds being deposited in the lake’s shoreline by inflowing streams. During water collection, the preferential hydrogen (pH) was measured using a pH meter and temperature measured using a thermometer at the three different locations since these parameters also help in defining the concentration of microorganisms in water.

Fresh fish samples from the fish landing beaches were obtained from fishermen from different fishing boats that were handpicked randomly. Using random numbers, the first, second and fifth boats to arrive at the shoreline were selected so as a well-constructed survey could be administered to the selected boats and the results tabulated. One kilogram of fresh fish samples (500 - 600 pieces of *R. argentea*) were collected from each fishing boat and placed in sterilized plastic bags. Three fish samples were obtained from three different fishing boats and thus nine fish samples were collected from each fish landing beach per visit to the beach.

All the collected water and fish samples were placed in clearly labeled bottles and plastic bags, respectively, and were then transported in cooler boxes with ice packs within four hours of collection to Maseno University Department of Zoology Laboratory for analysis.

# 3.4 Analyses of Fish and Lake Water Samples

On arrival at the laboratory at Maseno University, Department of Zoology, *R.* *argentea* and water samples from the field were unpacked and coded for laboratory analyses. Depending on the beach visited, the number of visit to the beach keeping in mind that four visits were made to each beach, the distance from the shoreline and the boat selected, the samples were coded e.g. DF1A, DF1B and DF1C which implies three *R. argentea* samples collected from the first boat in Dunga beach during the first visit to Dunga beach and UW3a, UW3b and UW3c implying water samples collected at 100m inshore in Uyoma Naya beach during the second visit to Uyoma Naya beach. An aliquot of 100ml from each of the 250ml water samples, and weighed ten grams of fish samples using analytical balance i.e. whole pieces of *R.* *argentea*, were used for analyses.

After coding and measuring the right quantities of the samples, 100ml of buffered peptone water (HiMedia Lab. Pvt. Mumbai, India) used for the non – selective pre – enrichment of *Salmonella* spp. from food was added into 500ml bottles. Buffered peptone water was chosen for its attributes in PCR compatibility, its speed and reliability of enrichment and its low relative cost. The loaded 500ml bottles were sterilized by autoclaving at 115 lbs for 15 minutes. The bottles were allowed to cool and then inoculated with the measured 100 ml of water and weighed 10 grams of fish samples, each sample per bottle. The bottles were incubated at 370C (Gallenkemp Germany). The bottles were examined and reactions recorded at 24 ± 2 h for turbidity. The turbid-negative bottles were then re-incubated for an additional 24 h and reactions recorded again at 48 ± 2 h (APHA, 1998). The next analyses were done on all positive (turbid) bottles.

A loopful of suspension from each turbid buffered peptone water bottle was transferred onto MacConkey agar (HiMedia Lab. Pvt. Mumbai, India) used for selective isolation and differentiation of coliform organisms and other enteric pathogens. The plates were incubated at 370C for 24h. A positive test was the presence of Lactose positive colonies (red/pink colonies).

Mueller Hinton agar (HiMedia Lab. Pvt. Mumbai, India) plates were inoculated each with red/pink colonies from the previous test and incubated at 370C for 24h. This was to differentiate *E. coli* which does not swarm on the media from those organisms that swarm on the media.

All colonies that didn’t swarm were then tested for the Indole, Methyl - Red, Voges – Proskauer and Citrate utilization (IMViC) reactions as below.

**3.5 Biochemical Reactions**

**3.5.1 Indole Production**

A tube of tryptone broth was inoculated with samples from the cultures appearing positive and then incubated for 24 ± 2 h at 35°C after which 0.2-0.3 ml of Kovacs' reagent was added to the tube to test for indole. Appearance of distinct red color in upper layer was a positive test.

**3.5.2 Voges-Proskauer (VP)-Reactive Compounds.**

Methyl Red - Voges-Proskauer (MR-VP) broth tube was inoculated with samples from the cultures that didn’t swarm on Mueller Hinton agar and then incubated for 48 ± 2 h at 35°C. 1 ml was transferred to 13 x 100 mm tube. 0.6 ml α-naphthol solution and 0.2 ml 40% KOH were added and the tube shaken. A few crystals of creatine were then added and the tube shaken again and let to stand for 2 h. Test was positive if eosin pink color developed.

**3.5.3 Methyl Red-Reactive Compounds**

After VP test, MR-VP tube was incubated for an additional 48 ± 2 h at 35°C then 5 drops of methyl red solution were added to each tube. Distinct red color was positive test. Yellow was negative reaction.

**3.5.4 Citrate**

A tube of Koser's citrate broth was inoculated with samples from the cultures that didn’t swarm on Mueller Hinton agar; The tubes were incubated for 96 h at 35°C. Development of distinct turbidity was a positive reaction.

**3.5.5 Gas from Lactose**

Lauryl Sulfate Tryptose (LST) Broth containing tube was inoculated with samples from the cultures that didn’t swarm on Mueller Hinton agar and then incubated for 48 ± 2 h at 35°C. Gas production (displacement of medium from inner vial) or effervescence after gentle agitation was a positive reaction.

**3.6 Interpretation:**

All cultures that (a) fermented lactose with gas production within 48 h at 35°C, (b) appeared Gram-negative non-spore forming rods and (c) gave IMViC patterns of ++-- (biotype 1) or -+-- (biotype 2) were considered to be E. coli.

The analytical profile index (API) 20E is a classification of based on experiments allowing fast identification of a limited number of gram negative Enterobacteriaceae or non – Enterobacteriaceae. The test systems are stored in 20 small reaction tubes which includes the substrates. Identification is only possible with microbiological culture. Before the test, one must confirm the culture is of an Enterobacteriaceae. The API 20E test was performed as a confirmatory test for the identified *E. coli* isolates.

All the confirmed isolates of *E. coli* were then streaked on tryptic soyagar plates and incubated at 370C for 24hrs in readiness forantimicrobial susceptibility testing.

# 3.7 Antimicrobial Susceptibility Testing

The identified isolates of *E. coli* were tested for antimicrobial susceptibility by the standarddisc diffusion method on Mueller-Hinton agar (HiMedia Lab.Pvt. Mumbai, India) (which relies on the zone size) andresults interpreted as described by National Committee for Clinical Laboratory Standards protocol (1999). From the tryptic soy agar plates,discrete colonies were picked using a sterilized wire loop andtransferred to a tube containing 5 ml of sterilized saline, vortexedand adjusted to 0.5 McFarland standard which is prepared by mixing 0.05ml of 1.175% barium chloride dehydrate with 9.95ml of 1% sulfuric acid so as to have the required number of bacteria; approximately 1.5 X 108 colony forming units per milliliter. Sterilized cotton swab werethen dipped, rotated and pressed firmly on the tube wallsabove the culture to remove excess inoculums from the swab.This was then evenly swabbed on the dried surface ofMueller-Hinton agar plates ensuring even distribution of thebacterium. Choosing the antimicrobials to be included in sensitivity tests depended on the pathogen (*E. coli*), the range of locally available antimicrobials, their bactericidal or bacteriostatic nature and the degree of the microorganism’s susceptibility to various drugs*.* The antimicrobial agents and their concentrations were used as follows: ampicillin/cloxacillin 10μg, chloramphenical50μg, nalidixic acid 30μg, gentamicin 10μg, cefuroxime30μg, tetracycline 30μg, and co-trimoxazole 25μg, (HiMedia Lab. Pvt., Mumbai, India). The antimicrobial loaded discs were placed on the bacteria lawn using sterilized forceps and incubated at 370C for 18 to 24 hrs. Isolates were classified as sensitive, intermediate or resistant as described by Clinical and LaboratoryStandards Institute (2007).

**3.8 Genotypic Analyses of *E. coli***

**3.8.1 Bacterial Strains and Culture Conditions**

The *E. coli* 0157:H7 strains that were used to conduct the optimization of monoplex PCR conditions were obtained from the Kenya Medical Research Institute - Kisian. The strains were stored at -20˚C in modified Tryptic Soy Broth (mTSB (TSB + novobiocin); Merck, Darmstadt, Germany) containing 25% glycerol. For experimental purposes, the strains were then incubated in modified Tryptic Soy Broth at 37˚C overnight. The strain was used as a positive control for the *E. coli* strains that were isolated for molecular analysis experiments.

# 3.8.2 DNA Template Preparation

A modified boiled cell method (Chai *et al.,* 2007; Tunung *et al.,* 2007) was used to extract the genomic DNA from the grown strains. One milliliter of the culture broth was centrifuged at 13,200 x g for 2 min. The supernatant was discarded and the cell pellet was resuspended in 200 μl of sterile distilled water followed by vigorous vortexing. Next, the homogenized cell suspension was boiled for 10 min; cooled at -20˚C for 10 min; and centrifuged again at 13,200 x g for 2 min. The supernatant, comprising DNA, was used for the monoplex PCR conditions.

# 3.8.3 PCR Amplification

A 96-Well Thermal Cycler (ARKTIK Thermal Cycler; Thermo Fisher Scientific Oy, Finland) was used to perform the monoplex PCR protocol in a volume of 25 μl of reaction mixture containing a master mix, 5.0 μl of 5 x reaction buffer, 0.5 μl of the *vt1* primer which had 5’CGCTGAATGTCATTCGCTCTGC3’ nucleotide base sequence and 2μl of each DNA template. Sterilized distilled water was added accordingly to top up the 25μl reaction mixture. Thermal cycling consisted of a 2 min initial denaturation at 94˚C and followed by 35 cycles of denaturation at 94˚C for 20 s, 1 min of annealing at 60˚C, and extension for 1 min at 72˚C, with a 10 min final extension at 72˚C and stored at 4˚C.

# 3.8.4 Agarose Gel Electrophoresis

From each PCR product an aliquot of 4μl was subjected to 1.0% agarose gel electrophoresis containing 0.5 x TBE buffer (pH 8.0) and 1x working ethidium bromide which is 0.5ug/ml was used to stain the gel. Electrophoresis was carried out at 130 Volt, 400mA for 40 min with 4μl of 100bp DNA marker. The DNA bands were observed under ultraviolet (UV) light using gel documentation system (Syngene).

# 3.8.5 Optimization of Monoplex PCR

The monoplex PCR parameter that was optimized was the annealing temperature from 620C to 600C.

# 3.9 Data Management and Analyses

All data on bacterial culture characteristics on the different media were recorded in a Microsoft Excel worksheet. The percentage of *E. coli* isolates from each beach was calculated by dividing the number of samples contaminated with *E. coli* per beach by the total number of samples from each beach then multiplying the result by one hundred. SPSS package was used for ANOVA to analyze the differences in *E. coli* contamination between the means of the two beaches (Dunga beach and Uyoma Naya beach) and a 95% confidence level was applied to establish the significance of the test. The number of MDR *E. coli* was calculated as a percentage of the total *E. coli* isolates.

**CHAPTER FOUR**

**RESULTS**

**4.1 Introduction**

After isolation of *E. coli* from *R. argentea* and water from Dunga and Uyoma Naya beaches in Lake Victoria Kenya, a total of 41 *E. coli* isolates were obtained. The results also included the average pH and temperature of water at the different distances from the shore line to the lake interior.

**4.2 pH and Temperature Analysis**

In any water designed studies, the temperature and pH concentrations should be determined for these factors also affect the concentration of *E. coli* in water possibly prohibiting certain cell functions, intracellular stability, and lengthening lag times. In cold temperatures, cell metabolism starts to slow, and growth is limited. At higher temperatures, cell parts start to fall apart and growth completely stops. Therefore, these water parameters were determined accordingly. The pH was found to be 7.2, 7.8, and 8.4 at 0m, 50m, and 100m from the shoreline in Dunga beach while in Uyoma Naya beach it was recorded as 7.5, 7.7 and 7.9 at 0m, 50m, and 100m from the shore line (Table 1). In addition, the temperature of water was recorded as 26°C, 25°C, and 24°C at 0m, 50m, and 100m from the lakeshore in Dunga beach while in Uyoma Naya beach it was 27°C, 26°C, and 24°C (Table 2). The mean average pH and temperature of the individual sites was 7.8 pH, 25oC temp for Dunga. The mean average pH and temperature of Uyoma Naya was 7.7 and 25.70C respectively.

The pH and temperature results from the two beaches were within the normal range 6.5 – 8.5 and 20oC – 30oC according to WHO parameters of water.

**TABLE I. Average pH concentration levels of water in Dunga and Uyoma Naya beaches**

|  |  |  |  |
| --- | --- | --- | --- |
| **SITES** | **0 M** | **50 M** | **100 M** |
| **Dunga Beach** | 7.2 | 7.8 | 8.4 |
| **Uyoma Naya Beach** | 7.5 | 7.7 | 7.9 |

* pH are an average of quadruplicates (Which is an average of the four visits to the beach)

**TABLE 2. Average temperatures of water in Dunga and Uyoma Naya beaches in 0C**

|  |  |  |  |
| --- | --- | --- | --- |
| **SITES** | **0 M** | **50 M** | **100 M** |
| **Dunga Beach** | 26 | 25 | 24 |
| **Uyoma Naya Beach** | 27 | 26 | 24 |

* Temperatures are an average of quadruplicates (Which is an average of the four visits to the beach)

**4.3 Microbiological Analysis**

**4.3.1 Data of water contamination with *E. coli* in the respective beaches**

Analysis of water samples from three sampling locations (0m, 50m, and 100m) were done microbiologically for the presence of *E. coli* and the isolates confirmed by API 20E (Biomerieux Inc) test. After analysis, *E. coli* isolates were found to be 41.6% (n = 5), 25% (n = 3), and 8.3% (n = 1) at 0m, 50m, and 100m from the shoreline in Dunga beach while in Uyoma Naya beach, it was 33% (n = 4), 25% (n = 3), and 8.3% (n =1) at 0m, 50m, and 100m from the shoreline. A total of 9 water samples from Dunga beach were found to be contaminated with *E. coli,* while 8 water samples from Uyoma Naya beach were contaminated with *E. coli*, (Table 3). The average number of *E. coli* isolates per sampling site from water in Dunga beach was 3 while in Uyoma Naya was 2.67.

**TABLE 3. Water samples from Dunga and Uyoma Naya beaches contaminated with *E. coli* at the different sampling distances from the shore line in percentage.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **SITES** | **0 M** | **50 M** | **100 M** | **TOTAL** |
| **Dunga Beach** | 41.6% (n = 5) | 25% (n = 3) | 8.3% (n = 1) | 21.95% (n = 9) |
| **Uyoma Naya Beach** | 33% (n = 4) | 25% (n = 3) | 8.3% (n = 1) | 19.51% (n = 8) |

# 4.3.2 *R. argentea* contamination with *E. coli* in the respective beaches

During the analysis of *R. argentea* contamination with *E. coli* in the respective beaches it was found that 55.56% (n = 5), 44.44% (n = 4), 44.44% (n = 4), and 33.33% (n = 3) *E. coli* were isolated during first visit, second visit, third visit and fourth visit to Dunga beach respectively while in Uyoma Naya it was found to be 33.33% (n = 3), 11.11% (n = 1), 11.11% (n = 1) and 33.33% (n = 3) during first visit, second visit, third visit and fourth visit respectively (Table 4)*.* The total percentage of *E. coli* isolates from *R. argentea* was 44.44% (n = 16) for Dunga beach and 22.22% (n = 8) for Uyoma Naya. The mean average percentage *E. coli* isolation per visit was 11.11% (n = 4) for Dunga beach while that for Uyoma Naya beach was 5.56% (n = 2)

**TABLE 4. Percentage of *R. argentea* samples contaminated with *E. coli* from Dunga and Uyoma Naya beaches per visit per beach in percentage.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **SITES** | **% *E. coli* isolated in *R. argentea* samples from Dunga and Uyoma Naya beaches** | | | | |
| **First visit** | **Second visit** | **Third Visit** | **Fourth Visit** | **Total** |
| **Dunga Beach** | 55.56  (n = 5) | 44.44  (n = 4) | 44.44  (n = 4) | 33.33  (n = 3) | 44.44  (n = 16) |
| **Uyoma Naya Beach** | 33.33  (n = 3) | 11.11  (n = 1) | 11.11  (n = 1) | 33.33  (n = 3) | 22.22  (n = 8) |

# 4.4 Antimicrobial Susceptibility Testing

After subjecting all 41 *E. coli* isolates to antimicrobial susceptibility testing, 100% (n = 41) of the *E. coli* isolates were found to be resistant to ampicillin/cloxacillin (10 μg), 65.85% (n = 27) were resistant to tetracycline (30 μg), 4.88% (n = 2) were resistant to cefuroxime (10 μg), and 2.44% (n = 1) was resistant to nalidixic acid (30 μg). There was intermediate resistance in some of the drugs; 75.61% (n = 31) of the isolates were intermediately resistant to cefuroxime (30 μg), 9.76% (n = 4) to chloramphenicol (50 μg), 9.76% (n = 4) to nalidixic acid (30 μg) and 26.81% (n = 11) to tetracycline (30 μg). From the test, 100% (n = 41) of the isolates were susceptible to gentamicin (10 μg) and Co-trimoxazole (25 μg) while 19.51% (n = 8) were susceptible to cefuroxime (30 μg), 90.24% (n = 37) were susceptible to chloramphenicol (50 μg), 78.05% (n = 32) were susceptible to nalidixic acid (30 μg) and 7.32% (n = 3) were susceptible to tetracycline (30 μg). A total of 25 (100%) *E. coli* isolates from Dunga beach were subjected to antimicrobial susceptibility testing and resistance was recorded as 100% (n = 25) for ampicillin/cloxacillin, 4% (n = 1) was resistant to cefuroxime and 64% (n = 16) was resistant to tetracycline. Intermediate resistance was recorded as 80% (n = 20) to cefuroxime, 8% (n = 2) to chloramphenicol, 16% (n = 4) to nalidixic acid and 28% (n = 7) tetracycline. Susceptibility was recorded as 16% (n = 4) to cefuroxime, 92% (n = 23) to chloramphenicol, 100% (n = 25) to co-trimoxazole, 100% (n = 25) to gentamicin, 84% (n = 21) to nalidixic acid and 8% (n = 2) to tetracycline (Table 5). A total of 16 (100%) *E. coli* isolates from Uyoma Naya beach were subjected to antimicrobial susceptibility test and resistance was recorded as 100% (n = 16) for ampicillin/cloxacillin, 6.25% (n = 1) was resistant to cefuroxime, 6.25% (n = 1) was resistant to nalidixic acid and 68.75% (n = 11) was resistant to tetracycline. Intermediate resistance was recorded as 68.75% (n = 11) to cefuroxime, 12.5% (n = 2) to chloramphenicol, 25% (n = 4) to nalidixic acid and 25% (n = 4) tetracycline. Susceptibility was recorded as 25% (n = 4) to cefuroxime, 87.5% (n = 14) to chloramphenicol, 100% (n = 16) to co-trimoxazole, 100% (n = 16) to gentamicin, 68.75% (n = 11) to nalidixic acid and 6.25% (n = 1) to tetracycline (Table 6).

**TABLE 5 Percentage antimicrobial resistance patterns of *E. coli* from *R. argentea* and lake water from Dunga beach represented in percentage**

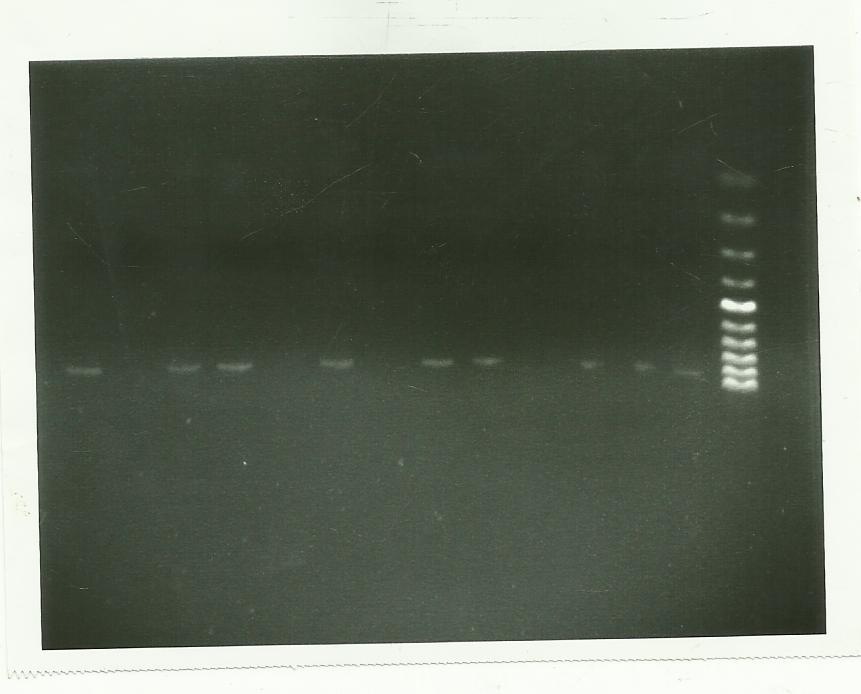
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Antibiotic agent** | **Disc concentration in micrograms (μg)** | **Antimicrobial distribution patterns of the *E. coli* isolates from Dunga Beach (n = 25)** | | |
| **%**  **Resistant** | **% Intermediate** | **% Susceptible** |
| **Ampicillin/Cloxacillin** | 10 | 100 (25) | 0 | 0 |
| **Cefuroxime** | 30 | 4 (1) | 80 (20) | 16 (4) |
| **Chloramphenicol** | 50 | 0 | 8 (2) | 92 (23) |
| **Co-Trimoxazole** | 25 | 0 | 0 | 100 (25) |
| **Gentamicin** | 10 | 0 | 0 | 100 (25) |
| **Nalidixic Acid** | 30 | 0 | 16 (4) | 84 (21) |
| **Tetracycline** | 30 | 64 (16) | 28 (7) | 8(2) |

**TABLE 6 Antimicrobial resistance patterns of *E. coli* from *R. argentea* and lake water in Uyoma Naya beach represented in percentage.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Antibiotic agent** | **Disc concentration in micrograms (μg)** | **Antimicrobial distribution patterns of the *E. coli* isolates from Uyoma Naya beach (n = 16)** | | |
| **% Resistant** | **% Intermediate** | **%**  **Susceptible** |
| **Ampicillin/Cloxacillin** | 10 | 100 (16) | 0,0 | 0,0 |
| **Cefuroxime** | 30 | 6.25 (1) | 68.75 (11) | 25 (4) |
| **Chloramphenicol** | 50 | 0 | 12.5 (2) | 87.5 (14) |
| **Co-Trimoxazole** | 25 | 0 | 0 | 100 (16) |
| **Gentamicin** | 10 | 0 | 0 | 100 (16) |
| **Nalidixic Acid** | 30 | 6.25 (1) | 25 (4) | 68.75 (11) |
| **Tetracycline** | 30 | 68.75 (11) | 25 (4) | 6.25 (1) |

# 4.5 Molecular characterization of Antimicrobial resistant *E. coli*

There were 65.85% (n = 27) *E. coli* isolates that displayed both tetracycline and ampicillin/cloxacillin resistance from the two beaches (Dunga and Uyoma Naya), which were later analyzed for the presence of verotoxin gene that is responsible for the toxin, that causes systemic symptoms. Monoplex PCR was performed on samples that were resistant to tetracycline and ampicillin/cloxacillin. Figure 2 below shows the amplicons obtained.



bp

900

800

500

200

100

M 13 12 11 10 9 8 7 6 5 4 3 2 1

**Fig 2.** Agarose gel electrophoresis image of amplicons showing the DNA bands. Lane M: DNA ladder (100bp), lane 1: positive control (*E. coli* 0157:H7), lanes 2, 5, 7 and 10: Negative control, *E. coli* isolates were on lanes 3 (WUIB), 4 (FU2A), 6 (FU1C), 8 (DF1A), 9 (DF3B), 11 (DF3C), 12 (DW2ai), and 13 (DF1e)

Key

1. Positive control (*E. coli* 0157:H7 strain ATCC 43895), since it contains *vt1* gene.
2. Negative control, - Bromophenol blue loading dye.
3. WUIB – *E. coli* isolate from water collected for the second time at 0 meters in Uyoma Naya

beach during the first visit to the beach.

1. FU2A – *E. coli* isolate from *R. argentea* collected for the first time at 50 meters in Uyoma

Naya beach during the first visit to the beach.

1. FU1C - *E. coli* isolate from *R. argentea* collected for the third time at 0 meters in Uyoma

Naya beach during the first visit to the beach.

1. DF1A - *E. coli* isolate from *R. argentea* collected for the first time at 0 meters in Dunga

beach during the first visit to the beach.

1. DF3B - *E. coli* isolate from *R. argentea* collected for the second time at 100 meters in Dunga

beach during the first visit to the beach.

1. DF3C - *E. coli* isolate from *R. argentea* collected for the third time at 100 meters in Dunga

beach during the first visit to the beach.

1. DW2ai - *E. coli* isolate from water collected for the first time at 50 meters in Dunga beach

during the second visit to the beach.

1. DF1e - *E. coli* isolate from *R. argentea* collected for the second time at 0 meters in Dunga

beach during the first visit to the beach.

Genotype data was available for eight of the *E. coli* isolates that were resistant to both ampicillin/cloxacillin and tetracycline. After performing the monoplex PCR using the one pair primer (*vt1*) which had 5’CGCTGAATGTCATTCGCTCTGC3’ nucleotide base sequence and *E. coli* 0157:H7 strain ATCC 43895 as the control, the amplicons obtained showed that the selected isolates that were resistant to both ampicillin/cloxacillin and tetracycline also had the verotoxin gene that is responsible for toxin production and for the determination of verocytotoxin producing *E. coli*. This was revealed by the appearance of a band size of 800 – 900 bp on the gel in all the isolates and the control (*E. coli* 0157:H7). Therefore, the pathotype of the isolates was verotoxin producing *E. coli* (VTEC).

There was a significance difference in the total number of *E. coli* isolated from water between the two beachesas indicated by ANOVA Critical value F = 9.55 and Test statistic value Ts = 36.9928 at 95% confidence level, out of the 144 samples analyzed from the two beaches, 28.5% (n=41) were contaminated with *Escherichia coli.* Dunga beach had the highest number of *E. coli* isolated 58.5% (n=24) while 41.5% (n=17) of the isolates were from Uyoma Naya beach.

**CHAPTER FIVE**

**DISCUSSION**

Microbiological pollution through wildlife, agricultural activities, forestry, agric-based industries, rural and urban settlement as well as surface runoff and storm water in the Kenyan catchment side of Lake Victoria, have negative effects on water quality of rivers draining into Lake Victoria leading to deterioration of Lake Victoria water (Rwabinge, 2005).

This study has shown that water temperature decreased and pH increased inshore. This also could be a reason why more *E. coli* isolates were recovered on the shore line where the water temperature and pH permits their survival. The higher temperatures on the shoreline almost always occur as a result of discharge of municipal or industrial effluents and runoff that flow over hot asphalt and concrete into the lake. The pH on the other hand is lower at the shoreline because of the dissolved biological and chemical elements from municipal or industrial effluents and runoff that flow into the lake this is supported by the findings by Lotze *et al.,* (2006). E. coli is of concern because only a few cells are needed to cause illness. The illness can progress quickly to cause severe consequences in susceptible people, particularly young children and the elderly. Also, E. coli is quite hardy. It can survive for extended periods in water and soil, under frozen and refrigerated temperatures, and in dry conditions. It also can adapt to acidic conditions. The organism is destroyed by thorough cooking or pasteurization (Lynch *et al.,* 2009). Like all bacteria, the survival and growth of E. coli is dependent on the interactions of various factors such as temperature, pH and water availability. It is, however, easily destroyed by heat (Lynch et al., 2009). From the study, the temperature and pH at 0m favored greatly cell functions, enzymatic activities and growth *E. coli*.

These findings could be attributed to the fact that Dunga beach has a closed shoreline that leads to poor water circulation in the area. The effects of urbanization also have had negative effects on Lake Victoria along Dunga beach in Kisumu city. As observed by Wandili *et al.,* (2011), Kisumu city is littered with pools of sewage spills from broken sewage pipes and tanks that form part of the surface run off after heavy rainfall that ends up into the lake providing a good environment for the survival of *E. coli.* These results also concur with findings by Onyango *et al.,* (2009) who found out that *E. coli* is one of the serious pathogens associated with fish from Dunga beach. Similar findings have been reported by Sifuna *et al.,* (2008) where it was found that fish handling procedures greatly contributed to fish quality in regards to microbial contamination and distribution within Winam gulf. These aspects of lake water microbial contamination were also reported by Wandili *et al.,* (2011) where *Salmonella*, *Shigella*, and *E. coli* were isolated from fish harvested from waters subjected to human sewage pollution. This is an indication that the lake water is contaminated with microbial population and hence occasional surveillance needs to be done which supports this cause of this study and findings.

Uyoma Naya beach in Lake Victoria is an open gulf far from an urban setting and faces the open waters of Lake Victoria Uganda side. It thus had low *E. coli* distribution that can be attributed to adequate water circulation and dilution effects at the shoreline. Similar to what was observed by Onyango *et al.,* (2009). Since Uyoma Naya beach is not close to an urban setting and major industries, there isn’t discharge of city council effluents into the beach. Also from personal observation, there were dug pit latrines in the region as a directive from the administrative authorities- Chiefs of the region. In both Dunga and Uyoma Naya beaches, the results showed that there was a decrease in *E. coli* contamination in water from the shoreline going into the lake and thus the shoreline in both beaches is more contaminated than 100m into the lake interior from the shoreline.

The results from this study showed that *R. argentea* collected from the beaches were contaminated with *E. coli*. This contamination may arise from the contaminated water in which they live in and further be enhanced by transportation of fish in dirty fishing boats and dirty packaging baskets by the fisher folks. The fish can also be contaminated with *E. coli* when feeding on zooplankton, phytoplankton including algae as results from previous studies have shown that zooplanktons, algae and phytoplanktons are normally found in the intestines of fish and together with the harvested fish in the nets hence possibility of fish contamination (Worden *et al,.* 2006). These results are consistent with those of Wandili *et al.,* (2011) study which indicated the presence of certain species of *Enterobacteriaceae* like *E. coli*, *Salmonella* species at some fish landing sites along the shores of Lake Victoria. Also established were fecal or thermo tolerant coliforms and therefore their prevalence were higher at the shoreline where beaches are located than offshore water, especially beaches which are densely populated with inadequate sanitary facilities. Also the washing by the locals on the beaches would be hypothesized to contribute to the point contamination of the beaches and this should be studied further to ascertain this hypothesis.

*R. argentea* contamination revealed that good hygiene practices such as transporting the fish in clean boats and clean packaging baskets are not operational and these may explain such findings. Good hygienic practices are important tools for managing and ensuring that food products are produced under hygienic conditions and that food safety measures are in place to address all possible risks that may be associated with the food product (Sifuna *et al.,* 2008).

Antibiotics are recommended for treating bacterial infections, to shorten the duration of infection, decrease morbidity and mortality, and reduce the duration of bacterial shedding (Lynch et al., 2009). Antimicrobial resistance may be due to [overuse of antibiotics](http://en.wikipedia.org/wiki/Overuse_of_antibiotic) in humans, but some of it is probably due to the use of antibiotics as growth promoters in animal feeds (Johnson *et al.,* 2006). According to Sifuna *et al.,* (2008) bacteria in the soil can acquire resistance to tetracycline from environmental exposure; possibly creating a reservoir of resistance factors generated outside host animals. In sub-Saharan Africa, repeated prolonged outbreaks of diarrhea with high case fatality rates have increased the demand for antibiotics. The emergence and dissemination of antimicrobial resistance among *E. coli,* is an increasing global health problem that is complicating the therapeutic management of severe diarrhogenic diseases (Sirinavin and Garner, 2000). The high prevalence of resistance to tetracycline and ampicillin in *E. coli* in the region has also been reported by Sifuna *et al.,* (2008), in which *E. coli* demonstrated resistance mostly to ampicillin, and tetracycline. Similar results were reported by Sack *et al.,* (2001) which attributed resistance to use of tetracycline for mass prophylaxis during cholera outbreaks. Tetracycline has also for a long time been used as a first-line antibiotic for many different species of domestic animals. Resistance to tetracycline is plasmid mediated and ribosomal, with a wide variety of genetic determinants, making it more prone for susceptible bacteria to acquire these resistance factors. The resistance pattern reported in this study can also be linked to use of these drugs as first line drugs in veterinary practice leading to resistance in human as previously reported by Lynch et al., (2009).

Results from this study have also shown that the enteric bacteria isolated from water and fish were resistant to Ampicillin/Cloxacillin, Cefuroxime, Tetracycline antibiotics. All the bacteria isolated from water were sensitive to Chloramphenicol, Co-Trimoxazole and Gentamicin; and all the bacteria isolates from *R. argentea* were sensitive to Chloramphenicol, Co-Trimoxazole and Gentamicin. In this study therefore, a multidrug resistance pattern was observed for *E. coli,* with Ampicillin/Cloxacillin, Cefuroxime, Nalidixic Acid and Tetracycline. The differences in levels of resistance and resistance patterns could be due to environmental factors and acquired resistance as a result of transposon and integron resistance gene acquisition as reported elsewhere (Antunes *et. al.,* 2011). These levels of exposure to the agents or other factors may have increased or decreased the likelihood of the development and conservation of resistant bacteria. *R. argentea* is not exposed directly to antibiotics but may be contaminated with antibiotic resistant bacteria through personnel, polluted fishing grounds or animal droppings. *E. coli* resistance patterns to ampicillin/cloxacillin, cefuroxime and tetracycline was similar between the two beaches revealing a widespread resistance to the readily available and commonly used antibiotics. This is an indication of contamination of the beaches by resistant *Enterobacteriaceae* populations that would be of human or animal origin (Sifuna *et al.,* 2013).

Despite *E. coli* being commensal bacteria found in intestinal microflora of a variety of animals including humans, not all the strains are harmless, and some can cause debilitating and sometimes fatal diseases in humans as well as mammals and birds. The capacity of *E. coli* to cause disease in humans is associated with its specific virulence factors and phenotypic traits. Verocytotoxin producing *E. coli* has the ability to express verocytotoxin gene (*vt*) and to form attaching and effacing lesions in the intestine (Smaijlovic *et al.,* 2007). The verocytotoxin gene results in the production of verocytotoxin which has two parts; The A part which damages gut [epithelium](http://en.wikipedia.org/wiki/Epithelium) through inhibiting its [protein](http://en.wikipedia.org/wiki/Protein) synthesis, facilitating entry to the [blood stream](http://en.wikipedia.org/wiki/Blood_stream) and the 5 B part which is adapted to inserting the A part into epithelial cells causing acute renal failure in children as a result of hemolytic uremic syndrome. Certain strains of VTEC produce an enterohaemolysin that may also contribute to VTEC pathogenicity (Smaijlovic *et al.,* 2007). There are two major types of *vt* (*vt1* and *vt2*) and *vt2* can be divided into at least five subtypes. Among VTEC serotypes, O157:H7 is associated with both outbreaks and sporadic cases of severe disease and thus has dangerous health implications, it has an unusual persistence in the environment and has very small infective dose as observed by Coombs *et al.,* (2011). After performing molecular characterization of *E. coli* that were resistant to ampicillin/cloxacillin and tetracycline using the one pair primer (*vt1*) which had5’CGCTGAATGTCATTCGCTCTGC3’ nucleotide base sequence and *E. coli* 0157:H7 strain ATCC 43895 as the control, the appearance of a similar band size on the gel in all the isolates and the control (*E. coli* 0157:H7) was indicative that the isolates had *vt1* gene of VTEC strains, a common property of verocytotoxin producing *E. coli* that may be associated with increased pathogenicity as *vt1* gene is considered to be major virulence factor involved in the pathogenesis of VTEC-induced disease. E. coli O157:H7, the serotype most commonly associated with human infection, typically carry the gene encoding *vt2*, and approximately two-thirds of these strains have the gene encoding *vt1* (Posse *et al.,* 2007).

**CHAPTER SIX**

**CONCLUSIONS AND RECOMMENDATION**

**6.1 Conclusions**

* Water in Dunga and Uyoma Naya beaches in Lake Victoria Kenya used by the locals in their daily activities is contaminated with *E. coli*. Also, *R. argentea* which is caught for food by many people living around Lake Victoria due to its nutritive value and high protein content is contaminated with *E. coli.*
* There is presence of antimicrobial-resistant *E. coli* in the Lake Victoria region. These antimicrobial-resistant bacteria are resistant to the cheaper and commonly used antibiotics, thus illustrating the effect of longstanding, unregulated antimicrobial use.
* There is the presence of verocytotoxin *E. coli* in both Dunga and Uyoma Naya beaches. This strain of *E. coli* which is a zoonotic pathogen associated with food and waterborne diseases that culminate to diarrhea.
* This suggests that the serotypes circulating in the human population are also found within the aquatic and fish environment. This study therefore, provides useful information to the ministry of medical services and public health, fisheries and other agencies in making policy decisions that aim to reduce microbial contamination of fish and water and the indiscriminate use of antibiotics.

# 6.2 Recommendation

The emergence and presence of antimicrobial-resistant *E. coli* in *R. argentea* which has high demand as food, posses a greater health challenge and therefore, continuous research on antibiotic susceptibility surveillance in the aquatic environments where *R. argentea* and water are obtained for human consumption should be undertaken.

Factors responsible for the establishment of antimicrobial resistance in *E. coli* should be determined so as to aid in the development of antibiotics that *E. coli* will be susceptible.

Public health workers to create awareness among the fishermen on the need to ensure that *R. argentea* is handled and processed under hygienic conditions and that food safety measures are in place.

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# APPENDIX

Statistical analysis on the levels of water contamination with *E. coli* between Dunga and Uyoma Naya beaches presented as percentages.

One way ANOVA was used to test for significant differences in *E. coli* levels between the two beaches as below.

|  |  |  |  |
| --- | --- | --- | --- |
| SITES | SAMPLING AT 0 M | SAMPLING AT 50 M | SAMPLING AT 100 M |
| Dunga Beach | 5 | 3 | 1 | |
| Uyoma Naya Beach | 4 | 3 | 1 | |

NOTE: Let sampling at 0 M represent x1, Sampling at 50 M represent x2 and sampling at 100 M represent x3

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| SITES | x1 | x12 | x2 | x22 | x3 | x32 |
| Dunga Beach | 5 | 25 | 3 | 9 | 1 | 1 |
| Uyoma Naya Beach | 4 | 16 | 3 | 9 | 1 | 1 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | x1 | x2 | x3 | TOTAL |
| Number (n) | 2 | 2 | 2 | 6 |
| x | 9 | 6 | 2 | 17 |
| Mean | 4.5 | 3 | 1 | 2.83 |
| x2 | 41 | 18 | 2 | 61 |
| Variance | 0.50 | 0.00 | 0.00 |  |
| Std. Dev. | 0.707 |  |  |  |
| Std. Err. | 0.500 | 0.00 | 0.00 |  |

**ANOVA RESULTS**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | SS | df | MS | F |
| Between | 12.333400 | 2 | 6.1667 | 36.9928 |
| Within | 0.5 | 3 | 0.1667 |  |
| Total | 12.833400 | 5 |  |  |

Critical value : F0.05 (2, 3) = 9.55

Test statistic : 36.9928

Cumulative probability: (F < 36.9928) There is significant difference in the number of *E. coli* contamination in water between the two beaches.