AN EVALUATION OF BACTERIOLOGICAL QUALITY OF WATER FROM READY-TO-EAT FOOD OUTLETS IN MASENO TOWNSHIP, WESTERN KENYA

BY

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DECLARATION

Declaration by the student

I declare that this is my original thesis and that it has not been presented to any other institution for a Degree or any other award.

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Approval by the Supervisors

This research has been carried out under our supervision and has been submitted to Maseno University School of Graduate Studies for examination by my approval as the candidate's supervisor.

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DEDICATION

To my lovely wife Violet, our sons Mwebi and Orina for their moral support and perseverance during my long absence from home. Also the thesis is dedicated to my mum, brothers and sisters for their prayers and motivation.

To my late brother Okara Melkzedeck and my late Dad Mr. Mwebi Moogi David who passed on during my course work in November 2013 and January 2014 respectively. Also to my friend, classmate, and nephew the late Jared Moogi and my uncle Francis Keraita Moogi who passed on, August and December 2015 respectively. May their souls rest in peace.

ABSTRACT

Water is a commodity which is essential for life as it has long been singled out globally as the most important factor in sustaining human health. Among other factors linked to human health; water has been implicated in the transmission of waterborne infectious diseases such as typhoid, bacillary dysentery and cholera in Kenya. Potable water scarcity in Maseno Township is common; moreover ready-to-eat food outlet operators rely on water vendors whose water sources are hardly known by the operators. Although waterborne disease cases such as cholera and typhoid have been reported in Maseno Township, the bacteriological quality of water used in ready-to-eat food outlets at Maseno Township remains unknown. The observation could be due to the ever increasing population which strains the little water resources available leading to increased chances of microbiological contamination due to poor hygiene and scarce sanitation facilities. The general objective of the study was to evaluate the bacteriological quality of water used in the ready-to-eat food outlets within Maseno Township. The specific objectives were; to establish the knowledge of food outlet operators about the source of water, modes of water treatment practices and determine the bacteriological quality of potable water in Maseno Township. A cross sectional study design was used. The sampling units were ready-to-eat food outlets and there were a total of 35 where 33 were randomly selected for the study. Three sets of water samples; hand-washing, ready-to-drink and stored water at each outlet was collected on three different occasions at an interval of one week. A questionnaire was used to get information on water sources and treatment practices from the operators while laboratory experiments such as bacteriological culture techniques were used to analyze water samples for microbial contamination. Quantitative data was analyzed using content analysis where 21 (70%) of the ready-to-eat food outlet operators knew sources of water. There was a significant statistical variation of the water sources as was mentioned by the operators (χ^2=15.435, p = 0.031). Ready-to-drink and hand washing water treatment had no statistical significant difference (χ^2=2.057, p = 0.561). The data of average faecal thermotolerant coliforms (*E. coli*) $\log_{10}$ transformed colony forming units/100 ml against the various sources of water was analyzed using MANOVA and a significant high variation of *E. coli* coliform count in the various sources of water reported (p=0.024). This result indicates that the water at some of the ready-to-eat Food Outlets in Maseno Township is not potable. In conclusion, the study showed that the operators who knew the sources of their water were more than those who did not know. Secondly, water treatment practices at the outlets were boiling and chemical treatment with some operators opting not to treat their water at all. Finally, there was contamination with both faecal thermotolerant coliforms (*E. coli*) and other thermotolerant coliforms. The outcome of the study can be used by public health officials and the food outlet operators to understand the bacteriological quality of water used and also know the risk level in relation to waterborne diseases especially having isolated serovariant *Salmonella* Typhimurium (i-H) which is found in warm blooded animals and is responsible for gastroenteritis in human. This can in turn help the Maseno community device better techniques and policies of improving and monitoring microbial quality of water they use.

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ABBREVIATIONS AND ACRONYMS

API 20E	Analytical Profile Index for 20 biochemical tests for enterobacteriaceae
CFU	Colony Forming Unit
DWAF	Department of Water Affairs and Forestry
MDG	Millennium Development Goals
POU	Point-of-Use
SCHIMS	Sub – County Health Information Management Systems
SPSS	Statistical Package for the Social Sciences
UNICEF	United Nations International Children Education Fund
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

DEFINITION OF TERMS

Coliform is a facultative anaerobic, rod shaped, gram-negative, non-sporulating bacteria which generally originate in the intestines of warm-blooded animals. In addition, they are capable of growth in the presence of bile salts or similar surface agents and are oxidase negative.

Colony forming unit (CFU) is a unit used to estimate the number of viable bacterial cells (which have the ability to multiply not dead) in a sample.

Faecal thermotolerant coliforms are types of bacteria (usually *E. coli*) which produce acid and gas from lactose within 48 hours at 44 ± 0.5°C -used to detect and indicate faecal contamination of water, hence estimate health risk.

Bacteriological quality of water estimation of the level of bacterial indicators present or absent in potable water.

Potable water is water which is microbiologically suitable for drinking and food preparation.

Safe water is water with 0/100 ml CFU faecal thermotolerant coliform count and <10/100 ml CFU of total thermotolerant coliform count as per the WHO standards.

Snack is a portion of food, smaller than a regular meal, generally eaten between meals and the individual eating does not necessarily need to wash their hands.

Thermotolerant coliforms are a class of micro-organisms excluding faecal coliforms commonly used as bacterial process indicators of sanitary quality of water, that is, they show the efficacy of water treatment practices.

Total thermotolerant coliforms are a group of coliform organisms including faecal coliforms.

Water chlorination is the process of adding chlorine (Cl_2) with the aim of killing certain bacteria and other microbes in potable water as the chemical is highly toxic which ultimately prevents the spread of waterborne diseases such as cholera, dysentery and typhoid.

Water treatment is the removal of microbial contaminants by use of chemical, physical or mechanical means - from water to produce potable water.

Waterguard is a dilute sodium hypochlorite (chlorine) solution used to disinfect water at the household level.

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CHAPTER ONE: INTRODUCTION

1.1 Background Information

Water is a commodity which is essential to life, but many people do not have access to clean and potable water and hence are prone to infection by waterborne pathogens. WHO data on the quality of disease indicates that approximately 3.2% of deaths (1.8 million) worldwide are attributable to microbiologically contaminated water, sanitation and poor hygiene (WHO, 2003). Moreover, in sub-Sahara Africa, about 80% of illnesses are linked to high microbial water quality and poor sanitation conditions (Kindhauser, 2003). Ensuring safety of potable water is an ongoing process. In developed countries, potable water regulations require the monitoring of numerous parameters key among them being microbiological and chemical parameters (Cabral, 2010).

The assumption is that the main source of microbiological contamination in the natural water environment in Kenya is of human or animal origin. The different population structures may be attributed to the different rates of growth and survival of these microorganisms in water (Onyango *et al*., 2009), and this necessitates a study to determine the identity of the bacterial pathogens associated with potable water. It is important to note that, *Salmonella typhimurium,* for example, is the clinically important serovar mostly identified in water, which attests to its capacity of adaptation and survival in water environment (Baudart *et al*., 2000). The presence of diverse enteric bacteria in the food outlet water environments suggests that strict hygiene procedures should be followed during the handling and processing of foods such as fish to prevent the transfer of potentially pathogenic bacteria to humans. Thus there is need for a code of practice for water handlers in food outlet systems to ensure safe water handling (Clasen *et al*.,

2007b). In addition, food handlers - especially those relying on water vendors - should know the source of water they use in their premises

Diarrheal diseases have been linked to water becoming contaminated from faeces being passed or washed into rivers, streams or pools or being allowed to seep into wells or boreholes (Medema *et al*., 2003). The most important aspect of analysis is therefore to determine whether faecal contamination is present. Bacteriological analysis of water has been used to confirm whether a water supply is faecally contaminated or not (Onyango-Ouma and Gerba, 2011). In piped water distribution systems, a sanitary inspection will often not detect problems occurring during distribution, for example pipes buried underground might be damaged, allowing in pollution. Analysis is also used to assess the effectiveness of disinfection processes. It is also a useful way of keeping communities interested in their water supplies' quality and justifying requests to health authorities for improvements in water quality. According to the Kenya standards (KEBS, 1996), for potable water requires that the total and faecal thermotolerant coliform counts (TTC) must be 0 colony forming units per 100 millilitre (CFU/100 ml) of water. While no Kenyan standard has been formulated for untreated water, the World Health Organization (WHO, 2008b) requires that for untreated water the total TTC must not be more than 10 CFU/100 ml for three consecutive samples and the faecal coliform count must be 1 CFU/100 ml of water. Water sources also influence the level of microbial contaminants which led to WHO classifying sources as either improved or unimproved and the world body has sponsored activities which create awareness in communities about the classification (WHO and UNICEF, 2000).

Maseno Township is a small upcoming urban center in Western Kenya. In the last two decades its population has risen from 500 to the current over 15,000 people (KNBS, 2010; Maseno, 2014). This growth has been attributed to the continued growth of Maseno University and other

institutions in the township. It has over the years not had a steady supply of water, where the ready-to-eat food outlets heavily rely on water vendors. Nevertheless the operators of these ready-to-eat food outlets are uncertain about the sources of the water supplied by the vendors.

Numerous studies have clearly shown that improving the microbiological safety of house-hold water on site, or point-of-use treatment and safe storage in improved vessels reduces diarrhoeal and other waterborne diseases in communities and households in developing countries (Makutsa *et al*., 2001; Hsu *et al*., 2002; Clasen *et al*., 2006b). In Maseno Township, however, point-of-use water treatment practices by the operators of the ready-to-eat food outlets to reduce or eliminate the microbes in water used in their premises remains unknown.

Faecal thermotolerant coliforms (sometimes called thermotolerant coliform organisms of *Escherichia coli* species) are the most appropriate indicators of faecal pollution. It is less useful to test for 'total coliforms' because they are not directly related to the presence of faecal contamination and so not to the potential presence of pathogens. The most valuable test for the routine quality control of water supplies is the faecal coliforms count (Nkere *et al*., 2011). Generally no study has tried to investigate the microbial quality and safety of water offered by the ready-to-eat food outlets within Maseno Township. These food outlets have continued to offer food including potable water to a large population of Maseno University students and people working within the town. According to the Ministry of Health report there were four (4) confirmed cases in Kisumu County - one (1) from Maseno division - of cholera in 2014 (MOH, 2015). In Maseno division, water borne illnesses have been attributed to low access to tap water due to irregular water flow. Unpublished data from the health facilities in Maseno Township indicates that typhoid fever was about 15% annually based on serological diagnosis of *Salmonella* antigen (SCHIMS, 2013). Gastroenteritis and dysentery had been reported in these

areas but no laboratory diagnosis had been made to determine the main cause. *Shigella* species and amoeba are the causative agents of dysentery and are both water transmitted hence the need for continued surveillance of potable water to prevent outbreaks. Understanding the microbial quality and practices employed by the food outlets to ensure safe water for use is important as it serves to document the levels and types of microbes present in the water and practices employed to minimize any water related disease outbreaks.

Water can be contaminated at the source, at the point-of-use and during storage. The study analyzed the microbial quality of water at three important sampling points at the food outlets; namely at storage, hand washing and drinking. The information generated from this study would be useful to the Maseno township community and food outlets as it offered an understanding of the bacteriological quality in water. The information may also be useful to the local public health department as it may help in the development of relevant training and sensitization programs targeting the Maseno township community with respect to water and sanitation.

1.2 Statement of the Problem

Potable water scarcity in Maseno Township is common. The ever increasing population in this township strains the little water resources available. The scramble for this important commodity leads to people using water whose source is not known or people may resort to using water from unprotected sources that are easily accessed by both domestic and wild animals hence highly faecally contaminated. Ready-to-eat food outlets rely highly on the water vendors who fetch water from sources mostly not known to the food outlet operators. In addition, water treatment practices at the point of use vary due to cost and in some cases no water treatment at all. In situations where water is treated, the food outlet operators may have not been assessing the

effectiveness of the water treatment method used. Microbial water quality assessment data within the township was lacking hence there was difficult of linking waterborne diseases to possible water contamination in Maseno.

1.3 Overall Objective

To evaluate the bacteriological quality of water used in the ready-to-eat food outlets within Maseno Township.

1.3.1 Specific objectives

1. To establish the knowledge of food outlet operators about the source of water used in their food outlets within Maseno Township.
2. To establish the modes of water treatment practices in the food outlets within Maseno Township.
3. To determine the bacteriological quality of water used in the ready-to-eat Food Outlets within Maseno Township.

1.3.2 Research questions

1. What are the sources of water used in the ready-to-eat food outlets in Maseno Township?
2. What are the modes of water treatment practices in the food outlets within Maseno Township?
3. What is the bacteriological quality of water used in the ready-to-eat food outlets within Maseno Township?

1.4 Significance of the Study

The microbiological contamination levels can assist any stakeholder involved in the regulation; operation and use of the food outlets understand microbial quality of their water. The regulators

of water supply and public health would strengthen and enforce policies to prevent transmission of gastrointestinal tract pathogens among users of ready-to-eat food outlets. Food outlet operators can have a chance to change water handling and treatment techniques at the point-of-use. In addition, interested scientists can come up with research proposals of water microbiological health risk assessment to determine the potential health risks associated with water in Maseno Township.

1.5 Study Limitations

Approaches to microbial water quality verification include testing of source water, water immediately after treatment, water in distribution systems or stored water at the point-of-use, but the current study did not cover all these areas. The limitation of microbiological indicators is lack of correlation between certain indicators and disease-causing organisms in humans, as well as the uncertain relationship between indicators and different sources of pathogens. Furthermore, enteric viruses and protozoa are more resistant to disinfection; consequently, the absence of *E. coli* will not necessarily indicate freedom from these organisms. Finally, the determination of health risk involves a multi-disciplinary approach and it requires a long time to monitor several parameters which was beyond the scope of this study. Also another challenge was that some ready-to-eat food outlets were shutting down business forcing the research to start sampling from another food outlet hence delaying the study for a few weeks. For this study, however, some of the study limitations were mitigated due to the fact that the study was a beginning of identifying microbial quality of water which normally forms a basis of designing a comprehensive study in relation to faecal water contamination.

CHAPTER TWO: LITERATURE REVIEW

2.1 Sources of Potable Water

Majority of the population of the developing countries obtain their water via non-piped systems and of the remainder most are supplied through systems that require some form of storage or handling before use, hence increasing the possibility of contamination. Even in areas where a reliable piped supply is the norm, occasionally interruptions occur (for example, harsh weather events that cause major lines to break and force adoption of household handling). From a public health perspective it is therefore essential to respond to contamination which occurs through the chain of supply up to the point-of-use and to consider all water supply forms used by the population (Clasen *et al*., 2007a).

According to the international classification, point sources of water such as tube wells, dug wells and protected springs are a representative of very significant proportion of the 'improved' water supplies provided to communities in developing countries (WHO and UNICEF, 2000). On the other hand unimproved water sources include unprotected/uncovered dug wells, springs, water pans, streams and rivers. Such supplies are very common in rural areas and may also represent a very significant proportion of the water supplies available and used for domestic purposes (including drinking) by low-income urban populations (WHO and UNICEF, 2000). The quality of such sources is often very variable and they frequently show high faecal contamination, particularly during wet seasons (Jim *et al*., 2004).

The water source classification helps people to understand the kind of water they are using and also give a hint of the contamination risk involved. The consequences of consumption of contaminated water from point sources can be severe both in relation to outbreak of endemic and

epidemic diseases. The monitoring of the bacteriological quality of domestic use water from such sources is important in reducing community health risks, but requires greater emphasis on support to point-of-use water management in order to improve operation and maintenance of water safety, and also significant user education sensitizes the community on water safety. Moreover, the WHO water source classification does not address post-delivery recontamination which is a potential source of health risk to the community. In Maseno Township the operators of ready-to-eat food outlets have acknowledged that they mostly depend on water from vendors whose source they do not know. The current study was to ascertain this particular claim.

2.2 Modes of Water Treatment

Treatment of water at point-of-use requires a variety of technologies, including physical and chemical methods, which have been developed and are often used in many parts of the world. Some of the treatments used to reduce the bacteriological quality of potable water at the point-of-use include chlorination, filtration, solar disinfection and flocculation together with disinfection (Clasen *et al*., 2006a). According to a recent study in Kenya, these methods can be used to ensure that potable water at the point-of-use is free from microbial contamination (Onyango-Ouma and Gerba, 2011).

In western Kenya - Kisumu, a project initiated by Care-Kenya a non-governmental organization, facilitated construction of shallow wells and pit latrines, but still the bacteriological quality of the water did not have any significant improvement. As a result, in 2001, Care implemented the Safe Water System (which consisted of point-of-use water treatment with sodium hypochlorite, safe storage, and behavior change techniques) within the established project infrastructure, using existing community structures in combination with a social marketing approach that introduced

affordable products. The project led to adoption rates of 33.5% for chemical water treatment and 18.5% for clay pots modified for safe water storage (Makutsa *et al*., 2001).

The persistent and consistent interventions applied in water microbial quality reduction in western Kenya has shown to have a positive impact. The cheap chemical water treatment, modified storage pots and social campaign approach are the key interventions which can gain easy adaption. However, there is need for constant monitoring of water quality at the point-of-use and checking the efficacy of every intervention before water is used especially in ready-to-eat food outlets as water is largely handled by different people. The efficacy data should always be readily accessible to the public.

2.3 Bacteriological Water Quality

Many environmental strains of coliform bacteria such as *Citrobacter*, *Enterobacter* and *Klebsiella* may be found in water distribution systems. However, it is generally agreed that water temperatures and nutrient concentrations are not suitable enough to support the growth and maintenance of *E. coli* (or enteric pathogenic bacteria) in water distribution biofilms. Thus the presence of *E. coli* is evidence of recent faecal contamination of potable water (Medema *et al*., 2003).

Microbiologically contaminated water plays an important role in transmission of waterborne bacteria to humans. Constant microbial water monitoring using accurate, sensitive, and specific diagnostic methods in water purification facilities can have a significant role on ensuring the reduction of microbial pathogens in water at the point-of-use (Momtaz *et al*., 2013).

Several studies have confirmed that water-related diseases not only remain a leading cause of morbidity and mortality worldwide, but that the spectrum of disease is expanding and the

incidence of many water-related microbial diseases is increasing (WHO, 2003). Since 1970s, several species of microorganisms from human and animal faeces from environmental sources, including over-flow water have been confirmed as pathogens (Jim *et al*., 2004). Examples of the pathogens include *Vibrio cholera*, *Shigella* species, *Escherichia coli* O157 and *Salmonella* species (WHO, 2003). Others include *Cryptosporidium* species, *Legionella* species, rotavirus, hepatitis E virus and norovirus (Fewtrell *et al*., 2005). Furthermore, the importance of water in the transmission of recognized pathogens is being continually assessed as new tools become available through advances in scientific technologies. *Helicobacter pylori* is an example of a recently emerged pathogen that may be transmitted through water (WHO, 2003).

Safe potable water access by all has long been a central aim of public health and international development policy. The Millennium Development Goals (MDGs) included target 7c to reduce by half the proportion of the population who lack sustainable access to safe potable water by 2015. The target was considered to achieve improved access based on water quantity and quality (safety) (WHO and UNICEF, 2013). The WHO and UNICEF through their Joint Monitoring Programme (JMP) were tasked with monitoring progress against the MDG target and adopted an indicator, 'use of an improved source' (WHO and UNICEF, 2013). The indicator is based on a facility type classification with sources such as boreholes and piped supplies classified as improved and unprotected sources, such as uncovered dug wells, classified as unimproved. By the year 2012, it was reported that the target had been met three years ahead of schedule (WHO and UNICEF, 2013).

Indicator organisms are a fundamental monitoring tool used to measure both changes in water microbial quality or conditions and the potential presence of pathogenic organism which are hard to get. An indicator organism provides evidence of the presence or absence of a pathogenic

organism surviving under similar physical, chemical, and nutrient conditions (Bain *et al*., 2014). For faecal contamination, indicator organisms should have certain characteristics which make them suitable as good indicators. First, they should be easily detected using easy to do laboratory tests such as indicator tests and culture. Secondly, they should generally not be present in unpolluted waters. Thirdly, they should appear in concentrations that can be correlated with the extent of contamination. Finally, they should have a die-off rate that is not faster than the die-off rate of the pathogens of concern (Petterson *et al*., 2001).

Data on bacteriological water quality may be divided into a number of categories; the levels of contamination associated with each category are to be selected based on the local circumstances. For this study however, WHO classification was used. A typical classification scheme is presented in table 1.0, based on increasing orders of magnitude of faecal contamination and levels of risk associated with it (WHO, 2007).

Table 2-1: Risk assessment of water for faecal coliforms.

Colony forming units count per 100 mL (CFU/100 ml)	Risk level
<1	Low risk/In conformity with WHO guidelines
1–10	High risk
>10	Very high risk

(**Source:** Robert and Lars, 2010).

Indicator bacteria in water are usually harmless, more plentiful, and easier to detect than pathogens. Specific methods are not currently available to culture or enumerate all the disease-causing organisms that might be present in water. Viruses and protozoans, for example, are generally not used as indicators because of difficulties associated with isolating them in the laboratory and detecting their presence in water samples. The coliform-bacteria species used as

indicators are normal flora in the intestines of warm-blooded animals and indicate the potential presence of dangerous pathogens that can cause human illnesses.

2.4 Bacterial Pathogens Associated with Water

2.4.1 *Salmonella* species

Salmonella enterica is one of the etiologic agent causing enteric infections in the world (Onyango *et al*., 2009). Raw foods and cross-contamination of ready-to-eat products are some of the main routes of *Salmonella* transmission. According to a study (Onyango *et al*., 2009) it can be concluded that isolation of *Salmonella* in fish harvested from the lake is an indication of contamination of the waters by the pathogen, which in turn is a potential source of contamination of water at the point-of-use. The sources of *Salmonella* are poorly understood, and more studies are necessary to provide vital data that is critical in assessing and controlling the risk associated with use of salmonellae contaminated water sources and household storage points. In addition, quantitative risk assessment is necessary for scientists to be able to gauge the potential pathogenicity of the pathogens isolated in water.

Further behind in importance are typhoid and paratyphoid fevers (caused by *Salmonella typhi* and *S. paratyphi*, respectively), resulting in an annual incidence of about 17 million cases world-wide (Kindhauser, 2003). Both typhoid pathogens are passed in the faeces and urine whereby people become infected after eating food or drinking beverages that have been handled by a person who is infected or by drinking water that has been recently contaminated or mix of potable water with raw sewage containing the bacteria. Once the bacteria enter the person's body system they multiply and spread from the intestines, into the bloodstream. Even after recovery from typhoid or paratyphoid, a small number of individuals (called carriers) continue to shed the bacteria (Ashbolt, 2004).

According to a study (Onyango *et al*., 2009) fish in Winam Gulf of lake Victoria have *Salmonella* species (31.7%), which explains partly the source of *Salmonella* species in potable water in Kenya as they are shed by the fish into the water. The *Salmonella* species distribution was as follows: 14.3% *Salmonella typhimurium*, 11.1% *S. typhi* and 6.3% *S. enteritidis*. These pathogens can also be introduced into ready-to-eat food outlets water through the fish handling during cooking (Makutsa *et al*., 2001).

2.4.2 *Shigella* species

This is a Gram negative; non-lactose fermenting and non-motile rod. The genus has four species namely; *S. dysenteriae, S. boydii, S. flexneri* and *S. sonnei*. The four species are closely related to *E. coli*. Many share common antigens with one another and with other enteric bacteria (for example, *Hafnia alvei* and *Plesiomonas shigelloides*) (Geo *et al*., 2007). *Shigella* species have a worldwide distribution with high cases being reported in developing countries where sanitation is poor. The bacteria can get into groundwater and private wells through discharges or sipping from faulty septic systems or sewage treatment plants. Wells are likely to be more vulnerable to such contamination after flooding, particularly if the wells are shallow, have been dug or bored, or have been submerged by floodwater for long periods of time.

Shigella species infects only humans. Transmission is mainly by the faecal-oral route with poor sanitation, unhygienic conditions, and overcrowding, facilitating the rapid spread of infection. Only a few organisms are required to cause disease (Cheesbrough, 2006). The high number of waterborne outbreaks of bacillary dysentery have been recorded (WHO, 2008b). The organisms are not particularly stable in water environments, if isolated from domestic water it is an indication of recent human faecal pollution. The control of *Shigella* species in domestic water

supplies is of public health importance in view of the severity of the dysentery caused (WHO, 2008a).

Most studies have identified risk factors and protective effects of bacillary dysentery incidences and fatality (DWAF, 2005; WHO, 2008b; Onyango *et al*., 2009). Despite the gradual improvements in water supply, bacillary dysentery continues to be endemic in low-income populations especially in the tropics, often among displaced populations following natural disasters and political crises globally. In Guatemala, people of extreme age groups (children and elderly) and middle aged males were found to be most susceptible to *S. dysenteriae* serotype 1. In war torn in the late 1990's Sierra Leone, on the other hand, the attack rate was higher among children under 5 years of age than in the rest of the population. While in rural Bangladesh (floods prone), shigellosis was most common in children aged between 1 - 2 years old and in people greater than 60 years. In Kenya *Shigella* species has been isolated from fish in Lake Victoria (39.7%) (Onyango *et al*., 2009). *Shigella flexneri* is the only species among the four known others which has been isolated in a large water mass - Nairobi River (Abednego *et al*., 2013).

2.5 Methods for Detection and Identification of Bacterial Pathogens Associated with Water

2.5.1 Conventional detection of bacterial pathogens associated with water

Escherichia coli count is the most useful test for detecting faecal contamination of water supplies in water quality analysis. Two main techniques are used for counting faecal coliforms, namely membrane filtration technique and most probable number. In membrane filtration technique, a 100mL water sample or a diluted sample is filtered through a membrane filter. The membrane, with the suspected coliform organisms on it, is then cultured on a pad of sterile selective media broth containing lactose and an indicator. After incubation for 18 – 24 hours at 35 – 37^0C, the

number of coliform colony forming unit (CFU) can be counted after subculture onto equally selective solid media. This gives the presumptive viable number of faecal coliforms in the 100mL water sample. Multiple tube/most probable number (MPN), on the other hand, 100mL water sample is distributed (five 10mL amounts and one 50mL amount) in bottles of sterile selective culture broth containing lactose and an indicator. After incubation, the number of bottles in which lactose fermentation with acid and gas production has occurred is counted. The lactose is fermented by the faecal thermotolerant coliforms if present in the water. By reference to probability tables, the most probable number of faecal thermotolerant coliforms in the 100mL water sample can be estimated (Cheesbrough, 2006).

The 3M© petrifilm plate is an all in one plating media containing system. This technique is currently widely used in the food and beverage industry throughout the world to monitor the microbial quality of products and test the efficacy of cleaning processes. Foods, beverages and surfaces can be tested for the presence of harmful bacteria (pathogens), indicator bacteria (that indicate the possible presence of pathogens), and spoilage organisms that can affect the shelf-life for products. Water samples are tested directly by inoculating the petrifilm plate with 1mL of water and incubated at the right conditions. If the count is too high, the sample is diluted then corrected for the dilution factor when expressing the result. There are several commercially available devices used in this technique but there are only three which are applicable in water analysis (Nero *et al*., 2006).

First, Coliform Count (CC) is designed to enumerate coliform bacteria in 24 hours. The CC plate works because it contains violet red bile salts (VRB) lactose nutrients, a cold water gelling agent and triphenyl tetrazolium chloride (TTC), an indicator that colours bacterial colonies red. Gas (indicated by bubbles) produced by faecal thermotolerant coliforms is trapped between the two

sheets of film, providing a confirmation test. Secondly, there is *E. coli*/Coliform Count (EC) which counts all faecal thermotolerant coliforms in a sample and differentiates *E. coli* as a subset of the total. Incubation is at 35 – 37^0C for 24 to 48 hours. The EC count is an indicator of faecal contamination, particularly in meat products and water. Thirdly, Enterobacteriaceae Plate (EB) enumerates faecal thermotolerant coliforms plus potential pathogens; it provides a broader picture of potential contamination in 24 hours. EB is a sample-ready culture medium system which contains modified violet red bile glucose (VRBG) nutrients, a cold water soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration. On the plate, Enterobacteriaceae will appear as red colonies with yellow zones and/or red colonies with gas bubbles with or without yellow zones (Nero *et al*., 2006).

Enumeration of coliform colonies is done directly on the petrifilm as it has subdivisions which enable the counting similar to that of the standard colony counter. The colony forming units per milliliters of sample (cfu/100 ml) is recorded. Picking out individual colonies is done to recover the microorganisms for interpretation and further identification. Quality/potable water does not contain any coliform colony forming units per ml of water analyzed (Nero *et al*., 2006).

The recovered bacteria are cultured routinely to study their characteristics in normal culture media. Some of the media is MacConkey agar, deoxycholate citrate agar, xylose lysine deoxycholate agar, motility indole medium and many others. *Salmonella* are motile rods that characteristically ferment glucose and mannose without producing gas but do not ferment lactose or sucrose. Most salmonellae produce hydrogen sulphide (H_2S). They are often pathogenic for humans or animals when ingested. Arizona is included in the *Salmonella* group (Geo *et al*., 2007). *Shigella* species, on the other hand, are non-motile and usually do not ferment lactose but do ferment other carbohydrates, producing acid but not gas. They do not produce H_2S. Based on

antigenic structure and biochemical reactions, *Shigella* species organisms are divided into four subgroups corresponding to the following species: Subgroup A, *Shigella dysenteriae,* which has 12 distinct serotypes. Serotype 1 was formerly called *S. shiga* while serotype 2 was formerly called *S. schmitzii.* Subgroup B, *Shigella flexneri*, contains 6 related serotypes and 4 serotypes divided into sub-serotypes. Subgroup C, *Shigella boydii*, on the other hand, contains 18 distinct serotypes. Finally subgroup D, *Shigella sonnei,* contains one serotype (Cheesbrough, 2006). Selenite-F Broth is used as an enrichment medium for the isolation of *Salmonella* species and *Shigella* species from faeces, urine, water, foods and other materials of sanitary importance. The media contains some additives which make it suitable to serve this purpose (Cheesbrough, 2006).

2.5.2 Biochemical identification of bacterial pathogens associated with water

The main biochemical tests used for the identification of *Salmonella* species and *Shigella* species include triple sugar iron (TSI) agar and lysine iron agar (LIA). These tests have proved to be crucial in enterobactericeae identification. The TSI test is a bacteriological test roughly named for its ability to test microorganism's ability to ferment sugars and to produce hydrogen sulfide. It is often used in the selective identification of enteric bacteria including but not limited to *Salmonella* species and *Shigella* species. Bacteria that ferment any of the three sugars in the medium will produce by-products. These metabolic by-products are usually acids, which will change the color of the red pH-sensitive dye (phenol red) to a yellow color. Position of the color change distinguishes the acid production associated with glucose fermentation from the acidic by-products of lactose or sucrose fermentation. Many bacteria that can ferment sugars in the anaerobic butt of the tube are Enterobacteriaceae. Some bacteria utilize thiosulfate anion as a terminal electron acceptor, reducing it to sulfide. If this occurs, the newly formed hydrogen sulfide (H_2S) reacts with ferrous sulfate in the medium to form ferrous sulfide, which is visible

as a black precipitate. Examples of sulfide-producing bacteria include *Salmonella*, *Proteus*, *Citrobacter* and *Edwardsiella* species. The blackening of the medium is almost always observed in the butt of the medium. The lysine iron agar slant or LIA slant test, on the other hand, is used to distinguish bacteria which are able to decarboxylate lysine and/or produce hydrogen sulfide from those that cannot. This test is particularly useful for distinguishing different Gram-negative bacilli - especially among the Enterobacteriaceae (Cheesbrough, 2006).

2.5.3 The API-20E enteric identification system

The Analytical Profile Index (API) is a miniaturized panel of biochemical tests which are compiled for identification of closely related bacteria. Different test panels are prepared in dehydrated forms which are reconstituted upon use by addition of pure bacterial suspensions. After incubation, positive test results are read and scored as a seven-digit number (profile). Identity of the bacterium is then easily derived from the database with the help of the relevant cumulative profile code book or software (bioMerieux, Inc., Hazelwood, MO).

API 20E which was preferred for this study is a biochemical panel for identification and differentiation of members of the family Enterobacteriaceae, provides an easy way to inoculate and read tests relevant to members of this family and associated organisms. The plastic strip holds 20 micro-test chambers containing dehydrated media having chemically-defined compositions for each test. The cytochrome oxidase (OX) test is performed separately from the API 20E panel using a portion of a bacterial colony on a paper strip impregnated by the oxidase reagent which turns blue if cells possess oxidase enzyme (bioMerieux, Inc., Hazelwood, MO).

2.5.4 Serological typing

Serotyping is the final phenotyping method where antisera are used against the pure isolates. Microorganisms produce a variety of antigens including structural components of the cells (cell

wall constituents, capsules or envelopes, flagellae, fimbriae); secretion products of the cells (toxins, extracellular enzymes) or antigens contained in the interior of the cells. Chemically the antigens used for such purposes are of two main macromolecules: proteins and carbohydrates (including mixtures of both components). The *Shigella* species are by definition non-motile, as such, only the somatic (O) antigens are utilized for the determination of serotype. Flagellar (H) antigens are not expressed by this species. *Salmonella* species express both O and H antigens. The O antigen consists of repeat units of oligosaccharide, and is part of the lipopolysaccharide (LPS) of the outer membrane of Gram negative bacteria and contributes to the main antigenic variability on the cell surface (Cheesbrough, 2006).

Serotypes of *Salmonella* species are defined based on the antigenic structure of both somatic or cell wall (O) antigens and flagellar (H) antigens. The flagellar (H) antigen has further been discovered to have seven sub-serotypes (a, b, c, d, I, z10 and z29). These antigens are detected using slide agglutination with commercially produced antisera; the O antigens using a suspension of growth from an agar plate while the H antigens using a suspension of broth culture. The serotype is deduced from the specific pattern of agglutination reactions using the Kauffmann-White classification scheme. Polyvalent antisera for specific organisms are used in the serotyping the isolates. O and H antisera are commercially available and manufacturer's instructions are used in the testing and interpretation of results (Geo *et al*., 2007).

Shigella species are sero-grouped by their O antigens using polyvalent group antisera and when indicated, monospecific (monovalent) anti-serum for example monovalent *S. dysenteriae1* antiserum is required to identify Sd 1 (Cheesbrough, 2006).

Bacteriological quality of water involves the analysis of adequacy of treatment practices and the level of microbial contamination if any. The current study was focusing on total thermotolerant coliforms and assessing for the presence of pathogenic bacteria, that is, *Salmonella* species and *Shigella* species.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Area

Maseno is a town in Kisumu West Sub-County of Kisumu County, Kenya. It is located 34^0 36′ 45″ E, 0^0 00′ 50″ S and 0^0 00′ 04″ N of the equator along Kisumu-Busia highway 20 kilometers northwest of Kisumu town, the county capital. A road connecting Maseno to Vihiga town is located 15 kilometers east of Maseno. Kombewa is located 10 kilometers west of Maseno. The altitude of Maseno is 1,503 metres or 4,934 feet above sea level. The town is the headquarters of Maseno Division, one of the four administrative divisions of Kisumu West Sub-County. Maseno division has a population of 46,802, of whom 2,199 are classified as urban residents (KBS census, 2009). In addition, Maseno Township serves a population of over 10,000 students of Maseno University, pupils of various primary, secondary and special schools. Maseno is part of Kisumu County Council and Kisumu Rural Constituency. The study covered areas of Nyawita, Emabungo and Maseno town villages (Fig. 1). Most inhabitants of the township are either employees of various institutions such as schools, training institutions and research stations or students. The ready-to-eat food outlets offer meals especially breakfast, lunch and super to this category of Maseno residents.

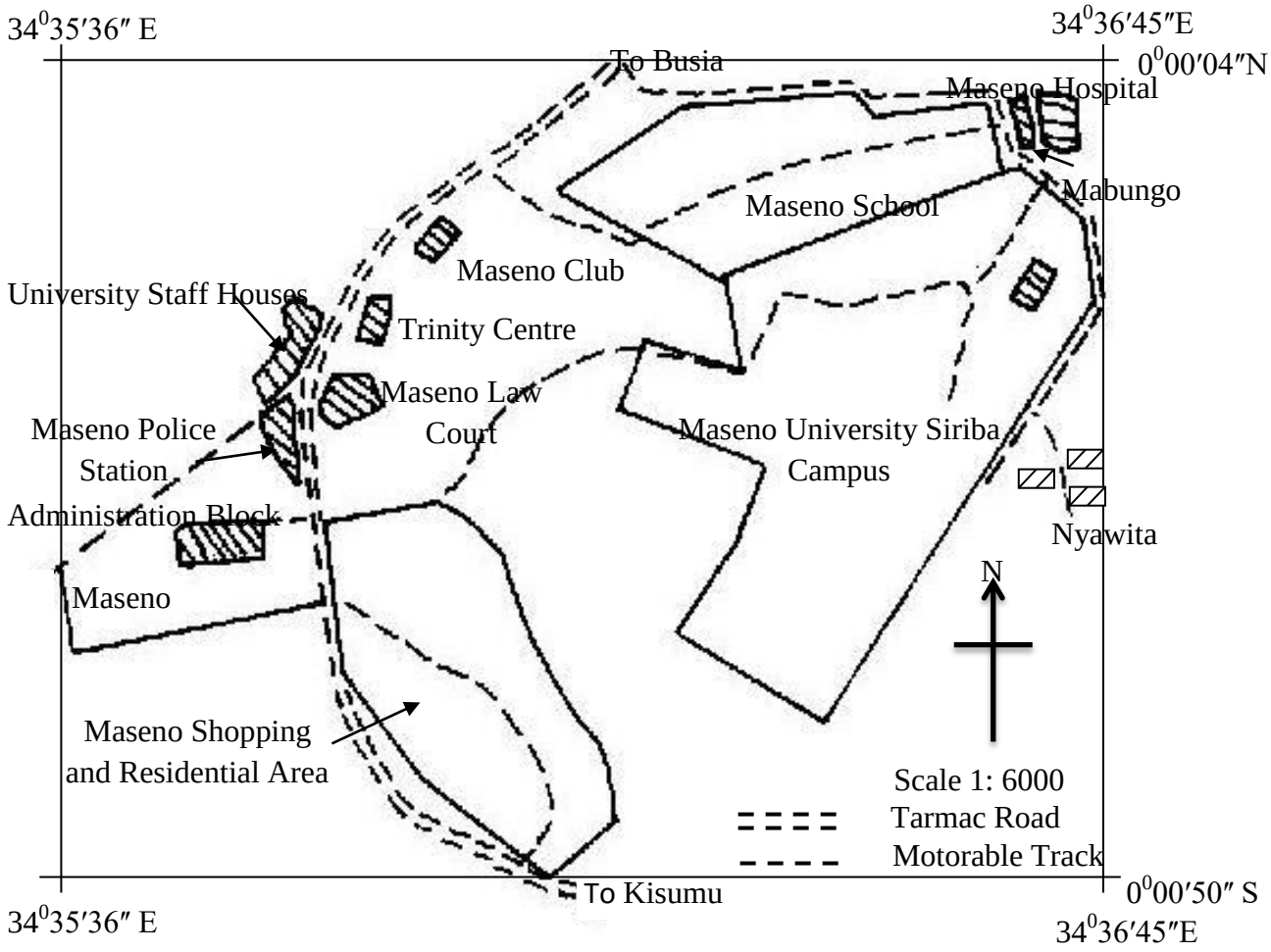

Figure 3.1: Map of Maseno Township

3.2 Study Population

The ready-to-eat food outlets within Maseno Township were inspected and the sampling criteria were determined. The outlets were 35 in total. The operators mainly were cooking a variety of foods within their premises and clients came in at various times of the day to take their meals. These types of outlets used a lot of water for their operations such as cooking, washing utensils, drinking and hand washing. A few operators had water connections to their premises but the supply was not frequent and if any it was not adequate for their use. They used tanks and plastic super-drums for their water storage. Some operators had modified the storage containers to have taps, while others used deep-in containers to get water from storage. Open storage containers were low enough to be reached by animals and children hence had high chances of recontamination. Residual microbial load start to multiply after the chemical deteriorates with time posing a risk to the clients and operators.

3.2.1 Inclusion criteria

Food outlets where foods were cooked and served within the premises was the key consideration. The clients were walking in any time of the day and being served various ready-to-eat main dishes. The outlets were identified during the mapping of the study area by the researcher and research assistant.

3.2.2 Exclusion criteria

Fast-foods outlet where mainly the ready-to-eat foods were snacks, that is, foods which one can eat without necessarily an individual washing his hands. Also food outlets where they served mineral water for drinking were excluded from the study.

3.3 Study Design

This was a cross sectional study where a questionnaire was adopted to collect data on the knowledge of food outlet operators about sources of potable water and water treatment practices. Laboratory experiments using conventional microbiological procedures were used to determine the bacteriological quality of water samples. The laboratory experiments were done in the Microbiology and Cell Culture Research Laboratory at Biomedical Science and Technology Department, Maseno University.

3.4 Sample Size

Sample size was determined by the formula shown below (Kothari, 2002):

$$n = \frac{z^2.p.q.N}{e^2(N-1) + z^2.p.q}$$

Where *p* is sample proportion, *q* = 1- *p*.

e = acceptable error (the precision). For this study the significance level was 5%.

z = standard variate (at 95% confidence is 1.96).

n = sample size.

N = population size. For this study there were 35 food outlets in Maseno Township.

Since *p* is what the study was trying to estimate, the value of *p* was taken as 0.5 whereby the sample was expected to yield atleast the desired precision. This was taken as the most conservative sample size.

$$n = \frac{(1.96)^2(0.5)(1-0.5)(35)}{(0.05)^2(35-1) + (1.96)^2(0.5)(1-0.5)} = 32.2 \cong 33$$

3.5 Sampling Procedure

As per the sample size calculation the minimum sample was 33 ready-to-eat food outlets which were sampled by simple random sampling method. During the familiarization tour, a sampling frame was prepared and then numbers were assigned to each ready-to-eat food outlet. The numbers were written in small pieces of paper, rolled into small balls and pooled in a bowl. The papers were thoroughly mixed before randomly picking one at a time until a minimum of 33 samples were picked. There were three sets of water samples from every food outlet in Maseno town. The collection for each set was done thrice at an interval of one week. These included 100mL water from ready-to-drink containers on the tables, hand washing water dispenser and storage container respectively. The water collection container was a sterile 200 mL capacity whirl pack (NASCO International, Inc., FT Atkinson, WI, USA) pre-marked 100 mL. The collection containers were handled aseptically before, during and after water collection. The samples were put in a cool box with ice packs and then transported to the laboratory whereby processing was done within four hours after collection. The operators sampled water from point-of-use source so as to get the real scenario how they fetched their water; the researcher or the trained research assistant then transferred the sample to whirl packs. Collection techniques depended on the type of holding container at the time of collection. If the holding container was small the samples were poured aseptically into the sterile collection containers. But if the holding container was big whatever jug or scooper used by the food outlet operators was used in the collection. Water containers with taps or even where the water is fetched directly from distribution taps was collected directly into the sterile sample collection containers.

3.6 Assessment of Knowledge on Water Sources and Water Treatment Techniques

A questionnaire was administered to each of the operators to establish if they knew water sources fetched by the vendors and the mode of water treatment undertaken on water used in their premises. The questionnaire was interviewer-administered whereby the manager or his/her representative responded and the answers provided were ticked without the respondents seeing the possible answers listed in the questionnaire. This was done after the study objectives and what is expected had being explained to them by the researcher. The questionnaire sought to establish sources of water, water treatment techniques employed by the operators among other information (Appendix VI).

3.7 Water Bacteriological Quality Determination

The petrifilm (3M® Corporation, USA) was used to enumerate total coliform and *E. coli* (faecal thermotolerant) levels in water as per manufacturer's instructions. Water (100 ml) from each set was filtered using a sterile millipore filter 0.45µL pore size (Pall Corporation, USA) with the help of a vacuum pump (Rocker, Japan). The filtration was done in such a way that about 1.5 ml was left on the filter; the petrifilm was labeled with the food outlet number and the sample point-of-use source. The labeled petrifilm was then inoculated, by lifting the top layer to expose the plating surface of the petrifilm, and using a sterile pipette, 1 mL of the sample was aseptically added. The top film was then slowly rolled down and the "spreader" was gently pressed on top of the film for even distribution, and allowed for about a minute to gel. The inoculated petrifilms were then incubated at 44.5^0C for 18 – 24 hours, after which characteristic colonies of interest namely blue in colour, with an air bubble surrounding and those with red spots were enumerated as *E. coli* (faecal thermotolerant) counts and thermotolerant coliforms respectively (see appendix

II). Enumeration was done using the standard grids on the petrifilm with the help of a tally counter. The colony forming units per 100 mL of sample (CFU/100 mL) were recorded.

3.8 Isolation of Bacterial Pathogens Associated with Water

3.8.1 Selenite F broth sub-culture and culture

The filter, after inoculation of the petrifilm, was then transferred into selenite F broth (Himedia, India) – using sterile forceps - for enrichment purpose. Selenite broth culture was incubated at 37^0C for 18 to 24 hours and then sub-cultured on a combination of greater and lesser inhibitory selective solid media namely: MacConkey (Himedia, India) and Salmonella/Shigella agar (Himedia, India). After incubation at 37^0C for 18 – 24 hours the characteristic colonies (colourless) of interest were stained using Gram's staining technique whereby Gram negative bacilli were processed further for identification purpose, that is biochemical tests (Cheesbrough, 2006).

3.8.2 Biochemical tests

In triple sugar iron agar - TSI - (Himedia, India) and lysine iron agar - LIA - (Himedia, India) the medium was put in a tube, sterilized and slanted so that a short slant and deep butt were formed. Characteristic single colonies from the solid agar were inoculated in both tubes with a straight needle by stabbing the butt and streaking the slant. The caps of the tubes were replaced loosely so that aerobic conditions prevailed on the slant, and then incubated at 37^0C overnight. The characteristic organisms was picked by a straight wire and then subjected to further identification process (Cheesbrough, 2006).

3.8.3 The API - 20E enteric identification system

A wire loop was used to scoop a loopful of the characteristic organisms from TSI and a suspension of the pure isolate was made using normal saline. A plastic strip holding twenty mini-

test tubes was inoculated with the saline suspension (as per manufacturer's insert directions). This process also rehydrates the desiccated medium in each tube. A few tubes were completely filled and some were overlaid with mineral oil such that anaerobic reactions could occur. After incubation in a humidified chamber for 18-24 hours at 37^0C, the color reactions was read (some with the aid of added reagents), and the reactions (plus the oxidase reaction done separately) were converted to a seven-digit code which is called the Analytical Profile Index - API - (bioMerieux, Inc., Hazelwood, MO, France). The code was then interpreted through a book which had a list for the identification for most Enterobacteriaceae (see appendix III).

3.8.4 Serotyping of *Salmonella* species

Polyvalent antisera (Beckton Dickson, USA) for specific organisms was used in the serotyping the isolates in a slide test. O and H antisera which were commercially available and manufacturer's instructions were used in the testing and interpretation of results. This was a rapid agglutination test whereby a pure colony of the organisms' suspension was prepared using buffered normal saline. A drop of the suspension was added onto the pre-labeled (O, H - a, b, c, d, I, z10 and z29 - and sample number) transparent test tile and a pre-warmed to room temperature antisera was added 1 drop each to O and to the specific H circles and mixed well with an applicator stick. The mixture was then rocked well for two minutes while checking for agglutination. The test was positive if there was visibly clear agglutination in both O and specific H within the two minutes.

3.8.5 Serotyping of *Shigella* species

Shigella antisera typing (Beckton Dickson, USA) was used for serotyping organisms which had cultural characteristics of *Shigella* species where the API 20E outcome was not conclusive. The antisera represented the different species of shigellae organisms, that is, *S. dysenterae* (A), *S.*

flexneri (B), *S. boydii* (C) and *S. sonnei* (D). The well isolated specific colony was picked aseptically and then a suspension of the organism was prepared using buffered normal saline supplied by the manufacturer. A drop of the suspension was added to a pre-labeled (A, A1, B, C, C1, C2 and D) transparent test tile and a pre-warmed antisera was added to the specific circles and mixed well with an applicator stick. The mixture was the rocked for two minutes and agglutination within the two minutes was to be interpreted as positive but in this case there was no visible agglutination, hence negative for *Shigella* species.

3.9 Data Management and Analysis

Data generated was stored in a computer with a password only known by the principal investigator. Content analysis was used for quantitative data generated by the questionnaire. Chi-square (χ^2) was used to test for statistical significance for objective one and two. While in objective three MANOVA was used to determine whether there were variations between bacteriological quality of water samples and the different water sources. Post hoc test least significant difference (LSD) helped in comparing a pair of sources of water if there was a significant statistical variation against the average $\log_{10}$ (*E. coli*) faecal thermotolerant colony count.

3.10 Ethical Considerations

The ethical considerations were emphasized in order to safeguard the integrity of the study. Permission to conduct the study within Maseno Township was granted from District Public Health Officer Kisumu West Sub-County. Ethical approval from the Maseno University Ethical Review Committee (MUERC) was granted. The operators on the other hand were sensitized on the study; in addition, they were given a consent letter to sign before sample collection began.

The data collection tools with raw data were kept in protected area accessed by the researcher only. The confidentiality of the study participants was guaranteed by the researcher not disclosing the research outcome to any other person before their consent was sought.

CHAPTER FOUR: RESULTS

4.1 Socio-Demographic Profile

The ready-to-eat food outlet operators in Maseno Township are adults whose average age was 37 years ranging 20 – 65 years old and 18 (54.5%) of them were male. Food production and or management related course had 17 (51.5%) of the operators while six (18.2%) have other trainings like education and business related courses. Based on the level of education, 10 (30.3%) of the operators whose education was below secondary education none had any professional training (Table 4-1).

Table 4-1: Socio-demographic profile of the ready-to-eat food outlet operators in Maseno Township

Level of Education of the In-Charge		
	Frequency (n)	**Percent (%)**
Primary	2	6.1
Secondary	8	24.2
College	23	69.7
Total	33	100.0
Professional Training of the In-Charge		
	Frequency (n)	**Percent (%)**
None	10	30.3
Catering	9	27.3
Food Technology	1	3.0
Nutrition	7	21.2
Any Other	6	18.2
Total	33	100.0
Gender of the In-Charge		
	Frequency (n)	**Percent (%)**
Female	15	45.5
Male	18	54.5
Total	33	100.0

The table shows the frequency of the socio-demographic profile of the operators in the ready-to-eat food outlets in Maseno Township. The variables considered include education level, professional training and gender.

4.2 Knowledge of food outlet operators about the source of water they use in their food outlets within Maseno Township

The study shows that 30 (91%) of the food outlet operators buy water from vendors whereas three (9%) have their own protected wells within their premises (Figure 4-1).

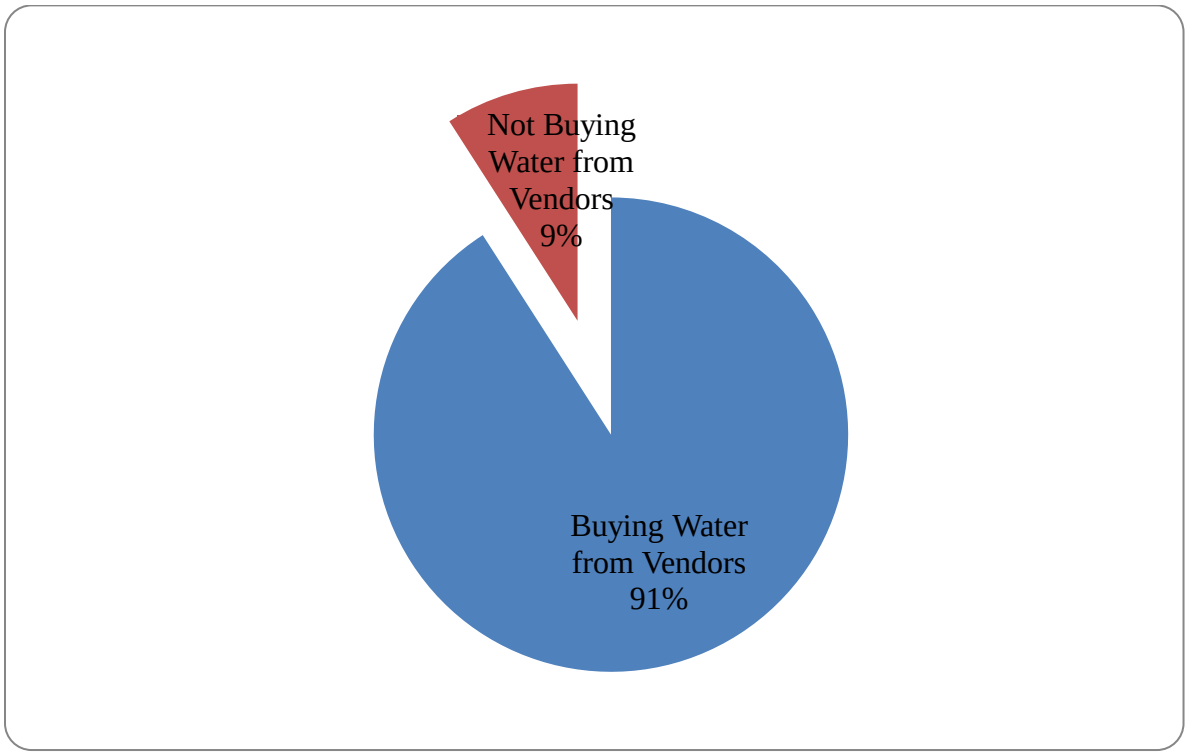

Figure 4-1: Ready-to-Eat food outlet respondents in relation to buying of water from vendors

A total of 21 (70%) of the ready-to-eat food outlet operators' knew sources of water they were using while nine (30%) did not (Figure 4.2). Those who did not know the source of water did not inquire from the vendors where they got their water from.

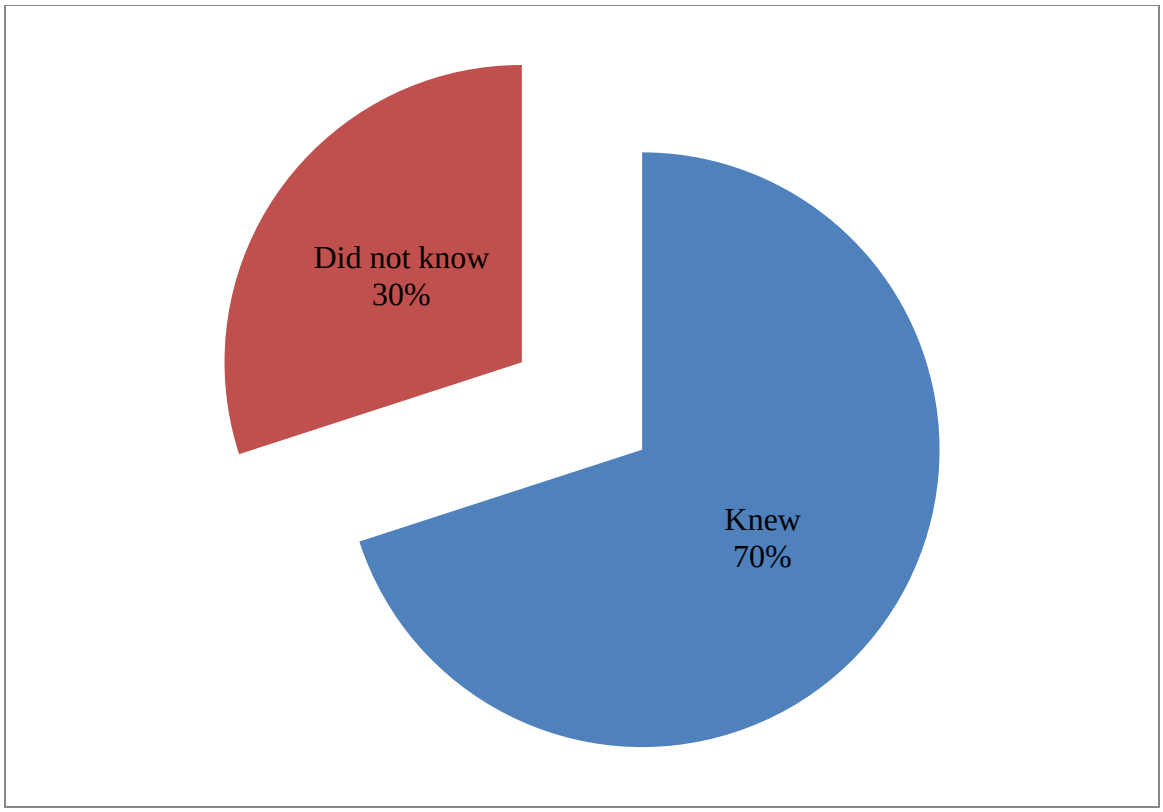

Figure 4-2: Knowledge of where water is fetched from by the operators

Water from public tap/standpipe was the most mentioned by eight (24%) of the ready-to-eat food outlet operators which was followed by four (12%) protected spring. Piped water to yard/plot and protected dug well were each mentioned by three (9%) of the respondents whereas tube-well or borehole, unprotected spring and surface water were the least mentioned, two (6%).

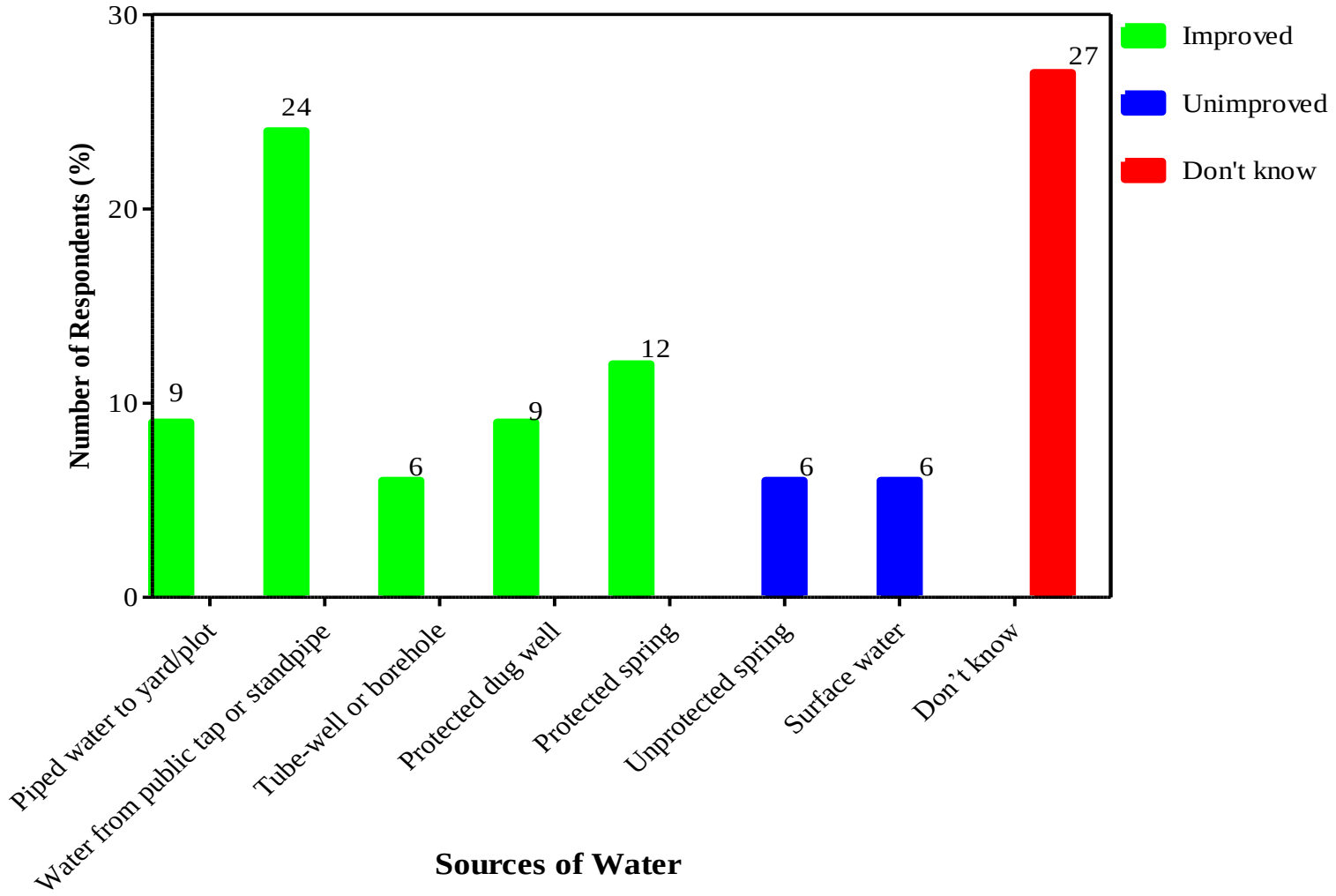

Figure 4-3: Sources of water used by food outlet operators in Maseno Township

The figure shows the sources of water as reported by the ready-to-eat food outlet operators (%). The classification of sources is based on WHO criteria. The "don't know' bar whows the number (%) of operators who did not know the sources of their water.

Statistical evidence showed significance of association between the classifications of the sources of water into improved, unimproved and those not known by the operators of the ready-to-eat food outlets in Maseno Township. The χ^2 value was (df = 7) 15.435, p = 0.031 (Table 4-2).

Table 4-2: Statistical analysis of sources of water classification as reported by the ready-to-eat food outlets operators

	Value	Degree of freedom	Asymptotic Significance (2-sided)
Pearson Chi-Square	15.435	7	0.031

The table shows the χ^2 relationship of the sources of water as was reported by the ready-to-eat food outlet operators in Maseno Township. Those who did not know where their water was fetched from was indicated as don't know.

4.3 Modes of Water Treatment Practices in the Food Outlets within Maseno Township

Water treatment at the point of use was slightly less 16 (48%) than the non-treatment 17 (52%) (Figure 4-4).

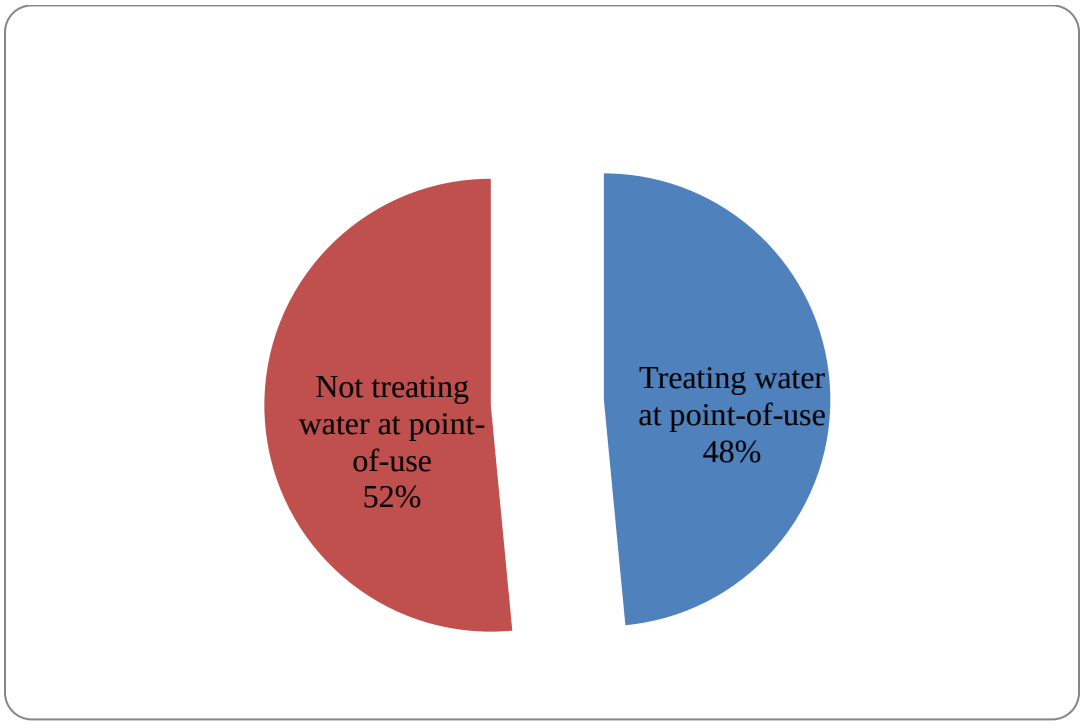

Figure 4-4: Treatment of water at the point-of-use by the food outlet operators

Ready-to-drink water was not treated at point of use by 17 (52%) respondents while three (9%), two (6%) and 11 (33%) used boiling, chlorination and waterguard respectively for water treatment (Figure 4-5). Hand washing water, on the other hand, had a slight increase of operators who did not treat the water 20 (61%) while boiling, chlorination and waterguard which was four (12%), three (9%) and six (18%) respectively was used by the ready-to-eat food outlets to treat hand washing water (Figure 4-5).

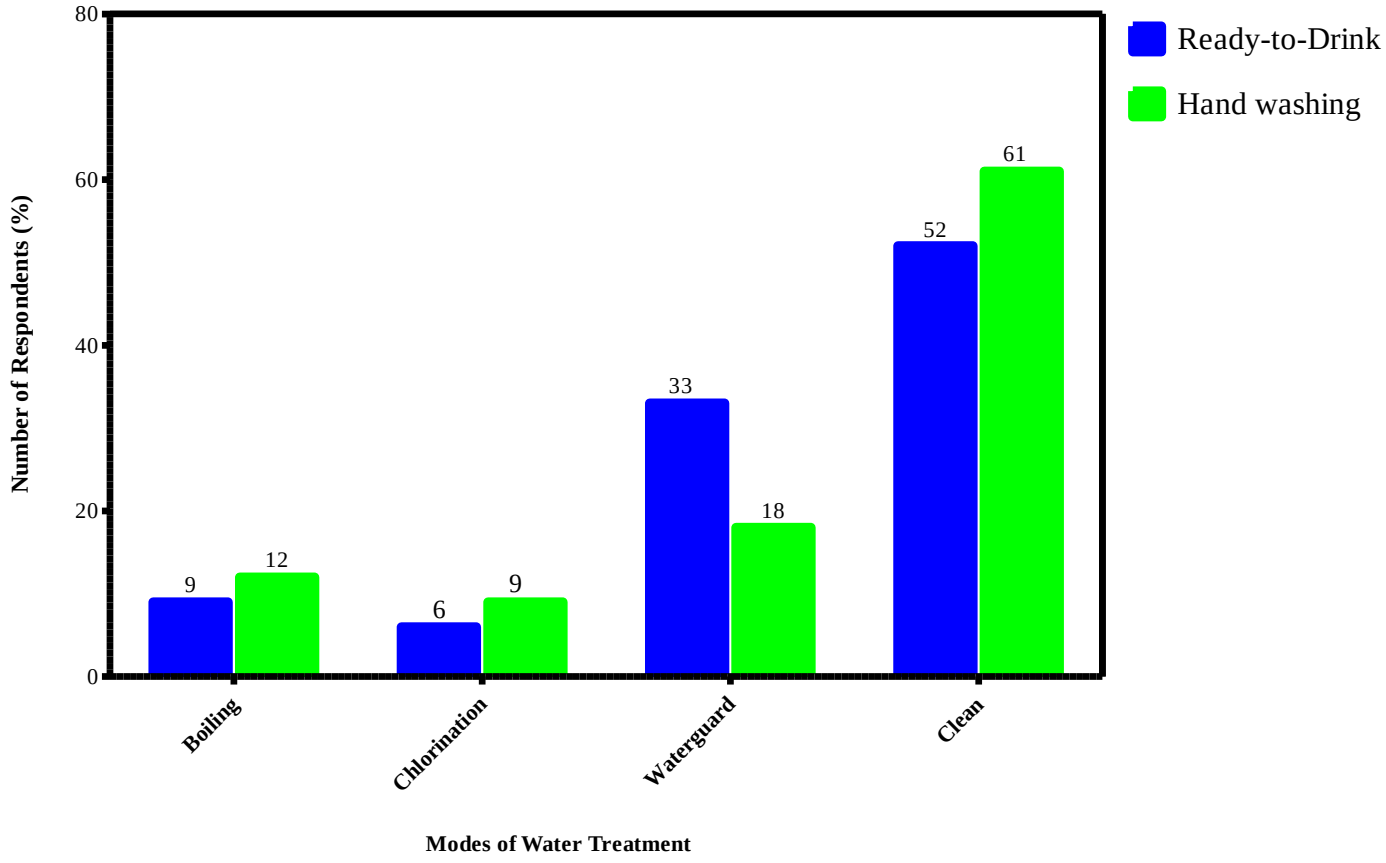

Figure 4-5: Treatment of ready-to-drink and hand washing water at the ready-to-eat food outlets in Maseno Township

The figure shows the number of food outlet operators (%) who reported the modes of water treatment at the point of use. The operators who did not treat water at the point of use are indicated as 'clean' in the figure above.

There was no statistical evidence of significant variation of the modes of water treatment at the point of use in the ready-to-eat food outlets in Maseno Township. The statistical analysis was on ready-to-drink and hand washing water. The χ^2 value was (df = 3) 2.057, p = 0.561 (Table 4-3).

Table 4-3: Statistical analysis of treatment of ready-to-drink and hand washing water at the point-of-use

	Value	Degree of freedom	Asymptotic Significance (2-sided)
Pearson Chi-Square	**2.057**	**3**	**0.561**

The table shows the χ^2 relationship of ready-to-drink and hand washing water treatment in the ready-to-eat food outlets in Maseno Township.

4.4 Bacteriological quality of water used in the ready-to-eat food outlets within Maseno Township

Bacteriological quality of water in this particular study was in relation to faecal thermotolerant coliform counts (*E. coli*) and thermotolerant coliform counts from a petrifilm. The faecal thermotolerant coliforms are used in water analysis as indicator models while other thermotolerant coliforms are process indicators. Indicator models act as predictors of presence of pathogenic microorganisms in water while process indicators are used to indicate if the treatment process was adequate or not.

4.4.1 *E. coli* (Faecal thermotolerant) colony forming unit count

Colony forming unit count in various types of water samples showed that there was contamination of water (Table 4-4). Also see appendix I for thermotolerant and total thermotolerant colony forming unit counts.

Table 4-4: Average *E. coli* count in hand washing, ready-to-drink and stored water

Hand Washing Water										
		Sources of Water								Total
		Piped water to yard/plot	Water from public tap or standpipe	Tubewell or borehole	Protected dug well	Protected spring	Unprotected spring	Surface water	Don't know	
E. coli CFU/100 ml	<1	4	4	0	2	1	1	2	6	20
	1 – 10	3	0	1	1	1	1	0	3	10
	>10	0	0	1	0	2	0	0	0	3
Total		7	4	2	3	4	2	2	9	33

Ready-to-Drink Water										
		Sources of Water								Total
		Piped water to yard/plot	Water from public tap or standpipe	Tubewell or borehole	Protected dug well	Protected spring	Unprotected spring	Surface water	Don't know	
E. coli CFU/100 ml	<1	7	4	2	3	3	1	2	6	28
	1 – 10	0	0	0	0	1	1	0	3	5
Total		7	4	2	3	4	2	2	9	33

Stored Water										
		Sources of Water								Total
		Piped water to yard/plot	Water from public tap or standpipe	Tubewell or borehole	Protected dug well	Protected spring	Unprotected spring	Surface water	Don't know	
E. coli CFU/100 ml	<1	6	3	1	3	3	1	2	6	25
	1 – 10	1	1	1	0	1	1	0	3	8
Total		7	4	2	3	4	2	2	9	33

In the MANOVA tests Wilks' Lambda was used for the analysis since the count in each category was unequal. There was a statistical significance in the overall multivariate analysis of the variance of various sources of water against the $\log_{10}$ transformed average faecal thermotolerant colony count; F (3, 21) =1.917, p = 0.024 (Table 4-5). This shows that sources of water differed significantly in respect of average $\log_{10}$ faecal thermotorelant coliform count.

Table 4-5: Multivariate test of bacteriological quality of water used in the ready-to-eat food outlets within Maseno Township.

	Value	F	Hypothesis degree of freedom	Error degree of freedom	Significance level (*p*-value)	Partial Eta Squared
Wilks' Lambda	0.257	1.917	21.000	66.594	0.024	0.364

The table shows the overall *p* value of the MANOVA test for sources of water.

The average $\log_{10}$ *E. coli* colony count in the three point of use, that is, ready-to-drink, hand washing and stored water was statistically analyzed by MANOVA. Hand washing water differed significantly in respect of the water sources; F (7, 25) = 3.036, p = 0.019.

Table 4-6: Tests of effects of bacteriological quality of water used in the ready-to-eat food outlets within Maseno Township.

Dependent Variable	Degree of freedom	F	Significant	Partial Eta Squared
Average $\log_{10}$ *E. coli* colony count in hand washing water.	7	3.036	0.019	0.459
Average $\log_{10}$ *E. coli* colony count in hand washing water.	25			

The table shows the outcome of MANOVA test where the average $\log_{10}$ *E. coli* count of the three point of use water sources (ready-to-drink, hand washing and stored water) where compared. Hand washing water was found to have a significant *p* value.

A post hoc test to assess the interaction of various sources indicated that there were several statistically significant variations in the *E. coli* colony count (Table 4-7). For example, water

from public tap or standpipe against protected spring, had the highest statistical significance (p = 0.002), while tube-well or borehole against piped water to yard/plot had the least statistical significance (p = 0.039).

Table 4-7: Multiple comparisons of bacteriological quality of water used in the ready-to-eat food outlets within Maseno Township

Least Significance Difference (LSD)							
Dependent Variable	**(I) Sources of water**	**(J) Sources of water**	**Mean Difference (I-J)**	**Std. Error**	**Significance level (*p*-value)**	**95% Confidence Interval**	
						Lower Bound	**Upper Bound**
Average $\log_{10}$ *E. coli* colony count in hand washing water.	Tubewell or borehole	Piped water to yard/plot	0.4950*	0.22659	0.039	0.0283	0.9617
		Water from public tap or standpipe	0.6650*	0.24474	0.012	0.1609	1.1691
		Protected dug well	0.5983*	0.25798	0.029	0.0670	1.1297
		Surface water	0.6650*	0.28260	0.027	0.0830	1.2470
		Don't know	0.5017*	0.22092	0.032	0.0467	0.9567
	Protected spring	Piped water to yard/plot	0.5225*	0.17713	0.007	0.1577	0.8873
		Water from public tap or standpipe	0.6925*	0.19983	0.002	0.2809	1.1041
		Protected dug well	0.6258*	0.21584	0.008	0.1813	1.0704
		Surface water	0.6925*	0.24474	0.009	0.1884	1.1966
		Don't know	0.5292*	0.16982	0.005	0.1794	0.8789
Average $\log_{10}$ *E. coli* colony count in potable water.	Unprotected spring	Piped water to yard/plot	0.3907*	0.14280	0.011	0.0966	0.6848
		Water from public tap or standpipe	0.4050*	0.15424	0.015	0.0873	0.7227
		Tubewell or borehole	0.4050*	0.17810	0.032	0.0382	0.7718
		Protected dug well	0.3717*	0.16258	0.031	0.0368	0.7065
		Protected spring	0.3800*	0.15424	0.021	0.0623	0.6977
		Surface water	0.4050*	0.17810	0.032	0.0382	0.7718

The table shows a post hoc test outcome where a pair of sources of water was compared and the multiple *p* values obtained indicated the sources which contributed to the overall significant p value in MANOVA test.

*The mean difference is significant at the 0.05 level.

Table 4-8: Identity of suspected pathogenic water isolates from ready-to-eat food outlets in Maseno Township.

SAMPLE NUMBER	API 20 E RESULTS	SEROVARIANTS
012	*Salmonella* species	*Salmonella* Typhimurium (i-H)
023	*Salmonella* species	*Salmonella* Typhimurium (i-H)
025	*Salmonella* species	*Salmonella* Typhimurium (i-H)

The table shows isolates with cultural characteristics suspected to be of enteric pathogens. They were tested using API 20 E and then serotyped using specific antisera to give the serovariants.

Seven isolates were indicative of pathogenic microorganism, that is, either *Salmonella* species. In serotyping there were three (9.1%) serovariants of *Salmonella* Typhimurium (i-H) identified (Table 4-8).

4.4.2 Risk level categorization

Based on the colony forming unit count for faecal thermotolerant coliforms water was classified in relation to the potential risk to waterborne disease outbreak. Bacteriological indicators have different implications where faecal thermotolerant coliforms are surrogates of possible presence of pathogenic microorganisms in water. All samples were used in the classification without transforming (log_{10}) the data; no averages were used. There were nine sample categories so as to indicate individual risk potential (figure 4.6 and Table 2.1). Generally all three hand washing water sample categories showed very high risk level, that is, three (9%), two (6%) and three (9%) ready-to-eat food outlets respectively. Stored water on the other hand, only first sample category showed very high risk level one (3%) ready-to-eat food outlets), while only first sample of the ready-to-drink water showed very high risk one (3%).

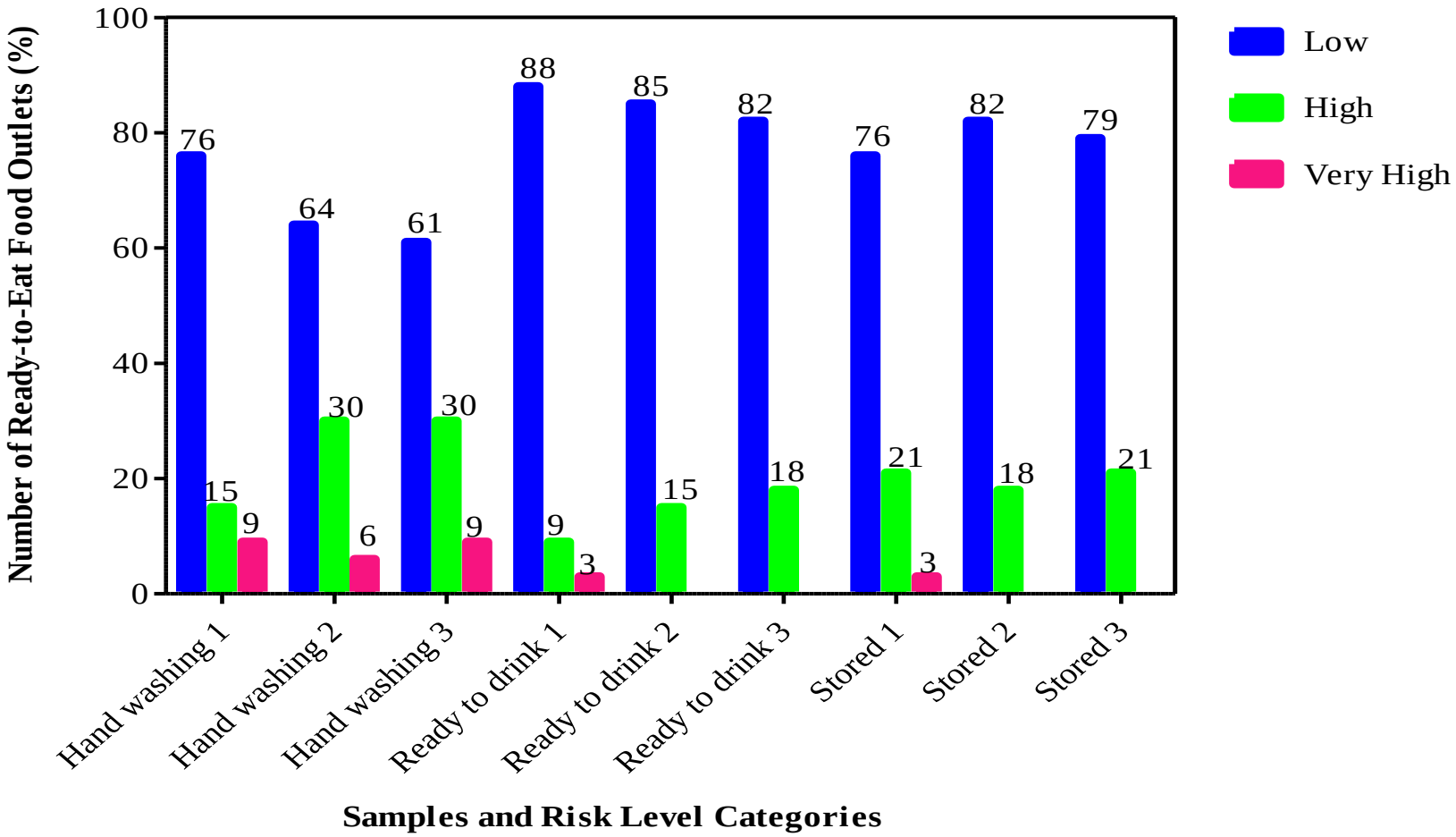

Figure 4-6: Categories of bacteriological risk levels in water from ready-to-eat food outlets in Maseno Township
The figure shows the risk classification based on table 2-1 where the colony forming units were categorized without transforming the count into $\log_{10}$.

CHAPTER FIVE: DISCUSSION

5.1 The Knowledge of Food Outlet Operators about the Source of Water they use in their Ready-to-Eat Food Outlets in Maseno Township

The operators who knew about the source of water in this study was high (73%) unlike the knowledge of study participants in Uganda (60%) in which they did not know where their water was fetched from and if they did they had no idea about the classification of the sources (Parker, *et al*., 2010). The high use of improved sources of water by the food outlet operators is very encouraging as this is a great achievement towards the millennium development goal of safe water supply by the year 2015 and 91% is the global population which is using an improved potable water source (WHO/UNICEF, 2015). The indicator for the MDG (7c) was the proportion of the population with access to improved sources of potable water. The target was reported to have been achieved in the year 2010, earlier than the 2015 deadline (WHO/UNICEF, 2015). The classification of water sources as improved and unimproved is based on prevention of contamination (Medema *et al*., 2003; Momba *et al*., 2006; Magali & Emmanuel, 2007). This is achieved by having sanitation facilities in a recommended distance away from water sources, no accessibility by children or animals and prevention of back-overflow of water from the environment (Parker *et al*., 2010).

Sources of water are important as they influence microbiological contamination depending on the improvement of the surrounding which prevents accessibility by animals and dirty water back-flow which are sources of microbiological contamination (Stauber *et al*., 2013; Bain *et al*., 2014). Some of the water vendors could at times be crafty since they are after profits which might influence them to fetch water from anywhere as there is no immediate indicator of

microbial contamination for the operators to scrutinize the water supplied. Awareness of the sources of water and their classification can help the operators be confident of the kind of water they are using in their operations. Also this will help the public health officials estimate the percentage of the population with access to improved water sources as per the millennium development declaration (goal 7).

Rain water is classified as improved source, hence potable, but none of the operators was using it for their operations. This could be explained as either they feared revealing about this particular source or they are ignorant about this source. The gutters and collection containers if managed hygienically well the water should be ready for drinking and use in other domestic cleaning.

The limitations of this study are that the assessment did not cover an area of accompanying the operators to the sources to find out the surrounding. It also did not cover the water vendors to see if there is a discrepancy between the operators' responses and the vendors. Also this study did not assess the knowledge about the classification of sources. The transportation of this water was not assessed despite the fact that it is an important factor in water recontamination.

5.2 The Modes of Water Treatment Practices in the Ready-to-Eat Food Outlets in Maseno Township

Findings of the current study indicate that almost half of the participants were aware of the risks posed by water hence they adopted the point-of-use treatment. Previous studies have shown that point-of-use treatment is effective in improving bacteriological quality of water as a result reducing water borne diarrhoreal diseases (Arnold & Colford, 2007). This however, does not mean that the public health officials should rest and watch but they should enhance campaigns to sensitize the operators so that there can be 100% adoption of point-of-use treatment. The

variation of water treatment, that is, hand washing against ready-to-drink water indicates that people have different preferences for water treatment with regard to water microbial quality verses infection. Hands can be good reservoirs of microorganisms as they attach on hands and remain in the nails which can be a vehicle of infection or recontamination of stored water (Trevett *et al*., 2005). Most studies have concentrated on household water treatment and forgotten about this important sector which serves the ever increasing population which prefers eating in food joints due to their busy outdoor activities. Since these food outlets serve different clients there is a likelihood of disease outbreak as compared to household recontamination as the faecal contaminants may be circulating in the same household members leading to herd immunity (Trevett *et al*., 2005; Onyango-Ouma and Gerba, 2011).

Water treatment at the point-of-use has been adopted by some of the operators as per the study. They are using chemical and boiling methods. Whereas there was low response in the use of boiling, this is a big contrast to an earlier study which showed a 90% response in households sampled (Makutsa *et al*., 2001). The big disparity may be attributable to the cost of fuel for boiling as a large amount of water is required by clients in a day while household members are few. Chemical treatment on the other hand, had chlorination and waterguard where the later had a high (33%), adoption rate. Boiling is the best for water treatment as there are no resistant microorganisms to heat as compared to chemical resistant microorganisms. Another advantage of boiling is that it can be used effectively in even turbid water whereas chemical treatment requires either filtration or sieving to make it effective.

The combined rate of chemical use for water treatment by the food outlet operators is higher compared to 33.5% of the households who used chlorine disinfectant product developed by CARE Kenya project (Makutsa *et al*., 2001). This was achieved by the project through a planned

campaign to promote the chemical water treatment after it emerged that there was very low adoption rate of chemical disinfection. This was attributed to high cost and unpleasant smell of chlorine but at 1 % chlorine it was acceptable by the community. The current study found that most of the respondents did not know the difference between waterguard (has low concentration of chlorine) and chlorine (has a higher concentration of chlorine). In addition, turbidity can be managed by use of flocculation, sedimentation and disinfection for the removal of microorganisms. Pur® Water Purifier, which has proved to be effective POU application, is a water treatment product (manufactured by Procter & Gamble, Cincinnati, Ohio, USA) which is composed of a coagulant, an alkaline agent, flocculation aids, a flocculent and a chlorine-based disinfectant. A sachet of the product is used in 10 L water (Souter *et al*., 2003). This product can prove to be of importance in water turbidity management in Maseno Township if adapted.

A study carried out to evaluate a point-of-use interventions in households indicated that, *Escherichia coli* levels in stored household water were <1 CFU/100 ml in most households where water was treated during research (interventions) but readily detectable at high levels in control households (no intervention) (Sobsey and Bartram, 2003). Water treatment at the point-of-use has shown to reduce diarrhoeal illnesses in the community (Sobsey and Bartram, 2003; WHO, 2008b).

Filtration is a form of water purification which removes dirt and some microorganisms from water - especially if a 0.45 microns pore size in vacuum system. However, none of the operators were using it. This could be explained by the ignorance of the operators as there are cheaper options of water filtration which the operators can afford to use. The process makes the subsequent water treatment especially chemical treatment, easier and effective.

The study did not incorporate measures to check the efficacy of the various modes of water treatment. Boiling water for example is a technique which is practiced by many but they understand it differently. Also the chemical treatment is effective at different concentrations hence there was need for the operators to be assessed on how they use the various chemical methods. Also there was need for observation on the application of the various modes of water treatment at the ready-to-eat food outlets. This will give a rough indication of the understanding of the operators and also the efficacy of the various methods used. Observing rolling boiling water, for example, assures that sufficiently high temperatures have been reached to achieve microorganism destruction which very few people practice. Free chlorine residue stays in water for slightly after 24 hours hence the need to test for the chlorine residue as a means of assessing the efficacy of the chlorine based disinfectants and form a basis of advising the operators on the duration of water storage after treatment.

5.3 The Bacteriological quality of Water used in the Ready-to-Eat Food Outlets in Maseno Township

Water in the ready-to-eat food outlets was contaminated with thermotolerant coliforms. The contamination of water at the point-of-use after its collection from the source and during storage has been documented (Trevett *et al*., 2005). WHO (2011) potable water quality guidelines make reference to water supply situation which is common in most developing countries where access to microbiologically safe water is scarce hence the need for water to be collected from a source, transported to the point-of-use and then stored for daily domestic use. Water transported or stored unhygienically may be re-contaminated and most of this is as a result of behavioural patterns; which when improved the health risk can be reduced or eliminated. Most of these

studies, however, are on household water not food outlets. The techniques, nonetheless, for bacteriological quality count are similar.

Coliforms that are able to grow and ferment lactose with the production of acid and gas at 44.5^0C in a bile salts environment are grouped as faecal thermotolerant coliforms (Figueras and Borrego, 2010). However, some thermotolerant coliform bacteria that conform to these defined characteristics belong to the genus *Klebsiella* and have been isolated from the environment in the apparent absence of faecal contamination (Fenwick, 2006; Figueras and Borrego, 2010). Potable water at the point-of-use should have <1 CFU/100 ml of faecal thermotolerant coliforms (WHO, 2011). Faecal thermotolerant coliforms are normally found in warm blooded animals, so when found in potable water, it is a cause of concern. This indicator microorganisms, however, are not pathogenic in non-immunocompromised persons (Fenwick, 2006; Fong *et al*., 2007).

Risk classification enables the stakeholders to device mechanism of planning on how to combat waterborne disease outbreaks. According to world health organization's toolkit for monitoring and evaluating water for domestic use very high risk (>10 CFU/100 ml), for example, requires an immediate attention to prevent disease outbreaks(WHO/UNICEF, 2012). Studies have shown that even in very high risk category there are cases of no major disease outbreaks. This may be due to strong herd immunity and the pathogens having stabilized, that is, the community having developed immunity to the particular pathogens (Younes and Bartram, 2001).

No major intervention at the point-of-use is required other than the environmental and behavioural actions to maintain low bacteriological quality of water. Some studies, however, have shown that the absence of indicator organisms does not rule out pathogens in potable water (Sobsey and Bartram, 2003; WHO/UNICEF, 2012). Quantitative risk assessment on microbial

water safety and source monitoring by use of molecular techniques is important in order to give a specific probability to predict waterborne disease outbreak to the community (Gundry *et al*., 2004).

There were three isolates of serovariants *Salmonella* Typhimurium (i-H) and no *Shigella* species was isolated from water at the ready-to-eat food outlets. The pathogenic isolates *S. typhimurium* and *Shigella* species have been isolated previously from fish in Kisumu's Winam Gulf (Onyango *et al*., 2009). Fish is one of the foods processed by the food outlet operators which can easily be the source of pathogenic bacteria, for example, causative agents of bacillary dysentery and typhoid fever (Onyango *et al*., 2009). Water at the point-of-use has been shown to be a source of waterborne diseases (Leclerc *et al*., 2002; Momba *et al*., 2006; Frost *et al*., 2009; Cabral, 2010; Abednego *et al*., 2013).

Bacteriological water quality estimation has been widely done on household water but little on food outlets yet they serve a great number of people. The current study showed that there was a significant ($p=0.024$) variation among the average faecal coliforms (*E. coli*) count compared to the sources of water. A post hoc test; LSD (least significance difference) showed several significant variations (Table 4-7) when two means were compared. This encompassed both improved and unimproved sources. Isolates of three *Salmonella* Typhimurium (i-H) organisms were isolated and this indicates that there is need for an immediate intervention to avert a public health problem. For instance, in risk level categorization there were cases of high and very high risk (Figure 4-6). The risk categorization was based on individual samples as averages could not reveal specific risk to the community. A reservoir of any given microbiological infection however small may be dangerous to the community as microorganisms multiply at a very high rate if the environment is conducive.

This shows that at the point-of-use there is re-contamination of water as improved sources are not supposed to be harboring microorganisms as measures have been put in place to minimize contamination. Water is microbiologically safe if distributed by pipes from an improved source to the point-of-use but lack of this makes residents to transport and store water which are catalysts of re-contamination. These findings serve as a warning to public health officials to intensify measures of awareness, training on water management at point-of-use and constant monitoring of water bacteriological quality to avoid any major public health problem related to water. There is need for the public health officials to act and improve water as there are potential pathogens as well as high classification. Water at point-of-use after interventions should not have any contaminants but, despite water being fetched from improved sources and treated there was still faecal contamination. High risk categorization should not be arising in such a place as it serves many people. Bacteriological indicators (faecal thermotolerant coliforms) are surrogates for the presence of pathogenic bacteria in water. This analysis does not indicate the viability of pathogenic bacteria if present in water and also does not indicate if there are inactivated or destroyed pathogens in water when water treatment is effective.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Some of the operators knew about the sources of the water they are using. Water treatment at the point-of-use was lower among the operators than expected with increased accessibility to information it was expected all operators treat water at the point-of-use. Contamination of water was noted as this is supposed to be very minimal contrary to the findings.

6.2 Conclusions

1. The ready-to-eat food outlet operators who knew the source of water they use in their food outlets was higher than those who did not know.
2. The modes of water treatment practices at the point-of-use was boiling and chemical treatment only but still there were those who assumed that treatment was not necessary.
3. Water at some of the food outlets was contaminated with both faecal thermotolerant coliforms (*E. coli*) and other total thermotolerant coliforms. Also *Salmonella* Typhimurium (i-H) was isolated but no *Shigella* species was isolated.

6.3 Recommendations

1. Sensitization on sanitation and classification of sources of water is necessary as this will help the operators to make right decisions about where to fetch their water from. Rain water is an improved source hence need for the operators to use it, but they should be sensitized on how to properly collect and manage rain water after collection.
2. Filtration is a good technique of water purification which can also be encouraged as the first way of reducing turbidity and removal of organic matter to allow application of other methods of treatment. In addition flocculation, sedimentation and sieving should be

encouraged by use of Pur® Water Purifier or any other related product for combined water treatment. Education on water management and interventions, including behavioural, is necessary in order to increase the uptake of safe water management methods at the point-of-use. Finally, need to test residual chlorine in water and observe any interventions in water treatment. This will enable the public health official or any other stakeholder to confirm the interventions.

3. A planned and regular monitoring and evaluation of bacteriological quality of water used in the ready-to-eat food outlets in Maseno Township and any other areas of the Republic of Kenya. Proper medical health check records as per public health requirements for operators and workers of ready-to-eat food outlets must be kept properly and inspected regularly for compliance. Due to limited financial resources these can be done in collaboration with individual or corporate stakeholders.

6.4 Recommendation for Future Studies

1. Further studies to be done on the modes of storage and duration of storage which will give a picture of the post-collection water management.
2. Molecular studies to be carried out in water in order to identify and quantitate pathogenic organisms in water.
3. Quantitative risk assessment to be done in order to estimate the probability of waterborne disease outbreak in Maseno Township.

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APPENDICES

Appendix I: Colony Forming Unit Count for Thermotolerant Coliforms

Average Thermotolerant Coliform Count in Hand Washing Water * Sources of Water										
Count										
		Sources of water								Total
		Piped water to yard/plot	Water from public tap or standpipe	Tubewell or borehole	Protected dug well	Protected spring	Unprotected spring	Surface water	Don't know	
Colony forming unit per 100 ml (CFU/100 ml)	<30	2	3	0	1	2	1	0	4	13
	31 – 60	2	0	1	1	2	1	0	1	8
	61 – 90	1	0	1	0	0	0	1	1	4
	91 - 120	2	0	0	0	0	0	0	2	4
	>120	0	1	0	1	0	0	1	1	4
Total		7	4	2	3	4	2	2	9	33
Average Thermotolerant Coliform Count in Stored Water * Sources of Water										
Count										
		Sources of water								Total
		Piped water to yard/plot	Water from public tap or standpipe	Tubewell or borehole	Protected dug well	Protected spring	Unprotected spring	Surface water	Don't know	
Colony forming unit per 100 ml (CFU/100 ml)	<30	5	2	1	1	3	0	1	4	17
	31 – 60	1	1	0	2	1	1	0	3	9
	61 – 90	0	1	0	0	0	0	1	0	2
	91 - 120	1	0	0	0	0	0	0	2	3
	>120	0	0	1	0	0	1	0	0	2
Total		7	4	2	3	4	2	2	9	33

Average Total Thermotolerant Coliform Count in Hand Washing Water * Sources of Water										
Count										
		Sources of water								Total
		Piped water to yard/plot	Water from public tap or standpipe	Tubewell or borehole	Protected dug well	Protected spring	Unprotected spring	Surface water	Don't know	
Colony forming unit per 100 ml (CFU/100 ml)	<30	2	3	0	1	2	1	0	3	12
	31 - 60	2	0	1	1	2	1	0	2	9
	61 - 90	1	0	1	0	0	0	1	1	4
	91 - 120	2	0	0	0	0	0	0	2	4
	>120	0	1	0	1	0	0	1	1	4
Total		7	4	2	3	4	2	2	9	33

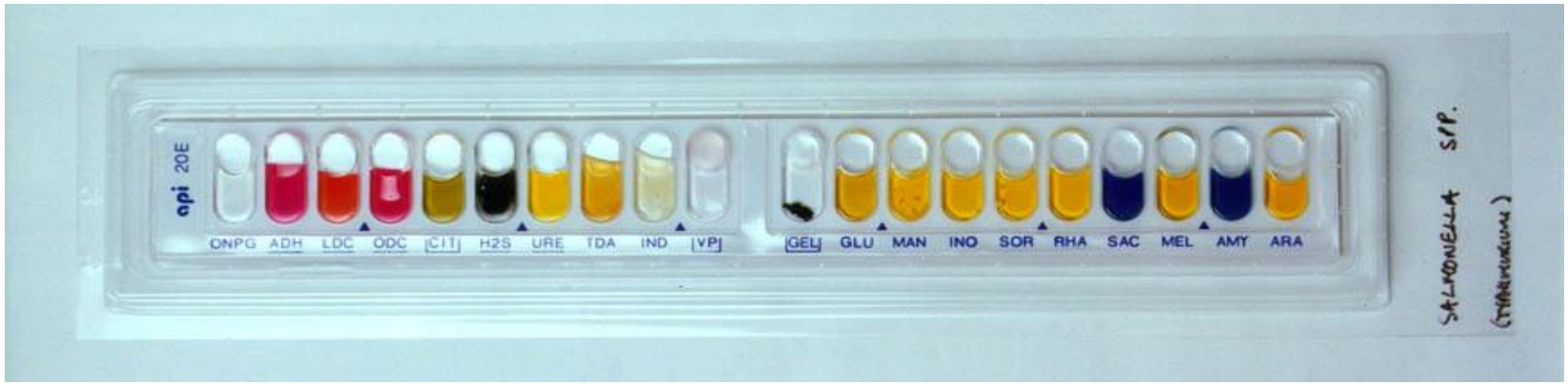
Average Total Thermotolerant Coliform Count in Ready-to-Drink Water * Sources of Water										
Count										
		Sources of water								Total
		Piped water to yard/plot	Water from public tap or standpipe	Tubewell or borehole	Protected dug well	Protected spring	Unprotected spring	Surface water	Don't know	
Colony forming unit per 100 ml (CFU/100 ml)	<30	5	2	2	1	4	0	1	4	19
	31 - 60	2	0	0	1	0	1	0	1	5
	61 - 90	0	1	0	1	0	0	0	0	2
	91 - 120	0	0	0	0	0	0	1	2	3
	>120	0	1	0	0	0	1	0	2	4
Total		7	4	2	3	4	2	2	9	33

Average Thermotolerant Coliform Count in Ready-to-Drink Water * Sources of Water										
Count										
		Sources of water								Total
		Piped water to yard/plot	Water from public tap or standpipe	Tubewell or borehole	Protected dug well	Protected spring	Unprotected spring	Surface water	Don't know	
Colony forming unit per 100 ml (CFU/100 ml)	<30	4	2	2	1	4	0	1	4	18
	31 - 60	3	0	0	2	0	1	0	1	7
	61 - 90	0	1	0	0	0	0	0	0	1
	91 - 120	0	0	0	0	0	0	1	2	3
	>120	0	1	0	0	0	1	0	2	4
Total		7	4	2	3	4	2	2	9	33
Average Total Thermotolerant Coliform Count in Stored Water * Sources of Water										
Count										
		Sources of water								Total
		Piped water to yard/plot	Water from public tap or standpipe	Tubewell or borehole	Protected dug well	Protected spring	Unprotected spring	Surface water	Don't know	
Colony forming unit per 100 ml (CFU/100 ml)	<30	5	3	1	1	3	0	0	4	17
	31 - 60	1	0	0	2	1	1	1	3	9
	61 - 90	0	1	0	0	0	0	1	0	2
	91 - 120	1	0	0	0	0	0	0	2	3
	>120	0	0	1	0	0	1	0	0	2
Total		7	4	2	3	4	2	2	9	33

Appendix II: Petri film

The diagram shows the colonies of thermotolerant coliforms from water at the point-of-use in the ready-to-eat food outlets in Maseno Township. The blue colonies with a bubble gas surrounding are positive for faecal thermotolerant organisms (*E. coli*).

Appendix III: Analytical Profile Index (API) 20 E



This diagram indicates positive API 20E reactions of *Salmonella* species isolates from water of the ready-to-eat rood outlets in Maseno Township.

Appendix VII: Consent Letter

CONSENT LETTER

I have been given information and I have had the opportunity to ask questions. I understand that my participation is voluntary in this particular research study and that I am free to withdraw at any time, without giving a reason and without cost. I voluntarily agree to take part in this study.

Participant's Signature _____Date _____

Investigator's Signature _____Date _____

Appendix VIII: Questionnaire

Hello my esteemed respondent. Please fill this questionnaire honestly and independently. Note that whatever information you give was treated highly confidential and the data was used for learning purpose and where necessary assist in advising you appropriately concerning your water. Thank you in advance.

This questionnaire is given to you to assist the researcher to assess the mode used by you in the treatment and storage of water. In addition, this will help in assessing the sources where vendors fetch water.

Please tick in the boxes provided.

Household demographics, including education and socioeconomic status

1. What is your age? _____

2. Gender of respondent

☐ Male

☐ Female

3. What is your (in-charge) level of education?

☐ Primary

☐ Secondary

☐ College

4. What is your (in-charge) professional training?

☐ Catering/Food production

☐

Food technology

Nutrition

Any other_____

5. Do you buy water from vendors?

Yes No

6. Do you know where the water is fetched from?

Yes No

7. Identify if it is from any of the following sources

a. Piped water into food outlet

b. Piped water to yard/plot

c. Water from public tap or standpipe

d. Tube-well or borehole

e. Protected dug well

f. Protected spring

g. Bottled water

h. Rain water

i. Unprotected spring

j. Unprotected dug well

k. Cart with small tank/drum

l. Tanker-truck

m. Surface water

8. Do you treat water at the point-of-use?

Yes ☐ No ☐

9. If yes to question 4, you treat potable water by:

a. Boiling ☐

b. Chlorination ☐

c. Waterguard ☐

d. Filtration ☐

e. It is clean from the source no need of treating ☐ (Tick if applicable)

f. Any other method _____

10. If yes to question 4, you treat water for hand washing by:

a. Boiling ☐

b. Chlorination ☐

c. Waterguard ☐

d. Filtration ☐

e. It is clean from the source no need of treating ☐

f. Any other method _____

We have come to the end of the interview. Any question?

Thank you.

Appendix IX: Public Health Authorization

REPUBLIC OF KENYA

MINISTRY OF HEALTH

WHEN REPLYING QUOTE
mohkisumuwestsubcounty@gmail.com

KISUMU WEST SUB COUNTY
P.O. BOX 2104-40100
KISUMU

REF: KSM/W/MOH

24TH JULY, 2014

TO WHOM IT MAY CONSERN,

Dear Sir/Madam,

RE: MR. JACOB SERONI MSC/PH/00060/13

The above named person is a registered postgraduate student in the department of Biomedical Sciences and Technology at the School of Public Health and Community Development of Maseno University.

He has done his entire course work and is due to begin a project which will lead to the award of Master of Science in Medical Microbiology.

Permission has been granted to him by this department to carry out his study in Maseno Township.

Kindly accord him the necessary assistance to be able to realize his research project and write a thesis.

Thanks you.

SUB COUNTY PUBLIC HEALTH OFFICER
KISUMU WEST

Joel Abongo
SUBCOUNTY PUBLIC HEALTH OFFICER

Appendix X: Data Collection Approval

MASENO UNIVERSITY
SCHOOL OF GRADUATE STUDIES

Office of the Dean

Our Ref: MSC/PH/00060/2013

Private Bag, MASENO, KENYA
Tel:(057)351 22/351008/351011
FAX: 254-057-351153/351221
Email: sgs@maseno.ac.ke

Date: 16th June, 2015

TO WHOM IT MAY CONCERN

RE: PROPOSAL APPROVAL FOR MWEBI JACOB SERONI—MSC/PH/00060/2013

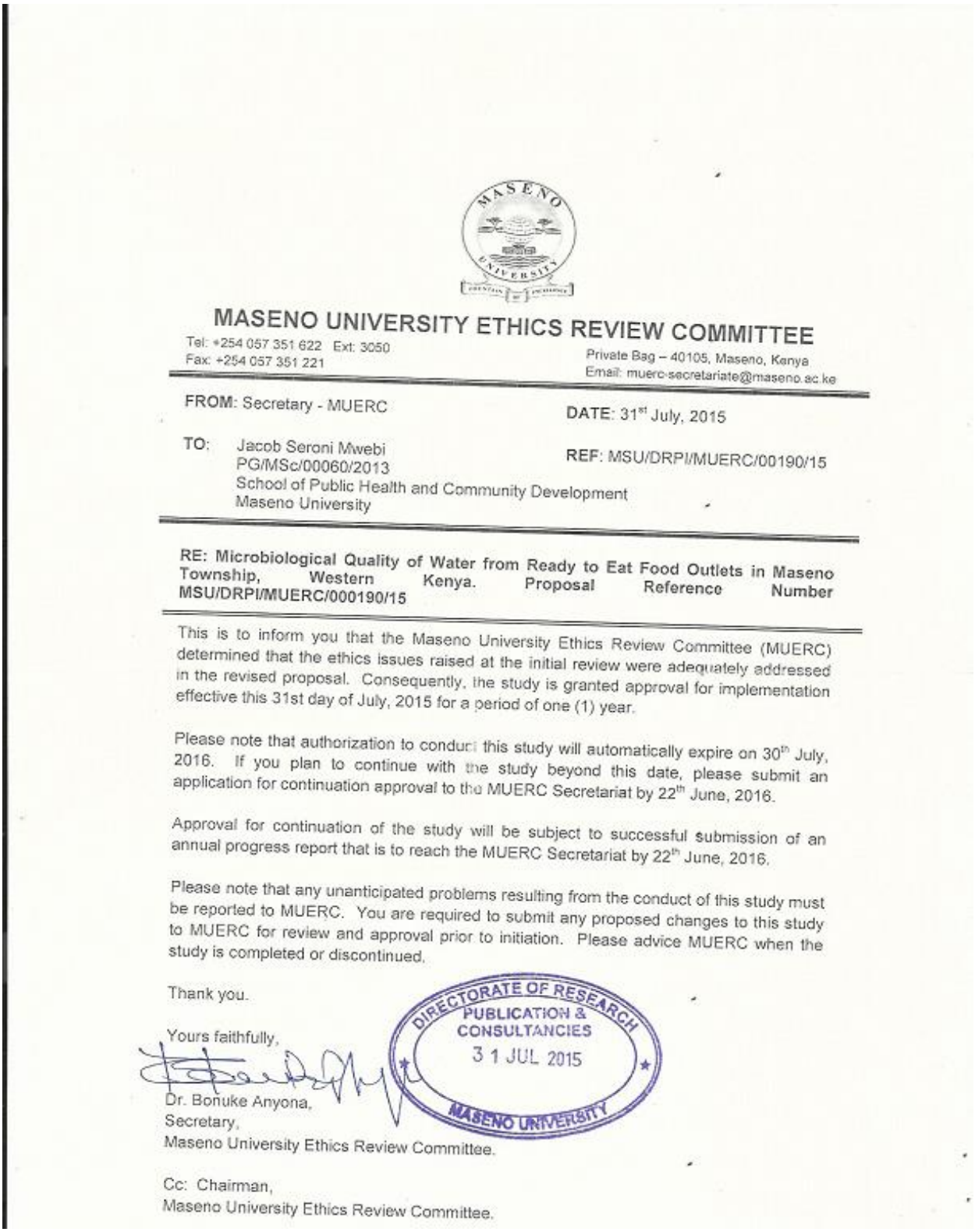
The above named is registered in the Master of Science of the School of Public Health and Community Development, Maseno University. This is to confirm that his research proposal titled "Microbiological Quality of Water from Ready to Eat Food Outlets in Maseno Township, Western Kenya" has been approved for conduct of research subject to obtaining all other permissions/clearances that may be required beforehand.

17 JUN 2015

Prof. P.O. Owuor
DEAN, SCHOOL OF GRADUATE STUDIES

Maseno University *ISO 9001:2008 Certified*

Appendix XI: Ethical Approval



MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

Tel: +254 057 351 622 Ext: 3050
Fax: +254 057 351 221

Private Bag – 40105, Maseno, Kenya
Email: muerc-secretariate@maseno.ac.ke

FROM: Secretary - MUERC

DATE: 31st July, 2015

TO: Jacob Seroni Mwebi
PG/MSc/00060/2013
School of Public Health and Community Development
Maseno University

REF: MSU/DRPI/MUERC/00190/15

RE: Microbiological Quality of Water from Ready to Eat Food Outlets in Maseno Township, Western Kenya. Proposal Reference Number MSU/DRPI/MUERC/000190/15

This is to inform you that the Maseno University Ethics Review Committee (MUERC) determined that the ethics issues raised at the initial review were adequately addressed in the revised proposal. Consequently, the study is granted approval for implementation effective this 31st day of July, 2015 for a period of one (1) year.

Please note that authorization to conduct this study will automatically expire on 30th July, 2016. If you plan to continue with the study beyond this date, please submit an application for continuation approval to the MUERC Secretariat by 22th June, 2016.

Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 22th June, 2016.

Please note that any unanticipated problems resulting from the conduct of this study must be reported to MUERC. You are required to submit any proposed changes to this study to MUERC for review and approval prior to initiation. Please advice MUERC when the study is completed or discontinued.

Thank you.

Yours faithfully,

DIRECTORATE OF RESEARCH PUBLICATION & CONSULTANCIES 31 JUL 2015 MASENO UNIVERSITY

Dr. Bonuke Anyona,
Secretary,
Maseno University Ethics Review Committee.

Cc: Chairman,
Maseno University Ethics Review Committee.