ESCHERICHIA COLI CONTAMINATION AND RISK EXPOSURE ASSESSMENT OF HUMANS CONSUMING WATER FROM UNPROTECTED WELLS IN CHAONA COMMUNITY, MWACHISOMPOLA AREA OF CHIBOMBO DISTRICT OF ZAMBIA

BY

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A DISSERTATION SUBMITTED TO THE UNIVERSITY OF ZAMBIA IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN FOOD SAFETY AND RISK ANALYSIS.

THE UNIVERSITY OF ZAMBIA SCHOOL OF VETERINARY

MEDICINE LUSAKA

DECLARATION

I, **DORIS ZGAMBO**, do hereby declare that this thesis represents my own original work. It has been presented in accordance with the guidelines for master's dissertation of the University of Zambia. This document has not been submitted before for the award of any degree or examination in any other University.

Signed _____ Date: _____

CERTIFICATE OF APPROVAL

The University of Zambia approves the dissertation submitted by **DORIS ZGAMBO**, as fulfilling the partial requirements for the award of the Master of Science Degree in Food Safety and Risk Analysis by the University of Zambia.

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ABSTRACT

A quantitative cross-sectional study was conducted to detect the presence of *E. coli* in unprotected water wells of Chaona community in Mwachisompola area, Chibombo District. A total of 48 wells drawn from four villages were sampled from the study area and an exposure assessment was done by use of add-in Model risk app in excel for risk assessment. The occurrence of the bacterium in well water was confirmed by laboratory processes of culturing, isolation and identification of *E. coli.* The identified *E. coli* was subjected to microbial resistance testing and the resistant genes were further detected by use of PCR. Out of 48 unprotected wells tested, 38 were indicative of *E. coli* presenting 79% (95% CI: 77.3 – 80.7%). The individual variation results that were positive to *E. coli* are Chilumbwa 5/38 (32%) (95% CI: 2.3 – 23.7%), Chabwa village 10/38 (26%) (95% CI: 12.1– 39.9%), Kafwilo 11/38 (29%) (95% CI: 14.6 – 43.4%) and Katobole12/38 (32%) (95% CI: 17.2 – 46.9%). Meanwhile, 16/48 (33.3%; CI: 31.4 – 35.2%) samples were found with an average number of CFU of between 1000 and 10,000 which was the highest range. *E. coli* isolates were also tested for Multi Drug Resistance (MDR) of which one isolate was indicative of being resistant to eight antibiotics and another to five antibiotics presenting (5.88%; CI: 3.2 – 8.6%) for each. Meanwhile, seven isolates were resistant to four antibiotics (41.2%; CI: 35.5 – 46.9%) and eight isolates were resistant to three antibiotics (41.1%) (CI: 35.4 – 46.9%). In addition, 30.9% (17/55) of the isolated *E. coli* organisms were found to be resistant to three or more classes of antibiotics primarily ampicillin, streptomycin, tetracycline, cefotaxime, nalidixic acid, norfloxacin and ciprofloxacin. The probability to be exposed to *E. coli* was revealed to be at 79.5% (95%; CI: 66.5 – 86.7%) when consuming water from unprotected wells in the study area. In conclusion, the study revealed that *E. coli* contamination was highly possible and it is recommended that water be boiled and or treated with chlorine before use at household level.

DEDICATION

This study is dedicated to my Children (Anderson A. Banda and Saviour A. Banda), my sisters, (Mary Zgambo and Elizabeth Zgambo) and my parents (Mr. Simeon Zgambo and Mrs. Ruth Zgambo) for the support rendered during my period of study. To them all, I say thank you very much and may Jehovah God continue to bless you.

ACKNOWLEDGEMENTS

I thank Jehovah God for making it possible for me to reach this level of study. Great appreciation goes to the research team at all levels of participation. I am highly obligated to Professor Bernard Hang'ombe, my principle supervisor who wholeheartedly gave me mammoth support and guidance throughout the research work. I further thank Professor Musso Munyeme, my co-supervisor for the treasured support and tireless effort rendered to me throughout my research work.

I thank World Bank through African Center of Excellence for Infectious Diseases of Humans and Animals (ACEIDHA) project for the scholarship and funding of my research work. Without this scholarship, pursuing this study was farfetched.

I wish to also extend my appreciation to the Laboratory technicians (Mr. E. S. Kabwali, Mr. L. Moonga, Ms. S. Mabhena, Ms. R. Sikasunge, Mr. B. Muvwanga and others) at school of Veterinary Medicine, Microbiology Laboratory for their technical support and guidance offered to me during Laboratory work. I salute the field research team; Mr. E.S Kabwali (Laboratory Technician), Mr. J. Mwashonge (Driver) and Mr. K. Moonga (Community Health Worker) for the good work done during data collection. Many thanks also go to Dr. Thendji Muila Lysa for the special guidance and assistance on Risk Assessment work rendered to me during data analysis

Special thanks also to my family; my parents, children, sisters, brothers and other family members for the full support offered during my period of study.

Finally, I would like to extend my heartfelt appreciation to all my course mates (Food Safety and Risk Analysis 2021/2022 intake) and other colleagues who spared their time and offered support with love during the entire period of my study.

To those not mentioned, I say thank you and May the Almighty God bless you all.

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LIST OF ABBREVIATIONS

AMP	Ampicillin
AMR	Antimicrobial Resistance
BHI	Brain heart infusion
CFUs	Colony forming units
CI	Confidence Interval
CIP	Ciprofloxacin
CLSI	Clinical Laboratory Standard Institute
COT	Cotrimoxazole
CTX	Cefotaxime
CTX-M	Cefotaximase-Munchen
DAEC	Diffusely adherent *Escherichia coli*
DNA	Deoxyribonucleic acid
EAEC	Enteroaggregative *Escherichia coli*
EHEC	Enterohemorragic *Escherichia coli*
EIEC	Enteroinvassive *Escherichia coli*
ELISA	Enzyme-linked immunosorbent test
EPA	Environmental Protection Agency of the United State of America
EPEC	Enteropathogenic *Escherichia coli*
ERES	Excellence in Research Ethics & Science Converge
ESBL	Extended spectrum beta-lactamase
ETEC	Enterotoxigenic *Escherichia coli*
FAO	Food and Agriculture Organization
GEN	Gentamicin
HUS	Hemolytic uremic syndrome
MDGs	Millennium Dvelopment Goals
MDR	Multi Drug Resistance
MF	Membrane Filter
MTF	Multiple Tube Fermentation
NHRA	National Health Research Authority

NOR	Norfloxacin
OD	Open Defection
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electrophoresis
QMR	Quantitative Microbiological Risk Assessment
SDGs	Sustainable Development Goals
SHV	Sulphydryl Variable
STEC	Shiga Toxic *Escherichia coli*
TEM	Temoniera
UN	United Nations
UNICEF	United Nations Children's Fund
UV	Ultraviolet light
VBNC	Viable but Non-Culturable
VTEG	Verocytotoxin *Escherichia coli*
WHO	World Health Organisation
WWF	World Wide Fund

CHAPTER ONE

INTRODUCTION

1.1 Background

Escherichia coli is a gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* and is mostly present in the lower part of the intestine of the warm-blooded organisms. Many *E. coli* strains live peacefully in the gut, to help keep the growth of more harmful microorganisms in check and helps to digest food (Percival et al., 2014). *E. coli* is also found in the environment; food and untreated water associated with living animals (Foster, 2022). *E. coli* is also found in the environment through the feacal-oral-route and constitute about 0.1 percent under aerobic conditions and can grow massively for a few days and later the numbers decline slowly (Ercumen et al., 2017).

Certain strains of *E. coil* however, can cause illness. There are six (6) different strains which are known to cause infections of which the first to be discovered was Enteropathogenic *E. coli* (EPIC) among other *Escherichia* strains in the 1940s. Shiga toxin-producing *E. coli* (STEC), also known as enterohemorrhagic *E. coli* (EHEC) and verocytotoxin producing *E. coli* (VTEG) mostly incriminated in food contamination. Enteroaggregative coli (EAEC), mostly ends up asymptomatically to the infected individuals, but have been implicated in the development of irritable bowel syndrome (Chen, 2005). Enteroinvassive (EIEC), commonly known as *E. coli* 0157:H7 have been incriminated as causative agent for bloody diarrhoea and hemolytic uremic syndrome (anemia and kidney failure). Diffusely adherent *E. coli* (DAEC), have also been considered a diarrhea causing *E. coli* with several potential virulence factors (Mansan et al., 2013). Lastly and not the least is Enterotoxigenic *E. coli* (ETEC) strain commonly known to cause traveler's disease and finds its occurrence more in contaminated water is another pathogenic strain (Percival et al., 2014). Generally, the most familiar strains of *E. coli* that cause infection, do so by producing a toxin called Shiga, and this toxin damages the lining of the small intestine and causes diarrhea (Juhna et al., 2007). *E. coli* is also the common cause of urinary tract infections such as systems, apart from the common diarrheal infections (Gwimbi, 2019).

Drinking water quality is one of the most important variables influencing human health. Poor drinking water quality, particularly in poorer nations, has led to an increase in waterborne infections (Pal et al., 2018). The most significant factor affecting drinking water quality is the presence of microorganisms that cause diarrheal and other illnesses in the water. Water is a vital resource for human life and well-being. It is a fundamental human right to have access to clean and safe drinking water, but a large portion of the world's population still struggles with poor water quality, which has serious health repercussions. Poor water quality directly endangers vulnerable groups, especially the elderly, pregnant women, children and the chronically ill, therefore its consequences on human health and Public Health cannot be understated (Babuji et al., 2023).

Assessing the risk of exposure to *E. coli* from consumption of water from unprotected wells involves several steps to evaluate the likelihood and potential consequences of exposure and includes: Hazard identification, characterizing the source of the hazard, evaluation of the contamination pathways, estimation of the exposure and quantification of the risk. *E. coli* are typically found in human and animal feces as well as bird's droppings and can contaminate water sources, especially those that are unprotected (Ford et al., 2017).

1.2 Problem Statement

Globally, some reports show more than 1.1 billion people lack access to safe water and over 2 million people die, among them under five children of age due to diarrhoeal diseases arising from unsafe drinking water and poor sanitation (UNICEF, 2015). Worldwide, provision of safe water and adequate sanitation are the most critical factors at ensuring human health and prevention of a wide range of diseases. The international Decade for Action: 'Water for Life' affirms the challenges of safe water provision in their journal that lack of safe water and sanitation is the largest cause of illness (Tenaillon et al., 2010). Furthermore, WWF (2006) report also signposted that lacking safe water is one of the main underlying factors of the millions of children that die every year due to repeated episodes of diarrheal diseases arising from contaminated water source of which *E. coli* maybe among the causative agents for these reported diarrheal diseases. Sustainable Development Goals (SDGs) seek to build on the Millennium Development Goals (MDGs) as the universal agenda with 169 targets of which some target at achieving universal and

equitable access to safe and affordable drinking water for all by 2030 (Bain et al., 2020). In spite of all these efforts made globally, reports compiled around developing countries showed about 1 billion people have limited access to safe drinking water as well as water for domestic purposes. This situation leads to diarrheal conditions mostly in children under five years of age and attributes to the most morbidity and mortality numbers in sub-Sahara Africa. Zambia being one of the developing countries, has over one-third of the population with limited access to safe drinking water as opposed to progress reports compiled on MDGs that 95% of the countries reported to have met the MDG target of achieving clean water for all by 2015 (United Nations, 2015). It is however, unfortunate that people in the study area are not part of this achievement as they have continued to source water both for domestic and drinking purposes from hand dug wells. Inadequate sanitation and poor water quality is the main cause of diarrheal diseases in Zambia because of consumption of contaminated water which may contain different pathotypes of *E. coli* and puts many people at risk directly or indirectly (Palaniappan et al., 2006). In Zambia, a study done by Reaver and others (2017) revealed that contamination of water with *E. coli* is mainly associated with fecal contamination thus, threatens food safety and may expose the community members to diarrheal diseases (Nowicki et al., 2021). Some studies further showed that most people in the rural parts of Zambia including Chibombo District still practice open defecation which is likely to cause contamination of ground water sources attributing to diarrheal diseases (UNICEF, 2015).

1.3 Justification of study

Studies conducted around the world clearly show that it is very difficult to attain provision of safe water in most areas of the developing countries (Bain et al., 2014). Some studies conducted in Zambia suggest that water sources for domestic use are largely contaminated with pathotypes organisms of *E. coli* responsible for disease (Peter, 2015). In addition, Morgan and others (2017) indicated that some studies conducted in Zambia discovered that most water sources don't meet the World Health Organisation (WHO) guidelines and local standards by the Zambia Bureau of Standards. Specific studies done in Chibombo District were on *E. coli* in food (Reaver et al., 2017). But no study was conducted in the study area to isolate *E. coli* in unprotected wells and determination of risk exposure factors creating a gap in knowledge in which the principal investigator pursued to fill. Further the study looked at finding the possible carriage of disease-

causing elements in the unprotected wells of Chaona community in Mwachisompola. Risk exposure factors related to the presence of *E. coli* in wells were also determined by use of Monte Carol simulation –model risk add-in app in excel. Conducting this study gave some hope in providing some form of solution to the provision of safe water in the study area and elsewhere.

1.4 Research Questions

1.4.1 Do *E. coli* organisms exist in unprotected wells in Chaona, Mwachisompola area of Chibombo district?

1.4.2 What levels of *E. coli* contamination does exist in the unprotected wells in Chaona community, Mwachisompola area of Chibombo district?

1.4.3 What are the antibiotic profiles of *E. coli* isolated from unprotected wells in Chaona communty, Mwachisompola area of Chibombo district?

1.4.3 What is the estimated risk of exposure from drinking water that is contaminated with *E. coli* isolated from unprotected wells in Chaona communuity, Mwachisompola area of Chibombo district?

1.5 General Objective

To detect the presence of *E. coli* and determine the risk of exposure from consumption of water from unprotected wells in Chaona community, Mwachisompola area of Chibombo District.

Specific Objectives

1. To isolate *E. coli* from water in unprotected wells of Chaona community of Mwachisompola area in Chibombo district.
2. To determine *E. coli* count in unprotected wells of Chaona community of Mwachisompola area in Chibombo district.
3. To evaluate the antibiotic profiles of *E. coli* isolates obtained from unprotected wells.
4. To estimate the risks of exposure to *E. coli* from consumption of water from unprotected wells.

CHAPTER TWO

LITERATURE REVIEW

2.1 General overview of *Escherichia coli*

Escherichia coli is a bacterium that live in the gastrointestinal tract of animals without creating any problems as they are important in keeping harmful microorganisms in check (Kendall, 2012). These organisms are commonly found in the gastrointestinal tract of humans and warm- blooded animals. Even though most of these organisms are harmless their presence in water suggests faecal contamination and a possible presence of pathogenic organisms (Choudhury, 2012). Among the *E. coli* organisms, some serotypes can cause serious food poisoning in their hosts (Begum et al., 2005).

Escherichia coli was discovered and implicated with infection of the gastrointestinal tract in 1885 during an investigation of microbial life of the human gastrointestinal tract. It is against this background that *E. coli* microorganisms were commended to be used as indicator organisms of microbial contamination in water (Choudhury et al., 2012). Figure 2.1 shows a pictograph of *E. coli* in water under microscopy.

Figure 2.1: Presence of *E. coli* in water under a microscopy (Source: *E. coli* bacteria istockphoto.com –ID 1325173791)

Despondently, among the contaminants that can be found in water, pathogenic microorganisms from human and animal faeces pose a greatest danger to public health. While modern

microbiological techniques have made the detection of pathogenic bacteria, viruses and protozoa possible, it may not always be practical to isolate these microbes from drinking water (Rice et al., 2000). Indictor organisms are therefore used to assess the microbiological safety of drinking water as indicated by other scholars because these indicators are less difficult, less expensive and less time consuming to monitor water quality (Bain et al., 2014).

There are two habitats for *E. coli* organisms namely: the primary and the secondary. The primary habitat consists of the gastrointestinal tract where the bacteria thrive naturally. While the secondary habitat is where the bacteria has been dispersed and these include water, soil and flora that comes into contact with faecal material the primary habitat. Since the year 2000, the assumptions of negative growth rate in secondary habitats were challenged by the recognition of negative naturalised *E. coli* populations (Tenaillon et al., 2010).

Escherichia coli has characteristics of colonising the gastrointestinal tract of the infants within a few hours after birth. There has been mutual benefit between the human host and the presence of *E. coli* in the human gut. This type of *E. coli* strain is known as commensal and they rarely cause disease. The niche of these commensal strains of *E. coli* is the mucous layer of the mammalian colon. It is a prosperous competitor at this congested site with other microorganisms (Williams et al., 2013)

In addition, Williams et al (2013) indicated that among the harmless strains of *E. coli*, there are six (6) strains that are responsible for causing disease and includes; Enteropathogenic *E. coli* (EPEC*)*, Enterohaemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC) and Diffusely adherent *E. coli* (DAEC). EHEC and ETEC are also implicated to cause disease in animals using the same virulence factors that are present in human strains and distinctive colonisation factors that are not found in human strains.

Further, Thani and others (2016) reported that pathotypes that have the capacity to cause disease could only be successful in combinations with virulence factors that are known to have continued to cause disease in the health individuals. These diseases would include: diarrheal diseases, urinary tract infections and sepsis among other conditions. Some strains of *E. coli* such as ETEC causes

watery diarrhea that can range from mild purging to severe. The organisms are seriously associated with diarrhea in children (Asada et al., 2022) and it was discovered to be the main cause of diarrhea in travelers in the developing world (Thani et al., 2016) of which Zambia is not an exceptional.

2.2. *Escherichia coli* transmission in water and food

Escherichia coli can get into food at all stages of the food chain such as production, manufacturing, processing, and distribution as well as during consumption (Kendall, 2012). For example, *E. coli* may survive, if the contaminated meat is not cooked to 71ºC. *E. coli* may also be present in raw milk, as a result of bacteria spreading from cow's udder to the milk produced (Kaper, 2004). Kaper (2004). Further it's indicated that *E. coli* can be introduced into food by the food handlers who could be infected with *E. coli* during the process of handling food.

As earlier mentioned, human and animal faeces do habour *E. coli* in both ill and healthy individuals' leading to contamination of both surface and ground water. This may occur mainly in the rain season. Another study on *E. coli* also indicated the possibility of contaminating water sources used for domestic purposes with *E. coli* through improper disposal of human and animal faecal waste (Bain, 2014). Some of the water sources meant for domestic purposes such cooking, bathing and washing of kitchen utensils (Schram et al., 2020) may include wells which may be hand dug, shallow wells or and boreholes.

2.3 Sources of *E coli* in Water Wells

Generally, sources of *E. coli* in water wells maybe humans and non-human reservoirs which includes; companion animals, food animals, retail meat products, sewage, and other environmental sources. All these sources mentioned have been identified as potential reservoirs for the *E. coli* strains that are responsible for the majority of human gastro-intestinal infections (Gwimbi, 2019). *Escherichia coli* may find itself in all the mentioned reservoirs through contamination from faecal matter. All warm-blooded animals are carriers of *E. coli*. This makes the bacteria to be ubiquitous in nature and this makes it easy to contaminate unprotected water wells everyday as a result of human and animal activities (Moral et al., 2017). To understand these sources further they are divided into extrinsic and intrinsic factors (Figure 2.2) as explained below;

2.3.1 Extrinsic sources

Extrinsic factors refer to external sources of contamination that can affect the quality of groundwater (Moral et al., 2017). These can include:

a. Latrine use in rural areas

The assessment done by World Bank in (2018) gave an estimation of about 2.5 billion people worldwide to be living without access to improved sanitation and 1 billion of this population practice open defection (OD) (Thys, 2015). Lack of or poor sanitation has a potential to deteriorate health as evidenced that each year diarrhea kills about 1.5 million children under five years of age. Further, Biran, (2003) indicated that OD has serious implications such as height deference in a child.

In general, a number of studies conducted in Africa found that lack of sanitary facilities was a risk factor for the presence of several pathotypes of *E. coli* in Wells (Yulyani et al., 2019). These findings were linked to a high prevalence of diarrheal illnesses in the region. In Sub-Saharan Africa alone, 70% of the population had limited access to proper sanitation (Phiri, 2016). In Sub-Saharan Africa, open defecation and drinking contaminated water have significantly contributed to degrading habitats, with an estimated 215 million individuals engaging in OD, which is a key mechanism of transmission of bacteria that cause diarrheal diseases (Gwimbi, 2019).

b. Use of unprotected water wells

Bacterial contamination of water is a major source of water-borne disease in rural areas of the most developing countries due to multiple faecal-oral transmission pathways in their environments. *E. coli* infections associated with drinking contaminated water remain the main Public Health concern (Gwimbi, 2019). Similarly, the World Health Organisation (WHO) estimates that diarrheal diseases are as a result of exposure to drinking unsafe water, inadequate sanitation and unhygienic practices which contribute to more than 25 percent of the reported global environmental burden of the diseases (Gwimbi, 2019).

Access to safe drinking water is an essential requirement not only for health but a right too. It is unfortunately estimated that over 1 billion people have no safe water in developing countries for domestic use that includes drinking, cooking, washing needs; foods, utensils and hands (Thani et

al., 2016). Few classic studies conducted worldwide further indicated that about 20 million deaths occur annually of which 80 percent children under five years of age due to waterborne diseases arising from unsafe water (Thani et al., 2016). To be specific, an assessment done on exposure of drinking water to faecal coliform revealed that globally, about 18 billion people have access to water that contains faecal coliforms and also *E. coli* which is against World Health Organisation (WHO) standards (Bain et al., 2014).

Studies conducted in the low-middle income countries and documented from 1990 to 2013 that assessed drinking water quality revealed the presence of *E. coli* from different water sources. Literature gathered from around African continent clearly indicates a significant association between gastrointestinal illnesses and consumption of water from unprotected Wells with presence of *E. coli* (Raina et al., 1999).

Different nations face a wide range of problems around the world when it comes to sourcing quality water for domestic use. Some of these problems include international and regional disputes over water, water shortage, poor quality of water and many more problems including the climate change threat. In most parts of the world, provision of water that is free from contamination has been a great challenge. As far back as year 2000, the United Nations (UN) in collaboration with other individual nations revealed that 89% of the world population around the world did not have access to "improved water supply" (WHO, 2015). For instance, in Zambia, water contamination has been documented in a number of studies and some studies suggested contamination of water with pathotypes organisms of *E. coli* responsible for causing disease (Reaver et al., 2017).

c. Free range of domesticated animals

Most rural areas of the developing countries are in habit of rearing free range domestic animals and birds such as chickens, pigeons and others. These domestic animals and birds contribute to the contamination of the environment and water sources with gastrointestinal pathotypes including *E. coli* through their droppings. In Africa, more than 80 percent of the chickens are kept free range in most rural parts of the continent. Local/village chickens are the common birds kept by households around the continent inclusive of Zambia (Hamisi, 2014) and other domesticated animals like pigs.

2.3.2 Intrinsic sources

The intrinsic factors refer to the properties of the water and the surrounding geology that contribute to contamination (Moral et al., 2017). For example, the type of rock or soil surrounding the Well can impact the quality of the water. If the soil or rock contains high levels of minerals or other contaminants, those substances can leach into the groundwater and contaminate the Well. Thus, intrinsic factors that would promote contamination in groundwater include; hydrogeology, depth of the well, age of the well, temperature, pH and gaseous environment. The optimum temperature for *E. coli* growth is 37ºC, optimum pH of 3.6 and an anaerobic environment. The water that meets these factors would therefore, enhance the growth of *E. coli*.

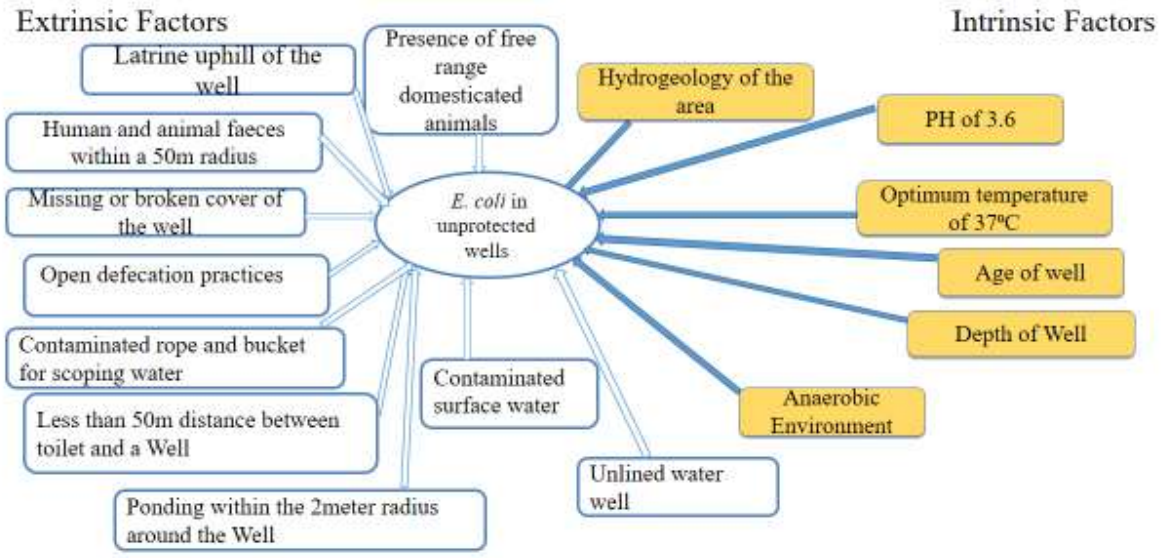


Figure 2.3: Extrinsic and intrinsic factors contributing to presence of *E. coli* in unprotected wells

2.4. Maintenance of E. coli in the Environment

Escherichia coli are normal intestinal habitant of both animals and humans thus a number of reservoirs exists (Martinson, et al., 2020). A study done in Bangladesh revealed a number of human activities such as waste water generation was attributed to the carriage of *E. coli.* In other studies, it was also revealed that shorter distances between the dug wells and pit latrines contributed to the existence and maintenance of *E. coli* in the water and environment (Muruka et al., 2014). Captivatingly, other studies revealed also that waste from farm settlements, hospitals and

pharmaceutical industries have the ability to cause contamination by the resistant bacteria (Montealegre et al., 2018).

On the other hand, *E. coli* was formerly believed not to survive well outside of the host due to its inability to multiply with external conditions (Ishii et al., 2006). New research however, discovered that this bacterium can proliferate in dirt, sand, and silt in tropical, subtropical, and temperate temperatures and survive for extended periods of time outside of the gastrointestinal system (Ishii, et al 2008). In these environments, some *E. coli* can naturalise, or integrate into the local microflora (Ishii et al., 2008). When regularly observed from the environment over months or years, naturalised *E. coli* genotypes were distinguished from animal-host origin (Rangel et al., 2005).

The persistence of pathogenic *E. coli* in the environment is influenced by a number of variables, including plant genotype, bacterial plant colonisation potential, and interactions with native phytobacteria (Montealegre et al., 2018). Both biotic and abiotic factors can affect *E. coli* growth and survival in their natural habitats (Ezeonu et al., 2012). Temperature, the availability of water and nutrients, pH, and sun radiation are examples of abiotic variables. Other microorganisms' existence and *E. coli* capacity to consume resources, out compete other microbes, and build biofilms in natural habitats are examples of biotic factors (Montealegre et al., 2018).

The most significant environmental element influencing *E. coli* growth and survival is probably temperature (Juhna et al., 2007). In the intestinal system of warm-blooded mammals, the temperature is steady and ideal for *E. coli* growth (36–40°C), whereas the temperature in the natural environment is typically low at about 30°C (Juhna et al., 2007). *E. coli* can grow in soil at temperatures above 30°C, albeit their mortality rate is higher at higher temperatures than at lower temperatures (below 15°C) (Ishii et al., 2006). For instance, *E. coli* survived for more than 6 months in sun-dried algal mats kept in airtight plastic bags at 4°C demonstrating its ability to endure prolonged exposure to a cold environment reduced compared to host bodies (Montealegre et al., 2018).

Another factor that will make the bacteria to have capacity to thrive depends on the water's natural or substrate-induced (salt or sugar) low water activity (or potential), provided that all other

parameters are within acceptable tolerance levels. One of the typical challenges to bacteria in natural habitats is desiccation (Jang et al., 2017). Rehydration can create an anoxic environment around the cells; therefore *E. coli* and other bacteria must modify their membranes and gene regulation to adapt to the desiccation and rehydration cycles (Jang et al., 2017). Desiccation of the soil had a negative impact on *E. coli* growth, but there was no difference in *E. coli* survival rates between wet and dry soils (Ishii et al., 2006). *E. coli* that survived in dry soil grew after being rehydrated, showing that water availability is essential for *E. coli* to proliferate (Jang et al., 2017).

The availability of nutrients like nitrogen and carbon in comparison to the intestinal tract of warm-blooded animals, natural environments often contain fewer easily available nutrients (Jang et al., 2017). Further, *E. coli* is a flexible energy acquiring organism that can break down a variety of carbon substrates, including aromatic compounds (Jang et al., 2017). Additionally, *E. coli* showed a catabolic flexibility in glucose-limited circumstances, leading to the effective uptake of several carbon sources (Montealegre et al., 2018). *E. coli* should be able to survive and thrive in the environment with such adaptability and flexibility in energy and carbon acquisition (Ishii et al., 2006).

The survival and proliferation in the soil can be affected by environmental pH (Russell et al 2001). *E. coli* vary with strain, according to the Society for Applied Microbiology, non-O157 *E. coli* strains and *E. coli* serotype O157:H7 strains demonstrated improved survival at low pH (Jarvis, et al 2001). Some *E. coli* O157:H7 bacteria can survive better at low pH than at relatively high pH, similar to acidophiles. Therefore, depending on the local pH of the environment, some *E. coli* strains may be able to survive. In order to survive low pH, *E. coli* utilises a number of well-studied mechanisms, such as the decarboxylase/antiporter-dependent acid resistance systems (Jarvis et al., 2001).

Another well-known element influencing *E. coli* persistence in natural habitats is biofilms generated by *E. coli* surfaces in aquatic environments, such as sediments (Percival et al., 2014). When exposed to harmful environmental factors including UV radiation, desiccation, protozoan predators, and chemicals like antibiotics and disinfectants, bacteria are shielded by biofilms (Juhna et al., 2007). They might also serve as a source of nutrients for microorganisms. Increased flow

rates in aquatic environments can transport bacterial cells that have been detached from mature biofilms to other locations and lead to the formation of new biofilms (Juhna et al., 2007), suggesting that biofilm-borne *E. coli* can be transported to different sites and observed without evidence of faecal contamination in the environment.

2.5. Diseases caused by *E. coli*

Diverse strains of *E. coli* that cause human disease are classified according to the type of disease symptoms that they produce in humans (Rock, 2018). Table 2.1 summarises the diseases and modes of transmission for the six (6) strains of *E. coli* that cause disease in humans.

Table 2.1: Strains of *E. coli*, disease and mode of transmission

Strain of *E. coli*	**Modes of Transmission**	**Disease**
Enterotoxigenic *E. coli* (ETEC)	Food or water ingestion	ETEC causes diarrhea without fever. It is common in infants and is often the cause of travelers' diarrhea
Enteropathogenic *E. coli* (EPEC)	Food or water ingestion, direct and indirect human contact	EPEC causes watery, sometimes bloody diarrhea. It is a common cause of infantile diarrhea in underdeveloped countries.
Enterohemorragic *E. coli* (EHEC)	Food/ingestion, direct or indirect human contact	EHEC strains cause bloody diarrhea and can sometimes damage the kidneys and progress to the potentially fatal hemolytic uremic syndrome (HUS).
Enteroinvassive *E. coli* (EIEC)	Food and water ingestion	EIEC causes watery, dysentery like diarrhea. Fever is another common symptom.
Enteroaggregative (EAEC)	Food and water ingestion	Development of irritable bowel syndrome
Diffusely adherent *E. coli* (DAEC)	Food and water	Bloody diarrhea, abdominal, dehydration and fever

It is important to note that the epidemiology of foodborne pathogenic *E. coli* varies throughout the world (Jang et al., 2017). Mostly in communities with poor sanitation and hygiene, Enterotoxigenic *E. coli*, Enteroinvasive *E. coli* (EIEC) and Enteropathogenic *E. coli* (EPEC) are prevalent (FAO, 2011). The transmission pathways differ for each strain and often include raw or undercooked meats or horticulture products, contact with animal manure, cross-contaminated raw foods and water (FAO, 2011).

There different sources of pathogenic *E. coli* which are most likely transmitted from the faeces of either the ill or healthy humans' habouring the bacterium. The human and wildlife hosts both appear to be the major reservoirs because of the wide distribution of human and animal faecal material into the environment (FAO, 2011). FAO reports (2011) indicated that the major challenge lied with bacteria having the potential to be present in areas used for food production, and this meant that most home environments are likely to be contaminated, increasing the risk of exposure for human beings.

2. 6. Management and prevention of *E. coli* associated Contaminants

Activities like lining of water wells, upgrading water sources to boreholes and keeping the environment free of faecal contaminants (from humans, animals & Birds) and health education can be employed in the community to improve water quality and help prevent contamination of water from *E. coli* and improve quality of water (Rock, 2018). Prevention of exposure to *E. coli* therefore, requires protection of both surface and ground water from contamination with *E. coli* (Montealegre et al., 2018). In addition, members of the public may need to get interested in knowing the sources of contamination in their areas; this would enable them to identify potential sources of water contamination and their means of prevention (Rock, 2018).

2.7. Treatment of *E. coli* associated conditions

Treatment of *E. coli* associated conditions are mostly based on the type of specific strain involved (Russell et al., 2001). *E. coli* infections mostly clear within a few number of days but generally, uptake of plenty of fluids is recommended for most cases. Antibiotics can be administered to shorten the period of illness (Brooks et al., 2005). Luckily, most *E. coli* infections go away on their own, especially when aided with plenty uptake of fluids (Thani et al., 2016). Thani and others (2016) further displayed that antibiotics are usually not important and should not be given for fear of worsening the condition in some individuals. Also, inhibiting diarrhea is not encouraged because *E. coli* can be kept in the body and increase the risk of hemolytic uremic syndrome (HUS).

Treatment of *E. coli* should involve antimicrobial susceptibility testing to know the exact antibiotic to use. Treatment without establishing antibiotic susceptibility may aid in the acquisition of

antibiotic resistance determinants as well as the selection and amplification of resistant *E. coli* (Russell et al., 2001).

2.8 Laboratory Detection of *E. coli* and Enumeration

Escherichia coli can be detected in water samples using a variety of techniques, including traditional Microbiology techniques, biosensors and cutting-edge techniques (Nasrull et al., 2018). Although they take several days and require specialists to perform, these traditional microbiological tests are thought to have a high degree of accuracy. These tests, which include the multiple tube fermentation (MTF), plate count enumeration method, and membrane filter (MF) approach, can quantify the number of bacteria present in water or other substances (Nasrull et al., 2018). The MTF works on the theory of dilution of the most likely number to count all bacteria forms. This method calls for many dilutions; typically, 1ml is decanted into several falcon tubes.

Although toxin-producing *E. coli* have been identified in clinical samples and cultures using real-time PCR (Chaudhuri et al., 2012). Real-time PCR has the advantage of allowing for the quantification of the bacteria in the sample and is typically more sensitive than conventional PCR when analyzing samples with low bacterial counts (Chen et al., 2014). The number of infectious bacteria in a sample may be overestimated by real-time PCR since it cannot distinguish between living or dead bacteria. The number of infectious bacteria in a sample may be overestimated by real-time PCR, even though it has been used for toxin detection. However, if the Viable but Non-culturable (VBNC) form of bacteria exists and are capable of causing disease. Traditional culture-based methods will undercount the true number of infectious bacteria (Chen, 2005).

2.8.1 Enumeration of Colony Forming units

The enumeration of colony forming units (CFUs) is a common method used to quantify the concentration of bacteria, including *E. coli*, in water samples. Water samples from the unprotected wells are collected aseptically into sterile containers to prevent contamination during collection.

Series of dilutions of water sample using sterile diluent (usually sterile buffered saline or distilled water) are prepared. This is done to reduce the concentration of bacteria in the sample to a countable range. Thereafter, 0.1m from each dilution is poured on MacConkey plate medium and

spread using a microbe spreader and incubated for 18 -24 hours at 37℃. After incubation, the grown colonies are counted on the plates and Calculate the number of CFUs per unit volume of the original sample, taking into account the dilution factor used during sample preparation as shown in the formula below;

$$\text{CFU/ml} = \frac{\text{Number of colonies counted}}{\text{Volume plated (ml)} \times \text{Dilution factor}}$$

The results reported as CFU/ml or CFU/100 ml, depending on the volume of water that was plated by enumerating colony forming units using this method, the concentration of bacteria is quantified in water samples, which is crucial for assessing the microbiological quality of water and determining the level of contamination from unprotected wells (Gayathiri, 2018).

2.8.2 Isolation and Culture of *E. coli*

Escherichia coli organisms can be grown and isolated on MacConkey agar, while those that are extended beta lactamase producers can be isolated using MacConkey agar supplemented with cefotaxime (CTX) or ceftriaxone added in concentrations of 1 or 2 mg/L (Chishimba et al., 2015). Pre-enrichment can be carried out in a general broth containing 1 mg/L of cefotaxime, ceftazidime, and cefoxitin, such as Muller Hinton, brain-heart infusion, or Luria-Bertani broth. ESBL are susceptible to cefoxitin, variable to cefotaxime, and resistant to ceftazidime (Chishimba et al., 2015). The National Committee for Clinical Laboratory Standards' breakpoints, which are used to assess an isolate's susceptibility or resistance, are displayed in the table 2.2.

Table 2.2: Defense zone diameters (source: performance standards for antimicrobial susceptibility testing 29th edition)

S/N	Antibiotic (µg)	Resistant	Intermediate	Susceptible
1.	Ampicillin (2)	≤13	14 – 16	≥17
2.	Cefotaxime (30)	≤22	23 – 25	≥26
3.	Gentamicin (10)	≤12	13 – 14	≥15
4.	Streptomycin (10)	≤11	12 – 14	≥15
5.	Tetracycline (30)	≤11	12 –14	≥15
6.	Ciprofloxacin (5)	≤21	22 – 25	≥26
7.	Nalidixic Acid (30)	≤13	14 –18	≥19

8.	Cotrimoxazole (25)	≤10	11 – 15	≥16
9.	Chloramphenicol (30)	≤12	13 – 17	≥18
10.	Norfloxacin (10)	≤12	13 – 16	≥17

2.8.3 Molecular Typing of *E. coli*

Molecular typing is a way of identifying specific strains of microorganisms. Serogrouping of *E. coli* based on somatic O antigen was used to detect *E. coli* genes for pathogenic bacteria such as Ribo typing, Plasmid typing and pulsed-field gel electrophoresis (PFGE) typing (Farber, 1996). But these methods were considered costly, time consuming and to some extent poorly correlated with the virulence factors as indicated by many scholars in the field of Microbiology.

Polymerase Chain reaction, however, has for some years been used now to detect genes for many pathogenic bacterial species (Alía et al., 2020). Only the PCR and ELISA procedures have been deemed to be established methods, despite the fact that numerous sophisticated techniques are utilized to identify *E. coli* bacteria in water. The two techniques are the most widely utilised ones that most scholars examined. Further, a study done discovered that combining both approaches can produce stronger outcomes (Nasrull et al., 2018).

Polymerase Chain reaction is a rapid and dependable tool for the molecular diagnosis of a wide range of infectious diseases (Lukman et al., 2020). PCR analysis has been reported for screening drinking water and environmental samples and has been used to identify *E. coli* in primary water specimens, stool specimens and outbreaks. When compared to standard culture and ELISA methods, PCR is an excellent method for *E. coli* detection because it is very sensitive, accurate, and promises real-time quantitative results. Despite its high sensitivity, PCR is difficult to use because it requires complex standardized protocols during the testing process. As a result, a skilled worker is required to operate the machine due to its complexity (Nasrull et al., 2018).

Polymerase Chain reaction analysis involves subjecting the isolates for confirmation of the resistance genes Temoniera (TEM), Sulphydryl Variable (SHV) and Cefotaxime (CTX). Temoniera was named after the patient from whose name the bacteria were taken. This was considered the first plasmid-mediated β-lactamase in gram-negative bacteria and was found in

Greece in the 1960s (Chishimba et al., 2015) There have been roughly 140 TEM-type enzymes described. The most common ones are TEM-10, TEM-12, and TEM-26 (Hedman et al., 2020). The most frequent β-lactamase found in *E. coli* is TEM-1, which has a 90% resistance to the antibiotic ampicillin. *K. pneumoniae* has been found to contain the TEM-53 and TEM-63 enzymes, while *S. typhimurium* has been found to contain the TEM-3 enzymes. Other kinds of Gram-negative bacteria have also been found to produce TEM-type β-lactamase, and its prevalence is rising as a result of its amino acid changes responsible for the extended spectrum beta-lactamase (ESBL) phenotype's localization to the enzyme active site (Montealegre et al., 2018).

Sulphydryl variable (SHV) refers to (SHV)-type viruses that are all derived from SHV-1 through point mutations, with more than 90 SHV ESBL variants described so far (Paterson et al., 2003). A wide variety of penicillins, including ampicillin, tigecycline, and piperacillin, are resistant to SHV-1, however oxyimino substituted cephalosporins are not always affected (Livermore, 1995). The two SHV-5 and SHV-12 enzymes, which have been found in a variety of *Enterobacteriaceae* and outbreaks caused by SHV-producing *Pseudomonas* and *Acinetobacter* species, are the most often found ones (Mshana et al., 2013). African nations including South Africa and Tanzania have identified SHV-2, SHV-5, SHV-19, SHV-21, and SHV-12 enzymes from *K. pneumoniae,* while Morocco has isolated SHV-12 enzymes from *S. enteric* serotype (Ndugulile et al., 2005).

The most common ESBLs in *Enterobacteriaceae* are those of the Cefotaximase-Munchen (CTX-M) type, a class A ESBL that is rapidly expanding across *Enterobacteriaceae* worldwide. These enzymes have significant activity against cefotaxime and ceftriaxone but typically not against ceftazidime (Rossolini et al., 2008). With a greater invasion into *E. coli*, CTX-M ESBLs have emerged more frequently (Rangel et al., 2005) A high frequency of the CTX-M-15 gene was discovered in the ESBL-producing *K. pneumoniae* in African nations like Tanzania (Mshana et al., 2013).

2.9. Antimicrobial Resistance (AMR) of *E. coli*

Antimicrobial Resistance happens naturally over time, typically through generic changes and no longer respond to medicines causing infections harder to treat and increase the risk of disease

spread, severe illness and death. Organisms that are considered AMR are found everywhere including in people, animals, food plants soil, air and water (Nowicki et al., 2021).

Since, *E. coli* is commonly found in the intestines of warm-blooded animals, it is subjected to frequent antibiotic encounters, providing it with high selection pressure that leads to resistance to antibiotics consumed by its hosts (Jang et al., 2017). This gives rise to the theory that host origin may be determined using the antibiotic resistance patterns of *E. coli* strains from various hosts. Specific *E. coli* phylogenetic groups were shown to exhibit various levels of antibiotic resistance, regardless of the acquisition of resistance, despite the fact that this method was later shown to be ineffective for its intended use. This suggests that the genetic background of *E. coli* also influences its antibiotic resistance pattern (Jang et al., 2017).

Furthermore, study done by Nowicki and others, (2021), attribute the many reasons of AMR to misuse and over use of antibiotics; lack of access to clean water, sanitation and hygiene for both humans and animals; poor infection prevention and control; poor access to quality affordable medicines; and lack of awareness and knowledge among other things. Globally, the emergence and spread of drug-resistant pathogens that have acquired new resistance mechanisms have continued to threaten the ability to treat common infections (Larson et al., 2019).

2.10 Microbiological Quantitative Risk Assessment of *E. coli*

CODEX Alimentarius Commission defines Microbiological Risk Assessment as ‘a scientifically based process’ consisting of the following steps: (i) hazard identification; (ii) hazard characterisation; (iii) exposure assessment; and (iv) risk characterization (FAO, 2023). This scientific process is used to approximate risk of injurious effects such as infection, illness and death due to exposure from pathogenic organisms arising from food, water, air and other exposure pathways that will allow pathogen transmission (Pernet, 2013). The characterizing of uncertainty and variability are however key to the risk assessment process (EPA, 2012). In this case uncertainty is defined as lack of or incomplete information, whereas quantitative uncertainty analysis endeavors to analyze and describe the degree to which a calculated value may diverge from the true value; Probability distributions maybe used and depends on the quality, quantity, and relevance of data, as well as the reliability and relevance of models and assumptions (EPA, 2012).

While, variability refers to true differences in attributes due to heterogeneity or diversity. It is usually not reducible by additional measurement or study, though it can be better characterised. Practically every facet of a risk assessment will have some level of uncertainty, usually due to gaps in data and incomplete knowledge. Variability is a natural part of biological systems and will always exist. Generally, variability cannot be reduced, but it can be better understood with more information and knowledge (EPA, 2012). QMRA further uses models such as Monte Carol simulation "model risk" to measure microorganisms in the environment as inputs and calculate associated risks as outputs (Kouamé et al., 2017).

2.10.1 Hazard identification

Hazard identification is the first step in QMRA after the preliminary stages of problem identification. A comprehensive list of hazards associated with identified problem are well defined and articulated at this stage, further this stage, determines the pathogens and human health outcomes of concern. Strictly speaking, the health outcomes considered are either infection or acute illness (Schijven et al., 2011). Pathways of exposure which could be air, water and environment as well as measure or model the pathogen exposure doses during defined exposure events. Direct measurements of pathogen levels are however preferred over modeled levels because they result in more empirical estimates of the exposure dose (Pradhan et al., 2009). Figure 2.3 shows suggested pathways of *E. coli* into unprotected wells.

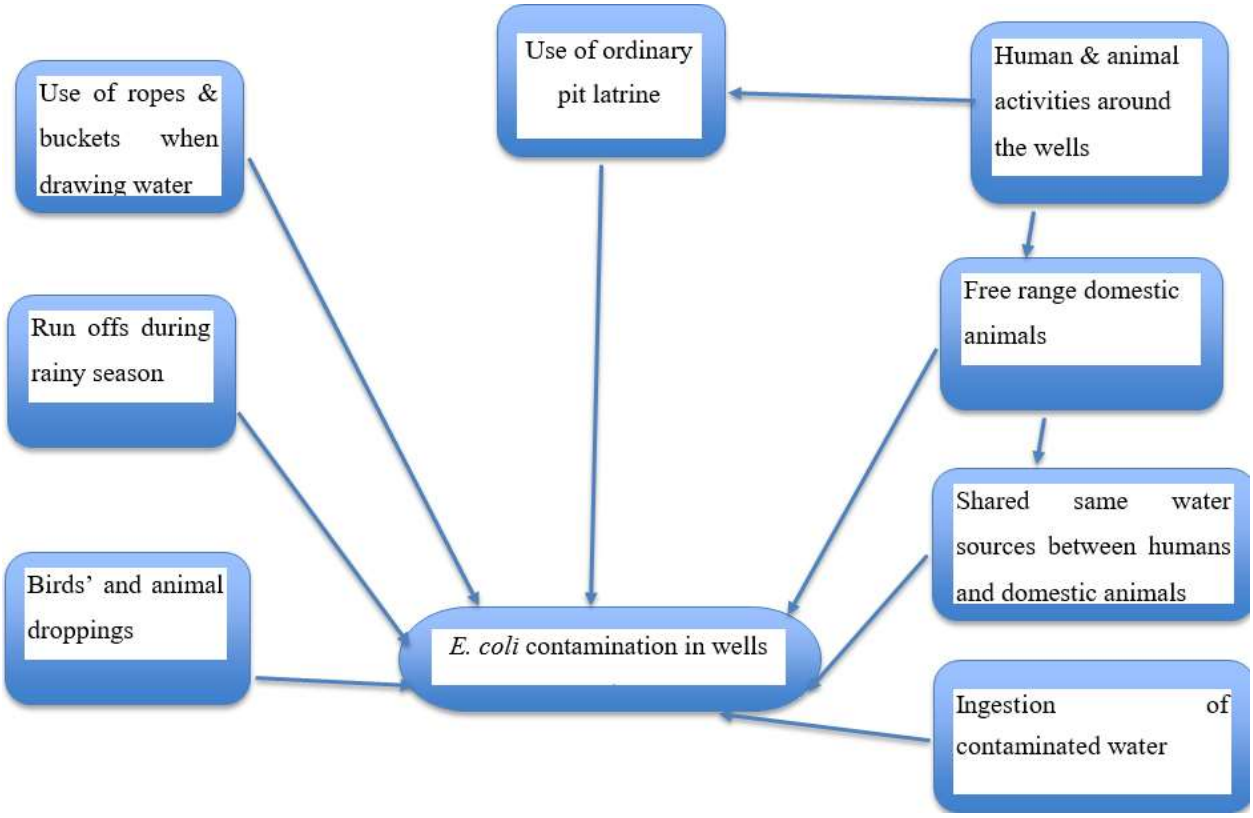


Figure 2.2 shows exposure scenario tree for *E. coli* pathways into unprotected wells

2.10.2 Exposure Assessment

Exposure assessment is one of the four components of risk assessment within the risk analysis framework adopted by Codex as the basis for all standard-setting processes. The estimated contribution of specific foods or food groups to the total dietary exposure to a contaminant as it relates to a quantitative health hazard endpoint. Exposure assessments must be guided by clearly articulated policies elaborated by Codex with the aim of increasing the transparency of risk management decisions (Babuji et al., 2023). Further, Codex Alimentations Commission (2006) defines Exposure assessment as the qualitative/quantitative evaluation of the likely intake of biological, chemical and physical agents through food and exposures from other sources. Thus, a Risk exposure assessment of humans consuming water from unprotected water wells involves evaluating the potential hazards associated with contamination and assessing the likelihood and severity of adverse health effects (Babuji et al., 2023).

Exposure assessment can also be done by assessing the water consumption patterns in individuals exposed to the contaminated water. Water consumption patterns vary among different age groups,

seasons, and countries as indicated in one study done in the United State of America between 1977 and 1978 (Banda et al., 2022). Another study still in the United State of America between 1994 and 1996 reported variations on the water consumption in winter and summer (Banda et al., 2022). Findings of the these studies were similar to Popkin et al (2010) study in the United State of America again that indicated water consumption varied among people of different age groups and between sexes. Furthermore, a study done by Lavallee and others (2021) in Sweden indicated water well consumption was associated more with females (Banda et al., 2022).

2.10.3 Hazard Characterisation

A probability of risk is estimated through the combination of data on the dosage received from the Exposure Assessment and the risk associated with various doses from the dose Response Assessment (Agunwamba, 2022). Hazard characterization techniques can be as simple as inserting a dose into a dose response function to obtain a single "point estimate" of risk or they can be more complex and take into account the uncertainty of the model's input parameters as well as individual and subpopulation variability (Hora et al., 2017).

2.10.4 Risk Characterization

Risk characterization is the determination of probability that outcomes specified when the hazard is identified will occur when exposed under different pathways. The different pathways could be air, water and the environment. These pathways could influence the exposure doses from pathogen presence and disease occurrence (Westrell et al., 2018). Monte Carlo simulations are frequently used in risk characterization to account for variability and uncertainty in projected health risks. Monte Carlo simulations also enable sensitivity analysis, which can uncover risk factors such as environmental factors, exposure variables and others that have the greatest influence on risk.

Similar studies done elsewhere (Gitter et al., 2023; Lutterodt et al., 2018; Percival et al., 2013) demonstrated the presence of *E. coli* in unprotected wells which are the major water sources. *E. coli* is used as a tool of risk assessment as it is a faecal indicator. Risk exposure assessment for consumers to *E. coli* contaminated water is a crucial element to be considered when looking at the microbiological quality of water. *E. coli* contamination in water poses significant risks to public health, as ingestion of contaminated water can lead to gastrointestinal illnesses such as diarrhea,

vomiting, and abdominal pain as well as urinary tract infections (Williams et al., 2013). Conducting a risk exposure assessment helps identify the extent of the contamination and the potential health risks associated with exposure to contaminated water. It has been established that assessing the risk of exposure to *E. coli* contaminated water, authorities could prioritise and target interventions to mitigate the risk (Babuji et al., 2023). It is therefore important that such studies are conducted in communities sourcing water from unprotected water wells. In totality, conducting a risk exposure assessment could also help authorities determine whether water sources meet regulatory standards and identify areas where improvements are needed to ensure compliance with both national and international standards.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Study design

A quantitative cross-sectional analytical study was conducted to investigate the occurrence of *E. coli* in unprotected water wells in Chaona community of Mwachisompola area in Chibombo district. Unprotected wells were sampled from the study area and an exposure assessment on the risk factors associated with the occurrence of the *E. coli* for the said wells were done by use of a semi-structured questionnaire and obtained responses were modeled in Monte Carlo simulation add-in Model risk app in excel for risk assessment. This app is a computerised mathematical technique that allows people to quantitatively account for risk in forecasting and decision-making. It is a way of repeating random samples of parameters to explore the behavior of a complex system. A Monte Carlo simulation is used to handle an extensive range of problems in a variety of fields to understand impact of risk and uncertainty. The app allows one to see all possible outcomes including actual probabilities, each will occur by running simulations with random variables thousands of times known as iterations. Further, these variables are described by their probability distribution which can be estimated by historical data or defined using expert opinion (Corbellini et al., 2017). This App was used to estimate the risks associated with the consumption of water from unprotected wells by simulating some scenarios that incorporated various uncertainties and parameters based on the collected data in the study area.

3.1 Study area

This study was conducted in Chibombo District which is among the Seven (7) districts of Central Province of Zambia situated along Great North Road. The Administrative offices are located at about 90 kilometers North of Lusaka the capital city of Zambia and 50kms from Kabwe the provincial headquarters of Central Province. It shares borders with Kabwe on the North, Lusaka, Shibuyunji and Chilanga districts are on the South while Chisamba is on the East. To the North-East is Chongwe District, and Mumbwa on the Western side. (Chibombo District Action Plan, 2021). To be specific, the study was conducted in Chaona community of Mwachisompola area located in Chibombo rural district of central province (14° 55' 0" South, 27° 57' 0" East), Zambia.

Figure 3.1 shows a map for Zambia, Chibombo district and Mwachisompola health facility Map to aid understanding of the study area.

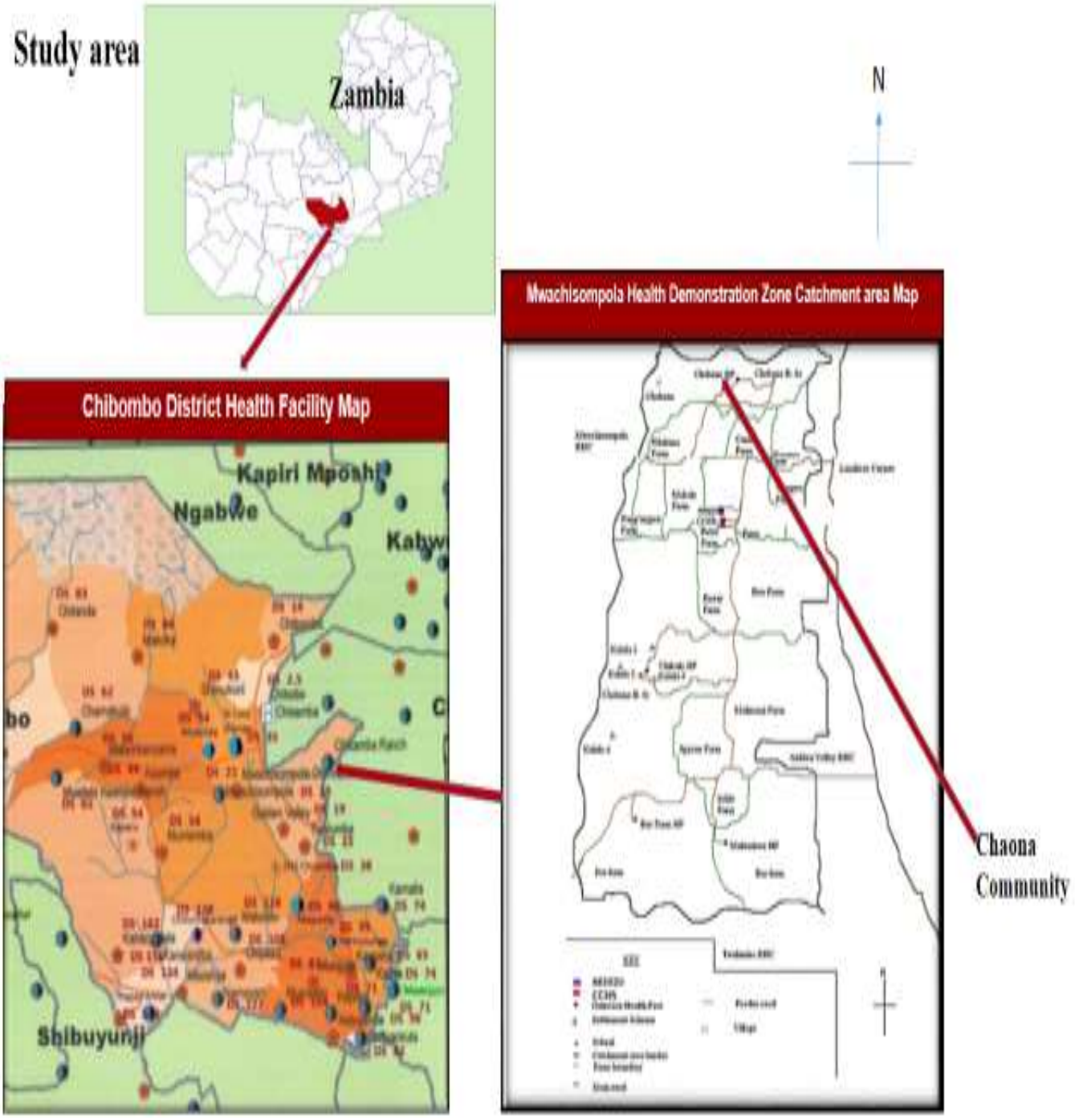


Figure 3.1: Map showing study area. (Source, Chibombo Health Office Action plan 2021)

3.2 Study Population

The study population were unprotected wells and humans consuming water from the same wells as shown in figure 3.2 in Chaona community of Mwachisompola area in Chibombo district of central province, Zambia as indicated on the map provided above.

Figure 3.2: Picture showing unprotected wells of Chaona community

3.3 Sample size determination and sampling

The researcher arrived at the required sample size by using a reasonable estimate of key proportions, rates, degree of accuracy and size of the population that the sample is going to represent in this study. Therefore, the sample size was calculated from the single proportion of fifty-five (55) unprotected water wells in the study area as described by (Charan et al., 2013).

$$n = \frac{z^2 . p . (1-p)}{d^2} = \frac{1.96^2 .0.5.0.5}{0.05^2} = 384$$

Therefore, to determine the sample size, the adjusted finite sample calculation was used. This formula below was used in order to arrive at the adjusted sample size;

$$N = \frac{N . x}{(x + N-1)} = \frac{55 \times 384}{(384 + 55-1)} = 48$$

Therefore, the final sample size was 48 unprotected Wells.

Convenient sampling was engaged for obtaining water samples because data was collected at time when community members in the study area were busy with cultivation. The research team managed to sample 48 wells as planned from the house owners who were found available during the visitation days from the four villages within the study area. However, twelve wells were sampled from each village to ensure representativeness of the results.

3.4 Inclusion and exclusion criteria

The study included sampling of unprotected (hand-dug hole with no lining and with/without cover) and untreated (without addition of chlorine at the time of sample collection or a week before) wells. All sampled wells were located in the study area. Protected wells and any other form of water source apart from the described 'unprotected' were excluded.

3.6 Isolation and identification of *E. coli*

3.6.1 Sample collection

Water samples were collected into sterile falcon tubes (50 mls) from the sampled wells directly using the same containers that the community members use to draw water from each Well. The collected samples were labeled carefully and transported to school of Veterinary Medicine Microbiology laboratory at the University of Zambia for analysis. Whilst in transit, the samples were stored in cooler boxes with ice packs (between 2º C to 8º C) to maintain the temperature of the samples from the time of collection up to the laboratory for analysis. Figure 3.3 shows field pictures of sample collection.

Figure 3.3: Pictures showing sample collection and temporal storage of samples in the field

3.6.2 Isolation and confirmation of *E. coli*

After obtaining 1 ml from each raw sample for serial dilution, 49 mls of each sample remained in falcon tubes and centrifuged at 3000*xg* for 5 minutes. After centrifugation, the supernatant was decanted to remain with 5mls of the sediment. Then the sediment was vortexed (Mixed) and 1ml was obtained and pipetted into a test tube containing 9 mls brain heart infusion (BHI) broth. The BHI tubes with the samples were incubated at 37ºC for 24hrs. After 24hrs, a loopful of the sample was inoculated on MacConkey Agar for 24hrs. From each of the 48 plates. Suspected *E. coli* colonies were gram-stained to observe a Gram reaction of the bacteria. *E. coli* which is Gram-negative, appearing pink/red under the microscope. Further confirmation of the suspected *E. coli* colonies were done by use of four different biochemical tests: 1. Triple sugar iron (TSI), 2. Sulfide-Indole-Motility (SIM), 3. Urease and 4. Citrate.

3.6. 2. 1 Triple sugar iron test

The test is used to differentiate bacteria based on their ability to ferment sugars and produce hydrogen sulfide gas. Suspected *E. coli* bacteria was streaked on TSI agar and due to fermentation of glucose and lactose, acid was produced turned the agar yellow (Uğurlu et al., 2021).

3.6.2.2 Sulfide-Indole-Motility test

This is used to detect hydrogen sulfide production, indole production from tryptophan, and bacterial motility. Suspected *E. coli* bacteria was stabbed into SIM medium, which contains iron salts for detecting hydrogen sulfide production and tryptophan for indole production is observed as the growth pattern radiating from the stab line. *E. coli* positive was indicated by a red colour after the addition of Kovac's reagent and motility (Alves et al., 2006).

3.6.2.3 Urease test

It determines whether bacteria can hydrolyze urea to produce ammonia and carbon dioxide. Suspected *E. coli* bacteria were inoculated onto urea agar, which contains urea and a pH indicator. After incubation, a color change indicates urease activity of no colour change was observed in the medium (Dahlén et al., 2018).

3.6.2.4 Citrate test

This test determines whether bacteria can utilize citrate as the sole carbon source.
Suspected *E. coli* bacteria was streaked onto simmons citrate agar, which contains citrate as the only carbon source. A pH indicator detects alkaline products of citrate metabolism of which no colur change was observed (Blount et al., 2012).

Suspected *E. coli* bacteria was further sub-cultured onto MacConkey Agar and incubated for 24hrs at 37ºC. Pure *E. coli* colonies (typically they are small, round, smooth, and opaque on agar plates with a slightly raised appearance as well as pink to red in color due to lactose fermentation) were grown on MacConkey containing cefotaxime (CTX) and incubated for 24hrs. The colonies that grew on CTX MacConkey were subjected to antibiotic testing using disc diffusion method (Kapena et al., 2020). Drugs used were Cefotaxime, Nalidixic Acid, Streptomycin, Co-trimoxazole, Ciprofloxacin, Tetracycline, Gentamicin, Chloramphenicol, Ampicillin and Norfloxacin. The colonies that were resistance to Cefotaxime were subjected to DNA extraction for polymerase chain reaction (PCR). Figure 3.4 shows samples in the laboratory prior to analysis and figure 3.5 summarises the process of *E. coli* isolation from sampled water.

Figure 3.4: Picture showing field samples in the microbiology laboratory prior to processing

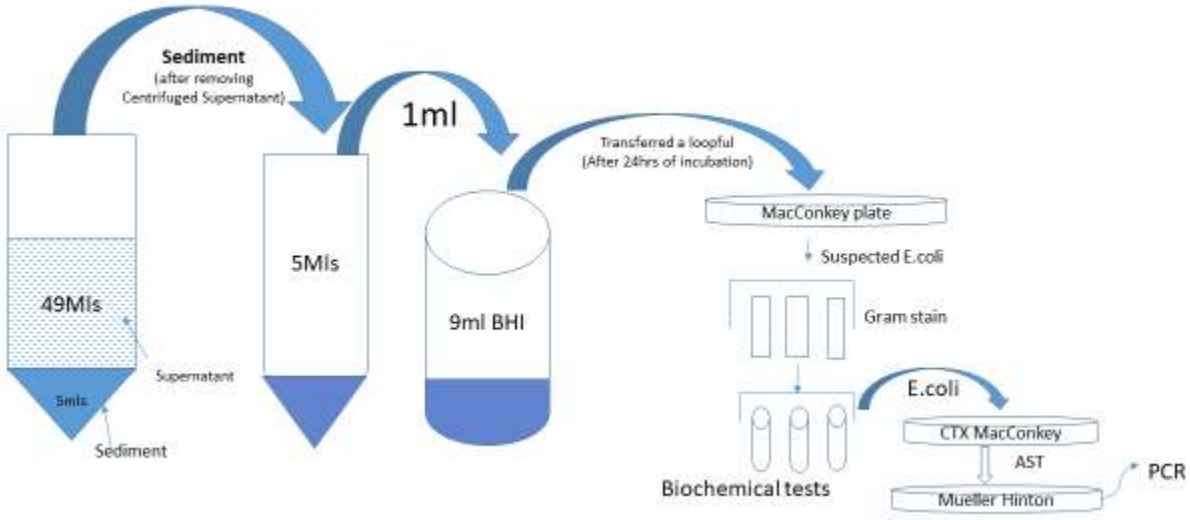


Figure 3.5: showing summary of the procedure from *E. coli* isolation to PCR

3.6.3 Serial dilution and Enumeration

Each collected sample was serially diluted to a 10^{th}, 100^{th} and 1000^{th} fold and later plated on MacConkey agar and incubated at 37º C for 24 hours. This was done to enable quantification of the colonies. The process started by obtaining 1 ml of the original sample and suspended it in 9 mls normal saline giving a 10^{-1} dilution. Secondly, 1ml from the first test tube containing 10^{-1}

dilution was pipetted into the second test tube containing 9 mls normal saline giving 10^{-2} dilution and thirdly, 1ml from the second test tube was pipetted into the third tube containing 9 mls of normal saline giving 10^{-3} dilution. Thereafter, 0.1ml from each of the three (3) test tubes were pipetted onto MacConkey plates and spread using a microbe spreader. The plates were incubated for 24hrs at 37ºC as shown in figure 3.6. After 24hrs, the developed colonies were counted on the MacConkey plate visually and multiplies by the reciprocal of the dilution factor. To find total colony forming units (CFUs) occurring for each sample, the average was calculated (Davidson., et al., 2014)

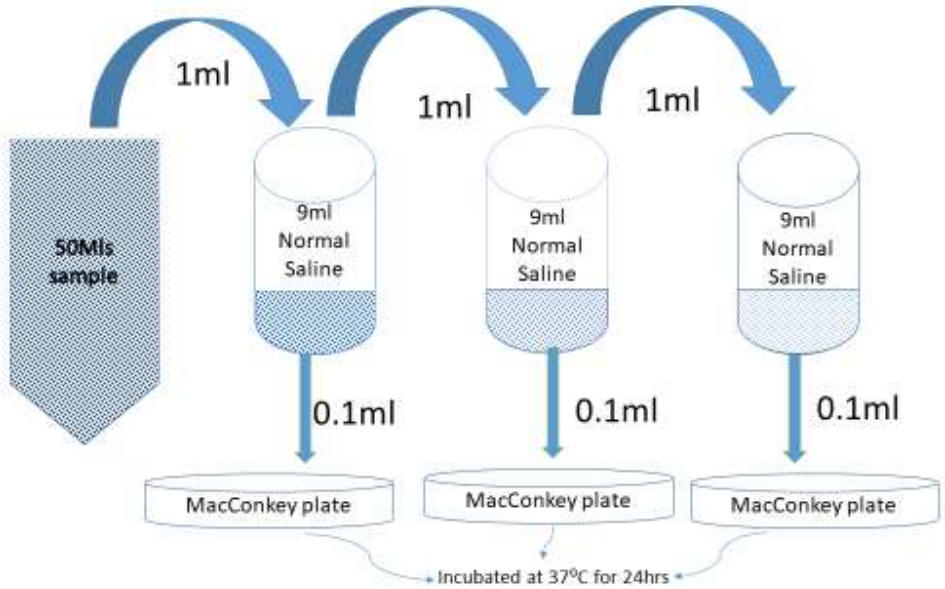


Figure 3.6: Illustrates the procedure for serial dilution and bacterial enumeration

3.6.4 DNA Extraction

After culture, 2-3 pure colonies from nutrient agar plate were placed in 1ml of distilled sterile tube. The Eppendorf tube were vortexed for 10 seconds. Bacteria DNA was extracted using heat- lysis protocol. Molecular confirmation was done by PCR, where the ß-glucuronidase gene (uidA) were used as *E. coli* reporter. *E. coli* (ATCC® 25922) were included as positive controls.

3.6.5 PCR Analysis

Polymerase chain reaction was used to confirm resistance of genes TEM (Temoniera), SHV (Sulphydryl variable), and CTX (Cefotaximime-Munich in *E. coli* isolates). A total reaction volume of 20 µl was used for the PCR (Finnzymes piko), which included 5 µl phusion, 2µl sterile distilled water, 2µl primers (forward and reverse), and 1µl bacterial DNA template. The rapid cycle DNA amplification method was used for the PCR reaction, which included an initial denaturation step at 94º C for 1minute, followed by 35 cycles of template denaturation at 94º C for 30 seconds, primer annealing at 60º C for 30 seconds, primer annealing at 72º C for 30 seconds, and a final extension at 72º C for 5 minutes. After electrophoresis through 1.5% agarose gel, the products were visualized with ethidium bromide, as described by Ranjar et al (2008).

3.6.6 Antimicrobial Susceptibility test

Based on recommendations from the Clinical Laboratory Standard Institute (CLSI), the Kirby-Bauer disc diffusion method was used for the antimicrobial susceptibility testing (CLSI, 2009). Ampicillin (10 µg), sulfamethoxazole/trimethoprim (1.25/23.75 µg), streptomycin (300 µg), ciprofloxacin (5 µg), tetracycline (30 µg), gentamicin (10 µg), nalidixic acid (30 µg), chloramphenicol (30 µg), ceftazidime (30 µg), norfloxacin (10 µg), and cefotaxime (30 µg) were among them. Using recommendations established by CLSI, gives break points matching to zone of inhibition diameter, interpretation of susceptibility patterns on different anti-microbial disks were performed. To prevent contamination, normal laboratory practices for quality control were carefully followed. The organisms utilised for quality control were *E. coli* (ATCC®) 25922.

3. 7 Investigation of Factors associated with *E. coli*

The investigation of factors associated with the presence *E. coli* in water was achieved by use of a semi-structured questionnaire which was administered verbally. The questions were translated into the local language (Tonga/Lenge). All 48 households where water samples were collected had questionnaires administered. Each household administered with a questionnaire had household head or any eldest member provided responses. All responses were recorded carefully and translated back into English. The questionnaire was pre-tested, ten households who had same conditions as in the targeted study area were sampled from a nearby community known as Mwachisompola village. The questionnaire determined the demographic data and factors

associated with presence of *E. coli* in water from unprotected wells as shown in table 3.2. In this table, each represents a specific question, including its number and description. The response options for each question are listed, and the frequencies/percentages of respondents selecting each option are provided in the subsequent columns. During the administering of the questionnaire, each participant was assigned a unique identifier to maintain anonymity and track the individual responses.

Table 3.1: shows data collected by use of a semi – structured questionnaire

Question number	**Question description**	**Response option/response theme for open ended questions**	**Frequency (%) n=48**
2.0 Demographic data			
2.1	Age	18 -30 31 – 45 Above 46	18 -30 = 9 (18.75) 31 – 45 =18 (37.5) Above 46 =21 (43.75)
2.2	Sex	Female, Male	Female =27(56.25) Male =21(43.75)
2.3	Level of Education	None, Primary, secondary	None = 2 (4.17) Primary =22 (45.83) Secondary =24 (50)
2.4	Marital status	Single, Married, Windowed, Divorced	Single =1(2.08) Married =43(89.58) Windowed =3 (6025) Divorced =1(2.08)
2.5	Occupation	Farmer	Farmer =48 (100%)
3.0 Factors associated with presence of E. coli in water from unprotected wells			
3.1	Is water for drinking and other domestics purposes sourced from well?	Yes, No	Yes = 48 (100) No = 0
3.2	Is well protected?	Yes , No	Yes = 0 No =48(100)
3.3	"Call of nature" is answered from?	Ordinary pit latrine, VIP, Bush, Other	Ordinary pit latrine =46 (95.83) VIP =0 (0) Bush =1(2.08) Other =1(2.08)
3.4	Approximation of the distance (M) from Pit latrine to unprotected well	20-29 30-39 40- 49 50-59 60-69	20-29 =14 (29.17) 30-39 =22 (45.83) 40- 49 =4 (8.33) 50-59 =5 (10.41) 60-69 =1 (2.08) NA = 2 (4.17)

3.5	Rearing of domestics animals/birds	Yes , No	Yes = 47 (97.92) No =1 (2.08)
3.6	How are animals/birds reared?	In pens, Free-range	In pens =0 Free-range = 47 (97.92) NA = 1(2.08)
3.7	Source of drinking water for animals/birds	Stream, shallow wells, other	Stream =0 shallow wells =3 (6.25) other (sharing same water) = 44 (91.67)
3.8	Do EHTs in the sample water for quality monitoring?	Yes, No	Yes =0 No =48 (100)
3.9	How often is water from unprotected well sampled	Monthly, quarterly, other	Monthly =0 Quarterly =0 Other (Not sampled) =48 (100)
3.10	Is well treated with chorine?	Yes. No	Yes =0 No =48 (100)
3.11	How often is well treated with Chlorine?	Monthly, quarterly, other	Monthly =0 Quarterly =0 Other (Not treated) =48 (100)

3.8 Determination of the Risk exposure factors

The data collected through questionnaires and results from the laboratory were both entered into excel sheets. With help of the add-in model risk Monte Carlo Simulation software tool in excel (Benamar et al., 2021). Data was modelled and analysed to assess the risk of exposure to *E. coli* from unprotected wells.

Input parameters for determination of the probability of exposure assessment to *E. coli* in unprotected wells was aided by data collected by a questionnaire and only three parameters were picked due to homogeneity of the responses which included type of toilets, free range rearing of domestic animals and use of same water source as per collected data from the study area. Meanwhile, distributions like Beta, Uniform and pert were considered and run at 10,000 iterations. Table 3.1 shows the input parameters used to determine exposure to *E. coli* due to consumption of water from unprotected wells in Model Risk with different probability distributions commonly used in Monte Carlo simulations and other statistical analyses. Each distribution has its own characteristics and is suitable for different types of data and modeling scenarios. Whereas, Beta distribution is a continuous probability distribution defined on the interval [0, 1]. It is commonly

used to model proportions or probabilities, making it suitable for representing uncertainty in proportions or rates. It can vary widely depending on the values of its two shape parameters, often denoted by α (alpha) and β (beta). It can further be symmetric or skewed, depending on the relative values of α and β. While, Uniform distribution is a simple probability distribution where all values within a given interval are equally likely to occur. It is characterized by a constant probability density function within the specified interval and zero probability density outside that interval. It is also defined by two parameters: the minimum and maximum values of the interval over which the distribution is defined. Pert distribution, also known as the Triangular distribution, is characterized by a range of values bounded by a minimum, maximum, and mode which is the most likely value.it is shaped like a triangle, with the highest probability density at the most likely value and decreasing density towards the minimum and maximum values.

Table 3.2 Risk exposure to *E. coli* from consumption of water from unprotected wells

Variable	**Parameter**	**Data**	**Distribution**
		38+1-38+1	
P1	Probability *of E. coli* in unprotected wells	N1*P1	Beta
P 2	Probability of *E. coli* /type of toilet	N*P2	Uniform
p 3	Probability to be exposed 1	P1*P2	Beta
N 1	Number of people exposed /500 cases/P3	500*N1*P3	
P4	Probability of *E. coli*/rearing domestic animals	N*P4	Uniform
P5	Probability to be exposed 2	N1*P5	Uniform
N2	Number of people exposed /500cases/P5	500*N2*P5	
P6	Probability of *E. coli*/source of animal water	N*P6*	Pert

P7	Probability to be exposed 3	N1*P7	Uniform
N3	Number of people exposed/500 cases/p7	500*N*P7	

3.9 Data management and Analysis

Both data from the questionnaires and laboratory results were entered into excel sheets and coded. Then the entered coded data was transported into Stata version 14.0 for analysis. Data collected from the questionnaire was checked for completeness and consistency before entering. To explore the data, descriptive statistical analysis of quantitative bacterial counts, measurements of location were used to describe the outcome. Results were presented in percentages/proportions and the difference in distribution of predictor variables was considered significant if p-value was less than 0.05.

3.10 Ethics consideration

Permission to conduct Research was sought from National Health Research Authority (NHRA) and ERES Converge. Further, Chibombo district health office gave authorization to conduct research in their catchment area. Consent and permission were sought from Village headmen, and household's heads before sample and data collection. Generally, there were no ethical issues involved since the collected information did not infringe on the rights of the participating households.

CHAPTER FOUR

RESULTS

4.0 General descriptors

4.1 Demographic information on the sampled population

The study presented various demographic characteristics of the study participants, including gender, age group, education level, marital status, and occupation. For gender, there were 27 female participants, accounting for 56.3% (CI: 42.2 – 70.3) of the total sample, while 21 participants identified as male, comprising 43.75% (CI: 29.7 – 57.8) of the sample. In terms of age group, the largest proportion of participants fell into the 31-45 age range, with 18 individuals (37.5%) (CI: 23.8 – 51.2). This was followed by the above 46 age group, which consisted of 21 participants (43.8%) (CI: 29.7 – 57.8. While, nine participants (18.8%) (CI: 7.7 – 29.8) were in the 18-30 age range. Regarding education level, the majority of participants had secondary education, with 24 individuals (50%) (CI: 35.8 – 64.2), while 22 participants (45.8%) (CI: 31.7 – 59.9) had primary education. Only 2 participants (4.2%) reported having no formal education. Marital status varied among participants, with the majority being married, comprising 43 individuals (89.58%) (CI: 80.9 – 98.2). Three participants (6.3%) (CI: -0.0071– 0.13) were widowed, one participant (2.08%) (CI: -0.016 – 0.1) was divorced, and only one participant (2.08%) (CI: -0.016 – 0.06) reported being single. In terms of occupation, all participants were identified as farmers, making up 100% of the sample. Generally, table 4.1 provides a comprehensive overview of the demographic characteristics of the study participants, offering valuable insights into the composition of the sample population.

Table 4. 1: shows demographic data collected from a questionnaire survey

Characteristic	Category	Frequency (n)	Percentage (%)
Gender	Female	27	56.25 (CI: 42.22 – 70.28)
	Male	21	43.75 (CI: 29.70 – 57.79)
Age group	18-30	9	18.75 (CI: 7.72 – 29.78)
	30-45	18	37.5 (CI: 23.82 – 51.18)
	Above 46	21	43.75 (CI: 29.70 – 57.79)
Education level	None	2	4.17 (CI: -0.015 – 0.10)
	Primary	22	45.83 (CI 31.72 – 59.94)
	Secondary	24	50 (CI: 35.83 – 64.17)
Marital status	Single	1	2.08 (CI: 0.016 – 0.06)

	Married	43	89.58 (CI: 80.93 – 98.23)
	Windowed	3	6.025 (CI: -0.0071– 0.13)
	Divorced	1	2.08 CI: -0.013 – 0.06)
Occupation	Farmer	48	100(CI: 1.0 – 1.0)

4.1.1 General descriptive results

A total of 48 unprotected wells were sampled across four villages which were independent of each other by distance. Out of 48 unprotected wells, 38 were indicative *E. coli* taking into account strata and within individual variation results were indicative of *E. coli* presenting 79% (95% CI: 77.3 - 80.7%). These 48 wells were sampled from 4 different villages. Figure 4.1 shows the results as follows; Chilumbwa 5/38 presenting 13% (95% CI: 2.3 – 23.7%), Chabwa had 10/38 presenting 26% (95% CI: 12.1 -39.9%), Kafwilo 11/38 presenting 29% (95% CI: 14.6 – 43.4%) and Katobole-12/38 presenting 32% (95% CI: 17.2 – 46.9%).

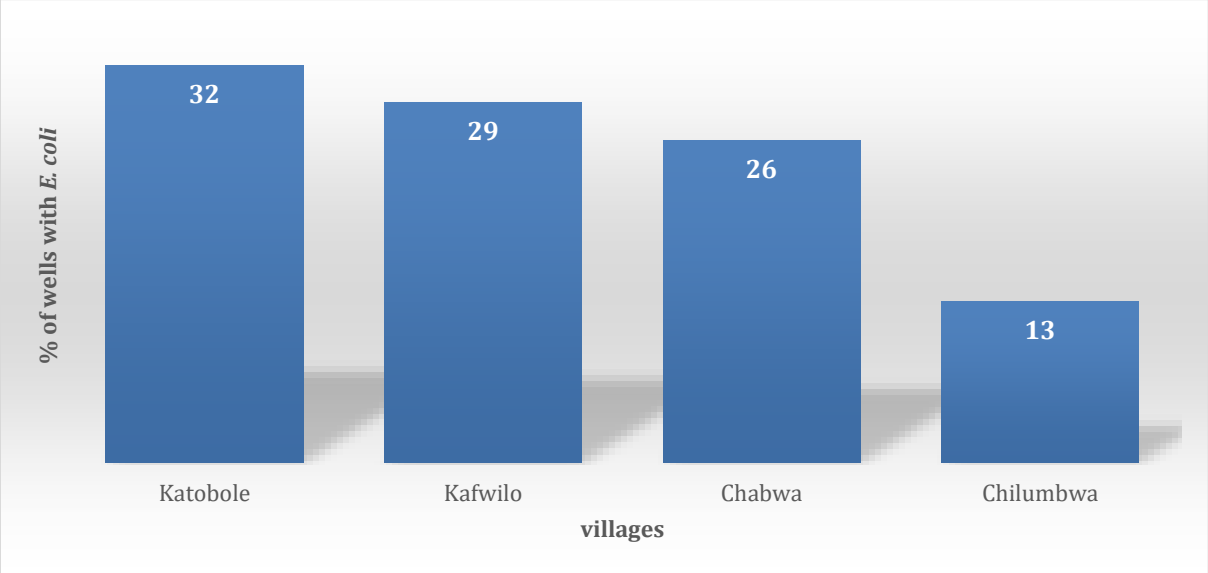


Figure 4.1: Shows percentage of contaminated wells with *E. coli* for each village

4.1.2 *Escherichia coli* positivity in relation to distance between pit latrine and unprotected well

The table 4.1 shows data on the distance range between pit latrines and unprotected wells, along with the corresponding number of unprotected wells, the number of wells tested positive to *E. coli*,

and the percentage of positive results. Only 46 out of 48 respondents were considered on estimating distance ranges between pit latrine and unprotected wells in meters, because two households reported not to have any type of toilets during the survey. Thus, the positivity rate was calculated out of 46. In the distance range of 20-29 meters, there were 14 unprotected wells, out of which 13 tested positive to *E. coli*, resulting in a 28% positivity rate. Within the distance range of 30-39 meters, there are 22 unprotected wells, with 14 testing positive to *E. coli*, leading to a 30% positivity rate. For the distance range of 40-49 meters, there are 4 unprotected wells, all of which tested positive to *E. coli*, resulting in a 9% positivity rate. Similarly, within the distance range of 50-59 meters, there are 5 unprotected wells, with 4 testing positive to *E. coli*, also resulting in a 9% positivity rate. Finally, in the distance range of 60-69 meters, there is 1 unprotected well, which tested positive to *E. coli*, resulting in a 2% positivity rate.

Table 4.2: Shows *Escherichia coli* positivity in relation to distance between pit latrine and unprotected wells (n=46)

Distance range between pit latrine and unprotected wells (M)	No. of unprotected wells	*E. coli* positive	%
20 - 29	14	13	28%
30 - 39	22	14	30%
40 - 49	4	4	9%
50 - 59	5	4	9%
60 - 69	1	1	2%

4.2 *E. coli* load determined in sampled water

The average number of colony forming units after dilution from raw samples collected from unprotected wells are shown in figure 4.2. The dilutions were in 10 folds, 100 folds and 1000 folds. 16/48 (33.3%; CI: 31.4-35.2%) samples were found with average number of CFU of between 1000 and 10,000 which was the highest range. Meanwhile, 14/48 (29.2%; CI: 27.3 -31.1%) of the samples were indicative of the average number of CFUs ranging from 1-1000 and 10,000 plus. `4/48 (8.3%; CI: 7.2 -9.4%) samples were found with Zero CFU.

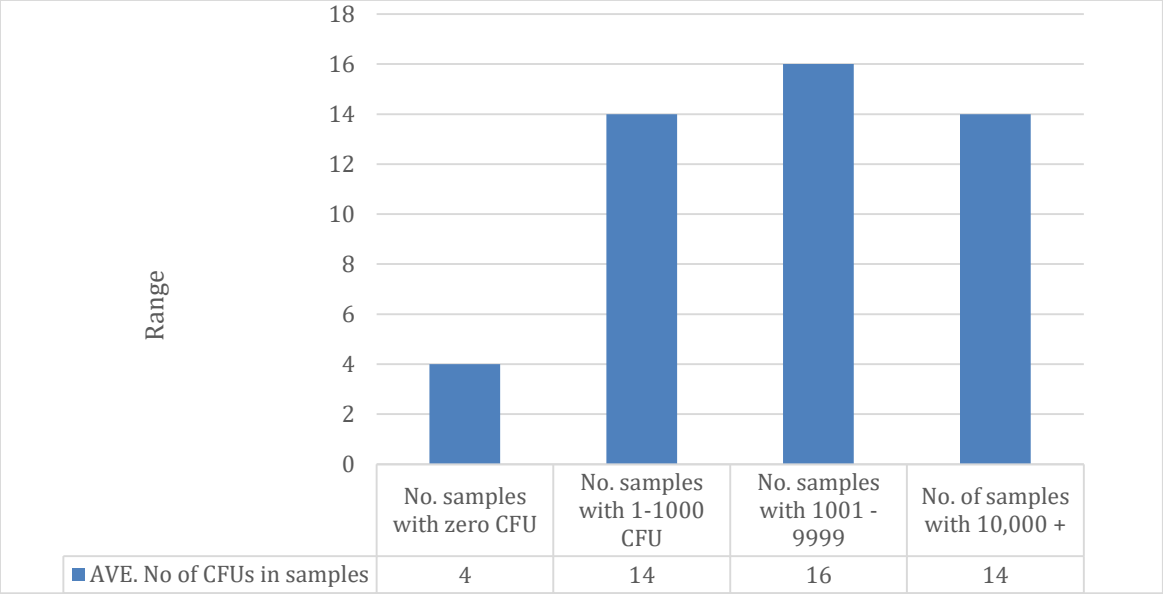


Figure 4.2: shows average number of CFUs in collected samples

4.3 Antimicrobial Susceptibility

All 55 isolates in table 4.2 were resistant to one antibiotic or more. All tested isolates were resistant to AMP (2) presenting 100%, CTX (30) had 22/55 giving (40% CI: 38.3 - 41.8%), N (30) 19/55 (34.6% CI: 32.9 - 36.2%) COT (25) 12/55 presenting (21.6% CI: 20.1 – 23%), TE (30) 8/55 (14.6%; CI: 13.3 – 15.8%) NOR (10) 3/55 giving (5.5%; CI: 4.6 – 6.26%), CIP (5) 2/55 presenting (3.6%; CI: 2.97 – 4.3%) and S (101) 1/55 giving (1.8%; CI: 1.3 – 2.3%).

Table 4.3: Antimicrobial susceptibility results (n=55)

Antibiotic	R (x)	I	S	% R=x/n*100
Ampicillin	55	0	0	100
Cefotaxime	22	0	33	40
Nalidixic Acid	19	8	28	35
Cotrimoxazole	12	4	39	22
Tetracycline	8	4	43	15
Norfloxacin	3	0	52	5
Ciprofloxacin	2	3	50	4
Streptomycin	1	0	54	2
Gentamicin	0	3	52	0
Chloramphenical	0	3	52	0

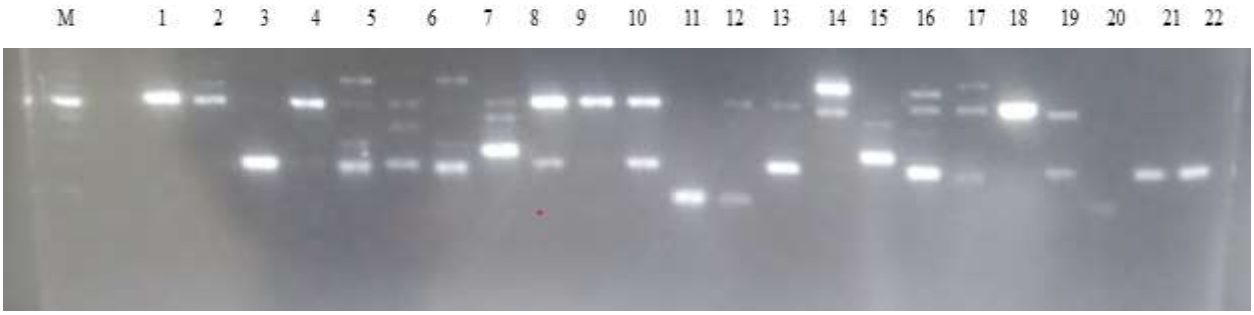
Escherichia coli isolates were also tested for Multi Drug Resistance (MDR) of which one isolate was indicative of being resistant to eight antibiotics and another to five antibiotics presenting (5.88%; CI: 3.2 – 8.6%) for each. Meanwhile, seven isolates were resistant to four antibiotics (41.2%; CI: 35.5 – 46.9%) and eight isolates were resistant to three antibiotics (41.1%; CI: 35.4 – 46.9%) as shown in table 4.4;

Table 4. 4: Multiple-Drug Resistant (MDR) profiles n=17

Isolate ID	MDR profile	Drugs	No=x	% (x/17*100)
28b	R3	TE, AMP & COT		
34b	R3	CTX, AMP & COT		
35a	R3	CTX, AMP &CIP		
35b	R3	CTX, AMP &NA/ACID	8	41.1
40d	R3	CTX, AMP &NA/ACID		
42c	R3	CTX, AMP &NA/ACID		
44c	R3	TE, AMP & COT		
48c	R3	CTX, AMP & COT		
30b	R4	TE, AMP, CTX & NA/ACID		
30a	R4	CTX, TE, AMP & NA/ACID		
30b	R4	TE, AMP, COT & NA/ACID		
31b	R4	CTX, TE, AMP & COT	7	41.2
32b	R4	CTX, AMP, COT & NA/ACID		
40b	R4	CTX, AMP, COT & NA/ACID		
42e	R4	CTX, AMP, TE & NA/ACID		
42d	R5	CTX, TE, AMP, COT &NA/ACID	1	5.9
8a	R8	CTX, TE, S, AMP, NOR, COT, CIP & NA/ACID	1	5.9

4.4 Genes detected on Molecular Analysis

The main genes detected on PCR were *bla*$_{TEM}$ cluster shown in Figure 4.4a, and *bla*$_{CTX-M}$ cluster in figure 4.4b which are beta lactamase coffering genes, while *bla*$_{SHV}$ gene was not detected on any isolates. The twenty-two *E. coli* isolates that were further analysed on PCR revealed that (72.7%; CI: 68.7 – 76.7%) sixteen isolates were Extended Spectrum Beta Lactamase (ESBL)-producing *E. coli* isolates carrying β-lactamase genes of *bla*$_{TEM}$ and (18.2%; CI: 14.7 – 21.6%) four isolates were ESBL-producing *E. coli* isolates carrying the β-lactamase genes of *bla*$_{CTX-M}$. These results have also been shown in Table 4.4.



(a.)

Figure 4.4a: Picture shows agarose gel with results for PCR products on *bla*$_{TEM}$ gene

(b.)

Figure 4. 4b: Picture of agarose gels showing results for PCR products on *bla*$_{CTX-M}$ gene

Polymerase Chain Reaction products are shown on agarose gels after amplification from the DNA of ESBL producing *E. coli*. (a) *bla*$_{TEM}$ gene and (b) *bla*$_{CTX-M}$ gene detected with and as negative and positive controls respectively. Lane M is a molecular marker, whereas lane 1 to 22 were samples being analysed.

Table 4.5: Shows *E. coli* isolates analysed on PCR (n=22)

Detected genes	No. of *E. coli* isolates	% of *E. coli* isolate
TEM	16	72.7
CTX-m	4	18.2

4.3 Risk exposure assessment of *E. coli* in unprotected wells

The probability to be exposed to *E. coli* was at 79.5% (95%; CI: 66.5 – 86.7%). This means that there is a highly likelihood (approximately 4 out of 5 times) of encountering *E. coli* when using water from unprotected wells. The risk due to use of water from unprotected wells is depicted in figure 4.5 where the probability of *E. coli* exposure is indicated. From these results, it is evident that there is significant risk of *E. coli* exposure associated with the water from unprotected wells.

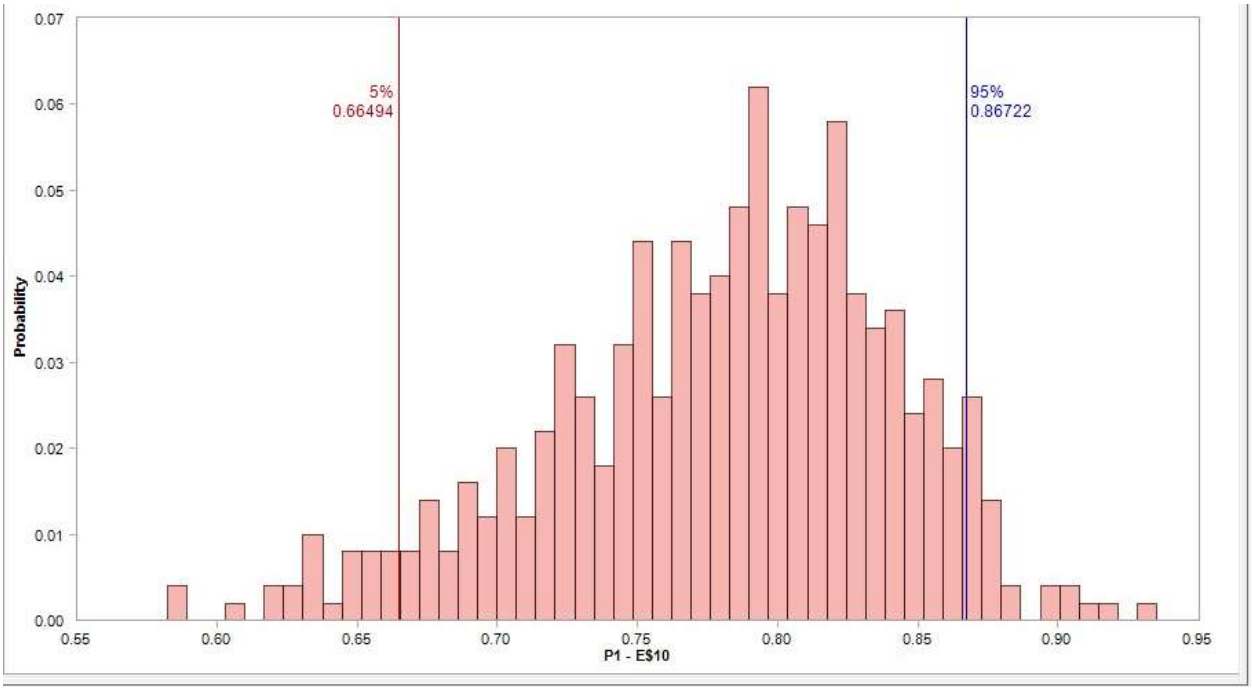


Figure 4.3: Shows probability of *E. coli* exposure due unprotected water source

The probability to be exposed to *E. coli* contamination was 48.8% (95%; CI: 4.9 – 83.4%) occurrence due to unprotected wells in relation to type of toilets being used by the community members in the study area. This result would mean there is a moderate likelihood (approximately

1 in 2 times) of encountering *E. coli* contamination when using water from unprotected wells. The wide range of the confidence interval shown in figure 4.6 suggests considerable uncertainty around the estimated possibility.

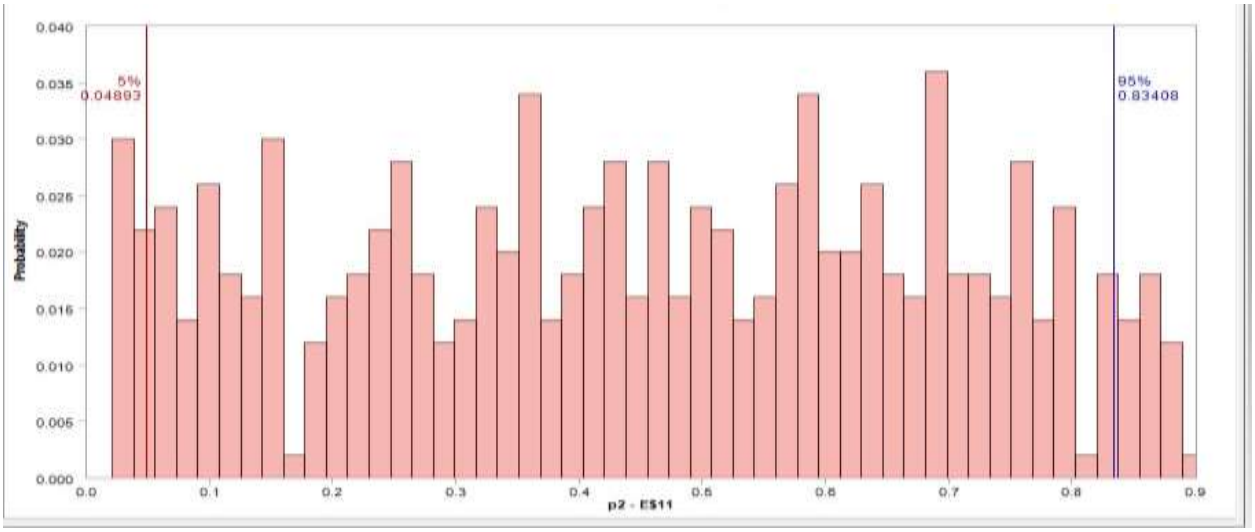


Figure 4.4 shows probability of *E. coli* exposure due to type of toilet

The probability to be exposed to *E. coli* was 49.8% with (95%; CI: of 0.02 – 84.8%) occurring in unprotected wells due to rearing of domestic animals. The occurrence of *E. coli* contamination due to the use of unprotected wells attributed to the rearing of domestic animals was moderate (approximately 1 in 2 times) of encountering *E. coli* contamination as shown in Figure 4.7. This confidence interval obtained however, suggest that while there is a moderate overall probability of *E. coli* contamination from unprotected wells, the actual risk can vary widely depending on specific factors such as the presence of domestic animals.

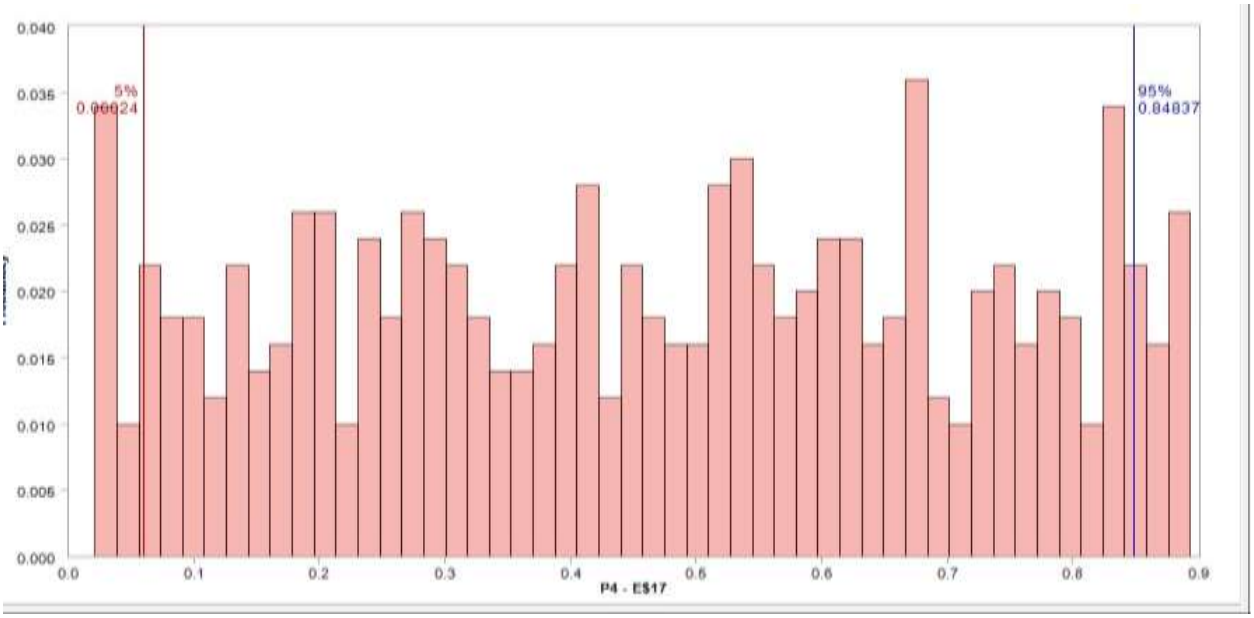


Figure 4.5: Shows probability of *E. coli* in unprotected wells in relation to domestic animals

The probability to be exposed to *E. coli* was 10.6% with (95% CI: 2.4 – 40.0%) occurring in unprotected wells when same water is shared between domestic free-range animals and community members as shown in figure 4.8. This probability and confidence interval both indicate relatively lower likelihood of encountering *E. coli* contamination when sharing same water source with domestic animals.

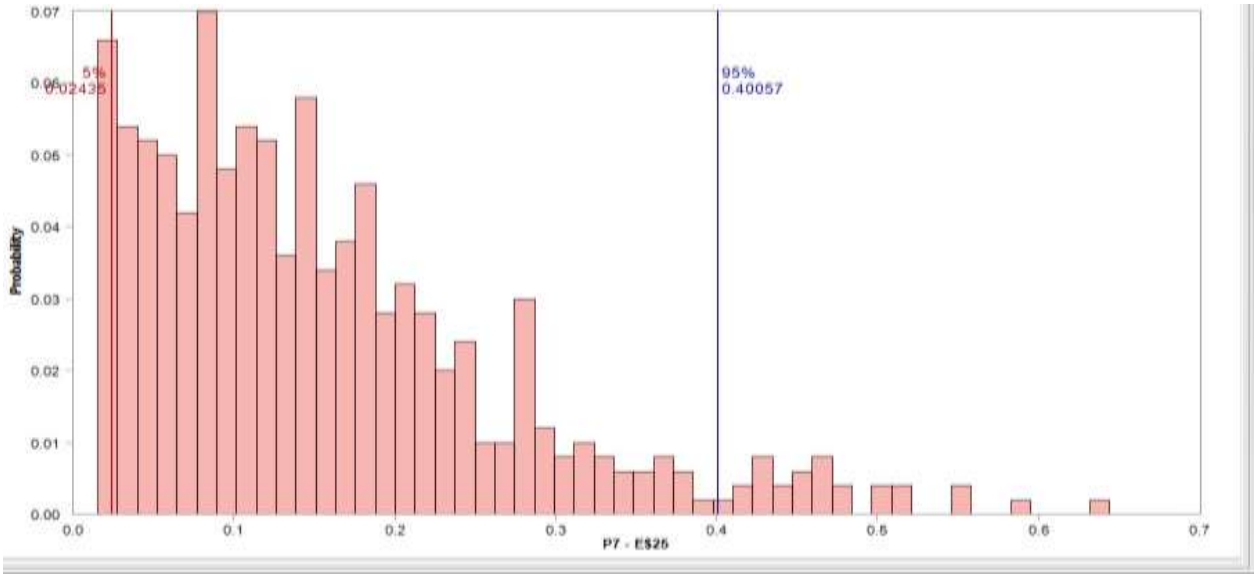


Figure 4.6: Shows the probability of *E. coli* exposure in unprotected wells in relation to use of same water for humans and domestic management.

CHAPTER FIVE

DISCUSSION

5.0 Demographical information of the sampled population

A detailed analysis of the demographic characteristics of the study participants includes; gender, age group, education level, marital status, and occupation. The study found a relatively balanced representation of gender among the participants. About half of the participants predominantly had secondary education. The majority of participants were married with smaller proportions of some being widowed, divorced, and single. It was also interesting to note that all participants were identified as farmers. It was observed that the youngest age range of 18-30 years old recorded the highest positivity rate on the presence of *E. coli* in their wells followed by 31-45 years old and the age range of 46 years old and above had the lowest percentage. These results make an assumption that the older age range could have the knowledge and experience on the manner their wells are kept following certain standards and hence reduce contamination while the younger age range lacks experience and knowledge. The other attribute about this observation could be that the elderly are more disciplined in the way they conduct their household activities. The presence of *E. coli* in their water sources is an indication of faecal contamination that would lead to diarrheal diseases in agreement with a study done by Begum and others (2005) on the isolation of pathogenic *E. coli* ETEC from surface water in rural areas of Bangladesh.

5.1 General descriptive results

The results of the study indicate that out of 48 unprotected wells sampled across four villages, 38 were found to be indicative of the presence of *E. coli*. This suggests that approximately 79% of the sampled wells were contaminated with *E. coli*. These findings are concerning as they highlight a high prevalence of bacterial contamination in the sampled wells, posing potential health risks to the communities relying on them for water. The results vary of *E. coli* contamination across all four villages. The village-specific contamination rates provide insights into the spatial variability of *E. coli* contamination within the study area. The differences observed among the villages may be attributed to various factors such as geographical location, population density, land use practices, and proximity to potential sources of contamination as observed by Begum et al (2005) on the isolation of *E. coli* from surface water in rural areas of Bangladesh.

These wells are usually prone to bacterial contamination as they are not covered and have no form of concrete lining. The water is not treated and is usually exposed to activities of the daily-living of humans and animals. The lack of covering exposes the wells to bird's droppings and dust, which may be drawn into the wells by wind, whereas the lack of concrete lining allows seepage of water from unexplained ground water sources. The ground water can be contaminated with feacal matter if the toilet depths are comparable to the depth of the wells. Animal activities such as dogs or cattle can have the desire to drink water and as they move close to the wells, they may drive dust or any other associated contaminants as reported by a study done (Lutterodt et al., 2018) on Microbial Groundwater Quality Status of Hand-Dug Wells and Boreholes in Ghana.

Generally, these results underscore the importance of water quality monitoring and the implementation of appropriate interventions to address bacterial contamination in drinking water sources, particularly in rural communities where access to safe water may be limited. Effective strategies for water treatment, sanitation improvement, and community education are essential for safeguarding public health and preventing waterborne diseases.

5.1.1 *E. coli* positivity in relation to distance between pit latrine and unprotected wells

Generally, the results obtained on the relationship between distances that exist between pit latrines and unprotected wells indicates a clear trend that *E. coli*-positivity rate in wells increases with short distances between pit latrines and unprotected wells and *E. coli*-positivity rate decreases as the distance increases. This correlation suggests that proximity to pit latrines is indeed associated with the contamination of wells with *E. coli* and correlates with study done by (Muruka et al., 2012) on The Relationship between Bacteriological Quality of Dug-Wells & Pit Latrine Siting in Kenya. Subsequently, it underlines the importance of maintaining a safe distance between sanitation facilities like pit latrines and water sources to lessen the risk of waterborne diseases caused by fecal contamination

5.1.2 *Escherichia coli* load determined in sampled water

Among the samples analysed, the majority fell within the range of 1000 to 10,000 CFUs after dilution. This range represents the high CFU counts, indicating a significant presence of bacterial colonies in these samples. These high percentages of CFUs found in sampled wells was in line

with a study done by Phiri (2016) on risks of domestic underground water sources in informal settlement in Kabwe, Zambia that revealed 70% of poor water quality in Africa. Addressing water quality issues in these areas is crucial for safeguarding public health and reducing the risk of waterborne diseases associated with bacterial contamination.

5.2 Antibiotic sensitive tests and genes detected

Antibiotic sensitive testing revealed fascinating resistant patterns in that all tested isolates were found to be resistant to three (3) or more classes of antibiotics. These findings were similar to a study conducted in Lusaka, Zambia by Chishimba and others (2015) on ESBL where high antibiotic resistant profiles were established and also a study done by Larson and others (2019) on antibiotic resistance *E. coli* in drinking water in Peru. These resistant patterns observed in this study could be attributed to high misuse of antibiotics in treatments as indicated by WHO (2021). These enteric bacteria organisms that may acquire resistance through this system may end up in a pit latrine increasing the risks of water contamination with antibiotic resistant bacteria in unprotected water wells. AMR is of great concern as the issue was deliberated upon in the tripartite joint Secretariat for AMR where a political declaration was made to which head of states at the United Nations general assembly in New York in 2016 committed to. These heads of states agreed to focus on broad coordinated approach that engage human, animal, plant environment and health in what is known as "One Health Approach" (WHO, 2021).

This study further revealed high prevalence of the isolates analysed on PCR were carrying β-lactamase genes of bla_{TEM} cluster. Meanwhile a relatively low percent of isolates were carrying β-lactamase genes of $bla_{\text{CTX-M.}}$ This picture was not very different from the study done by Chishimba and others (2015) in Lusaka, Zambia despite that β-lactamase genes for bla_{SHV} in this study were not detected on any isolates analysed by PCR. The difference could be due to differences in sample size. It was further interesting to comprehend that the predominant gene detected in this study was bla_{TEM} as opposed to the other study done by Chishimba (2015). The occurrence of these ESBL-producing *E coli* bacteria in water wells is of great concern because bacterial pathogens have been reported to be associated with serious human infections with symptoms of diarrheal globally and especially in children as indicated in a study conducted by Thani and others (2016) on isolation and characterization of *E. coli* on phenotypic factors associated with well and borehole water

contamination in India. These findings necessitate that water drawn from unprotected wells could play a major role in the spreading of ESBL producing *E. coli* hence the need to improve water sources in the study area.

5.3 Risk factors involved in *E. coli* exposure

The results for *E. coli* exposure assessment of unprotected wells as simulated in Monte Carol Model risk revealed a strong likelihood of the people in the study area to be exposed to *E. coli* due to consumption of contaminated water. The study also revealed that there could be a strong likelihood that the population in question could be exposed to *E. coli* when sharing the same source of water with their domestic free-range animals and birds. The two parameters however, showed a less likelihood of the community members in the study area to be exposed to *E. coli* when consuming water from unprotected wells in relation to type of toilets being used. These results are slightly different from other studies done on latrine use and associated factors among rural communities in Indonesia by Yulyani and others (2019) which reported a stronger likelihood of *E. coli* contamination in groundwater due to use of ordinary pit latrines. Maybe this was so because of a small sample size used in this study as compared to other studies as well as the homogeneity of the data set. By employing a Monte Carlo model, stakeholders can systematically evaluate the risks associated with consuming water from unprotected wells, account for uncertainty, and make informed decisions to protect public health and ensure access to safe drinking water. The findings of risk exposure assessment however, suggest that measures should be taken to mitigate the risk of *E. coli* contamination in water from unprotected wells and decision makers to be made aware of such results so that action could be put in place for improving water quality and ensure safety of the consumers.

5.4 Synthesis of contamination pathways of water observed in this study

This study revealed a number of possible contamination pathways that may include dust as the wells are uncovered; human contamination arising from use of contaminated buckets and ropes used to draw water from the same water wells; run-offs when drawing water and during the rainy season; and fecal droppings from domestic animals and birds as indicated in the study done by Bain, (2014). Following these identified possible pathways, the community members wash their dishes (plates, cups and other kitchen utensils) and bath using the same water. These activities

expose the community members to higher risk of contamination as the washed plates and other cooking utensils would habour *E. coli* when using the dishes. Oral contamination is also anticipated during bathing. In addition, the study also revealed that the water from unprotected wells was being used for washing fruits, vegetables and cooking. In some case the same water is used for drinking especially that there are few boreholes in the community. These observations were also made by Woolf et al., 2023 in the report done at American Academy of peadiatrics. It is however, fortunate that there has been no serious reported diarrheal disease outbreak in the area.

5.5 Socio-economic implications and Significance of the study

Escherichia coli in drinking water can have noteworthy socio-economic implications, particularly in developing countries where access to clean water and sanitation is limited, with the following effects;

1. **Health impact**: *E. coli* contaminated water can lead to waterborne diseases like diarrhea which is the major cause of illness and death especially in the under five children of age (Ahmed et al., 2020).
2. **Economic Loss**: Illness due to *E. coli* contaminated water may lead to lost workdays, reduced productivity, and increased healthcare costs. In countries like Zambia where there is a free healthcare policy, other costs may be incurred such as transportation to and from the health care facility (Genter et al., 2022).
3. **Educational Impact**: School absenteeism due to Children suffering from *E. coli* associated waterborne illnesses may miss school days, affecting their education. Chronic absenteeism can lead to lower educational attainment and reduced future opportunities (Williams et al., 2013)).

Water that has been contaminated with *E. coli* has far-reaching consequences, affecting health, education, and economic well-being thus, addressing water quality is crucial for sustainable development and improved livelihoods as targeted in the SDGs.

Communicating the results of both the sampled wells and risk exposure assessments to the public would raise awareness about the potential health risks associated with *E. coli* contamination in water and in turn, individuals would be empowered to take appropriate precautions to protect themselves and their families from such exposure. Generally, conducting a risk exposure assessment to *E. coli* contaminated water is essential for protecting public health, ensuring

regulatory compliance, optimizing resource allocation, and supporting effective water quality management strategies.

5.6 Study limitations

1. Field samples were only collected in December and due to limited resources it was not possible to go back in the field at different times of the year to make a good comparison of the results
2. Samples collected at a time when community members were cultivating their fields making it difficult to use systematic random sampling
3. Worked with trial version of Monte Carol Model risk software excel-add.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.0 Conclusion

1. *Escherichia coli* was isolated from water in 38 (79%) out of the 48 of the unprotected wells in Mwachisompola community under Chibombo District.
2. The *E. coli* counts were found above the normal standard set by the WHO water guidelines.
3. The study also revealed high (91 %) colony forming units (CFUs) an indication of fecal contamination in the sampled wells.
4. The isolated *E coli* organisms showed antibiotic resistance and some were even multi-drug resistant.
5. The likelihood of risks of exposure to *E. coli* as a result of consuming water from unprotected wells was high (79.5%)

6.1 Recommendations

The recommendations to be put into consideration following the findings of this study are:

1. To advocate for improved water sources such as; protected wells and boreholes in the area.
2. To educate the community members on the importance of observing distances between pit latrines and water sources as guided by WHO and Public Health Act cap 295; Drainage and latrine regulation no. 81 of the laws of Zambia.
3. To subject water sources to routine water quality monitoring by collecting water samples on regular basis.
4. Routine treatment of wells to interject the frequent contamination of water wells and to be in compliance with the requirements of the law on water quality.
5. Further study in the area: to focus on determining water contamination at household level and comparing results for seasonal variations.

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APPENDICES

APPENDIX 1: QUESTIONNAIRE

QUESTIONNAIRE

Questionnaire number: _____

1.0. Identification

1.1 **District**: _____1.2 **ward** _____

1.3 **Health Facility Catchment area** _____

1.4 **Community**: _____1.5 **Landmark**_____

2.0. **Demographic Data**

2.2 **How old are you?**

18-30 □ 31-45 □ Above 45 □

2.3 **Sex:**

Female □ Male □

What is your level of education?

None □ Primary School □ High School □

2.2. **What is your marital status**?

Married □ Single □ Widow/Widower □ Divorced □

2.3 **What do you do for your living (Occupation)? _____**

_____

3.0 **Factors associated with presence of *E. coli* in water**

3.1. **Do you get your water for drinking and other domestic purposes from a well?**

Yes □ No □ Other (please specify) _____

3.2. **If the answer is yes to the previous question, is the well protected?**

Yes □ No □

3.3**. Where do you go to answer the "call of nature"?**

Ordinary Pit latrine □ VIP latrine □ Bush□ □Other (Please specify) _____

3.4. **Approximately, how far is the pit latrine from the water source? _____**

3.5. **Do you own any domestic animals (Chickens inclusive)?**

Yes □ No □

3.6. **If answer is yes to the previous question, how do you rare these domestic animals?**

In pens □ free range □ other (specify) _____

3.7. **If answer is yes to question 3.5, where do animals drink water from?**

Streams □ shallow wells □ Other (Please specify) _____

3.8 Is the water from wells sampled by the EHTs?

Yes □ No □

3.9 If the answer is yes, to the previous question, how often is the water sampled?

Monthly □ quarterly (3 months) □ Other (specify) _____

3.10 Do EHTs treat the water in the wells?

Yes □ No □

3.11 If answer is yes to the previous question, how often are the wells treated?

Monthly □ quarterly (3 months) □ Other (specify) _____

APPENDIX II: INFORMED CONSENT IN ENGLISH

A. PARTICIPANT'S INFORMATION FORM

My name is Doris Zgambo a master's student at the University of Zambia, School of Veterinary Medicine conducting a study on **"*Escherichia coli* contamination and risk exposure assessment of humans consuming water from unprotected wells in Mwachisompola of Chibombo District, Zambia"**. *E. coli* is one of the germs that are found in contaminated water and may lead to diarrhoeal diseases mainly affecting children.

The information to be collected will be useful in understanding factors that may lead to the presence of germs in water which may be used for domestic purposes. The results of this study are expected to provide some form of solution to the study area and add to the body of knowledge on the factors that would lead to the presence of germs in the water, in this case *E. coli.*

The study has no physical risks to participants. I however, recognize some information you may tell me or fill in in the questionnaires maybe personal or/ and sensitive to other stakeholders. In addition, I would like to assure you that the information we will get from you will not be shared with anyone outside the research team.

The answers that you will give will be treated with utmost confidentiality and your name will not appear anywhere because this study is of an academic purpose only.

For any clarification call the following: Principal Investigator (Doris Zgambo); Physical address: University of Zambia (UNZA) main campus along Great East Road. +260978935250/+260966294509; Email: *doris.zgambo@gmail.com*; Co-Principal Investigator (Prof. B. Hang'ombe) +260977326288; Email: *mudenda68@yahoo.com / bhangombe@unza.zm*

Call or contact the ERES Ethics Committee office for any ethical queries. The Ethics Committee contact information is: +260 955 155633/+260 955 155634

CONSENT SHEET

You have been selected randomly to participate in this study but you have the right to withdraw or refuse to participate in the study before or during the course of the interview.

Thank you for your willingness to participate in the study.

Name of interviewer…….. Signature……..

The above information has been explained to me clearly and fully understand and consent myself to participate in the research.

SIGNATURE……/ | Thumb print | DATE……/……/2022.

B. PARTICIPANT'S INFORMATION FORM IN TONGA

Izyina Langu ndime Doris Zgambo sichikolo waku Univesrity of Zambia na Chikolo Chipati na (UNZA) kuchikolo chilanganya nseba zyabanyama na Veterinary medicine. Mubuvuntauzi oobu tulanganya kazunda katengwa "***Escherichia coli (E. coli*) KusofwazyaMeendaAkunywa BantuKuzwa Mumigoti ItakwabilidweKwa Mwachisompola Muboma Lya Chibombo, Zambia".** Aka kazunda kajanwa mumenda akunywa inga kalaletela bulwazi bwaku-soomona na kunyanya kapati kubana bashoonto.

Mibuzyo eyi iyakubeleshengwa kuluhibo lwakazunda ka **(*E. coli*)** kukubona nzila mbokanjila mumenda anyungwa muchoko ka Mwachisompola. Bumvunauzyi bweesu bwelede kungwasha na kupa mizezo mibotu kubantu boonse alimwi akuchikolo kutengwa bantu bakwabililwe. *E. coli.*

Buvuntauzi oobu bunyina bukaka buli bonse kubantu, nkambo tutela nchomwamba mumibuzyo njotwamupa na tamuyandi kutola lubazu mubuvuntauzi obu inga mwamba tauli mulandu pee. Twamushomezya kuti twambo tutwabweza kulindinywe tatukatubiki muluwo na i*nternet* tubeleshengwa boo kubuvuntauzi oobu.

Bwinguzi mbumuyakupa buyakulemekwa alimwi izyina lyanu talikalembwi mupaper eli na mubulembo bwesu nkambo oobu buvuntaunzi mbwachikolo buyo.

Kumibuzyo minji njomungamwaba anjiyo inga mwatuma luwaile kumuvuntauzyi mupati Doris Zgambo ukala kuchikolo chipa cha UNZA munsi amugwagwa mupati uya kujwe na *Great East Road.* Inga mwatuma kumanambala aya +260978935250/+260966294509; na Email: *doris.zgambo@gmail.com*; Inga mwatuma kumuvuntauzi uchilila na ba (Professor B. Hang'ombe) balo bali mbayi bapati ku UNZA kunambala eyi +260977326288; na Email: *mudenda68@yahoo.com. / bhangombe@unza.zm.*

Inga mwatuma alimwi ku ERES Ethics Committee balanganya buvuntauzyi bwazyikolo a basichikolo mubboma lya Lusaka. Inga mwabatumina kunambala eyi +260 955 155633/+260 955 155634

CONSENT SHEET

Mwasalwa kutola lubazu mubuvuntauzyi bwesu amuzyibe kuti mulijisis nguzu zyakuleka na timwasalalilwa kumibuzyo yeesu muciindi chakubuzyingwa.

Twalumba mukulisungula kweenu kukutola lubazu mubuvuntauzi bwesu

Inzyina Lya Simubweza twambo

…….. Kusaina …….. Buzuba……

Twaambo toonse tuli atala twapandululwa kulindime ndatelela alimwi ndalisungula mukutola lubazu mubuvuntauzyi oobu.

Kusaina ……Buzuba.....