PHYSICO-CHEMICAL AND BACTERIOLOGICAL QUALITY OF WATER, AND ANTIMICROBIAL SUSCEPTIBILITY OF PATHOGENIC ISOLATES FROM SELECTED WATER SOURCES IN SAMBURU SOUTH.

BY

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DECLARATION

I, Jeophita June Mwajuma, declare that this thesis is my original work and has not been presented for the award of a degree in any other University or any other award.

Signature……... Date……..

We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

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DEDICATION

For my girls, Neema and Wema. Babies, the sky is the limit!

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~~1.~~ ACKNOWLEDGEMENT

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LIST OF ABBREVIATIONS AND ACRONYMS

XLD	Xylose lysine deoxycholate agar
EMBA	Eosin methylene blue agar
MPN	Most probable number
SS	Salmonella Shigella agar
MAC	MacConkeys agar
TSI	Triple sugar iron
MIO	Motility indole ornithine agar
TCBS	Thiosulfate citrate bile salts sucrose agar
API	Analytical profile index
ATCC	American type culture collection
BSAC	British society of antimicrobial chemotherapy
MIC	Minimum inhibitory concentration
TS	Trimethoprim/sulphamethoazole
CH	Chloramphenicol
AML	Ampicillin
AMC	Amoxacillin
CTX	Cefutaxime
CN	Gentamicin
CIP	Ciprofloxacin
SXT	Cotrimoxazole
CXM	Cefuroxime
PFGE	Pulsed-field gel electrophoresis

ABSTRACT

Water is the most critical resource in the Samburu District of Northern Kenya. The region has one permanent river, the Uaso Ng'iro. Use pressure by man, domestic and wild animals is high in all water sources, which include dams, laggas, and dry river bed wells. The primary objective of this study was to investigate the bacteriological and physic-chemical quality of water from these sources, so as to shed some light on the causes of perpetual diarrheal diseases and their likely responses to commonly used antibiotics. Samples were collected quarterly over 1 year. During sample collection, the physico-chemical conditions of water such as temperature, pH, conductivity, alkalinity, salinity and water color were recorded. A total of 207 water samples were collected and their microbial quality determined based on the most probable number (MPN) of coliforms, total plate count, feacal coliform counts and presence or absence of *E. coli.* To isolate water borne bacterial pathogens, samples were inoculated in to appropriate enrichment and selective media and the recovered bacteria characterized using relevant biochemical and serological tests. The pathogenic isolates were subjected to the Kirby Bauer and Epsilometer tests to determine their sensitivity to antibiotics commonly used in first line treatment of enteric infections. The color of water from most source types except boreholes and springs was not aesthetically acceptable for direct drinking. Alkalinity, pH and conductivity values in all water sources except the boreholes were within the recommended limits for potable water, set by World Health Organization (WHO) and Kenya Bureau of Standards (KBS). Dissolved oxygen was within acceptable levels while only springs had salinity levels less than 250 mg L^{-1}, the set limit for potable water. On the basis of total plate count 85% of the water samples were unfit for human consumption. Mean MPN was highest in dams at 643coliforms mL^{-1} and lowest in springs and boreholes at 35coliforms mL^{-1}. As such, the water from dams, rivers, springs, laggas and dry river bed wells was unfit for human consumption. However, on the basis of presence of feacal coliforms, all water sources do not meet the standards for potability. The bacterial pathogens isolated were *Shigella flexineri, Shigella boydii, Aeromonas hydrophilla,* and *Salmonella spp.* (non-typhi). Pathogenic isolates from springs and permanent rivers were mainly *Klebsiella* and *Pseudomonas spp*., considered to be of little clinical significance. Recovered *S. flexineri* isolates were susceptible to cefotaxime, gentamicin, ciprofloxacin and cefuroxime, but resistant to cotrimoxazole and, chloramphenicol. However some of the recovered *S. flexineri* isolates were resistant to ampicillin, the antibiotic recommended for treatment of dysentery. *S.boydii* and *Aeromonas. spp*. displayed complete resistance to ampicillin. *S. flexineri* isolates were compared using Pulsed-field gel electrophoresis with *Not*I restriction enzymes. Isolates resistant to ampicillin had similar pulsotypes to those susceptible to it. Although boreholes and springs had better quality water, Samburu District water did not meet the WHO and KEBS requirement for potability. Most pathogenic isolates were susceptible to commonly used antibiotics. Pulsed field gel electrophoresis revealed no genetic evidence of difference in strains, where resistance to ampicillin occurred. Water monitoring in Samburu should be carried out over a longer period of time using more rapid and discriminatory procedures. Appropriate and affordable water disinfection techniques such as boiling and filtration ~~for the area~~ should be designed.

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CHAPTER ONE

÷ INTRODUCTION

1.1 Background to the study

Waterborne pathogens often cause diarrheal disease, a serious international problem. Poor water quality continues to pose a major threat to human health. Diarrheal disease alone amounts to an estimated 4.1% of the total global burden of diseases and is responsible for the deaths of 1.8 million people every year (WHO, 2004). 88% of this burden is attributable to unsafe water supply, sanitation and hygiene and is mostly concentrated on children in developing countries. 2 million children under the age of five die every year from diarrhea. Most of these diarrheal cases are caused by use of unsafe drinking water and poor sanitation (CDC, 2004c).

Lack of access to safe water and sanitation contributes to diarrheal morbidity and mortality in developing countries (Garret *et al*., 2008). WHO estimates that in 2002, 38% of Kenyans lacked access to safe drinking water. However, when looking only at rural areas, this number increases to 54% (WHO, 2004). Poor water supplies, sanitation and hygiene pose a major threat to the health of children under the age of five years. Even a nonfatal diarrheal disease is a critical concern, especially for children. Diarrheal diseases among children under the age of five years, account for over 4.7% of all outpatient cases country-wide with the annual incidence of diarrhea being 3.5 to 4.6 episodes per child per year, making it one of the ~~of~~ top child killers (MOH, Kenya, 1999). The fourth Millennium Development Goal is to reduce child mortality. The target is to reduce by two thirds the mortality rate among children under the age of five by the year 2015. Diarrhea has lifelong effects on children, leading directly to a decrease in physical and cognitive development (CDC, 2005). In the 2005 – 2015 "Water for Life Decade", there has been a general shift

in water issues quantity alone, with the World Water Day on 22nd March 2010, campaign being to raise the profile of water quality (WHO and UNICEF, 2010).

~~No specific therapy~~ ~~is~~ The available therapy for treatment of enterobacterial diarrheal infections is not specific but rather varies with the aetiologic agent. The sulphonamides, ampicllin, cephalosporins, fluoroquinolones and aminoglycosides have marked antibacterial effects against the enteric, but variation in susceptibility is great and laboratory tests for sensitivity are essential for appropriate choice of therapeutic antibiotics (Geo *et al*., 1998). Antibiotic resistance in *Enterobacteriaceae* has accelerated over the past two decades (Bennet and Jarvis, 2007). Optimal means of control of enterobacterial disease depends on the bacterial genus and the mechanism of drug resistance developed.

Water is the most critical resource in the Samburu region of North-Central Kenya. Samburu District is classified as arid to semi-arid and receives a mean annual rainfall of between 250 mm and 500 mm. The relative humidity is typically low. The mean annual potential evapotranspiration exceeds 2000 mm (Griffiths, 1995). The Uaso Ng'iro is the only permanent river in the district. Additionally, numerous ephemeral laggas, which are water channels formed during the rainy season, and natural ponds, have water during the rain seasons (March to May and October to December). Man made dams for harvesting rainwater augment the water resources available for domestic, livestock and wildlife use.

In the Samburu region, animals will tend to be dispersed (GFS, 1995). With the onset of the dry season, animals will tend to migrate to areas with adequate forage and water. Over abstraction of river Uaso Ng'iro water and sinking of boreholes for the purpose of

irrigation water supply further reduces the overall amounts of surface water available for man, livestock and wildlife. As man will tend to dominate watering points, competition for available water will inevitably lead to increased human-wildlife conflict, especially at the end of the rain season and during the dry season. As the standing bodies of water start to dry, there will be a corresponding change in water quality (Kotut *et al.*, 1999) and the concentration of dissolved solutes will increase.

Most of the Samburu community consumes water directly without any special treatment or filtration procedures being employed on it. Similarly several cases of diarrheal diseases have been reported in Samburu district. Owing to the scarcity of water and sharing of watering points between man, domestic stock and wild animals definitely the water can be a good vehicle for infective bacteria and other disease causing biological agents.

1.2 Problem statement and justification.

Information on the bacteriological quality of water sources in Samburu District is scanty if any. Given that the majority of people in Samburu consume water directly without any treatment and that causes of diarrheal diseases are common in Samburu there was therefore need to document the water quality of various sources. In Samburu, both ephemeral and permanent water sources are shared between livestock, wildlife and man, and can easily serve as vehicles for transmission of diseases.

~~In the Samburu region, animals will tend to be dispersed during the rain seasons when water availability is not critical, (GFS, 1995). With the onset of the dry season, animals will tend to migrate to areas with adequate forage and water. Over abstraction of river~~

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~~Uaso Ng'iro water and sinking of boreholes for the purpose of irrigation water supply further reduces the overall amounts of surface water available for man, livestock and wildlife. As man will tend to dominate watering points, competition for available water will inevitably lead to increased human wildlife conflict, especially at the end of the rain season and during the dry season. As the standing bodies of water start to dry, there will be a corresponding change in water quality (Kotut *et al.*, 1999) and the concentration of dissolved solutes will increase.~~

~~Most of the Samburu communities consume water directly without using any special treatment or filtration procedures. Numerous cases of diarrheal diseases are reported in Samburu district every year. Water contaminated with infective bacteria and other disease causing biological agents is suspect, owing to the scarcity of water and sharing of watering points between man, domestic stock and wild animals.~~

~~1.2 Problem statement and justification~~

~~Information on the bacteriological quality of water sources in Samburu District is scanty. Given that the majority of people in Samburu consume water directly without any treatment and that cases of diarrhea diseases are common in Samburu, there was therefore need to document the water quality of various sources. In Samburu, both ephemeral and permanent water sources are shared between livestock, wildlife and man, and can easily serve as vehicles for transmission of diseases.~~

This study was therefore designed to determine, assess, and evaluate the quality of water in Samburu South. The documented data on water quality will be applied as below:

1. Establishment of baseline data, which can be used in both monitoring and future assessment of trends in water quality.
2. Assessment of the suitability of available water sources for domestic use by the Samburu communities.
3. Evaluation of the likely effects of contaminated water on domestic animals and wildlife.
4. Recommendations on pollution control, environmental protection and health measures necessary for the various water sources.

1.3 Research hypotheses

1. Water sources in Samburu South meet the physico-chemical standards for portable water.
2. Indicator organisms in the water sources are within acceptable limits.
3. There are no common bacterial pathogens in water from both permanent and ephemeral sources of Samburu South.
4. Pathogenic isolates from water obtained from permanent and ephemeral sources in Samburu district are sensitive to commonly used antibiotics.

1.4 Research questions

1. Does water from permanent and ephemeral sources in Samburu south meet the physic chemical standards for portable water?
2. Are bacterial indicators of water quality from these sources within acceptable limits?

3. What are the common bacterial pathogens in water from permanent and ephemeral sources in Samburu south?
4. Are the pathogenic isolates from water obtained from permanent and ephemeral sources in Samburu south sensitive to commonly used antibiotics?

1.5 The general objective

The general objective of this study was to determine the physico-chemical and bacteriological quality of water and the antibiotic sensitivity of pathogenic isolates from selected water sources in Samburu South.

1.6 Specific objectives

i). To determine whether the physico-chemical properties of water from different categories of water sources in Samburu south meet~~s~~ the criteria for portable water.

ii). To enumerate indicator organisms in the water sources.

iii). To isolate, identify and characterize common bacterial pathogens in water from permanent and ephemeral sources in Samburu south.

iv). To determine the sensitivity profiles of the pathogenic isolates to commonly used antibiotics.

CHAPTER TWO

÷ LITERATURE REVIEW

2.1 Biological hazards of water pollution

More than 3 billion people do not have clean water to meet their daily needs (WHO, 2000). Water sources available for consumption and recreation have been adulterated with agricultural as well as animal and human waste. Polluted waters contain vast amounts of organic matter that serve as an excellent nutritional source for growth and multiplication of the polluting microorganism. The increasing demands placed on currently available water resources can raise the potential of contaminating surface water and ground water by enteric pathogens. Potable water systems can become polluted with coliforms and pathogenic bacteria from normal, diseased or carrier human and animal excrements (Atlas and Bartha, 1993).

According to one estimate, more than 300,000 children are hospitalized in the United States each year at an annual cost of $ 1 billion because of infectious diarrhea (Glass *et al*., 1988). The global impact is enormous since more that 250 million new cases of waterborne diseases are reported each year, resulting in more than 10 million deaths (Snyder and Merson, 1982). Between 1991 and 1998, 230 waterborne disease outbreaks were reported in the United States (Gunther *et al*., 2001). Many waterborne disease outbreaks are classified as “unknown”. This is not a very helpful classification because little can be done in the way of prevention or abatement. The fact remains that in many instances, there is no clear evidence of what caused the illness. There are an estimated 4 billion cases of diarrhea and 2.2 million deaths annually (WHO, 2002). Lack of access to safe water and sanitation contributes to diarrheal morbidity and mortality in developing

countries (Garret *et al*., 2008). The consumption of unsafe water has been implicated as one of the major causes of these diseases (88%). Children account for the biggest share of that burden, with 2 million children under the age of five dying each year from diarrhea, most of them because of drinking unsafe water and poor sanitation (CDC, 2005).

Worldwide the most common bacterial diseases transmitted through water are caused by *Shigella*, *Salmonella*, Enterotoxigenic *Escherichia coli*, *Campylobacter jejuni* and *Vibrio cholera* (Mitchell, 1972). Members of the genus *Campylobacter* are important and frequently cause diarrhea. These bacteria occur in the excrements of birds, and thereby enter water. A gram of bird faeces contains up to 10^7 *Campylobacter* cells. Often *Mycobacterium tuberculosis* also occurs. Presence of the spores of pathogenic *Clostridia*, particularly those causing gas gangrene like *Clostridium perfringens*, *C. novyi* and *C. ss~~S~~epticum*, can nearly always be demonstrated in sewage loaded water (Seltzer, 1991). The spores of the causative agent of anthrax (*Bacillus anthracis*) are also very resistant to common procedures of water treatment (Rheinheimer, 1991).

Botulism of water birds is caused by *C. botulinum* (Kohl, 1989). The toxin produced by these bacteria is one of the most powerful poisons and induces paralysis resulting in death. Even the birds which are not infected with the bacterium can succumb to the toxin. This may occur because of maggots of carrion flies taking up the toxin from the bodies of dead birds and accumulating it. Other birds can be poisoned by consuming these maggots.

2.2 Transmission of enteropathogens

The common feature with enteropathogens is that the infectious organisms are shed in the faeces of sick individuals and clinically asymptomatic carriers (Grabow, 1996). Faecal contamination through untreated or inadequately treated sewage effluents entering lakes, rivers or ground waters that in turn serve as water supplies creates conditions for rapid dissemination of the pathogens (Atlas and Bartha, 1993). The primary route of infection is ingestion of drinking water. Fruits, vegetables and eating utensils washed with contaminated water are additional possible carriers.

All diseases that are spread by the feacal oral route could potentially be contracted by unintentional ingestion of polluted recreational water. Swimming associated outbreaks caused by *Shigella, Giardia*, Norwalk-like viruses and other enteropathogens have been documented (Sorvillo *et al.*, 1988, Turner *et al.*, 1987).

Interrupting direct transmission depends ~~on~~ not only on water treatment, but also improved personal and domestic hygiene. A major sanitation effort therefore is necessary to treat and safely distribute public water supplies. Such sanitation practices have led to the virtual elimination of waterborne infections in developed countries, but these infections continue to be major causes of sickness and death in undeveloped regions (Sobsey and Olson, 1983). In tropical countries, *Vibrio cholerae* ~~*comma*~~ the causative agent of cholera occurs epidemically and is commonly spread by water contamination (Rheinheimer, 1991).

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2.3 Bacteriological indicators of pollution

2.3.1 Concept of indicator organisms

To assess the suitability of water for domestic use, there is need for simple and rapid tests for the detection of possible presence of pathogenic bacteria (Ashbolt *et al*., 2001). The detection of actual enteropathogens such as *Salmonella* or *Shigella* in routine monitoring studies would be a difficult and uncertain undertaking. Unfortunately, no single procedure is available for the detection of all waterborne pathogens (Singh and Macfeters, 1992).

Microbial contamination of drinking water is a major factor in the spread of disease. Pathogens are the agents responsible for infectious disease, and can be grouped as protozoa, helminthes, bacteria, or viruses. Pathogens can infect humans via ingestion and inhalation, or contact with skin, wounds, eyes or mucous membranes (WHO, 2004). Most pathogens are introduced into drinking water sources by human or animal waste, and cannot proliferate in water (WHO, 2004). Pathogens transmitted through this route are referred to as "enteric" because they initially occupy a niche in the intestines, or enterons, of their host. Upon leaving their host, the viability and infectivity of pathogens tend to decrease exponentially over time (WHO, 2004). ~~Pathogens possessing a high resistance to decay are the most problematic parasites when it comes to waterborne diseases.~~

Recovery of pathogens from environmental samples is generally difficult and methods designed for processing clinical specimens are quite often inadequate (Grabow, 1996). Biological parameters have been utilized for a long time for the evaluation of water quality

in inland and coastal waters. Kolkwitz and Marsson (1908) used bacteria, besides other vegetable and animal creatures, as indicator organisms for characterization of water condition and cited, for example, in the polysaprobic regionn~~n,~~ are, *Zooglea ramigera* and *Sphaerotilus natans*.

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Due to the large variety of pathogens and the complexity of testing methods, it is unrealistic and often difficult to test for individual species. As such, the use of indicator organisms becomes attractive. The idea behind indicators is that certain nonpathogenic microorganisms are present in the faeces of all warm-blooded animals (Gerba, 2000). These microbes are easily isolated and quantified using simple microbial methods. Microbial testing included as part of operations and verification, including surveillance and monitoring, is usually limited to that for indicator organisms, either to measure the effectiveness of control measures or as an index of feacal pollution (Grabow, 1996).

The presence of microbial indicators reveals that fecal contamination has occurred and that enteric pathogens are likely to be present in the water (Gerba, 2000). Several criteria have been established for ideal microbial indicators. According to the Guidelines for Drinking-Water Quality (WHO, 2004), an indicator should be universally present in the feces of humans and animals in large numbers. It should not multiply in natural waters, persist in water in a similar manner to fecal pathogens, be present in water in higher numbers than fecal pathogens, respond to treatment processes in a similar fashion to fecal pathogens and be readily detectable by simple, inexpensive methods.

This criterion reflects an assumption that the same indicator organism could be used as both an index of feacal pollution and an indicator of treatment/process efficacy (APHA,

1990). However, it has become clear that one indicator cannot fulfill these two roles (Sueiro *et al*., 2001). In addition, greater reliance is being placed on parameters that can be used as indicators for the effectiveness of treatments and processes designed to remove feacal pathogens, including bacteria, viruses, protozoa and helminthes. An index organism is one that points to the presence of pathogenic organisms while an indicator organism is one that is used to measure the effectiveness of a process (WHO, 2002).

Several microorganisms have emerged as suitable indicators, but to date no single microorganism meets all the criteria. Some of the most frequently used bacterial indicators include *Escherichia coli*, intestinal enterococci, and *Clostridium perfringens*. Enteropathogens usually appear intermittently in low concentration in an aquatic environment that is not ideal for either their growth or extended existence (Atlas and Bartha, 1993). Hence, to guarantee the good health of a community, there is need for readily available methods to detect and enumerate pathogens from aquatic sources.

Several limitations render detection of waterborne pathogens difficult. The low microbial densities usually found in water necessitate the analysis of large volumes of water to detect pathogens effectively. This may limit the number of samples that could be processed at one time and make the procedure costly (Rose *et al*., 1990). For example, the detection of *Giardia* cysts requires filtering a minimum of 380 liters (APHA, 1990) and in the case of *Cryptosporidium* up to 1000 liters of water (Rose, 1990).

The concept of using indicator organisms as signals of feacal pollution is a well established practice in the assessment of drinking-water quality (Grabow, 1996). The major indicator organisms of feacal pollution are *E. coli*, feacal *Streptococci*, and spores of

sulfite-reducing *Clostridia*. However, their presence does not prove that pathogens are present (Gray, 1995). No single indicator provides assurance that water is safe for consumption. Nevertheless, tests for total and feacal bacteria and *E. coli* are useful because it is rare to isolate bacterial enteric pathogens in the absence of feacal contamination (Singh and MacFeters, 1992).

2.3.2 Total coliforms

Total coliforms are defined as all of aerobic and facultative anaerobic ~~g~~Gram-negative, non-spore-forming, rod-shaped bacteria capable of growth in the presence of bile salts or other surface-active agents with similar growth-inhibiting properties, oxidase-negative, fermenting lactose at 35-37°C with the production of acid, gas, and aldehyde within 24- 48 hours (Dufour *et al.*, 2003). Total coliforms have been used in the past as indicators of water contamination. However, because coliforms have the ability to survive and multiply in natural waters, their effectiveness as indicators of feacal contamination is compromised. Detection of coliforms has historically served this purpose (indicator) to some extent, although coliform-free potable water has been implicated in several waterborne disease outbreaks (Berry and Norton, 1979).

Coliforms consist of various genera and species, for example,~~e.g.~~ *Escherichia coli*, *Klebsiella spp.*, and *Citrobacter spp*. but the group is more heterogeneous and includes a wider range of genera, such as *Serratia* and *Hafnia*. The different genera in coliform groups are differentiated by Indole, Methyl red, Voges Proskaeur, Citrate tests (IMVC formulae). An arbitrary definition of total coliforms is aerobic or facultatively anaerobic ~~facultative~~, Gram negative non spore forming, rod-shaped bacteria~~l~~ which ferment lactose with gas formation within 48 hours at 35-^{o}C. Additionally, studies have shown that there is

no direct correlation between the presence of pathogens and the presence of total coliforms (Garrett *et al*., (2008). Instead, total coliforms can be better used to assess treatment methods; their presence in filtered or disinfected water reveals inadequate treatment (WHO, 2004). Heterophile plate count measurements detect a wider range of microorganisms and are generally considered a better indicator of distribution system integrity and cleanliness (Ashbolt *et al*., 2001).

The total coliform group includes both feacal and environmental species. Total coliforms include organisms that can survive and grow in water. Hence, they are not useful as indices of feacal pathogens, but they can be used as an indicator of treatment effectiveness and to assess the cleanliness and integrity of distribution systems and the potential presence of biofilms (Grabow, 1996). Total coliform bacteria (excluding *E. coli*) occur in both sewage and natural waters. Some of these bacteria are excreted in the faeces of humans and animals, but many coliforms are heterotrophic and able to multiply in water and soil environments. Total coliforms can also survive and grow in water distribution systems, particularly in the presence of biofilms (Sueiro *et al.*, 2001).

The atypical coliforms may grow in soil and vegetation and may often be present in water free from feacal pollution. Typical coliforms survive in water only for several days or weeks after leaving animal intestines (Atlas and Bartha, 1993). Coliforms are used as pollution indicators instead of waterborne pathogens because they are present in great abundance in the intestine of man; coliforms in human faeces amounts to more that 100 x 10^6 per gram of wet weight (Rheinheimer, 1991). In 1991, the United States Environmental Protection Agency (USEPA) eliminated the requirement for the

enumeration of coliform bacteria in water samples, instituting regulations based on the presence or absence of coliform bacteria (USEPA, 2000).

2.3.3 Faecal coliforms

Faecal coliforms, mainly comprising *E. coli* are a sub-group of the total coliform group and they occur almost entirely in faeces (APHA, 1990). *E. coli* is always present in faeces, and the majority of *E. coli* strains can cause diarrhea, (Caincross and Faechem, 1993~~,~~). All coliforms will be detected at 37^{o}C but feacal coliforms can only be detected at 44^{o}C (Gray, 1995). *Streptococcus faecalis* are regularly found in faeces though their number is much less than *E. coli*. Their presence in water is regarded as confirmatory evidence of ~~recent~~ feacal contamination of water in doubtful cases. The determination of the number of *Clostridium perfringens* spores is of hygienic interest in some countries (Bonde, 1977). *Clostridium perfringens* also occurs in faeces regularly. They are excreted in much smaller numbers than *E. coli*. Hence, the presence of spores of the organism in water supplies indicates feacal pollution. Presence of *Clostridium perfringens* spores and absence of coliforms is suggestive of feacal contamination of water at some point in the past.

2.3.4 *Escherichia coli*

Escherichia coli is~~are~~ a thermotolerant coliform that belongs to the total coliform group. Thermotolerant coliforms are capable of fermenting lactose at 44.5±0.2°C. *E. coli* are differentiated from this group by their ability to produce indole from tryptophan or by the production of the enzyme ß-glucuronidase (George *et al.*, 2001). It is believed to be of purely feacal origin, and has been found to be present in fresh faeces in concentrations as

high as 10^9 per gram. Its presence is detectable by simple, inexpensive methods (Dufour *et al*., 2003). For these reasons, and because environmental conditions are unlikely to support *E. coli* growth outside of the intestine, this organism has come to be the preferred indicator of choice for feacal contamination.

The concentration of *E. coli* is, under most circumstances, directly related to that of thermotolerant coliforms. Hence, their use in assessing water quality is considered acceptable for routine purposes. Specific detection of *E. coli* by additional confirmatory tests or by direct methods, as described in the research literature, should be carried out if high counts of thermotolerant coliforms are found in the absence of detectable sanitary hazards (APHA, 1990). *E. coli* is found in sewage, treated effluents, all natural waters and soils that are subject to recent feacal contamination, whether from humans, agriculture, or wild animals and birds. Recently, it has been suggested that *E. coli* may be found or even multiply in tropical waters that are not subject to human feacal pollution. However, even in the remotest regions, feacal contamination by wild animals, including birds, can never be excluded. As animals can transmit pathogens infective for humans, the presence of *E. coli* or thermotolerant coliform bacteria can never be ignored, because the presumption remains that the water has been faecally contaminated (Hoadley and Putka, 1997).

E. coli is also the organism of choice in monitoring programmes for verification, including surveillance of drinking-water quality (Craun *et al*., 2003). These organisms are also used as disinfection indicators, but testing is far slower and less reliable than direct measurement of disinfectant residue. In addition, *E. coli* is far more sensitive to disinfection than are enteric viruses and protozoa. *E coli* occur in high numbers in sewage and water that has been subjected to recent feacal pollution by human and animal faeces.

Water temperatures and nutrient conditions present in drinking-water distribution systems are highly unlikely to support the growth of these organisms (Ashbolt *et al*., 2001).

E. coli is a very common bacterium that exists in many varieties (CDC, 2004c~~e~~). Harmless varieties live in human (and animal) large intestines and are essential to normal digestion. Some types are harmful (*E. coli* O157:H7), producing a potentially lethal toxin when ingested through contaminated food or water. If symptoms appear, they include little or no fever, bloody diarrhea and abdominal cramping. Among children younger than five and the elderly, hemolytic uremic syndrome may develop, which causes red blood cells to rupture and the kidneys to fail.

Complete identification of *E. coli* is too complicated for routine use; hence certain tests have been evolved for identifying the organism rapidly with a high degree of certainty (Farmer, 1991). The presence of *E. coli* provides evidence of recent feacal contamination, and their detection should lead to consideration of further action, which could include further sampling and investigation of potential sources of feacal contamination.

2.4 Nuisance organisms

There are diverse organisms that have no public health significance, but which are undesirable in water (Atlas and Bartha, 1993). They produce turbidity, taste and odor, and are aesthetically objectionable because they appear as visible animal life in water. Seasonal blooms of cyanobacteria and other algae in reservoirs and in river water impede coagulation, filtration, cause coloration, and turbidity of water after filtration (WHO/WSH, 2003). Microbial growth in distribution systems is encouraged by the

presence of biodegradable and assimilable organic carbon in water, often released by oxidative disinfectants (chlorine, ozone) from another category of nuisance organism. This growth may include *Aeromonas spp*., which can give a positive reaction in the coliform test.

Opportunistic pathogens are naturally present in the environment and are not formally regarded as pathogens. They are able to cause diseases in people with impaired local or general defense mechanisms such as the elderly or the very young, patients with burns or extensive wounds, those undergoing immunosuppressive therapy, or those with acquired immunodeficiency syndrome (AIDS) (Nelson *et al*., 2004). Water used by such patients for drinking or bathing, if it contains large numbers of these organisms, can produce various infections of the skin and mucous membranes of the eye, ear, nose and throat. Examples of such agents are *Pseudomonas aeruginosa* and species of *Flavobacterium*, *Acinetobacter*, *Klebsiella*, *Serratia*, *Aeromonas* and certain "slow growing" mycobacteria.

2.5 Pathogenic bacteria

2.5.1 *Salmonella*

Salmonella is a bacterium that is most commonly associated with eating undercooked meat, poultry, and eggs, but can affect water supplies. It can be acquired directly from animals. *Salmonella* causes an illness, known as enteric fever which is usually uncomfortable but not dangerous, except for young children, the elderly, and the immuno-compromised, who may become dangerously dehydrated (CDC, 2004b~~d~~). Symptoms of enteric fever include diarrhea, fever and abdominal cramps.

Salmonella in water is highly variable; there are limitations and variations in both the sensitivity of accepted *Salmonella* isolation procedures for the detection of the more than 2300 Salmonella serotypes currently recognized. Owing to the small sample size used in routine analyses, a negative result by any of these methods does not imply the absence of *Salmonella*, nor does it imply the absence of other pathogens. These organisms are usually present in small numbers compared to ~~other~~ coliforms; hence, it is necessary to examine a relatively large sample to isolate the organisms (Gray, 1995).

Sample enrichment is essential, because the pathogens are usually present in low numbers and solid selective media for colony isolation are somewhat toxic, even to pathogens. No single enrichment medium can be recommended that allow optimum growth of all *Salmonella* serotypes. Usually, two or more selective enrichment media are used in parallel for optimum detection. Selenite F broth allows for optimum recovery of most *Salmonella* species. Further separation of pathogens from the remaining nonpathogenic bacterial population is facilitated by proper choice of incubation temperature for primary enrichment followed by secondary differentiation on selective solid media (Chen *et al.*, 1993). The increased recovery of *Salmonella typhi*, after 24 h~~ours~~ at 35~~–~~°C to 37~~–~~°C is accompanied by a slight decrease in selectivity when compared to selenite cysteine. The combination is optimum for recovery of all *Salmonella* serotypes. Method comparisons are encouraged to determine the best combination for a given circumstance.

Many enteric organisms with little or no pathogenicity share certain major biochemical characteristics with *Salmonella*. The identification of pathogens by colony characteristics on selective solid media has limitations inherent in the biological variations of certain organisms and cannot be relied on for even tentative identification. Suspected colonies

grown on selective solid media must be purified and further characterized by biochemical reactions, and finally verified by serological identification.

2.5.2 Shigella

Shigella is a genus of bacteria that cause illnesses very similar to *Salmonella* and *Campylobacter*, but usually spreads differently, through the fecal-oral route. In the developing world *Shigella dysenteriae* type 1 causes deadly epidemics whose symptoms include diarrhea, bloody diarrhea, cramping and fever. As with several other bacterial infections, *Shigella* may necessitate hospitalization of the very young or elderly because of profound diarrhea (CDC, 2005~~d~~). Some infected individuals show no symptoms but easily pass the illness on to others. About 3% of victims of *Shigella flexineri* suffer a serious, long-term complication known as Reiter's syndrome, which causes painful joints, irritation of the eyes, painful urination, and in some cases long-term arthritis. The syndrome may last for months or years. It is still an important public health problem, especially in developing countries, where there is substandard hygiene and unsafe water supplies (Niyogi, 2005). *Shigella* still accounts for a significant proportion of bacillary dysentery in many tropical and subtropical countries (Zafar *et al.*, 2005). It is the most prevalent etiologic agent in childhood diarrhea in most countries (Khan-Mohammed *et al*., 2005). In Ethiopia, *Shigella dysenteriae* and *Shigella flexineri* have been identified as the species that account for about 80% of *Shigella* isolates.

Shigellosis is a global acute gastrointestinal disease of humans, caused by four species or serogroups of the *Shigella*, which include *S. dysentariae* (Group C), and *S. sonnei* (Group D). *Shigella* invades the intestinal mucosa, producing dysentery characterized by

abdominal pain, fever and diarrhea (Farmer, 1991). Infections by *S. ~~higella~~ flexineri*, *S. dysentriae* and *S. boydii* are very common in developing countries, while *S. sonnei* is an important pathogen in developed countries (Dupont, 1990). The infectious dose for S*higella* ~~S~~*spp*. is low, and most cases result from person-to-person transmission. When outbreaks occur, they are usually associated with fecal contamination of foods and, less frequently, water.

Shigella survive up to 4 days in river water. It is improbable that S*higella* can be recovered from an environmental source, unless there is a continuous source of contamination such as wastewater seepage. *Shigella* can survive in a viable but non-culturable state after 21 days (Colwell *et al.*, 1985). Methods that have resulted in isolation of *Shigella* include membrane filtration and centrifugation (Dabrowski, 1982), with or without subsequent broth enrichment. Selenite F broth has successfully been used to recover S*higella* from water and sand.

2.5.3 *Escherichia coli*

E. coli are normal inhabitants of the human digestive tract. However, some cause diarrheal diseases in humans (Fenwick, 1995). These pathogenic *E. coli* fall under five groups: enterotoxigenic (ETEC), enterohemorrhagic (EHEC), enterroinvasive (EIEC), enteropathogenic (EPEC), and the newly recognized group called enteroadherent-aggregative *E. coli* (EA-AggEC) for its aggregate or "stacked-brick" – like adherence to cultured mammalian cells (Vial *et al.*, 1998).

Although pathogenic *E. coli* has most often been implicated in food-borne illness, several major waterborne outbreaks have been reported (Fenwick, 1995). Outbreaks have

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involved both water supplies and recreational waters. Pathogenic *E. coli* that ferment lactose and are not affected by elevated temperatures can presumptively be distinguished from nonpathogenic *E. coli* using the MPN fecal coliform procedure or the fecal coliform membrane filter method, followed by serotyping and virulence analysis. These methods, as well as methods from other sources (U.S Food and Drug Administration, 1995), have been modified to detect specific pathogenic groups. Regardless of the method, however, when testing for pathogenic *E. coli*, it is advisable to identify the isolates available using biochemical identification kits first, before serotyping and assaying for the virulence factors associated with respective pathogenic groups.

2.5.4 Campylobacter

Campylobacter are commonly found in the normal gastrointestinal and genitourinary flora of wild animals, birds and domestic animals including sheep, cattle, swine, goats and chickens (Ryan, 1990). The fecal oral route often acquires *Campylobacter* infections, ~~often~~ as zoonoses through exposure to infected animals. Large outbreaks have resulted from contaminated milk, uncooked meat or fowl, and contaminated water systems (Baron, 1994). Waterborne transmission of *Campylobacter* has resulted from drinking untreated surface water, contamination of groundwater with surface water, faulty disinfections, and contamination by wild bird faeces (Tauxe, 1992). The occurrence of the organisms in surface waters has proved to be strongly dependent on rainfall, water temperature and the presence of waterfowl (Frost, 2001).

2.5.5 *Vibrio*

Vibrio cholerae is the causative agent of cholera, a waterborne illness with symptoms ranging from mild to severe and potentially fatal diarrheal diseases (Oliver and Kaper, 1997). *V. cholerae* occurs as part of the normal micro flora in estuarine areas, with non-Ol/non-O139 strains being much more common than are O1 strains. The Ol serogroup is associated with epidemic and pandemic cholera, especially in developing countries.

2.5.6 Leptospira

Leptospira spp. prefer alkaline conditions, and ~~they~~ persist longest in warm, moist environments protected from sunlight. Generally, pathogenic leptospiras require an animal host and do not survive and propagate in the environment. Leptospirosis is a world wide zoonotic disease of wild animals (Atlas and Bartha, 1993). Reservoirs of leptospiras in wildlife include deer, foxes, raccoons, skunks, opossums, muskrats, and rodents. Domestic animals harboring *Leptospira spp.* include horses, cattle, goats, pigs, and sheep (Shaw, 1992). Dogs may become infected but cats are never affected. Humans are incidental hosts. Humans acquire leptospirosis (Weil's disease) directly from animals, and from occupational or recreational exposure to urine contaminated water (Venkataraman and Nedunchelliyan, 1992). They can also acquire leptospirosis from environmental surfaces. Outbreaks of leptospirosis associated with drinking water are extremely unusual, and are invariably caused by contamination of domestic water reservoirs with urine of infected rodents (Cacciapouti *et al.*, 1987).

Leptospira enter through imperfections in the skin, through mucous membranes, or by ingestion of contaminated water. Urine of infected animals and humans may contain 10^6 to 10^8 organisms per mL of urine. *Leptospira* may be shed into the environment up to 3 months after clinical recovery from disease. *Leptospira* are recovered from environmental sources with great difficulty (Baker and Baker, 1970). Since saprophytic pathogenic strains of *Leptospira* may be recovered from environmental samples, their presence has no public health significance apart from an epidemiological context.

2.5.7 *Legionella*

Legionella species also have been isolated in non-disease-related circumstances from a variety of aquatic systems such as lakes, streams, reservoirs and sewage (Cherry *et al.*, 1982). Recovery of *Legionella* from environmental water samples is sometimes difficult. *Legionella* may take up to a week to grow on plate media; even with acid pretreatment and the addition of antibiotics to the medium, faster growing organisms may overgrow *Legionella*. In addition, other organisms, including *Pseudomonas spp*. secrete into the surrounding media bacterial products that can inhibit *Legionella* growth (Paszko-Kolva *et al.*, 1993). Many wild, domestic, and farm animals are reservoirs of these organisms.

2.5.8 Yersinia

Yersinia enterocolitica has become recognized worldwide as an important human pathogen and is nearly as common as *Salmonella* and *Campylobacter* as a leading cause of acute or chronic enteritis (Fenwick and McCarty, 1995). *Yersinia* isolations do not correlate with levels of total and feacal coliforms or total plate count bacteria (Wetzler *et al.*, 1979).

2.5.9 *Aeromonas*

Aeromonas spp. are natural inhabitants of aquatic environments worldwide. These ~~g~~Gram negative, facultative anaerobic, glucose-fermenting organisms have been isolated from groundwater, treated drinking water, surface waters, wastewater, sludge, and sediment. Their populations are seasonal in all natural waters with the highest numbers present in warmer months. ~~ The group of m~~Mesophilli~~c~~c~~ic~~ motile aeromonads, which have ~~with~~ a single polar flagellum, ~~aeromonads is~~are ~~considered~~ of potential human health significance (Frost, 2001). These motile aeromonads have emerged as a serious microbial threat to human populations, especially the immune-compromised (Janda and Abbott, 1996). The epidemiological association between ingestion of untreated well water and subsequent *Aeromonas* gastrointestinal illness has been widely documented. Numerous cases and outbreaks of water- and food-transmitted illnesses caused by *aeromonads* have been reported (Janda and Abbott, 1996). *Aeromonas* contamination of drinking water has been documented as a cause of travelers' diarrhea (Shinde *et al.,* 2005).

The genus *Aeromonas* comprises 14 recognized and 2 proposed DNA hybridization groups with 13 named phenospecies and 4 unnamed genospecies. The extreme difficulty of DNA hybridization techniques in most laboratories have le~~a~~d clinical microbiologists to report aeromonds as *A. hydrophila*, *A. sorbia*, or *A. caviae*, according to published classification scheme (Oliver and Kaper, 1976). Environmental microbiologists usually combine all motile, mesophilic *aeromonads* into the *Aeromonas hydrophila* complex, or simply report isolates as *A. hydrophila* (WHO, 2002).

The ability to isolate, enumerate, and identify *aeromonads* from potable water and wastewater sources is important because of their role in causing human and animal

diseases, and their ability to colonize treatment plants and distribution systems (Huys *et al*., 1996). *E. coli*, or alternatively thermotolerant coliforms can not be used as an index for the presence or absence of *Aeromonas spp*. (Borchardt *et al*., 2003). Samples of water taken from a suspected source may test negative because the sampling technique failed to capture an organism. This can occur because of the dynamic nature of water systems. In each water body, environmental conditions such as currents and water quality may ~~be~~ markedly vary over a very short time lapses. Modern techniques in molecular biology are making it more feasible to trace agents to their sources. However, the techniques are new and constantly evolving (Gunther *et al*., 2001).

2.6 Antimicrobial sensitivity of common enteropathogens~~.~~

The emergence of antimicrobial resistance to members of the *Enterobacteriaceae* family is posing serious problems in the treatment of outbreaks of infections. Since its first report in studies conducted in the 1950s, multiple-drug resistance transmitted by plasmids among *Shigella* species has been reported from many countries (Geo *et al*., 1998, and Brito and Nij, 1994). Moreover, an increase in resistance to many different drugs has been observed in the last two decades. In one study~~,~~ carried out from 1988-89 to 1991-92, a significant decrease was observed in the susceptibility of *Shigella* to ampicillin and cotrimoxazole (Dan, 1993). In another report, it was shown that cotrimoxazole resistance of *Shigella* increased from 3% to 40% within a ten years ~~a~~ period of ~~ten years~~ (Heikkila and Siitonen, 1990).

Studies carried out in Madrid (Spain) show that the percentage of resistant *Shigella* strains increased from 39.6% to 97.9% for ampicillin, from 34.4% to 96.9% for cotrimoxazole, from 6.3% to 18.0% for Trimethoprim, and from 1.6% to 15.1% for chloramphenicol

(Lopez and Collado, 1983). In Ethiopia, strains of *Shigella* that were resistant to many commonly used drugs have been reported in different parts of the country by several studies (Senait *et al*., 1993). The strains were found to be most commonly resistant to trimethoprim (>80%), ampicillin (>65%), and cotrimoxazole (>70%). Multiple drug resistance to ampicillin, chloramphenicol, trimethoprim, and streptomycin ~~was also~~ was also observed in these studies. Besides the temporal changes in the antibiogram of *Shigella* species, it is well known that antibiotic susceptibility patterns in *Shigella* may differ between geographical areas. Such differences are never stable and may change rapidly especially in places where antibiotics are used excessively, particularly in developing countries (Leslie *et al.*, 1998). There is therefore, a need for frequent observation on the change in the pattern of antibiogram for this organism.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

3.1.1 Location

The study area was Samburu South, which is part of Samburu District situated in Rift Valley province of Kenya (Figure1). Samburu lies between latitudes 0° 40' and 2° 31'S and longitudes 36°E 2' and 38°E 10'N (Bussmann, 2006). The district covers an area of 20.804 Km^2 from the Southern shores of Lake Turkana, across Samburu, to Isiolo. Eighty four percent of the area is described as low potential and is semi arid. It borders Turkana District to the northwest, Baringo District to the southwest, Marsabit District to the northeast, Isiolo District to the east and Laikipia District to the south. The altitude varies from 1000 – 2,000 m. Out of this, the Samburu National Reserve, which was established in 1985, occupies an area of 390 Km^2. Only 8% of the district is considered to be of high to medium potential and able to sustain agriculture. Some of this land is under commercial wheat and barley production. A smaller portion is under food crop production for local consumption (GOK, 1994). The southern part of Samburu District covers Wamba, Ngaroni, Barsalinga and Ngutuk Ongiron divisions. Namunyak Conservancy, a community owned group ranch, is a protected area for wildlife which lies within this region. Two major seasonal laggas, Lengusaka and Lossesia, two permanent streams, Sere Wamba and Buffalo Springs as well as a part of river Uaso Ng'iro interpass this region.

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Actual sampling sites were: Seiya river, Lapus dam, Margwe wells, Serewamba stream, Namunyak dam, Loidikidiko dam, Nakoruworu wells, Naibelibeli dam, Uaso Ng'iro river, Ngutuk Ongiron dam, Lerata river, Mugur Omuny springs, Uaso Ng'iro river, Lkisin

borehole, Naisunyai borehole, Lodungokwe dam, Serewamba borehole, Ndikir, Nkwaas and, Enyangainito (Figure 1).

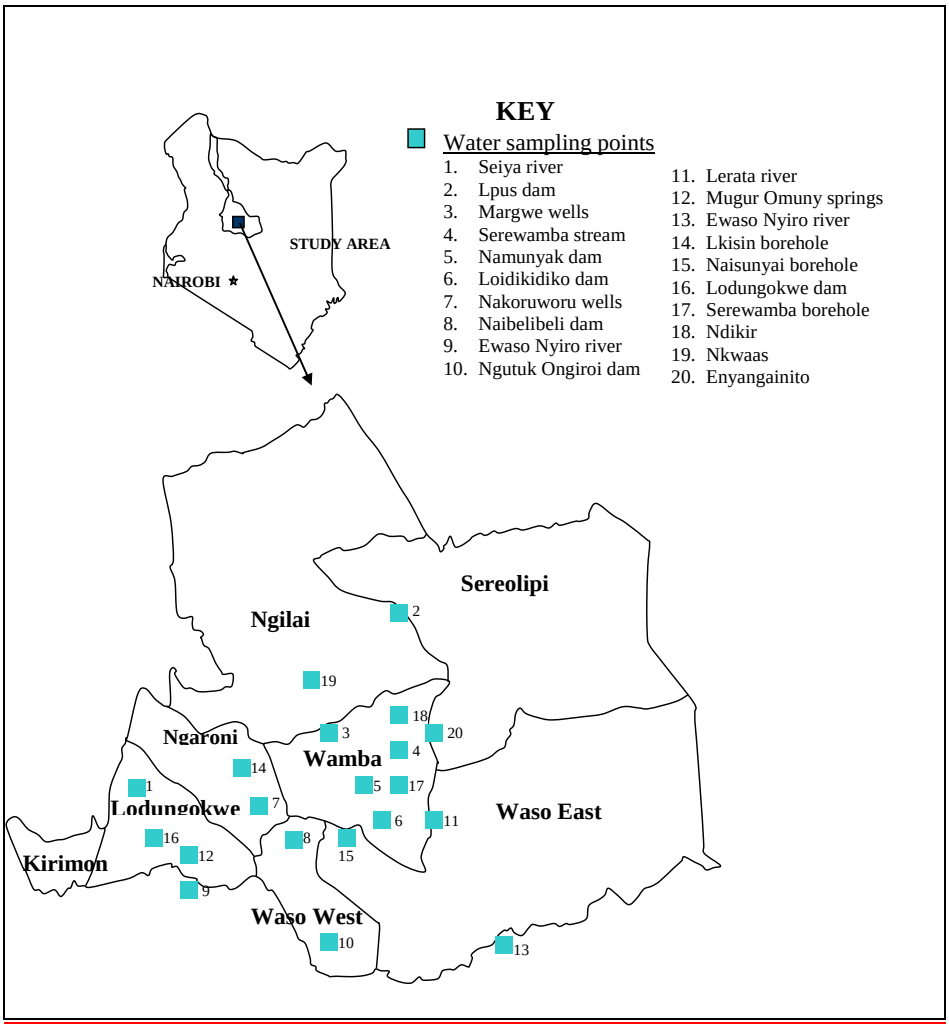

Figure 1. Map of the Samburu District showing the study area (Wamba Division)

3.1.2 Climate

Samburu District is classified as arid to semi-arid and receives a mean annual rainfall of between 250 mm and 500 mm per annum. Relative humidity is typically low. The mean annual potential evapotranspiration exceeds 2000 mm (Griffiths, 1995). Rainfall is

bimodal, peaking in April and November for most of the district. The average annual rainfall is between 200 – 450 mm, rising to 700 mm in higher areas of Lerroghi division. The Uaso Ng'iro is the only permanent river in the district. Additionally, numerous ephemeral laggas and natural ponds have water during the rain seasons (March to May and October to December). Man made dams for harvesting rainwater augment the water resources available for domestic, livestock and wildlife use. Most mountain areas like Mathews Range are covered with vegetation, which could be the result of humidity received from mist condensation and frequent cloud formation in the peak areas.

The soil is dry and sandy with a poor vegetation cover. For most of the year, the area experiences high temperatures during the day with no cloud cover. Temperatures vary with altitude and generally range between 24°C mean minimum and 33°C mean maximum. The highlands belts of the Lerroghi Plateaus are cool, while the central plains and the region east of the Mathews Range have the highest temperatures.

3.1.3 The People.

The inhabitants of Samburu district are mainly the Samburu people. Like their cousins the Maasai, they speak a "Maa" language and belong to the Chari-Nile branch of the Nilo-Saharan language family. The Samburu are mostly nomadic pastoralists, inhabiting the plains and highlands of Northern Kenya (Bussmann, 2006). They live mostly in group ranches governed at community level by appointed elders. Traditional nomadic lifestyle is still prevalent amongst the Samburu, who migrate from lowlands to the humid mountain areas in the dry season in search of grazing grounds (Spencer, 1965). Milk and blood from

cows, camel, sheep and goats, and soups derived from wild collected herbs are the main parts of the Samburu diet.

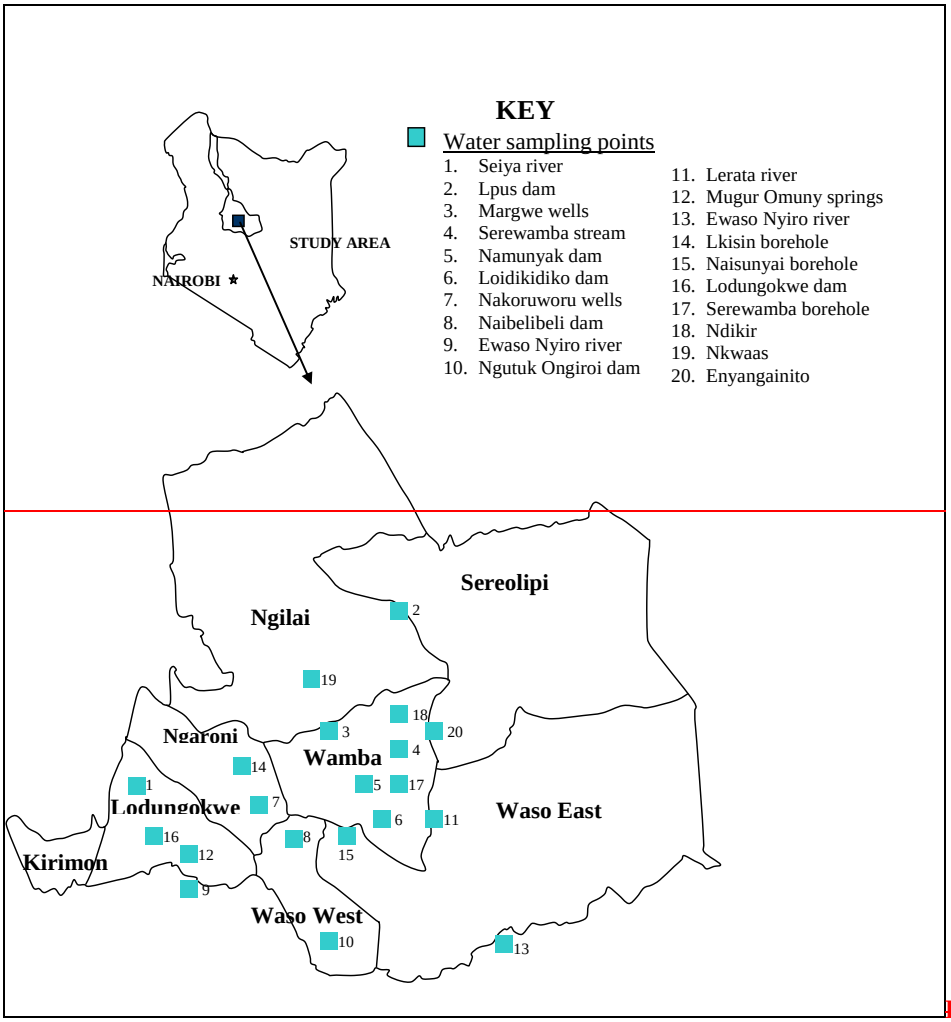

Figure 1. Map of the Samburu District showing the study area (Wamba Division)

3.2 Sampling

Purposive sampling targeting water sources for man, livestock and wildlife was carried out. At each water source, whenever possible, water samples were collected directly in pre-sterilized polypropylene bottles of 500 mL. At the water reservoirs, the bottles were

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opened aseptically, then held at their bases and submerged to a depth of about 20 cm with the mouth facing upwards. Samples were taken by filling the bottles to the top to exclude air. In case of a current, the bottles were tilted towards the current and filled. The samples were examined within 1 – 3 h. of collection. Where delays of 3 – 6 h. were anticipated, the bottles were kept on ice, preferably in an icebox until the time of analysis. Samples for measurement of physico-chemical properties were collected using a water scooper and poured in to a plastic bucket where the actual measurements were taken. The scooper was also used to fill a 500 mL plastic bottle, which was then labeled and carried for further laboratory analysis.

3.3 Field tests

Physical observations of water, such as clarity of water and the presence of plants and other aquatic life were recorded.

Air temperature and relative humidity were taken using a sling psychrometer (1330 PJ) and expressed in degrees Celcius (°C) and percentage of moisture in the atmosphere respectively. Water temperature, pH, salinity, alkalinity, dissolved oxygen and conductivity were measured *in situ* using a potable Universal Multiline P4 WTW (Wilheim, Germany) meter. The meter was calibrated and operated as per the manufacture's- instructions~~operating manual~~. At the sampling point, the measuring probes were lowered into the water and allowed to settle for 1 - 2 min.,~~utes~~ before the readings were taken. Electrical conductivity was measured using a conductivity probe and expressed in microSiemens per centimeter (µS cm^{-1}), while salinity was measured in milligrams per liter (mg L^{-1}).

3.4 Bioassay

3.4.1 Total plate count

An amount consisting of 1 mL of each water sample was transferred aseptically onto sterile petri dishes and approximately 20 mL of molten plate count agar (45°C) added and mixed. The plates were allowed to set then incubated at ~~(~~37°C~~)~~ for 48 h~~ours~~. (Method 9215B, APHA, 1990). Water samples that looked more polluted (>300 counts/mL) were serially diluted using peptone water or physiological saline before plating. Colony counts were made from plates with less than 300 but more than 30 colonies and results expressed as actual colony count multiplied by the dilution factor. Colony counts were expressed as colony forming units (cfu)/mL^{-1} of the sample.

3.4.2 Coliform tests

Coliforms were enumerated using the most probable number (MPN) of bacterial in water (McCrady, 1915). A series of lauryl tryptose broth (LTB) fermentation tubes were inoculated with 10, 1 and 0.1 mL of the sample (Method 9200A, APHA, 1990). Formation of gas at 35°C within 48 h~~ours~~ constituted a positive presumptive test. To confirm the test, inocula from positive tubes were transferred to tubes of 2% brilliant green bile lactose broth (Oxoid), dispensed in fermentation tubes fitted with inverted Durham tubes and incubated at 37°C for 24 h~~ours~~. Production of gas was taken as a positive test for the presence of coliforms.

3.4.3 Faecal coliforms

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A small proportion of the culture from each positive presumptive tube was recultured in brilliant green bile lactose broth 2% (Oxoid) in fermentation tubes and incubated in a water bath at 44.5 ± 0.2°C for 24 h. (Method 9222D, APHA, 1990)~~Production of gas after the incubation time was taken as a positive indicator of the presence of feacal coliforms.~~

3.4.4 Test for *Escherichia coli*

A loopful of the positive broth was streaked onto Eosin Methylene Blue agar (EMBA) and incubated for 24 h.~~ours~~ at 37°C.~~ Formation of green metallic sheen colonies confirmed the presence of *E. coli*.~~ The test was completed by making a thin smear for Gram staining from the green metallic sheen colonies and confirmed by biochemical tests (Method 9223A, APHA, 1990).~~. A negative Gram reaction confirmed *E. coli*.~~

3.4.5 Biochemical tests

Differentiation of coliform into subgroups was carried out on the basis of the result of four tests; Indole, Methyl red, Voges Proskauer and Citrate utilization, often referred to collectively as “IMVIC” tests.

3.4.5.1 Indole test

Testing for indole production was important in the identification of enterobacteria. Most strains of *E. coli, P. vulgaris, M. morganii* and *Providencia spp* breakdown the amino acid tryptophan to produce indole. Indole production is detected by Kovac’s or Ehlrich’s

reagent, which contains p-dimethylaminobenzaldehyde that reacts with indole to produce a red colored compound.

3.4.5.2 Methyl red test

A colony of the test organism from EMBA was inoculated in 0.5 mL of sterile glucose phosphate broth. After overnight incubation at 37°C, a drop of methyl red solution was added. Appearance of a bright red color indicated a positive methyl red test indicating presence of some enterobacteria. Some enterobacteria, when cultured in buffered glucose phosphate peptone water, produce much acid from the fermentation of glucose to give a red color with the indicator methyl red.

3.4.5.3 Voges-Proskauer (V-P) test

A loopful of the test organism obtained from EMBA (Difco), was cultured in glucose phosphate peptone water for 48 h~~ours~~ after which a drop of creatinine and sodium hydroxide were added. Under alkaline conditions and exposure to air, the acetoin produced from the fermentation of glucose is oxidized to diacetyl, which forms a pink compound with creatinine. This indicates the presence of some enterobacteria but not *E. coli*. Some strains of enterobacteria ferment glucose with the production of acetylmethycarbinol (acetoin) which can be detected by an oxidation reaction.

3.4.5.4 Citrate utilization test

A loopful of the test organism obtained from EMBA was cultured in a medium which contains sodium citrate, an ammonium salt and bromothymol blue indicator. Growth in the medium was indicated by turbidity and a change in color of the indicator from light green to blue due to the alkaline reaction following citrate utilization.

3.4.6 Faecal *Streptococci*

An amount of 100 mL of a water sample was filtered through sartorious memberane filters with a pore size diameter of 0.45 µm, and the filter transferred aseptically onto the Slanetz and Bartley agar (Oxoid), plate using sterile forceps. The plates were then incubated at 37°C for 24 h~~ours~~. After incubation, the plates were observed for typical maroon colonies which were counted and recorded as feacal streptococci per 100 mL of water sample.

3.5 Isolation of bacterial pathogens

Isolation of bacterial pathogens in water samples commenced upon arrival at the Mombasa Polytechnic University College microbiology laboratory. Water samples were plated on different selective media which included XLD agar (HiMedia), for non-lactose fermenting bacteria, Selenite F. broth (HiMedia), for *Salmonella spp*., MaConkeys agar (Difco), for non-lactose fermenting enterobacteria, SS agar (Difco), for *Salmonella* and *Shigella*, TCBS broth (Oxoid), for *Vibrio* and *Aeromonas* and Campy-blood free agar (Oxoid), for *Campylobacter jejuni*. The plates were then incubated over night at 37-°C, except for the campy-blood free agar, which was incubated for 48 h.~~ours~~ in a microaerophillic environment at 37-°C.

3.5.1 Identification of *Salmonella* and *Shigella*

Water samples were cultivated on selective and differential substrates in order to isolate *Salmonella* and *Shigella* species. Isolated colonies were further subjected to biochemical and serological tests for confirmation of genus. The water samples were plated on MacConkeys agar (Difco), Xylose lysine deoxycholate agar (HiMedia), Salmonella Shigella agar (SS), and Selenite F agar using a cotton swab and incubated overnight at 37ºC. MAC, XLD and SS plates were examined for typical non-lactose fermenting colonies. Such colonies were identified by morphology. Suspected colonies were inoculated into TSI agar (Difco), MIO agar (Oxoid), Simmon's Citrate agar (Oxoid), and Urea agar slopes and incubated at 37ºC for 12 - 18 h~~ours~~. The selenite F broth was subcultured on to XLD and SS agar plates and incubated at 37ºC for 12 – 18 h~~ours~~. Test results were examined by inoculating one set of API 20E (BioMerieux), system according to manufactures instructions, and slide agglutination.

3.5.2 Detection of vibrios and aeromonads

Thiosulfate Citrate Bile salts Sucrose agar (Oxoid), was inoculated with 0.1 mL of 100 mL water samples centrifugates. TCBS agar was checked for growth of typical yellow or blue-green colonies. *Vibrio* and *Aeromonas spp* were identified by the following tests: oxidase, motility, sensitivity to the vibriostatic agent O129 and finally confirmed by API 20NE (BioMerieux) system and serology.

3.5.3 Detection of *Campylobacter jejuni*

Concentrated water samples were cultured on Campylobacter blood-free agar under microaerophilic conditions. *C. —jejuni* appear as gray or colorless colonies on campylobacter blood free agar and have a negative reaction to the Gram test. The microscopic findings were confirmed by biochemical tests, such as the oxidase test, the hippurate hydrolysis test and the susceptibility to naxilidic acid.

3.6 Antibiotic sensitivity testing

3.6.1 Kirby-Bauer disc-diffusion method

Antimicrobial disk diffusion tests were done to determine the degree of sensitivity of pathogens isolated from water to a range of antimicrobial drugs (Bauer *et al.*, 1966). Discs impregnated with known concentrations of appropriate antibiotics (Cosmos), were placed on agar plates seeded with a lawn of the isolated pathogen. The antibiotics diffused into the media and the zones of inhibition were measured to express the activity of the antibiotics against test organism in-vitro.

Three to five well isolated colonies of the pathogenic isolates were each emulsified in normal saline to a turbidity of 0.5 (MacFarland standard). Sterile cotton swabs were dipped in to Bijou bottles containing the isolates. They were rotated several times and pressed firmly on the inside walls to remove excess inocula then streaked on to the dried surfaces of Muller Hinton agar (Oxoid) plates, from the centers outwards. The standardized antibiotic discs of ampicillin (10µg), amoxicillin (25µg), cefutaxime(10µg), gentamicin(10µg), ciprofloxacin(5µg), cotrimoxazole(25µg), chloramphenicol(30µg) and cefuroxime(5µg) from the British Society of Antimicrobial Chemotherapy (BSAC, 2000),

were distributed evenly so that they were not closer than 24 mm from center to center. Only 5 discs were placed on 100 mm plates.

After an overnight incubation, the diameters of the zones of complete inhibition were measured to the nearest mm using sling vernier calipers. The zone margins were taken as the areas showing no obvious, visible growth that could be detected with the unaided eye. The sizes of the zones of inhibition were interpreted using zone size break points provided by BSAC (2000) and organisms reported as either susceptible (S) intermediate (I) or resistant (R) to the antibiotics applied (Villanova, 1997).

3.6.2 Minimum inhibitory concentrations E- test

The Epsilometer Test (E-Test; Biodisk Sweden) is a new *in vitro* susceptibility testing method designed for quantitative determination of susceptibility to antimicrobial agents. The E-Test is a plastic strip containing a predefined continuous and exponential antibiotic gradient on one side and a graded continuous minimum inhibitory concentration scale that covers 15 two-fold dilutions on the opposite side. E-Test strips containing gradients of Trimethoprim/Sulphamethametoxazole (TS), (0.002-32), amoxicillin (AM), (0.016 – 256) and chloramphenicol (CH), (0.016-256), all from AB Biodisk were tested. The test procedure followed the manufacturer's instructions. The pathogenic isolates to be tested were emulsified in Muller Hinton broth (Oxoid) and the suspension turbidity adjusted to match that of the 0.5 McFarland standards. Sterile cotton swabs were used to inoculate the suspensions of organisms on Muller Hinton agar (Oxoid) plates, and then incubated overnight at 37°C. *E. coli* ATCC 25922 was used as a positive control for the effectiveness of the E-strips.

The MICs were read as the concentrations of antibiotics at the points where the zones of inhibition intersected with the strips. Isolates were classified as susceptible, intermediate or resistant according to the guidelines of the National Committee for Clinical Laboratory Standards (2004).

3.7 Pulsed-field gel electrophoresis (PFGE)

Many classic methods for distinguishing microorganisms have been used, such as finding the drug resistance pattern, phage typing, biotyping, and colicin typing (Liu *et al*., 1995). PFGE is considered to have a great discriminatory power in bacterial subtyping (Liebisch and Schwartz, 1996). It is a technique to separate DNA sequences of certain sizes and is an alternative means to regular gel electrophoresis, providing better separation of bands with sharper quality. The resulting electrophoretic patterns are highly specific for strains from a variety and provided an opportunity to examine for multiple variations throughout the genome of *S. flexineri* so as to identify specific strains. PFGE has great value in epidemiological analysis, in the differentiation of pathogenic strains, and in the monitoring of their spread among communities (Bannerman *et al*., 1995).

Methods for PFGE were as described by Maslow *et al.,* (1993). Isolated colonies of *Shigella flexineri* which are susceptible to ampicillin and those showing resistance to ampicillin were inoculated into tryptose soy broth and harvested by gradient centrifugation. Portions of the bacterial suspensions were then mixed with equal volumes of 1% low melting-point agarose and dispensed in a mould to solidify. The agar plugs were then incubated overnight in a solution of Tris buffer, EDTA, sodium lauryl sarcosine

and protinase K for lysis of the bacteria. The plugs were ~~then~~ washed in TE buffer slices (2.5mm) and incubated for 4 h. with 20 units of *Not*1 restriction enzyme. The slices were then loaded into wells of a 1.2% pulsed- field certified agarose (Bio Rad) plate in 0.5x TBE buffer.

Electrophoresis was performed with a counter-clamped homogeneous electric field apparatus (CHEF-DRI II, Bio-rad) at 14ºC with 200 V cm^{-1}. The pulse time were ramped from 5 to 50 seconds,~~onds~~ for 21.5 h~~ours~~. The gels were stained with ethidium bromide (0.5 µg mL^{-1}) for 30 minutes~~utes~~, and de-stained using distilled water for 3h. The resulting DNA bands were made visible by photographing under UV light.

The banding pattern in each lane was compared visually to that in every other lane. For band size determinations and lane comparisons a molecular weight marker was included on one end of the gel. Band differences were interpreted according to the table (Appendix iii), taken from the consensus paper for PFGE interpretation by Tenover, *et al.*, (1995).

3.8 Analysis of results

Data on the various physico-chemical and bacteriological properties were summarized in tables. Descriptive statistics, comprising of mean, median, range and standard deviation were used to measure variation and central tendency. One way ANOVA was used to determine whether there was significant difference in the physico-chemical properties and indicator organisms of the various water sources. The Tukey-Kramer test was used for the separation of means or medians. Correlation was used to determine whether there was a relationship between physico-chemical properties and microbial load of the water.

CHAPTER FOUR

RESULTS

4.1 Physico-chemical properties of water in Samburu district

Water sources in Samburu District had an average air temperature of 28.44ºC ± 3.39 (Table 1). This is very close to the average water temperature which is 26.66ºC ± 4.32. However, some sources recorded water temperatures as low as 10ºC and as high as 35.6 ºC. The average conductivity regardless of water source was 658.5 ± 713.57 µS.cm^{-1} while pH was in the neutral range of 7 – 8.

Table 1. Range and mean values of physico-chemical properties of water from different types of sources in Samburu South over the period of July 2004-July 2005.

Parameter	N	Range	Mean	Std. Deviation
Air temp. ºC	97	19.5 - 37.0	28.44	3.39
Rel humidity %	97	24 – 78	46.45	11.91
Water temp. ºC	95	10.0 - 35.6	26.66	4.32
El. cond µS cm^{-1}	97	147 – 2810	658.5	713.57
pH	97	5.50 - 9.20	7.814	.81
Salinity g L-1	97	0.0 - 1.4	0.4	0.41
Total alkalinity mg L^{-1} $CaCO_3$	97	144 – 1566	361.91	318.70
D O mg L^{-1}	97	0.5 -29	5.34	2.6

When the physico-chemical parameters were considered in categories according to source types using a one way analysis of variance (ANOVA), there was a highly significant variation among water sources in conductivity ($p<0.0001$) especially between dry river bed wells and laggas, and permanent rivers and boreholes. Alkalinity levels also differed significantly ($p<0.0001$) between all water source types and boreholes (Table 2).

Table 2. Mean values of selected physico-chemical properties of water and ambient environmental conditions at different types of sources in Samburu South.

	SOURCE					
PARAMETER	Dry river bed wells	Permanent rivers	Dams	Laggas	Boreholes	Springs
Air Temp (^{o}C)	27.6±3.4	28.2±3.1	28.4±2.9	31.6±2.4	30±1.6	26.8±3.6
Rel. Humidity (%)	43.5±9.4	47.7±14.7	45.3±13.9	50.7±11.4	44.2±12.2	50.1±6.9
Water color	Brown	Yellow	Brown	Yellow	Clear	Sparkling clear
Water Temp. (^{o}C)	27.5±3.7	25.9±4.8	27.3±3.6	27.7±3.9	28.2±0.5	22.3±5.9
Conductivity (μS cm^{-1})	1136.3^{ab}± 791.2	259.1^{c}± 183.7	348.8^{c}± 164.9	444.1^{bc}± 236.9b	2037.8^{a}± 63.5	552.8^{bc}± 535.0b
pH	7.7^{a}±0.7	7.9^{a}±0.8	8.4^{a}±0.6	7.6^{a}±0.8	7.5^{b}±0	7.1^{b}±1.0
D.Oxygen (mg L^{-1})	6.6(85.9)± 5.7(87.8)	6.88(79)± 2.5(31.9)	5.34(84.7)±2 .6(42.2)	4.12(51.5)±1 .9(22.2)	4.7(62.2)± 1.6(24)	6.8(53.2)± 5.4(27.9)
Salinity (g L^{-1})	0.6±0.4	0.3±0.4	0.3±0.3	0.5±0.4	0.9±0.2	0.2±0.2
Alkalinity (mg L^{-1})	357.6± 206.3^{b}	191.2± 141.5^{b}	257.6± 137.0^{b}	372.5± 168.9^{b}	1244.4± 572.2^{a}	475.5± 368.0^{b}

a-b: The same alphabet on different sample shows no significant difference ($p>0.05$) based on Tukey-Kramer test.

Air temperatures at the different water sources ranged from 19.5 ºC to 36.5 ºC. Spring water sources had the lowest mean temperatures readings of 26.8 ºC, while laggas had the highest mean temperatures of 31.6 ºC (Figure 2). There was no significant difference in air temperatures of the locations of different water sources (P=0.14, df =39) (Table 2).

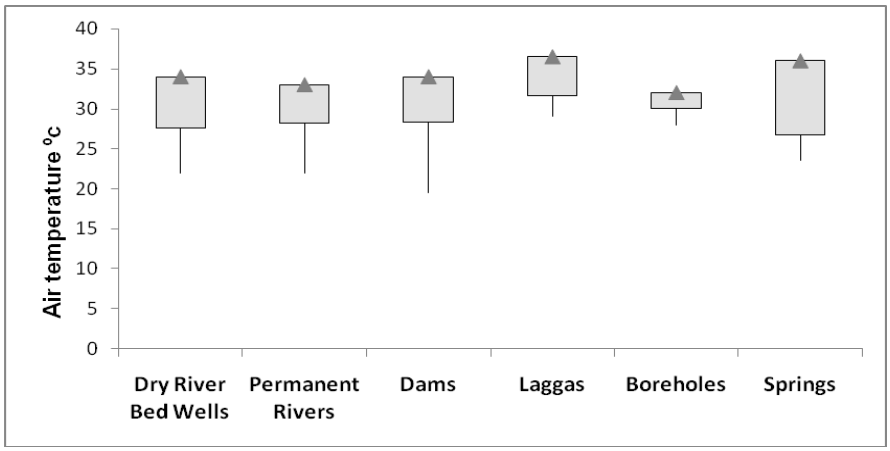

Figure 2. Air temperatures at different cartegories of water sources in Samburu South obtained over the period of July 2004-July2005.

~~Air temperatures at~~ a ~~fferent categories of water sources in Samburu South obtained over the period~~ ~~2004 July 2005~~**Legend**: a – Maximum value; b- Mean value; c – imum b lue

c

The relative humidity (RH) at the collection points of water was highest (78%) and lowest (24%) in dams (Table 2). Laggas, however, had the highest mean relative humidity of 50.8% while the lowest mean relative humidity was recorded in dry river bed wells (43.5%) (Figure 3). Based on one way ANOVA no significant difference in relative humidity among water sources was established (P=0.84, df =39).

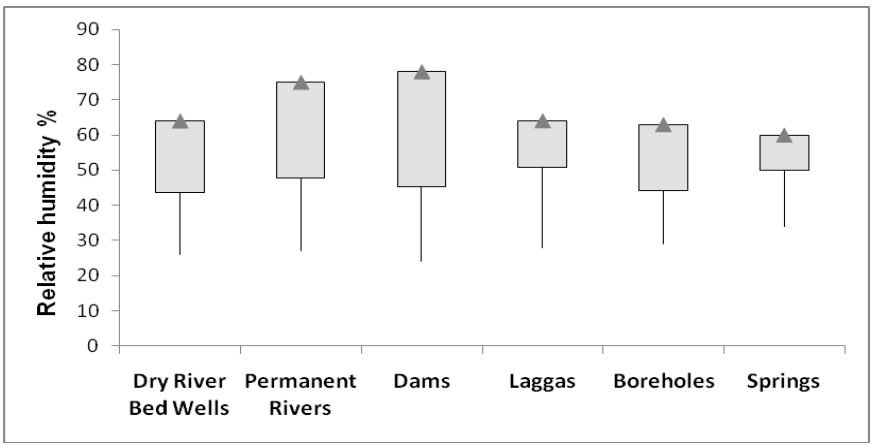

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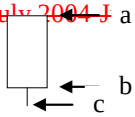
Figure 3. Relative humidity at different categories of water sources in Samburu South obtained over the period of July 2004-July 2005.

~~Relative humidity at different categories of water sources in Samburu South obtained over the period of July 2004-J a , 2005.~~

Legend: 

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Water temperature ranged from 35.6 ºC recorded in laggas to 16.4 ºC recorded in springs (Figure 4). Overall, boreholes had the highest mean water temperature of 28.2 ºC, while the lowest mean water temperature of 22.3 ºC was recorded in springs. Based on the one way ANOVA test, mean water temperature of the different categories of water sources was found to be insignificant (P=0.27, df =39).

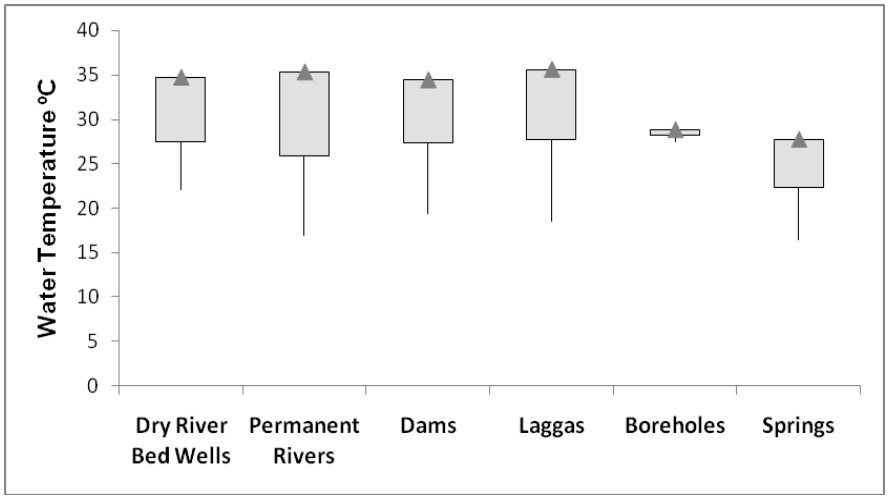

Figure 4. Water temperature of water from different categories of water sources in Samburu South during the period July 2004-July 2005.

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~~Water temp ture o f water from different categories of water sources in Samburu South during the period J c , 004-J~~**Legend**: a – Maximum value; b- Mean value; c – Minimum value

Electrical conductivity (EC) values ranged from 113 µS cm^{-1} to 2810 µS cm^{-1} (Figure 5). The lowest mean conductivity of 259.1 µS cm^{-1} was computed for the permanent rivers while the highest value of 2037.8 µS $cm^{-1}$was computed for the dry river bed wells. Using one way ANOVA test it was found that the difference in mean EC values of the different categories of water sources was highly significant (P=<0.001, df =39). Mean separation using (Tukey's) revealed that Boreholes and dry river bed wells had significantly higher conductivity than the other water sources while permanent rivers and dams had the lowest conductivity.

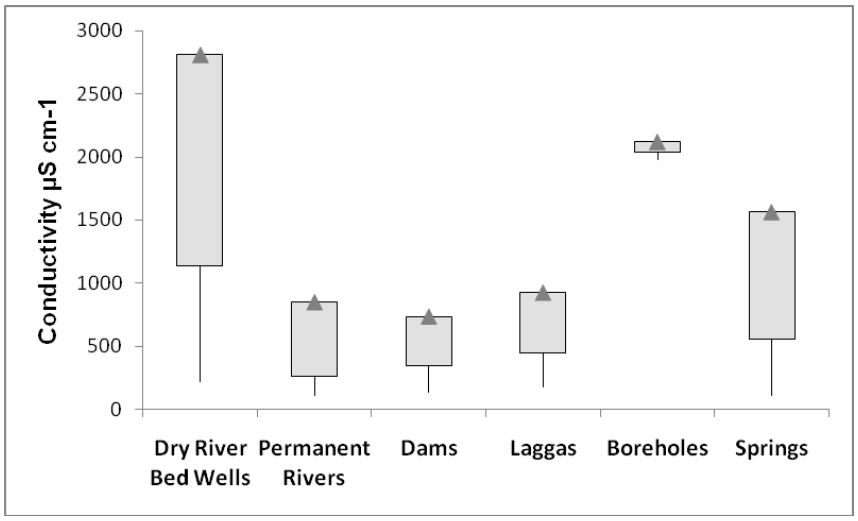
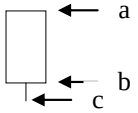


Figure 5. Electrical conductivity values of water from different categories of water sources in Samburu South obtained over the period July 2004-July 2005.

~~Electrical conductivity values of water from different categories of water sources in Samburu South obtained over the period July 2004-July 2005.~~

Legend: 

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Laggas had the lowest amounts of dissolved oxygen at 4.12 mg L^{-1}, while permanent rivers and springs had the highest mean amounts of dissolved oxygen, both at 6.8 mg L^{-1} ~~and 6.8 mg L^{-1} respectively~~. Springs, permanent rivers and boreholes had higher mean dissolved oxygen ratios compared to dry river bed wells 6.6 mg L^{-1}, dams 5.3 mg L^{-1} and laggas 4.1 mg L^{-1} (Table 2). There was no significant difference in amounts of dissolved oxygen among the water sources (P=0.3215, df=39).

Most pH readings of water from selected sources in Samburu South were in the range of 5.5 to 7.5 (Figure6). Dams and permanent rivers recorded the highest pH values (9.2) with a median pH of 8.6 for dams and 8.0 for permanent rivers. The lowest median pH values of 7.1 were recorded in springs ~~(Fig.6)~~.

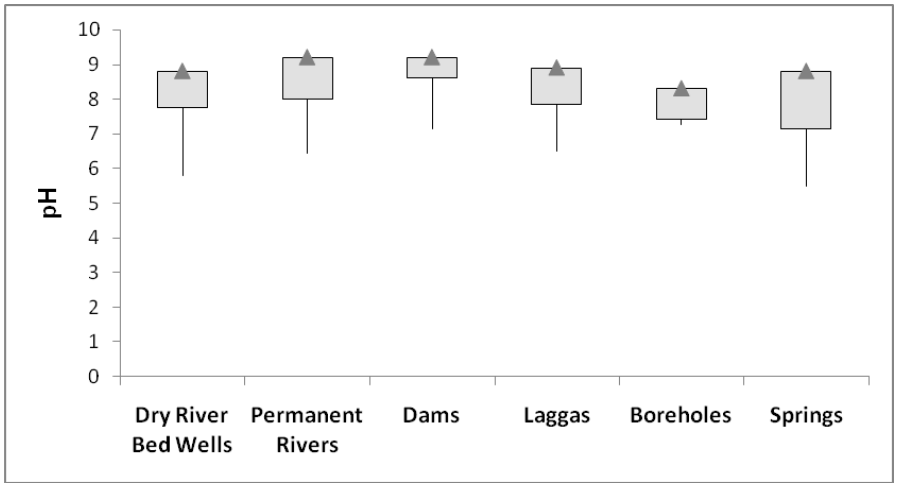

Figure 6. pH values of water from different categories of water sources in Samburu South obtained over the period of July 2004-July 2005.

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~~pH values of water from different categories of water sources in Samburu South obtained over the period of July 2004-July 2005.~~

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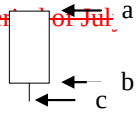
Legend:  a – Maximum value; b- Mean value; c – Minimum value

All categories of water sources except boreholes had salinity values ranging from below the limit of detection (0.0 g L^{-1}) to a maximum salinity of 1.4 g L^{-1} recorded in the dry river bed wells. All sources had mean salinity values less than 1 g L^{-1}. The highest mean salinity of 0.9 g L^{-1} was recorded in boreholes, while the lowest was 0.1 g L^{-1} among springs (Figure7). Based on the one way ANOVA test, mean salinity of water from the different categories of water sources was ~~found to be~~ not significant (P=0.19, df =39).

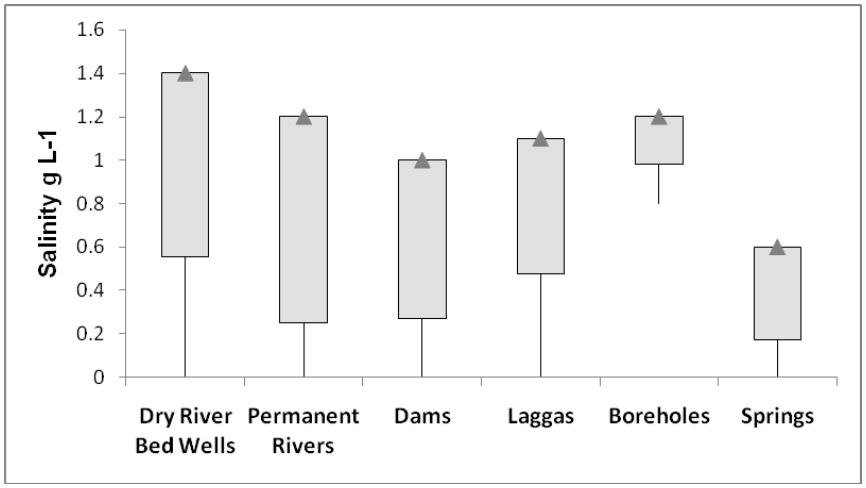
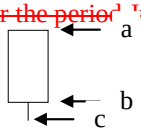


Figure 7. Salinity values of water from different categories of water sources in Samburu South obtained over the period July 2004-July 2005.

~~Salinity values of water from different categories of water sources in Samburu South obtained over the perior' 'uly 2004 July 2005.~~

Legend:  a – Maximum value; b- Mean value; c – Minimum value

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Alkalinity levels of water from different categories of water sources ranged from 144 mg L^{-1} to 1566 mg L^{-1}. Boreholes had the highest mean alkalinity (1244.4 mg L^{-1}) while permanent rivers had the lowest mean level (191.2 mg L^{-1}) (Figure 8). Using a one way ANOVA test, mean alkalinity levels of the different categories of water sources was found to be highly significant ($P<0.001$, df =39). Mean separation using Tukey's test revealed that boreholes had significantly higher alkalinity than the other water sources while permanent rivers had the lowest alkalinity.

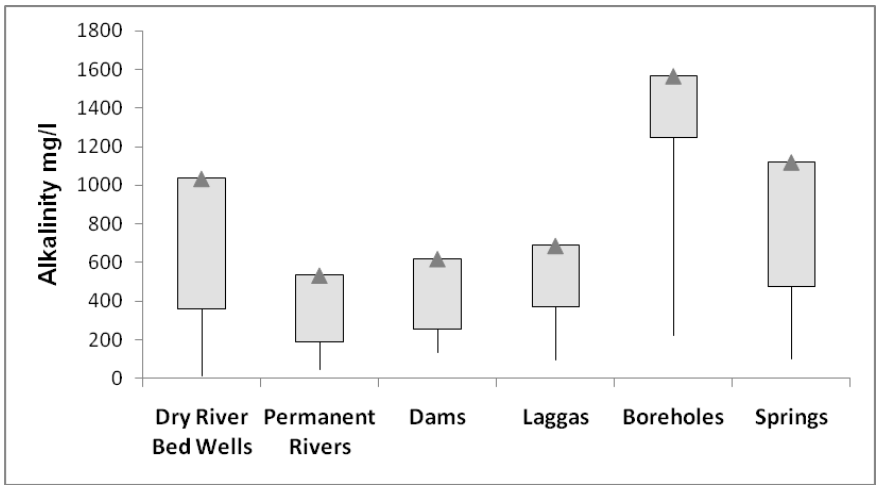
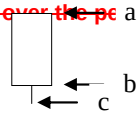


Figure. 8. Alkalinity level values of water from different categories of water sources in Samburu South obtained over the period July 2004-July 2005.

~~**Alkalinity level values of water from different categories of water sources in Samburu South obtained over the pe a rd July 2004-July 2005.**~~

Legend:  a – Maximum value; b- Mean value; c – Minimum value

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4.2 Bacterial quality of water

Total coliform numbers expressed as the most probable number (MPN) per 1 mL of water ranged from 4 in boreholes and springs to >2400 mL^{-1} in dry river bed wells, permanent rivers, dams and laggas (Figure9). Mean MPN per ~~1~~mL of water was highest in dams at 643 ± 444 and lowest in springs at 35 ± 186 (Table 3). Based on a one way ANOVA test it was found that there was a significant difference in MPN of water from the different categories of water sources (P=0.005, df=39). Mean separation using the Tukey- Kramer test revealed that the significant difference in MPN occurred between dry river bed wells and dams (Table 3).

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Total plate counts (TPC) in water from the various sources ranged from 2 cfu mL^{-1} in permanent rivers to 240 cfu mL^{-1} in laggas (Figure10). Mean TPC ranged between 13 ± 7 in boreholes to 241 ± 27 in laggas. A one way ANOVA test revealed a significant difference in TPC of water from the different categories of water sources (P<0.001, df = 39). Mean separation using the Tukey Kramer test revealed that the significant difference occurred between laggas and all the other categories of water sources (Table 3).

Table 3. Mean counts of selected water quality indicators in different categories of water sources investigated in Samburu South.

Source	MPN	TPC	FColi 44
Dry River Beds	485^{b}±438	71^{b}±55	16±14
Permanent Rivers	504^{b}±417	51^{b}±52	9±8
Dams	643^{a}±444	89^{a}±67	21±18

Laggas	186[b]±370	241[a]±27	21±13
Boreholes	35[b]±64	13[b]±7	1±2
Springs	35[b]±186	24[b]±12	4±3

a-b: The same alphabet on different sample shows no significant difference (p>0.05).

The most probable number of bacteria per 1 mL of water ranged from 4 in boreholes and springs to >2400/mL in dry river bed wells, permanent rivers, dams and laggas (Figure9). Based on a one way ANOVA test, it was found that there was a significant difference in the MPN of water from the different categories of sources (P=0.005, df=39). Mean separation using the Tukey- Kramer test revealed that the significant difference in MPN occurred between dry river bed wells and dams (Table 3).

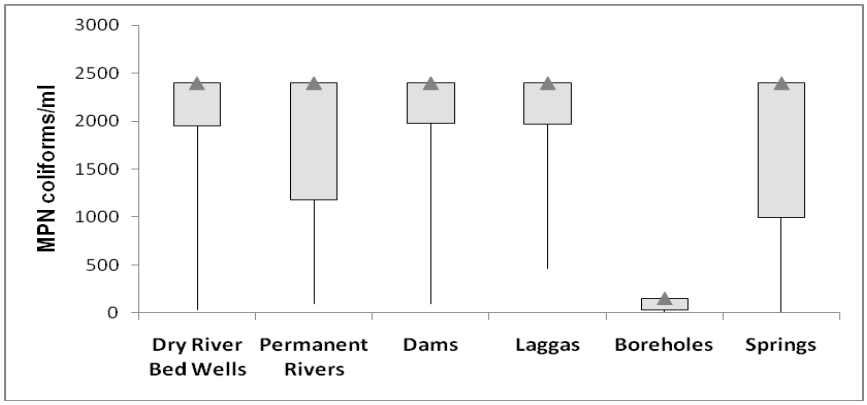

Figure 9. Most probable numbers (MPN) of coliforms in water from different categories of water sources in Samburu South over the period July 2004 - July 2005.

~~Most probable numb~~ a ~~s (MPN) of coliforms in water from different categories of water sources in S~~ ~~buru South over the period July 2004 - July 2005.~~

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Total plate counts ranged from 2 cfu mL^{-1} in permanent rivers to 240 cfu/mL^{-1} in laggas (Figure 10). A one way ANOVA test revealed a significant difference in TPC of water from the different categories of sources ($P<0.001$, df=39). Mean separation using the Tukey Kramer test revealed that the significant difference occurred between laggas and all the other categories of water sources (Table 3).

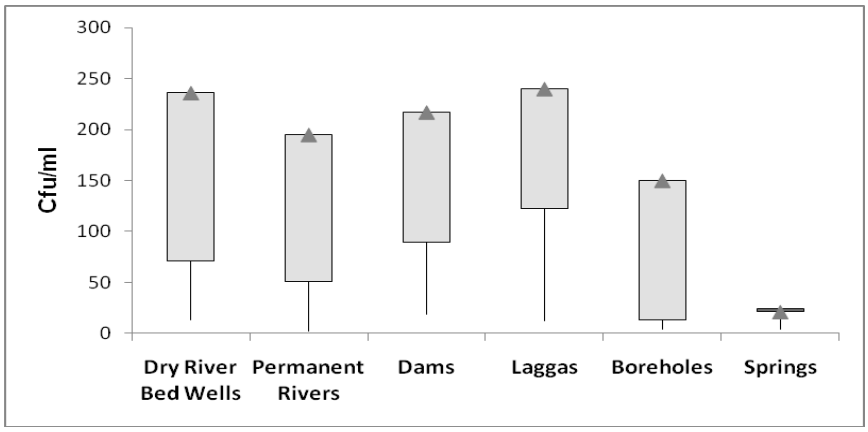

Figure 10. ~~**Total plate counts (TPC) in different categories of water sources in Samburu South over the period July 2004-July 2005.**~~ Total plate counts (TPC) in different categories of water sources in Samburu South over the period July 2004-July 2005.

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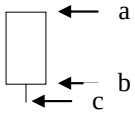

a – Maximum value; b- Mean value; c – Minimum value

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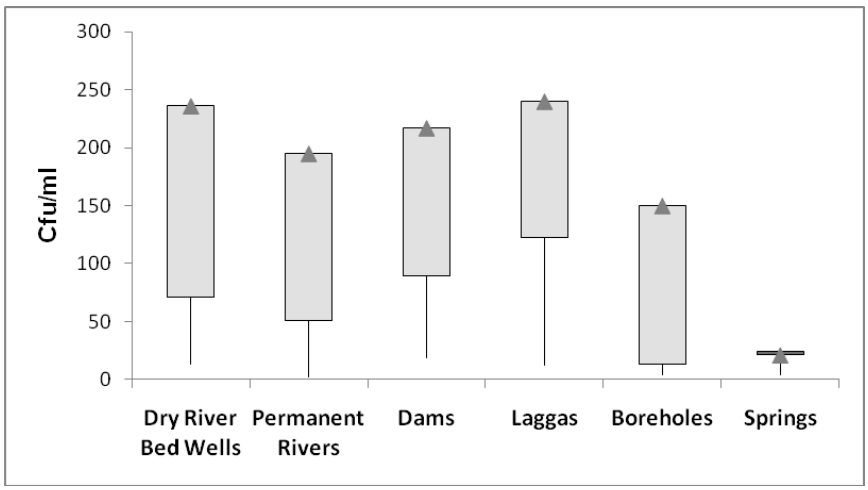
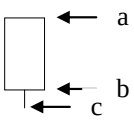
The highest number of faecal coliforms per 1 mL of water of 75 cfu mL^{-1} was recorded in a dam sample, while some water samples from springs, boreholes, dry rivers beds and permanent rivers registered no faecal coliforms. Mean faecal coliform counts were lowest in boreholes(1) and highest in dams (21), which are temporary water source(Figure 11).

Using a one way ANOVA test revealed that the mean faecal coliform counts in water from the different ~~categories of~~ sources was insignificant (p=0.726, df=39).



~~Figure 11.~~ Figure 11. Number of faecal coliforms in water from different categories of water sources in Samburu South obtained over the period July 2004-July 2005.

~~**Number of faecal coliforms in water from different categories of water sources in Samburu South obtained over the period July 2004-July 2005.**~~

Legend:  a – Maximum value; b- Mean value; c – Minimum value

Pearson's linear correlation test was carried out to compare the relations between the physico-chemical properties of water and its bacterial quality. The results of the correlation test revealed a correlation that was not significant (P >0.5) for all cases except between conductivity and TPC (Table 4).

Table 4. Correlation between MPN, TPC and feacal coliforms and the physico-chemical properties of water in Samburu district.

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	MPN		TPC		Feacal coliforms	
Parameter	r	P	r	P	r	P
Air Temp	0.048	0.891	0.162	0.386	0.073	0.189
RH%	-0.152	0.563	0.003	0.297	0.053	0.733
Water Temp	0.491	0.491	0.381	0.592	0.280	0.273
Conductivity	-0.003	0.186	-0.258	0.010	-0.259	0.476
pH	0.027	0.972	0.028	0.603	0.139	0.253
Dis. Oxygen	-0.042	0.604	0.029	0.816	-0.116	0.593
Salinity	0.069	0.812	0.065	0.537	-0.076	0.763
Alkalinity	0.026	0.553	-0.016	0.282	-0.253	0.558

The frequency of occurrence of *E .coli* in water from the different sources varied widely (Table 5). The highest occurrence was among the dry river bed wells with *E. coli* occurring in 20 out of the 28 wells, followed by dams with 17 out of the 20 dams. The least occurrence of *E. coli* was in springs, where they were present in 3 out of 8 spring water sources. *E. coli* was not detected in boreholes.

Table 5. Frequency of occurrence of *E. coli* in water from different categories of water sources in Samburu South over the period July 2004-July 2005

Source	Dry River Bed Wells	Permanent rivers	Dams	Laggas	Springs	Borehole
Present	20	7	17	8	3	0
Absent	8	13	3	4	7	6
Total	28	20	20	12	10	6

4.3 Pathogenic Bacterial Isolates in Samburu district water from various sources

S. flexineri was isolated in 19 dry river bed wells, 12 dams and 2 laggas. *A. hydrophilla* was recovered in 2 dry river bed wells, 1 permanent river, 2 dams and 1 lagga (Figure 12).

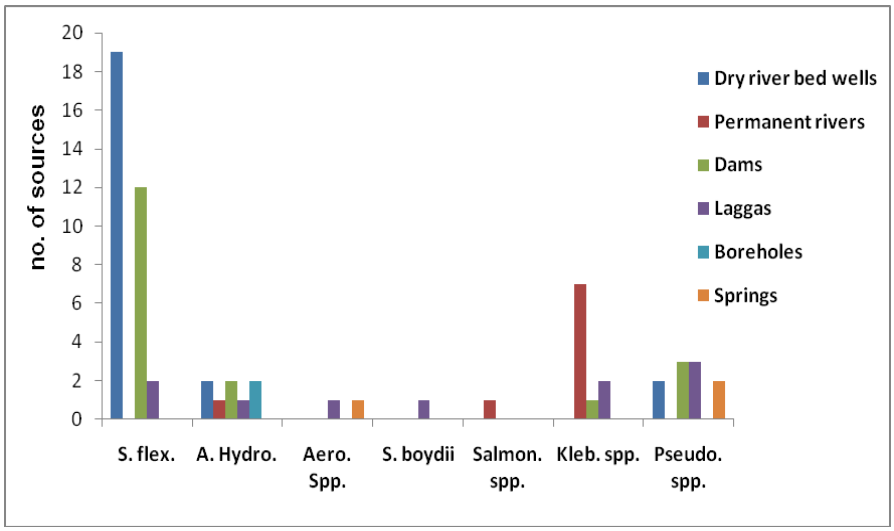

Figure 12. Number of water sources in each category from which specific pathogens were recovered.
~~Number of water sources in each category from which specific pathogens were recovered.~~

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On the other hand, water from 16 sources, (boreholes and springs) registered the presence of *Pseudomonas spp.*, *Aeromonas spp*. and had the least frequency of occurrence of pathogenic bacterial isolates. Thirty one out of the forty three sources that recorded brown water eventually had *E. coli* and 19 of these eventually yielded *S. flexineri.* Water was clear in 22 sources but only five of these had *E. coli* and *Klebsiella spp*. which were recovered in three of these sources (Table 6).

Table 6. Frequency of occurrence of pathogenic bacteria in various color categories of water from different sources

Color	*S. flex*	*E. coli*	*Pseudo. spp.*	*Kleb. Spp.*	*S. boydii*	*A. hydro.*	*Aero. Spp.*	*Sal. spp.*
Green (N=7)	1	2	2	1	-	1	1	-
Yellow (N=24)	12	17	4	4	1	2	-	-
Brown (N=43)	19	31	2	2	-	2	-	-
Clear (N=22)	2	5	2	3	-	1	1	1

4.4 Antibiotic sensitivity profiles

The results indicate that the highest average ampicillin, cefuroxime, ciprofloxacin, amoxicillin and gentamicin sensitivities were recorded in dams. The highest average cotrimoxazole and chloramphenicol readings are found in the permanent rivers while the highest average cefutaxime reading ~~wa~~were recorded in the dry river beds.

The clear areas around the discs (Plate 1) impregnated with antibiotics are as a result of inhibition of bacterial growth by the antibiotic. Growth begins at a distance from the disc at which the concentration is not sufficient to arrest bacterial growth. The diameters of the zones of inhibition of all the antibiotics assayed were above the lower limit of the breakpoints (Appendix I). The isolate (*Salmonella spp*.) is therefore susceptible to gentamicin, amoxacillin, ampicillin, cefutaxime and ciprofloxacin.

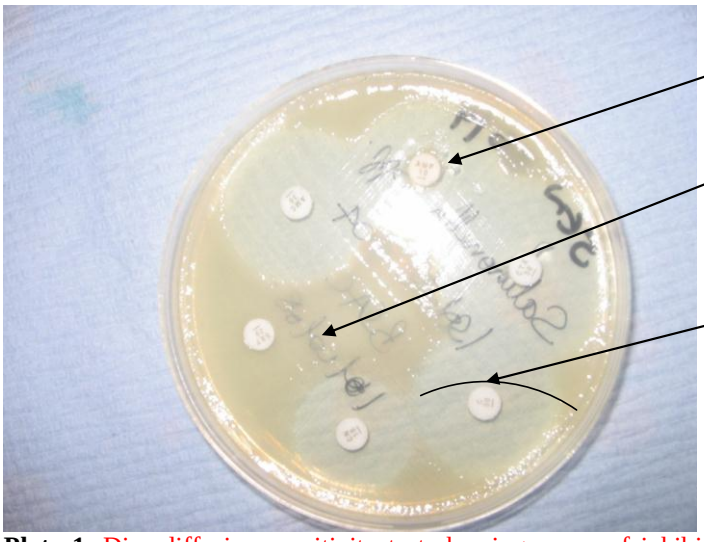

Plate 1. Disc diffusion sensitivity test showing zones of inhibition of *Salmonella* none *typhi* to commonly used antibiotics. ~~Disc diffusion sensitivity test showing zones of inhibition of *Salmonella* non *typhi* to commonly used antibiotics.~~

Legend: The clear areas around the discs impregnated with antibiotics are as a result of inhibition of bacterial growth by the antibiotic.

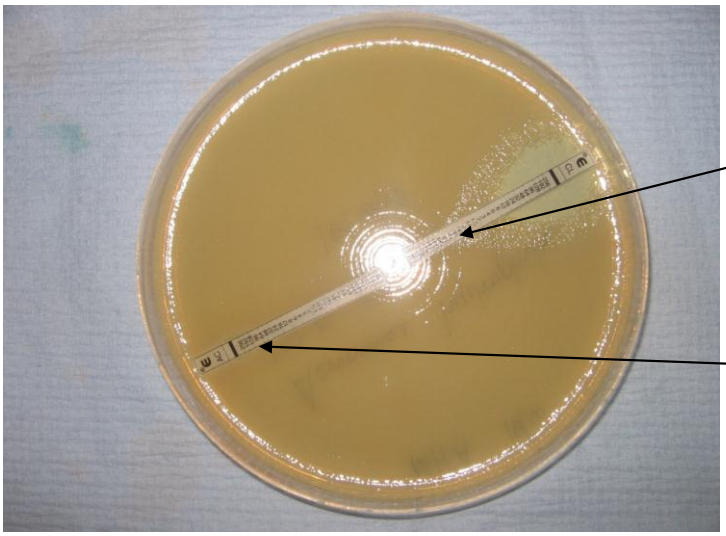

Plate 2. E-test showing *S. flexineri* isolate resistant to ampicillin and chloramphenicol. ~~E test showing *S. flexineri* isolate susceptible to ampicillin and resistant to chloramphenicol.~~

Legend: The Minimum Inhibition Concentrations are read as the concentrations of antibiotics at the points where the zones of inhibition intersect with the strips. Isolates were classified as susceptible, intermediate or resistant according to the guidelines of the National Committee for Clinical Laboratory Standards (2004).

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The E-strip is impregnated with antibiotics along a concentration gradient. The growth zones around the strip occurs at concentrations that can inhibit bacterial growth, while at the portion of the strip where antibiotic concentration are high enough to inhibit growth, clear zones of inhibition are formed (Plate 2).

The highest average MIC values were noted for isolates from dams, followed by dry river beds, laggas and lastly permanent rivers. Regardless of source category *S. flexineri* isolates were susceptible to ciprofloxacin whose lower limit breakpoint is 20 mg L^{-1} (Figure15).

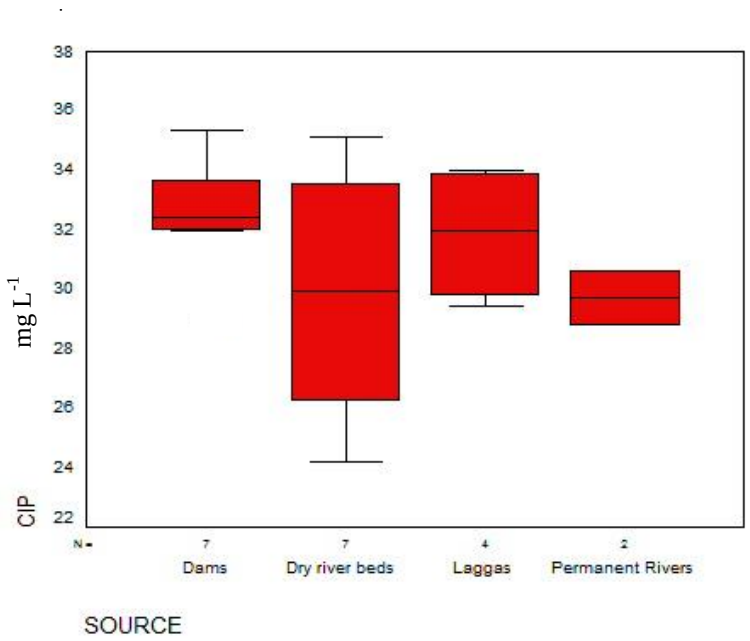
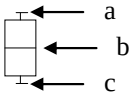


Figure 13. ~~Mean sensitivity of all types of pathogenic isolates from different categories of water sources to Ciprofloxacin~~. Mean sensitivity of all types of pathogenic bacterial isolates from different categories of water sources to Ciprofloxacin

Legend: 

a – Maximum value; b- Mean value; c – Minimum value

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S. flexineri was susceptible to Amoxacillin, Cefutaxime, Gentamicin, Ciprofloxacin, Cefutaxime and Trimethoprim. All isolates were resistant to Cotrimoxazole and Chloramphenicol. Of the 12 isolates assayed, 2 were resistant to Ampicillin, the antibiotic recommended for the treatment of dysentery. There was no significant difference in the sensitivity of *S. flexineri* to commonly used antibiotics (P=0.1102) (Figure14).

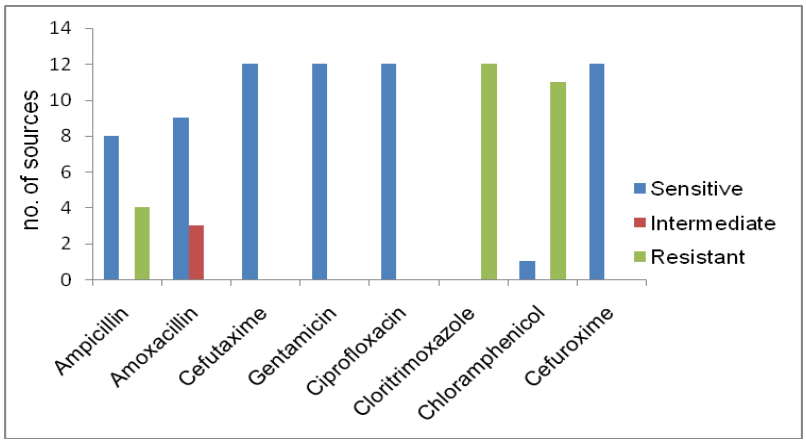

Figure 14.~~ *S. flexineri* isolates sensitivity to commonly used antibiotics.~~
S. flexineri isolates sensitivity to commonly used antibiotics.

Two isolates of *Aeromonas spp* isolated from a spring and lagga (Figure12) were resistant to ampicillin and amoxicillin, and susceptible to cefutaxime, gentamicin, ciprofloxacin, cotrimoxazole, chloramphenicol, cefuroxime and trimethoprim using both the E-test for MIC and the disc diffusion test (Figure 15).

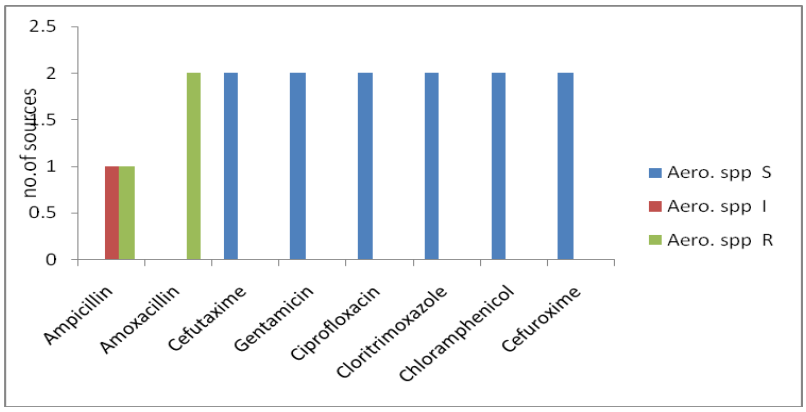

Figure 15. Sensitivity of *Aeromonas spp.* isolates to commonly used antibiotics.

~~**Sensitivity of *Aeromonas spp.* isolates to commonly used antibiotics.**~~

Isolates of *A. hydrophilla* from different categories of water sources had similar patterns of sensitivity to antibiotics. All the five isolates were resistant to Ampicillin and Amoxicillin, and susceptible to Cefutaxime, Gentamicin, Ciprofloxacin, Cotrimoxazole, Chloramphenicol, Cefuroxime and Trimethoprim using both the E-test for MIC and the disc diffusion test (Figure 16).

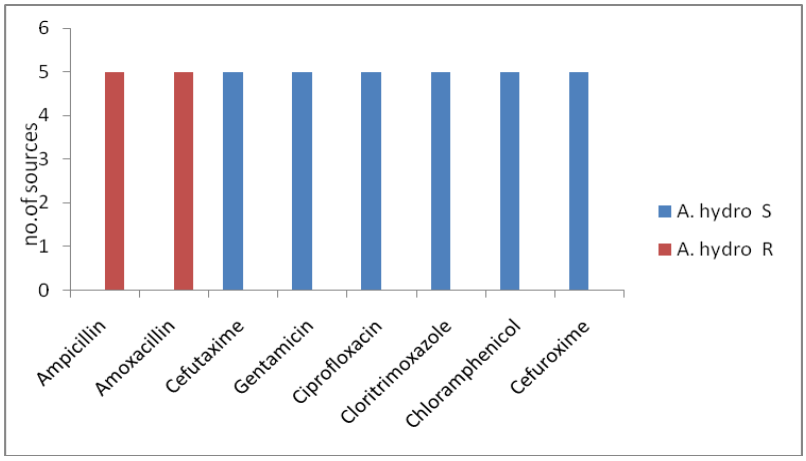

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Figure 16. Sensitivity of *Aeromonas hydrophilla* isolates to commonly used antibiotics. ~~Sensitivity of *Aeromonas hydrophilla* isolates to commonly used antibiotics.~~

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The *S. bodyii* from lagga was resistant to Ampicillin by disc diffusion antibiotic sensitivity test. It was, however, susceptible to Amoxacillin, Cefutaxime, Gentamicin, Ciprofloxacin, Chloramphenicol and Cefuroxime. Using the minimum inhibitory test, *S. boydii* was found to be resistant to Ampicillin and susceptible to Chloramphenicol and Trimethoprim (Figure 17).

The *Salmonella spp*. isolate from the permanent river source was susceptible to all the assayed antibiotics using both ~~both~~ the disc diffusion and minimum inhibitory concentration tests (Figure 17).

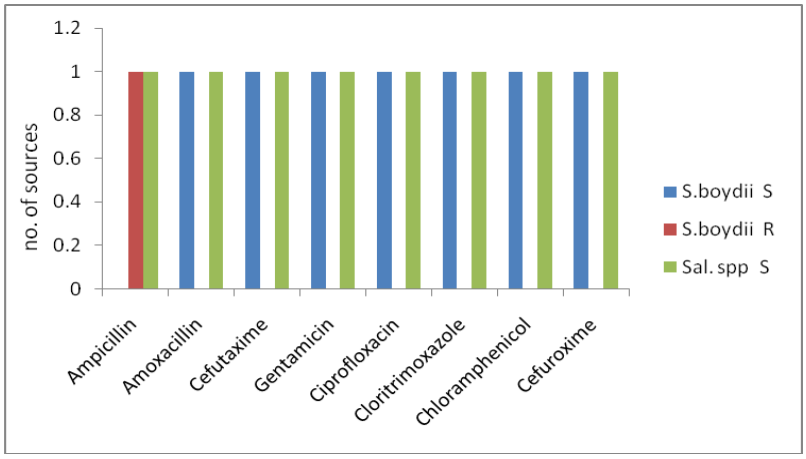

Figure 17. *Shigella boydii* and *Salmonella spp.* isolates sensitivity to commonly used antibiotics. ~~*Shigella boydii* and *Salmonella spp.* isolates sensitivity to commonly used antibiotics~~

~~.~~

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Using the minimum inhibitory concentration test, all isolated pathogens were sensitive to Trimethoprim except one *S. flexineri* isolate which displayed resistance. All *Shigella spp.*

displayed complete resistance to Chloramphenicol. However, Ampicillin had mixed outcomes; four *S. flexineri* isolates were sensitive while eight were resistant, *A. hydrophilla* and *Salmonella spp*. were sensitive to Ampicillin while one *S. boydii* isolate was resistant to Ampicillin (Figure 18).

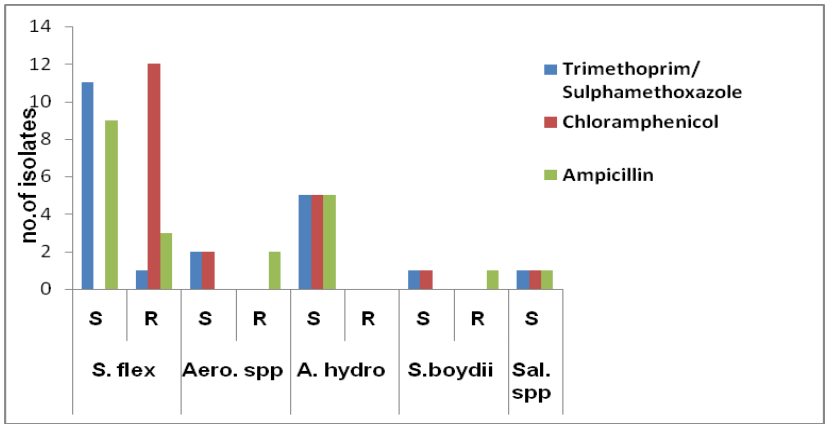

Figure 18. Sensitivity of pathogenic isolates to commonly used antibiotics using *E*-test.~~Sensitivity of pathogenic isolates to commonly used antibiotics using *E* test.~~

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All the isolates of *S. flexineri* recovered from laggas were sensitive to Ampicillin (inhibition zone diameters <8 mg L^{-1}). The isolates recovered from some dams and dry river bed wells however, displayed intermediate sensitivity as well as resistance to Ampicillin (inhibition zone diameters >16 mg L^{-1}) (Figure21).

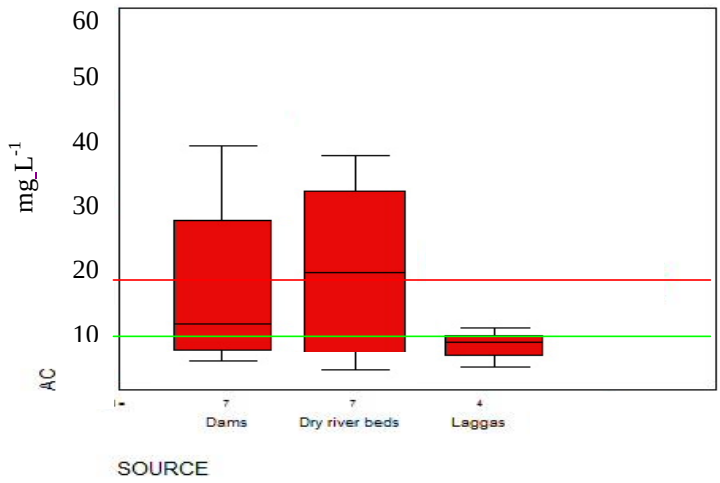

Figure 19. Mean sensitivity of *S. flexineri* from different categories of water sources to ampicillin ~~Mean sensitivity of *S. flexineri* from different categories of water sources to ampicillin~~

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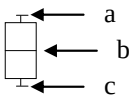
Legend: 

a – Maximum value; b- Mean value; c – Minimum value

4.5 Pulsed Field Gel Electrophoregram (PFGE) of *Shigella flexineri* isolates

The PFGE profile shows that the pulsed field profiles of *S. flexineri* isolates sensitive to Ampicillin and isolates displaying resistance to Ampicillin gave at least 12 to 14 resolvable fragments ranging from 31 kb to 725 kb. Isolates 4 and 6 had 14 visible fragments and the 2 additional unstable bands in their *Not*I PFGE types (Figure22).

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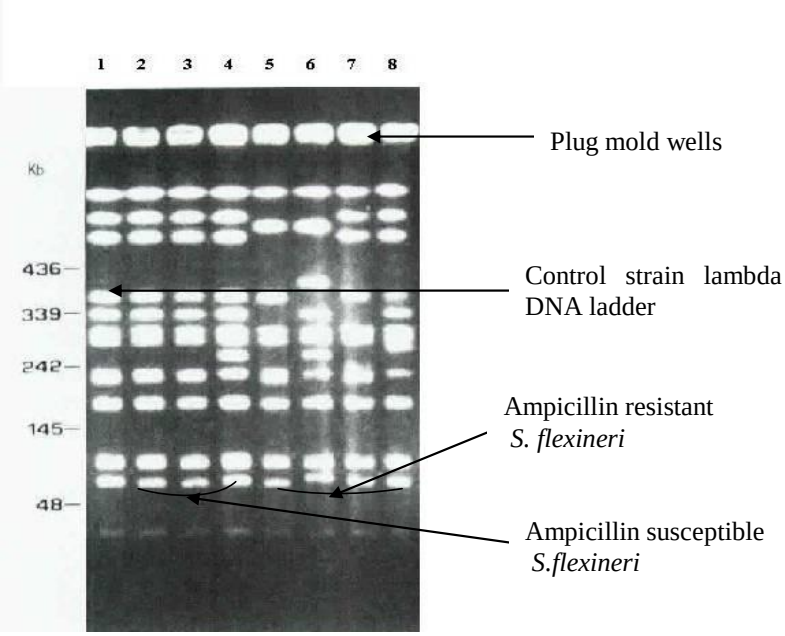

Plate 3. PFGE profiles of *S. flexineri* genomic DNA digested by *Not*I restriction enzyme.~~PFGE profiles of *S. flexineri* genomic DNA digested by *NotI* restriction enzyme.~~

Legend: Tracks1 – 8 contained: *S. flexineri* pulsed-field profiles (PFP). PFP 1 was control type. PFP 2-4 *S. flexineri* isolates susceptible to ampicillin. PFP 5-8 *S. flexineri* isolates resistant to ampicillin. PFGE patterns were compared visually for similarities.

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CHAPTER FIVE

÷ DISCUSSION

5.1 Physico-chemical properties

The physico-chemical properties of water such as pH, salinity, temperature, dissolved oxygen, conductivity and alkalinity provides useful information on water quality or the potential of water to support bacterial growth.

The color of drinking water is usually due to a variety of factors that include presence of colored organic mater associated with the humus fraction of soil. Color is strongly influenced by the presence of iron and manganese, humus and peat, plankton and weeds. pH also influences the color of water. No health-based guideline value is proposed for the color of drinking water (WHO, 2003).

Water from the various categories of sources yielded a wide range of color observations. A general assumption is that the less clear the water, the more likely it is to be contaminated with bacteria. In the present study, water sources with least bacterial contamination were springs and boreholes. This is because these sources exhibit a complete absence of *E. coli* and consequently with a result of no pathogen was isolated. However, water from some sources was clear but recorded a presence of *E. coli* and other bacteria such as *A. Hydrophilla*, *Salmonella* non *typhi* and *Pseudomonas spp*. There were also cases where nonclear water samples exhibited some characteristics of good quality water. For example, water samples from dry river bed wells were brown in color, yet *E. coli* was absent, with a limited result of *Pseudomonas spp*. However, due to its color, it is likely to be assumed to be contaminated. It is therefore important to note that water clarity does not necessarily translate to good water quality. A few water samples exhibited the characteristic of clarity

but failed to be free of pathogenic bacteria. Hence using water color to determine the water quality may not be a useful index of water quality but it may be the first alarm for a potential health hazard.

Cool water is generally more palatable than warm water. High water temperatures enhance the growth of microorganisms and may increase taste, odor and color (WHO, 2003). The average ~~water~~ temperature of water from different source types in Samburu district was 26.66 ºC + 4.32. This temperature is probably due to the high air temperature and reduced vegetation cover. There was no significant difference in the mean temperature of water from different sources since it had a narrow range of between 22.3^{0o}C+5.9 in springs and 28.2^{o0}C+0.5 in bore holes. Most springs occur in the hilly regions that have a higher vegetation cover which may lead to the lower temperatures. The borehole water may derive the higher temperatures from the heat retained in the subsoil after exposure to the sun. The highest water temperature recorded in laggas can be attributed to both the high air temperatures and the heat derived from the warming of the topsoil. Kenya bureau of standards, and WHO, have no set standards on the temperature of drinking water, and only recommend that it should be cool. The European Community, however, uses a guide of 12ºC and a maximum of 25ºC.

Changes in pH can be caused by a range of water quality changes such as acid sulfate runoff. Extremes of pH (less than 6.5 or greater than 9) can be toxic to aquatic organisms. Eye irritation and exacerbation of skin disorders have been associated with pH values greater than 11. Although pH has no direct impact on consumers, it is one of the most important operational water quality parameters (WHO, 2003). Median pH values of water

in Samburu district regardless of the type of source are in the neutral range of 7.8. The highest pH value recorded was in dams while the lowest pH values were recorded among dry river beds. The high water pH may probably be due to the photosynthetic uptake of CO_2. Dams recorded pH values as high as 9.2 with a median of 8.6. Higher pH values in dams are probably due to their exposed nature that allows for CO_2 uptake by photosynthetic organisms leading to a rise in pH. The slightly alkaline water pH recorded in dams is similar to that of other reservoirs in different parts of the world Thornton (1987) recorded a water pH range of between 6.4 and 9.1 in three Zimbabwean reservoirs. According to Moehl and Davies, (1993), the typical pH range in water reservoirs is usually between 5 and 10. However, significant short term variations can occur due to change in photosynthetic intensity. According to Hunter, (1993), the amounts of ions carried by ground water through seepage is typically much higher than through surface water and this could have a profound influence on water pH.

The proposed WHO guideline for pH is 6.5 - 9.0 while the Kenya Bureau of standards (2005) recommends a pH of 6.5 – 8.5 for drinking water. As such, 13% of the water sources in Samburu district do not meet the KEBS standards for drinking water.

The mean total alkalinity levels recorded in water from the various categories of sources in Samburu District was 361.914 mg L^{-1} $CaCO_3$. Mean total alkalinity levels were highest in boreholes (1244.4 mg L^{-1} $CaCO_{3)}$ and lowest in permanent rivers at 191.2 mg L^{-1} $CaCO_3$. There was a significant difference in total alkalinity among the water sources ($p < 0.0001$, df 39) with boreholes displaying significant difference from other water sources which may be due to accumulated ions leached in from the surrounding subsoil. The observations made during this study suggest~~s~~ that *S. flexineri* can survive in waters with low total

alkalinity values as its presence was recorded in samples with the lowest total alkalinity values of 144 mg L^{-1} $CaCO_3$. However, it is important to note that *S. flexineri* was more frequent in water samples with total alkalinity values of between 954 mg L^{-1} and 5254 mg L^{-1}.

The maximum observed level of water salinity was 1.4 g L^{-1} while the lowest was below the limit of detection by the meter used. Though concentrations of sodium in potable water are typically less than 20 mg L^{-1}, they can greatly exceed this in some countries. *S. flexineri* were recovered in sources with salinity levels ranging from 0.1 g L^{-1} to 1.4 g L^{-1}. The highest level of water salinity from which the *Klebsiella spp.* was recovered was 1.2 g L^{-1}, while the lowest was below the detection limit. In a study carried out in rivers in Ghana, salinity and alkalinity were 0.4 mg L^{-1} and 361.91 µS cm^{-1} $CaCO_3$ both in the range of fresh water respectively (Chapman, 1992). The proposed guideline by WHO and KEBS for drinking water salinity is not more than 250 mg L^{-1} and only springs meet this standard. Soils are often naturally saline in arid areas and in Samburu the soils serve as natural salt licks. Salts are likely to get leached into the subsoil accounting for the higher salinity levels in boreholes. Reservoir evaporation concentrates further the level of salts. The massive amounts of evaporation from the reservoirs caused by the high ambient temperatures are likely to concentrate the salts in open sources with large exposed surface areas.

Dissolved oxygen is essential for life processes of most aquatic organisms and is vital to enable bacteria to break down organic detritus. The presence of an adequate level of dissolved oxygen in a river is one of the main indicators of good water quality. Low concentrations of dissolved oxygen usually indicate the presence of organic matter in the

water system, while high values can indicate a high plant production (usually associated with eutrophication). Many aquatic organisms will suffocate if there is insufficient amount of dissolved oxygen and such water is unfit for drinking. During the study, mean dissolved oxygen varied between 4.1 mg L^{-1} in laggas and 6.81 mg L^{-1} in springs. Dissolved oxygen concentrations in unpolluted water are normally about 8.0 - 10 mg L^{-1} at 25^0C (DFID, 1999). High dissolved oxygen levels are probably sustained through the photosynthetic activity of both algae and submerged macrophytes which are known to improve dissolved oxygen content in water reservoirs. Lower dissolved oxygen recorded in laggas is probably because of the limited contact with air and low photosynthetic activity.

Conductivity is a measure of the amount of dissolved salts in the water, and therefore an indicator of salinity. Regardless of source type, the mean conductivity of water in Samburu south was 658 µS cm^{-1}. Lowest mean conductivity was in permanent rivers (259.1 µS cm^{-1}) and the highest was 2037.8 µS cm^{-1} in borehole water. The high conductivity was probably due to the high concentration of mineral salts in the boreholes which are in constant contact with the subsoil. Boreholes had the highest mean salinity of 0.9 g L^{-1} and correspondingly highest mean conductivity, which differed significantly from that of dams, laggas and permanent rivers. However, according to Chapman (1992) conductivity of most fresh waters range from 10- 1000 µS cm^{-1}, but may exceed 1000 µS cm^{-1} especially in polluted waters or those receiving large quantities of land runoff. Maithya (1998) recorded 640 µS cm^{-1} at Tinga dam near Lake Victoria. The differences are probably associated with environmental factors such as catchment geology and vegetation cover, climate and runoff quality. The low electrical conductivity of less than 1000 µS cm^{-1} suggests low ionic activity and possibly less dissolved materials (Mato, 2002). The low conductivities with no marked variability may also indicate that there is no

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contamination with effluent such as municipal or industrial wastewater. Thornton (1987) examined water quality of 64 reservoirs in Africa and found the overall conductivity range to be 10 - 800 μS cm^{-1}. All water source types in Samburu district meet the WHO standard of not more than 2000 μS cm^{-1} except boreholes.

5.2 Indicator organisms

Monitoring the levels of indicator bacteria in water provides a dependable safety factor because of their large numbers in contaminated waters; a feature that has been reinforced over many years of experience (WHO, 2003). On the basis of the most probable numbers of coliforms per ~~1~~ mL of water, only borehole water is fit for human consumption. The other sources had MPNs higher than 100 coliforms per mL of water with some having > 2400 coliforms per mL ~~of water~~ and therefore do not meet WHO and KEBS standards for drinking water.

Based on the total plate count, 85% of the water sources were found to be unfit for human consumption. Overall, only borehole and spring waters w~~a~~ere fit for human consumption while the rest do meet the WHO and KEBS standards for potability. Boreholes by their very nature are physically protected from contamination due to lack of accessibility by man and animals. Spring water also arises from underground and is therefore less likely to be contaminated compared to dams, rivers and laggas which are open to man and animals. Thermotolerant coliforms other than *E. coli* may also originate from organically enriched water such as industrial effluents or from decaying plant materials and soils (Grabow, 1996).

According to WHO (2004), fecal coliforms should not be detectable in potable water (should record zero cfu per 100 mL of water) (Appendix II). Hence the presence of feacal coliforms in all categories of water sources except boreholes, confirms that most water sources are not fit for human consumption. This therefore means that the direct use of natural water sources in Samburu district poses a health risk to the consumers. The study further revealed that there was a high chance of recovering *S. flexineri* and other pathogens in the presence of high levels of the feacal coliforms and where *E. coli* are present. Out of 45 samples of brown water, 31 showed presence of *E. coli* and 25 of these eventually yielded pathogenic bacteria, whereas of the 22 sources with clear water, only 3 recorded *E. coli*. Similarly pathogens were isolated from 8 sources although only 3 sources yielded pathogens of clinical significance. According to Sueiro *et al*., (2001), *E. coli* is a good indicator of feacal contamination, provides conclusive evidence of recent feacal pollution, and should not be present in drinking water.

5.3 Pathogenic bacteria

According to the guidelines for drinking water quality, there is no tolerable lower limit for pathogens in water intended for consumption, preparing food, drink or for personal hygiene; it should contain no agents pathogenic to humans (WHO, 2003 and KEBS, 2005). In the present study, *S. flexineri* was recorded in dams, laggas and dry river bed wells. Pathogenic isolates of very minimal clinical significance (*Klebsiella* and *Pseudomonas spp*.) were recorded in 70% of permanent rivers, springs and boreholes. *Pseudomonas spp*. is in the normal micro flora in human and animals (Shimeld, 1999). According to Hunter, (1993), *Pseudomonas* does not harm a healthy individual but may

cause problems in individuals with weak immune systems. However, it is more reliable and safe if the drinking water does not show the presence of *Pseudomonas spp*. According to Rosenberg (2003), the presence of *Pseudomonas spp*. in the water is due to contamination by humans themselves.

S. flexineri showed the most occurrences in brown and greenish colored water (Table 6) and was recorded usually where *E. coli* was present. Presence of *Shigella* in water sources of Samburu indicateds continuedal feacal contamination. This is because *Shigella* survives up to 4 days in river water. It is improbable that S*higella* can be recovered from an environmental source, unless there is a continuous source of contamination such as waste water seepage. *Shigella* can survive in a viable but non culturable state after 21 days (Colwell *et al.* 1985). *Salmonella* non *typhi* were recorded only in permanent rivers and their isolation may have been due to incidental contamination, since the organism had not been recovered in samples collected much earlier during this study.

*A. hydrop*hila and other *Aeromonas spp.* were recorded in all water sources except boreholes. Animals may also be the source of contamination by *A. hydrophilla* (Huys *et al*., 1996). Wildlife is not only abundant in Samburu, but share the watering points with man. Enteric pathogens are likely to be introduced into the water sources from feacal matter (Frost, 2001). The Samburu live in temporary shelters (Manyattas), and do not set up any special sanitary facilities such as toilets and bathrooms. It is possible therefore for feacal matter to be washed into the water bodies as surface run off during the rainy seasons. *Klebsiella spp.* and *Pseudomonas spp.* are generally abundant in water, and may not be unique to Samburu south (CDC, 1981). The highest levels of contamination occurred in dams, followed by dry river bed wells, laggas and finally permanent rivers. The extent of deterioration in water quality is in general related to the retention time of the

reservoir and its storage capacity in relation to the amount of water flowing into it. Water stored for many months or even years in a dam undergoes deterioration and may be lethal to most life.

5.4 Antibiotic sensitivity

Antibiotic sensitivity tests were done to determine the degree of sensitivity or resistance of a pathogen isolated from the water sources to commonly used antibiotics. Isolates were classified as susceptible, intermediate or resistant according to the National Committee for Clinical Laboratory Standard Guidelines (2004). All the 12 *S. flexineri* isolates recovered from ephemeral water sources were susceptible to CTX, CN, CIP and CXM. All isolates were resistant to SXT and Chloramphenicol regardless of the type of sensitivity test used. Three isolates displayed resistance to Ampicillin while two isolates displayed intermediate susceptibility to Amoxicillin. One isolate was resistant to TS. These responses to antibiotics are as would be expected for *S. flexineri* (Legros *et al*., 1998), except for cases of resistance to Ampicillin, one of the antibiotics recommended for prophylaxis against dysentery. Cases of resistance to Ampicillin have, however, been on the increase (Khan-Mohammed *et al.*, 2005). A study of antibiotic sensitivity of *S. flexineri* in Mbarara district in Uganda showed no isolate was resistant to Ciprofloxacin while 58% of the isolates were resistant to Ampicillin (Legros *et al*, 1998). These findings are similar to those for *S. flexineri* isolated in Samburu and may indicate that there is yet, no development of resistance factors to Ciprofloxacin ~~yet~~.

A study in Ethiopia showed a high sensitivity of *Shigella* isolates to Gentamicin (Belay *et al*., 2000). However, the study also demonstrated a 55% resistance of *Shigella* strains to Cotrimoxazole, while Khan-Mohammed *et al.* (2005) showed a 2.7% resistance rate in

Trinidad. A 100% resistance to Cotrimoxazole by *S. flexineri* reported in this study was in agreement with observations from Pakistan (Zafar *et al*., 2005) and Iran (Moez *et al.*, 2003), where increased resistance rates of 70.4% and 87.8%, respectively, by *Shigella* species to Cotrimoxazole were reported. The resistance of the majority of *Shigella* isolates to Cotrimoxazole is a cause for concern since this ~~drug~~ is one of the drugs of choice for the treatment of shigellosis in many countries (Patrick *et al*., 2002), including Kenya.

Another study conducted in six countries in the East African region also showed resistance of *Shigella* isolates to the commonly used antibiotics except for Nalidixic acid (Materu *et al*., 1997). A WHO scientific working group had reported a high prevalence of resistance to Ampicillin, Chloramphenicol, Trimethoprim, and Cotrimoxazole in developing countries because of the very high consumption of antibiotics from the open market in these countries (Leslie *et al*., 1998). The current treatment of choice for shigellosis is still Cotrimoxazole, Ampicillin and Ciprofloxacin (Geo *et al.*, 1998). *Shigella* are notorious for the rapid emergence and spread of multiple drug resistance among strains (Legros *et al.*, 1998)

Isolates of *A. hydrophilla* and other *Aeromonas spp.* recovered from all categories of water sources except boreholes and springs had similar patterns of antibiotic sensitivity. All the seven isolates were resistant to Ampicillin and Amoxicillin, and susceptible to CTX, CN, CIP, SXT, CH, CXM and TS using both the E-test for MIC and the disc diffusion test. A study carried out in Nagpur city India (Shinde *et al*., 2005) revealed multi drug resistance patterns in *Aeromonas spp*. The isolates showed complete resistance to ampicillin ~~(Shinde *et al*., 2005)~~. CTX, CN, CIP SXT, CH, CXM and TS can therefore be recommended for fighting infections caused by *A. hydrophill*a in the Samburu area.

The *Salmonella spp*. isolated from river Uaso Ng'iro was susceptible to all the assayed antibiotics using both disc diffusion and minimum inhibitory concentration tests. This implies therefore that any of the commonly used antibiotics can be used in the management of enteric fever. *S. boydii* was also susceptible to all assayed antibiotics except Ampicillin. The results based on the use of both MIC and Disc diffusion concur with the findings of Aneela and Rakhshanda (2007). All isolates subjected to sensitivity testing were susceptible to Ciprofloxacin. This may be due to its limited availability in village pharmacies and not being commonly used as a first line prescription drugs in hospitals. Ciprofloxacin would be highly recommended for management of diarrheal diseases in Samburu district.

5.5 Pulsed-Field Gel Electrophoresis

Seven similar patterns were found with 12 to 14 fragments, ranging from 31 to 725 kb from the *Not*I digestion of the *S. flexineri* isolates susceptible to and resistant to ampicillin using PFGE. *S. flexineri* isolates recovered in Taiwan for molecular subtyping, displayed similar pulsed-field profiles with 12 to 14 resolvable fragments (Chein-Sheng *et al*., 2001). *S. flexineri* isolates displaying resistance to Ampicillin had the same PFGE combination patterns with those displaying susceptibility to Ampicillin. There was no visible quantifiable evidence of genetic differences between PFPs generated from the isolates that were resistant to Ampicillin and isolates susceptible to Ampicillin. The "Tenover criteria" suggests that profiles differing from each other by up to three bands should be considered closely related, and up to six bands possibly related (Appendix iii). The basis of this was that a point mutation in a restriction site could result in loss of that site with two bands in one isolate

merging to form a larger band – i.e. a "three band difference". Using this logic, a three band difference could be the result of a single genetic event, and therefore isolates could be closely related. The antibiotic resistant and susceptible isolates are therefore similar strains.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The physico-chemical properties of water from most sources in Samburu district meet the recommended standards for potabilty set both by WHO and Kenya Bureau of Standards. All sources had acceptable levels of dissolved oxygen. Only springs met the proposed standards of salinity, while alkalinity, pH and conductivity were acceptable in water from all sources except boreholes. The color of water in many sources was not aesthetically acceptable for direct drinking.

2. Water from all sources in Samburu south except boreholes had total plate and coliform counts that exceed the permissible limits for potable water. Water from these sources evidently harbors *E. coli* and feacal coliforms and is therefore unfit for human consumption.

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3. The bacterial pathogens isolated from waters of Samburu district were *Shigella flexineri*, *Shigella boydii*, *Aeromonas hydrophilla*, and *Salmonella spp* (non-*typhi*) with a higher occurrence in temporary sources than permanent sources.

4. The antibiotic sensitivity profiles of pathogenic bacteria isolates recovered indicate thate pathogens are susceptible to the commonly used antibiotics and do not deviate from the expected standard antibiograms recorded. However, *S. flexineri* displayed increased resistance to Ampicillin.

5. Isolates of *S. flexineri* displaying resistance and those susceptibile to ampicillin are the same strain type.

6.2 Recommendations

1. The Samburu communities should implement relevant techniques for sterilizing the water before it is consumed. According to Fujioka Yoneyama (2001) and Moyo *et al.*, (2004), when thermotolerant (feacal) coliform counts in water are in categories of 20 and 2000 thermotolerant coliform concentration per 100 mL of water, the water could still be used for domestic consumption provided that simple physical treatment and disinfection such as filtration and boiling is carried out.

2. Since only boreholes and springs meet potability standards, more boreholes should be drilled and accessibility to springs should be improved by clearing surrounding bushes to make paths to the springs.

3. Considering that members of Samburu communities have been consuming this water over time, it is necessary to find out the strategies used by the community to minimize infections by waterborne bacteria.

4. Monitoring should be spread over longer periods (3 years) to capture seasonal variability with more replication of samples to increase the precision margin.

5. Transportation of samples over long distances is cumbersome, dangerous and may lead to loss of viability of fastidious bacteria such as *Campylobacter spp*. and *Listeria spp*., which are likely to thrive in the environmental conditions prevailing in the study area. Hence, methods of determining the presence and identity of the microorganisms, especially the molecular methods should be employed.

6. Tracing ancestry of isolates whether human or animal, will add information about the endemicity of the *S. flexineri* isolates by identifying the multiple circulating populations of *S. flexineri*. This should assist in developing preventive intervention strategies.

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APPENDICES

APPENDIX I: Antibiotic break-points

Appendix Ia. Minimum Inhibitory Concentration (mg L^{-1})

Antibiotic	Resistant (R >)	Intermediate Sensitivity (I)	Susceptible (S≤)
Trimethoprim/ Sulphamethoxazole(TS)	32	-	32
Cloramphenicol(CL)	8	-	8
Ampicillin(AC)	16	16	8

Appendix Ib. Disc diffusion inhibition zone diameters (mm).

Antibiotic	Resistant (R≤)	Intermediate Sensitivity (I)	Susceptible (S≥)
Ampicillin(AML)	11	12 – 14	15
Amoxacillin(AMC)	15	16 – 18	19
Cefutaxime(CTX)	29	-	30
Gentamicim(CN)	16	17 – 19	20
Ciprofloxacin(CIP)	16	17 – 19	20
Cotrimoxazole(SXT)	15	-	16
Cloramphenicol(CH)	20	-	21
Cefuroxime(CXM)	19	-	20

APPENDIX II: Standards for physico-chemical and microbial quality of water for human consumption

Characteristics	**WHO**	**KEBS**
pH	6.5-9.0	6.5-8.5
Conductivity	max 2000 μS cm^{-1}	max 2000 μS cm^{-1}
Total alkalinity	max 500 mg L^{-1}	
Salinity	max 250 mg L^{-1}	
Total bacteria count	10/mL	100/mL max
Total coliforms	0/100 mL	10/250 mL
Feacal coliforms	0/100 mL	0/100 mL
E. coli	0/100 mL	0/250 mL
All other pathogens	Not detectable	shall be absent

APPENDIX III: PFGE Interpretation by Ten over *et al.*, (1995).

No. Band Differences	No. Genetic Differences	Interpretation	Epidemiologic Significance
0	0	Indistinguishable	Isolates are same strain type
1-3	1	Closely Related	Isolates are probably related
4-6	2	Possibly Related	Isolates are possibly related
=>7	=>3	Different	Isolates are different