PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITY OF WATER DRAWN FROM LINED AND UNLINED WELLS IN ZARIA, NIGERIA

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DEPARTMENT OF MICROBIOLOGY,

FACULTY OF LIFE SCIENCES,

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ZARIA

NOVEMBER, 2017

DECLARATION

I hereby declare that this dissertation titled "PHYSICO-CHEMICAL AND MICROBIOLOGICAL QUALITY OF WATER DRAWN FROM LINED AND UNLINED WELLS IN ZARIA, NIGERIA"was prepared by me and it is the record of my own findings and has never been submitted anywhere for the award of any degree. Sources of information have been duly acknowledged by means of reference.

_____ _____

DANJUMA, Florence Yachim Date

CERTIFICATION

This is to certify that the research work titled "PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITY OF WATER DRAWN FROM LINED AND UNLINED WELLS IN ZARIA, NIGERIA" meets the regulations governing the award of the degree of Master of Science in microbiology of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Studies were carried out to assess the physicochemical and microbiological quality of water obtained from lined and unlined wells in Zaria. Zaria is a major city in Kaduna state in northern Nigeria. The city was divided into three sampling locations namely; Samaru, Sabon-gari and Tudun Wada. A total of 142 wells (53, 44 and 45 wells from Samaru, Sabon-gari and Tudun Wada respectively from both lined wells and unlined wells) were sampled for a period of six months encompassing both rainy and dry seasons (July to September for rainy season and November to January for dry season). Water samples from these wells were collected and analyzed for presence of cysts of *Entamoebahistolytica* and faecal contamination using membrane filtration techniques. Temperature,pH, conductivity and total dissolved solids were also measured. Temperature andpH had values that were within the normal ranges of 20^0C-32^0C and 6.5-8.5 respectively. ElectricalConductivity (EC) and Total Dissolved Solids (TDS) had values beyond the acceptable limits of 180-500µS/cmand 300-500mg/L respectively. A prevalence of 27.5% was recorded for *Entamoebahistolytica*cysts in the study area and the presence of the parasite was higher in lined wells (30.5%) than unlined wells (23.3%). The water samples contained high density of coliforms. A total of 168 coliforms bacteria belonging to10 species were isolated from the study which include;*E. coli* (48.3),*Enterobacteragglomerans*(11.2%), *Enterobactergergoviae*(10.5%), *Enterobacteraerogenes*(7.0%), *Klebsiella pneumonia* (9.9%),*Klebsiellaozanae*(10.5%), *Citobacterfruundii*(6.3%), *Proteus mirabilis* (2.8%),*SerratiaLiquefaciens*(9.9%), and*Acinebacteriwoffi*(1.4%). *E. coli* was the most frequently isolated bacterium. Isolates were subjected to antibiotic sensitivity tests and all the isolates tested (45) were susceptible to Nitrofurantoin, Chloramphenicol, Ciprofloxacin and Gentamicin except *E. coli* which showed resistance of 5.6 and 16.7% toChloramphenicol and Gentamicin respectively. The presence of *Entamoebahistolytica*cysts and significant counts of coliform bacteria in the well water samples is of great concern and suggest that the water obtained from these wells are not suitable for domestic(especiallydrinking) purposes without proper treatment.

CHAPTER ONE

1.0 INTRODUCTION

Water is extremely essential for the growth and survival of all living things including man. The provision of adequate supply of safe drinking water is a basic human right. Water quality can affect the health of people both physically and socially (Desalegn*et al.*, 2014). WHO estimated that about 80% of illnesses in developing countries are associated with lack of safe water and adequate sanitation (Adekunle*et al.*, 2004; Odeyemi*et al.*, 2010; Siyum and Woyessa, 2013). Most infectious diseases are caused by pathogenic bacteria, viruses and protozoa which have the ability to transmit disease through contact with or drinking of contaminated water. Waterborne infectious diseases caused by these pathogens can be life threatening (Siyum and Woyessa, 2013).

Nigeria is one of the countries in the world that has unsafe water supplies especially in the rural communities. Given the low quality of drinking water in Nigeria, millions of people have been trapped in the cycle of poverty and ill health, often rendering them unable to work or go to school due to water-related diseases which are preventable (National Academy of Science (NAS), 2008; Ocheri and Atu, 2012; Adeyinka*etal.*, 2014). About 90million peoplein Nigeria do not have access to safe drinking water and about 130,000 children under the age of five die every year from preventable waterborne diseases. Therefore lack of access to safe water supply is a serious threat to the country (Adeyinka*etal.*, 2014).

In rural communities of Nigeria, water for human consumption and domestic uses is primarily obtained from shallow (hand dug) wells and this constitutes the major source of ground water; other available sources are from rivers, streams, lakes, ponds and rainfall. Faecal contamination is the main source of contamination of these water sources

due to unhygienic human practices such as defaecation in open fields, animal wastes and seepage from septic systems.This has left the rural communities with no option but to use water from these sources, exposing them to various waterborne diseases (Desalegn*et al.*, 2014; Hammuel*et al*., 2014)

Groundwater is generally considered to be cleaner and safer than surface water because it is less susceptible to microbial contamination due to the lengthy time of their travel in subsurface environment, and the soils and rocks through which ground water flows screen out microorganisms, especially bacteria and protozoa. However, in recent years, an increasing threat to ground water quality due to human activities has become of great concern as microbial and chemical contaminants have been detected in ground water. Several factors such as discharge of industrial, agricultural and domestic wastes, land use practices and rate of infiltration affect the quality of ground water and once contamination occurs, it can persist for hundreds of years because water movement in aquifers is very slow (Ramirez *et al*., 2010; Nandkishor*et al*., 2014).

Isolation and characterization of enteric bacteria are of primary importance in the microbiological quality of water. The use of indicator organisms particularly the coliform group as a means to assess the potential presence of waterborne pathogens is of importance to public health. The presence of any coliform organism in drinking water is used as an indicator of faecal contamination because they are most sensitive in demonstrating faecal contamination of water (Aboh*et al,* 2015).

Waterborne gastrointestinal parasites such as *Crytosporidiumparvum, Giardia lamblia*, and *Entamoebahistolytica* are frequently associated with morbidity, particularly in children.Worldwide, these parasites are the most common cause of waterborne

infections. Outbreak of these infection have also been associated with feacal contamination (Bilal *et al,*. 2003; Desalegn*et al,*. 2014).

In the absence of regular fresh water supplies, people residing in Zaria communities rely on groundwater (from dug well) for their domestic and drinking purposes; and some of these wells are usually with hand-laid stones or concrete blocks orpre-cast concrete sections. Modern wells available are usually with pre-cast concrete rings. This study was designed to assess the physicochemical and microbiological quality of well waters and to compare the level of contamination of water fromlined and unlined wells in Zaria communities.

1.1. STATEMENT OF RESEARCH PROBLEMS

The quality of water is increasingly threatened by human activities. The availability of and accessibility to safe drinking water to the Nigeria populace has been and is still a serious issue as evidenced by high frequency of waterborne infections incidences reported. A large number of infectious diseases are transmitted primarily through water supplies contaminated with human and animal excreta. Outbreaks of waterborne diseases continue to occur throughout the world, especially in developing countries (Muhammad *et al,*. 2010).

In spite of efforts to improve access to safe drinking water, an estimated 1.1 billion people worldwide lack access to an improved water source. Over 3 million people die annually from water-related diseases. About 2 million of these deaths are the result of diarrhoeal diseases, which are caused by the ingestion of faecally contaminated water, as well as by inadequate sanitation and hygiene. Children under the age of five are the most affected and 11% of all under five deaths occur in Nigeria. (UNICEF, 2008; CDC, 2008).

The human pathogens that present serious risk of disease whenever present in drinking water include *Salmonella* species, *Shigella* species, *Yersinia enterocolitica*, *Campylobacter* species, various viruses such as Hepatitis A virus, Hepatitis E virus, Rotavirus and parasites like *Entamaebahistolytica*, *Giardia lamblia*, *Cryptosporidium parvum* and others (Muhammad *et al*,. 2010).

In addition to causing direct health impacts, unsafe drinking water can cause malnutrition and opportunistic infections (such as pneumonia) especially in children who are weakeaned by frequent diarrhoeal episodes. Water borne pathogens such as *Cryptosporidium, Pseudomonas, Klebsiella,* other heterotrophic microorganisms like heliobacteria, green non-sulfur bacteria and purple non-sulfur bacteria can cause serious complications in people with compromised immune system (UNICEF,2008).

1.2. JUSTIFICATION

Water and sanitation remain the major primary drivers of public health. Access to potable water supply and sanitation remain basic human needs that should be satisfied adequately to meet minimum health standards. Adequate supply of safe and potable water assist in preventing the spread of gastrointestinal diseases, supports domestic and personal hygiene, and improves the standard of living (Aremu*et al*., 2011).

Many organisms causing diseases like diarrhoea, gastroenteritis, Keratoconjunctivitis, respiratory infections, and viral hepatitis are often found in environmental samples including ground water, surface water, and coastal water (Borchardt*et al*., 2003; Borchardt*et a*l., 2004; Xagoraraki*et al.,* 2007).

Lack of access to safe drinking water eludes millions of people each and every day. As a result, waterborne diseases have become a serious threat throughout the world, especially in underdeveloped nations with poor conditions of public health.

Water sources are highly prone to contamination by pathogens and chemical agents. Assessing the quality of water sources through frequent monitoring is therefore extremely necessary for effective maintenance of water quality through appropriate control measures (de Kok*et al*., 2001; Yisa*et al.*, 2012).

Hand dug wells serve as the main source of water supply in most Nigeria's rural and parts of urban communities. However, most of these wells are improperly constructed or poorly situated and maintained. It is therefore necessary that these wells should be routinely checked to ensure safety of water obtained from them.

1.3. AIM

The aim of this study was to assess the physicochemical and microbiological quality of water drawn from lined and unlined wells in Zaria, Nigeria

1.4. SPECIFIC OBJECTIVES"

The specific objectives of this study were to:

1. Determine some physicochemical quality parameters of water from lined and unlined wells in Zaria
2. Detectand quantify cysts of *Entamoebahistolytica* from the well water samples
3. Determine the total bacterial count, coliform count and *E. coli count* of the water samples
4. Isolate and characterize members of the coliform group of bacteria
5. Determine the antibiotic susceptibility pattern of the isolates.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. WATER

Water is a transparent liquid composed of two elements; two hydrogen atoms and one oxygen atom (H_2O). Water is a liquid at ambient conditions, but it often co-exists on Earth with its solid state being ice, and gaseous state (being water vapour or steam). Water is a finite substance; there is the same amount of water now as there was when the earth was formed (United States Environmental Agency (U.S. EPA), 2016). While water is a renewable resource, its availability in space and time is limited by climate, geographical and physical conditions. Most human activities involve the use of water in one way or the other. It may be noted that man's early habitation and civilization sprang up along the banks of rivers. Water is useful for different needs which include agricultural, residential, manufacturing, community and personal needs. Increasing water demands and its ineffective management has led to crises situations in many parts of the world; crisis over the availability of adequate and appropriate quality water. It can be argued that water is everywhere but water for drinking purposes is not everywhere because water available for drinking and other domestic activities is very minute (Padma and Namrata, 2003; U.S. EPA, 2016).

2.1.1. Global Water Supply

Fresh water resources are unevenly distributed worldwide, with much of the water located far from human populations. Many of the world's largest river basins run through thinly populated regions. There are an estimated 263 major international river basins in the world, covering approximately 231,059,898 km^2 or 45.3% of the earth's surface area (excluding Antarctica).

Groundwater represents about 90% of the world's readily available freshwater resources, and some 1.5 billion people depend on groundwater for their drinking water. Agricultural water use accounts for about 75% of total global consumption, mainly through crop irrigation; industrial use accounts for about 20%, and the remaining 5% is used for domestic purposes. (Padma and Namrata, 2003).

Natural contamination of water resources mainly results from normal geological phenomena such as ore formation. Nevertheless, human activities are a major factor determining the quality of surface and groundwater. These activities include; atmospheric pollution, effluent discharges, use of agricultural chemicals, eroded soils and land use (Ruma*et al*., 2014).

In the past few decades, public concerns over the quality of drinking water have grown considerably. These concerns have risen as a result of increased awareness about environmental pollution and episodes of waterborne disease outbreaks. According to Oyegun (1983), a large number of miseries, sickness and deaths occur due to infectious diseases which are linked to open water supplies in the tropical developing countries. In such countries, there were many people without safe drinking water, which resulted in many water borne disease infections.

Worldwide, about 900 million people lack access to safe water supply, about 90% do not have access to adequate sanitation facilities and 4,500 children die every day from water borne diseases. WHO in 2002 predicted that by the year 2012 many African countries will experience water scarcity. The availability of good quality water is an indispensable feature for preventing diseases and improving quality of life (Ruma*et al*,.2014).

2.1.2. Water Supply in Nigeria

Nigeria, the largest and biggest exporter of oil in Africa, is characterized by low level of access to improved water sources and limited access to improved sanitation.

The responsibility for water supply is shared between the three levels of government. The Federal Government is in charge of water resources management, State Governments handle urban water supply while the Local Governments together with the communities are responsible for rural water supply. The responsibility for sanitation is not clearly defined. Water supply service quality and cost recovery are low. Water tariffs are low and many water users do not pay their bills. Service providers thus rely mostly on occasional subsidy to cover their operating cost. Investments are mainly financed by Governments and foreign donors and fall short of what is needed to achieve a significant increase in adequate supply (Federal Republic of Nigeria, 2000).

Access to an improve water source stagnated at 47% from 1990 -2006, but increased to 54% in 2010. In urban areas, access to an improved water source actually decreased from 80 - 65% in 2006, but it then recovered to 74% in 2010. Access to sanitation decreased from 39% in 2010 with a particularly marked decrease in urban areas. About 25% of Nigerians have to use shared sanitation facilities, which are considered as inadequate. About 22% are estimated to use other inadequate facilities and another 22% are estimated to defecate in the open. Adequate sanitation is typically in the form of latrines and septic tanks. Piped sewage is almost nonexistent. Except for some parts of Abuja and Lagos, no urban community has a piped sewage system (WHO/UNICEF, 2010).

Water resources available in Nigeria can be broadly classified into fresh and marine water resources. The former constitutes the fraction that should ideally be accessible to

all communities; they comprise of water from lakes, hand dug wells, boreholes, streams, rivers, wetlands and those available in underground reservoirs.

Freshwater represents the main source of safe water for household, agricultural and even industrial uses. They are required for drinking, cooking, recreational activities, farming, fishing e.t.c(Galadima*et al*.,2011). Freshwater resources available to the local inhabitants are either unsafe or difficult to obtain; and are severally stressed by poor management. These make access to clean water a serious problem.In some instances women and children need to walk for hours to fetch ordinary drinking water. A preliminary survey between 2007 and 2010 was carried out by Galadima*et al.,* and the results indicate that, there are many villages that have never seen the so called "treated tap water" in their communities. Despite the scarcity and the foreseeable global water crisis as previously noted by the United Nations, the available domestic water in the communities is increasingly polluted daily. (Galadima*et al*., 2011; Chukwu,2008).

2.2. SOURCES OF FRESH WATER

Rain, snow, hail and sleet are precipitated upon the surface of the earth and may be considered the natural source of all the water collected from roof run-off and ground surfaces (Center for Disease Control (CDC), 2009). Water for human consumption and other activities are primarily derived from two sources; surface water and ground water.

2.2.1. Surface Water

Surface water is water obtained from stream, river, lake, pond, reservoir or ocean. Surface water is naturally replenished by precipitation and naturally lost through discharge to the oceans, evaporation and ground water recharge. Surface water frequently contains substances that must be removed before it can be used as drinking water (CDC, 2009).

2.2.2. Ground Water

Groundwater is the water that saturates the tiny spaces between alluvial material (sand, gravel, silt, clay) or the crevices of fractures in rocks. The zone above the water table is known as the zone of aeration (unsaturated or vadose zone). Water in the soil (in the ground but above the water table) is referred to as soil moisture. Spaces between soil, gravel and rock are filled with water (suspended) and air. Most groundwater is found in aquifers—underground layers of porous rock saturated from above or from structures sloping toward it. Aquifer capacity is determined by the porosity of subsurface material and its area. There are two major types of aquifers: confined and unconfined (Conservation Technology Information Centre (CTIC), 2015).

Confined Aquifers: This is also known asartesian or pressure aquifers. They exist where the groundwater system is between layers of clay, dense rock, or other materials with very low permeability. Water in confined aquifers may be very old, even millions of years old. This water is under more pressure than water in unconfined aquifers**.** Thus, when tapped by a well, water is forced up, sometimes above the soil surface. This is how a flowing artesian well is formed (CTIC, 2015).

Unconfined Aquifers:They are more common than confined aquifers. Unconfined aquifers have a permeable deposit that leads into the aquifert. Water may have arrived by percolating through the land surface. This is why water in an unconfined aquifer is often very young, in geologic time frames. The top layer of the aquifer is also the water table. Thus, it is affected by atmospheric pressure and changing hydrologic conditions (CTIC, 2015).

2.3. WELLS

A Well is an excavation or a structure created in the ground by digging, driving, boring or drilling to access groundwater in underground aquifers. Wells can vary greatly in depth, water volume and water quality. Well water typically contains more minerals in solution than surface water and may require treatment to soften the water. (Philip, 2011).

There are basically three types of wells. They include hand-dug wells, driven wells and drilled wells.

2.3.1. Driven Wells

Driven wells are built by driving a small-diameter pipe into soft earth, such as sand or gravel. A screen is usually attached to the bottom of the pipe to filter out sand and other particles. They can only tap shallow water, and because the source of the water is so close to the surface, contamination from surface pollutants can occur(Philip, 2011).

2.3.2. Drilled Wells

Drilled wells require a fairly complicated and expensive drill rig. They use rotary drill bits that chew away at the rock, percussion bits that smash the rock. Drilled wells can be drilled more than 1,000 feet deep. Often a pump is placed at the bottom to push water up to the surface (Philip, 2011).

2.3.3. Hand-dug Wells

Hand-dug wells are constructed by hacking at the ground with pick and shovel to dig until the water table is reached. If the ground is soft and the water table is shallow, then dug wells can work. The well is with stones, brick, tile, or other material to prevent collapse, and is either covered with a cap of wood, stone, metal or concrete (Philip, 2011).

The traditional method of obtaining groundwater in rural areas of the developing world, and still the most common, is by means of hand-dug wells. Many of the wells found in homes are excavated until the water table is reached. They are described as shallow wells. The depth of the wells depends on how far the water table could be reached. Most of these Wells, while digging, the side of the excavations are usually supported.

2.4. METHODS OF LINING WELLS

There are several methods of supporting the sides of the excavation while digging proceeds:

2.4.1. Pre-cast concrete rings

The safest method is to excavate within pre-cast concrete rings which later become the permanent lining to the sides of the well. The first ring has a cutting edge, and additional rings are placed on it as excavation proceeds. As material is excavated within the ring, it sinks progressively under its own weight and that of the rings on top of it. This method should always be used in unstable ground. When construction has finished, the joints between the rings which are above the water table should be sealed with cement mortar (Water Aid, 2013).

2.4.2. In situ lining

In suitablystable ground, excavation may proceed for a short distance without support to the sides; these are then supported by means of concrete poured *in situ* from the top, between the sides of the excavation and temporary formwork, which becomes the permanent lining to the well. This process is repeated until the water table is reached (Water Aid, 2013).

2.4.3. Supported excavation (the 'Chicago' method)

In suitably stable ground, excavation may proceed within the protection of vertical close-fitting timber boards, supported by horizontal steel rings (the Chicago method). The timbers are hammered down as excavation proceeds and additional timbers are added progressively at ground level. The steel rings must be hinged, or in two parts bolted together, so that lower ones can be added as the excavation progresses. The vertical spacing between the rings will depend on the instability of the ground. The well is with bricks or concrete blocks, from the water table upwards, within the timbers as they are withdrawn (Water Aid, 2013).

However, in stable ground, wells are often excavated down to water level without a lining, and are with in situ concrete, or with pre-cast concrete rings, from this level upwards.

Wells safely dug during the dry season may become unstable when the water level rises in the wet season and therefore must be before this occurs to prevent collapse. In firm and stable ground, wells may be safely excavated without support and may give satisfactory service, although it is strongly recommended that a permanent supporting lining be installed. This will support the sides of the excavation, preventing them from collapse and preventing pollution by surface water (Water Aid, 2013).

2.5. WATER POLLUTION

The presence of contaminants in natural freshwater continues to be one of the most important environmental issues in many parts of the world, particularly in developing countries, where several communities are far away from potable water supply. Low income communities, which rely on untreated surface water and groundwater supplies for domestic and agricultural uses are the most exposed to the impact of poor water

quality. Several reports have revealed that human activities are a major factor determining the quality of surface and groundwater through atmospheric pollution, effluent discharges, use of agricultural chemicals, eroded soils and land use (Peter and Olusegun, 2006; Yusuf, 2007; Chukwu, 2008; Nwanta*et al*., 2010) Environmental pollution, mainly of water sources, has become public interest. Also the chemical composition of groundwater is controlled by many factors that include the composition of precipitation, mineralogy of the watershed and aquifers, climate and topography. These factors can combine to create diverse water types that change in composition spatially and temporally (Galadima*et al.*, 2011).

2.6. CAUSES OF WATER POLLUTION

2.6.1. Domestic Activities

A combination of increasing population and lack of adequate sewage and wastedisposal, has made many communities potential health hazard areas for their inhabitants. Sanitary and sewage systems are poor, and where they exist, are poorly managed. Improper waste disposal causes serious pollution problems in these communities. Heavy rainfall, poor drainage or lack of drainage system could lead to serious flooding. In most houses, only toilet wastes are discharged through septic systems and all other household liquids are discharged directly to storm drains where they flow into the streets. In addition, most houses lack indoor plumbing systems and adequate sources of potable water is limited; this has led some of the people to resort to drilling boreholes, or wells. Some of these wells are shallow, and require treatment before meeting the various standards for drinking water quality (Yusuf, 2007).

2.6.2. Local Market Commercial Activities

Local markets refer to shops, supermarkets, road side marketsand open field markets. These markets are consistently battered by the sellers of different food items and cosmetics. There are also other daily markets and the weekly community markets where rural dwellers and their city associates gather for the buying and selling of farm produces, cattle, donkeys, sheep, poultry and other household materials. These types of market constitute more than 90% of the market availability in Nigeria, being the only one accessible to most poor people, who live below the poverty line. During daily operations reasonable quantities of different wastes are consistently generated and dumped on fresh waterways. Road side sellers, the major culprits, dispose various items such as cans of soft drinks, banana and orange peels, wrappers of sweets, street mechanic dust e.t.c. The abattoirs are generally performing opposite (anti-sanitation) function. Blood, faeces and related wastes from animal slaughter find their way into gutters and the so called "drainage system", the final destination are rivers, lakes, hand dug wells and reservoirs used by people as sources of household water (Galadima*et al*., 2011; Chukwu, 2008).

2.6.3. Oil Spills

Oil spillage is a result of leakage of hydrocarbon from pipes. To a large extent, poor maintenance of oil pipelines and poor monitoring of pressure regimes of the fluids with respect to the strength of the pipe are the main causes of water pollution. Production of oil and gas is usually accompanied by substantial discharges of wastewater in the form of brines. Constituents of brines include sodium, calcium, ammonia, boron, trace metals, and high total dissolved solids (Galadima*et al*,. 2011).

2.7. WATER QUALITY

So many factors influence the quality of water, and so, the concept of water quality is complex. The concept of water quality is mostly tied to the different intended uses of the water; different uses require different criteria. Water quality is one of the important factors that must be considered when evaluating the sustainable development of a given region (Cordoba *et al.*, 2010) Water quality is defined based on a set of physical, chemical and biological variable that are closely related to the water's intended use. There is a range of chemical, physical, and biological components that affects water quality and hundreds of variables could be examined and measured. Some variables provide a general indication of water pollution, whereas others enable the direct tracking of pollution sources. For each component, acceptable and unaccepted values must be defined. Water whose component does not meet the pre-established standards must be treated before use (Cordoba *et al.*,2010). Water quality is considered the main factor controlling health and the state of disease in both man and animals.

2.7.1. Microbial Indicators of Drinking Water Quality

From a microbial perspective, the main public health concern associated with drinking water is enteric diseases. It is impractical to look for all known enteric pathogens that may contaminate drinking water because there are hundreds of pathogens implicated in water contamination. Microbial safety is assessed through detection of indicators of faecal pollution. Microbial indicators of faecal pollution are organisms that occur in high numbers in human and animal faeces. If detected, they indicate potential faecal contamination of the water body or distribution system under investigation and the consequent potential presence of enteric pathogens. These microbial indicators are generally not themselves human pathogens but their presence indicate potential presence of pathogens. The use of indicator bacteria, in particular *Escherichia coli* and the

coliform bacteria as a means of accessing the potential presence of water-borne pathogens has been paramount to protecting public health. Many pathogens are present only under specific conditions and, when present, occur in low numbers compared with other micro-organisms. Whilst the presence of coliform bacteria does not always indicate a public health threat, their detection is useful indication that treatment operations should be investigated (Environment Agency, 2002).

1. Total coliforms (TC) Bacteria

Coliform bacteria belong to the family Enterobacteriaceae and share similar cultural characteristics. Typical genera encountered in water supplies are *Citrobacter, Enterobacter, Escherichia, Hafnia, Klebsiella, Serratia*and *Yersinia.* Coliform bacteria are Gram-negative, non-spore-forming, rod-shaped bacteria which are capable of aerobic and facultative anaerobic growth in the presence of bile-salts or surface-active agents with similar growth-inhibiting properties. They usually ferment lactose at 37^{o}C within 48h, possess the enzyme B-galactosidase and are oxidase-negative.

Total coliforms (TC) are no longer used as an indicator of faecal contamination because advances in the science of taxonomy show that they are not specific to the intestine of humans or warm blooded animals and can also be found in the environment. The presence of TC in water samples can indicate the presence of a biofilm or can serve as an indicator of treatment efficiency because of their sensitivity to chlorine. However, heterotrophic plate count (HPC) is a better indicator for that purpose because it covers a wider range of bacteria (Environment Agency, 2002).

2. Faecal coliform (FC) or Thermotolerant coliform

The faecal coliform is a subset of the total coliform group. Faecal coliform bacteria possess the characteristics of coliform bacteria but are able to carry out lactose

fermentation at 44 ^{o}C. Although still in use in some jurisdictions, the lack of specificity of the coliform test to faecal pollution of drinking water has long been documented. For example, the presence of high *Klebsiella*spp counts has been associated with various vegetation and pulp and paper effluent in the absence of faecal contamination. This is why a positive result for FC should be interpreted with caution (Edberg *et al.,* 2000).

3. *E.coli*

E.coli is a coliform bacterium and has been historically regarded as the primary indicator of faecal contamination of both treated and untreated water. As a coliform bacterium it is a member of the family Enterobacteriaceae, and is capable of fermenting lactose or mannitol at 44^{o}C, usually within 24h, and produces indole from tryptophan. *E.coli* is the most reliable indicator of enteric diseases and is therefore the indicator of choice to indicate occurrence of recent faecal contamination in drinking water systems. One exception is in tropical climates where *E.coli* may be present and multiply in water not directly subject to faecal pollution (Edberg, *et al.,* 2000). Only some strains of *E.coli* bacteria are capable of causing disease and only under certain conditions (e.g., *E.coli* O157:H7). *E.coli* occurs in the faeces of all mammals, often in high numbers (up to10^{9} per gram of faeces). This wide spread faecal occurrence, coupled with the fact that methods for the recovery and enumeration of *E. coli* are relatively simple to conduct, has contributed to the detection of this bacterium as the cornerstone of microbiological quality water assessment for over 100years (Edberg, *et al.,* 2000 : Environmental Agency, 2002).

4. Faecal Enterococci

Faecal enterococci are defined as Gram-positive cocci that tend to occur in pairs and chains. They are non-spore-forming, oxidase-negative, and catalase-negative. They grow

aerobically and anaerobically in the presence of bile salts and in sodium azide solutions, at concentration of which are inhibitory to coliform and most Gram-negative bacteria (Layton *et al*., 2010). Enterococci include a number of species that occur in the faeces of human and warm-blooded animals. The main reason for their enumeration is to assess the significance of the presence of coliform bacteria in the absence of *E. coli;* or to provide additional information when assessing the extent of possible faecal contamination. As such, they are regarded as secondary indicators of faecal pollution (U S EPA, 2006). In human faeces, numbers of enterococci rarely exceed 10^6 per gram of faeces, while in animal faeces they are often more numerous than *E. coli* (Layton *et al*., 2010). Enterococci of faecal origin rarely multiply in water and are more resistant to environmental stress and chlorination than *E. coli* and coliform bacteria. They generally persist longer in the environment, with the exception of *Streptococcus bovi* and *Streptococcus equines*, which die off relatively rapidly once outside the intestinal tract. Testing for enterococci can be a useful additional indicator of water treatment efficiency (Goto and Yan, 2011)

5. Sulphite-reducing clostridia

Sulphite-reducing Clostridia are Gram-positive, anaerobic spore-forming rods that reduce sulphite to sulphate (Ashblot*et al.,* 2001).*Clostridium perfringens* is the key species of the sulphite-reducing clostridia and is commonly found in human and animal faeces. Most species of *Clostridium* are environmental bacteria. Many are saprophytic, normally inhabiting soil, water, and decomposing plant and animal material. *Clostridium perfringens* produces environmentally resistant spores that survive in water and the environment for much longer periods than the vegetative cells of *E. coli* and other faecal indicators. Clostridia are removed from water by coagulation and filtration, but the spores of these bacteria can be resistant to chlorine at concentrations normally used in

water treatment (Araujo, 2001). As *Clostridium perfringens* is generally present in faeces in much lower numbers than *E. coli* and enterococci, it is less sensitive as an indicator of faecal contamination. Low numbers may occasionally occur in water supplies, but they do not present a risk to health. These bacteria will not grow to a significant number, or produce toxins in water supplies, as conditions are usually unsuitable (Environment Agency, 2002).

6. The Heterotrophic bacteria

The heterotrophic groups of bacteria encompass a broad range of bacteria that use organic carbon source to grow. Colony counts of heterotrophic bacteria, referred to as heterotrophic plate count (HPC), provide an indication of general load of aerobic and facultative anaerobic bacteria of water sample (WHO, 2004). An important benefit of determining HPC, particularly if carried out regularly from the same site and location, is that it can provide an indication of seasonal and long-term changes in the general bacteriological quality of the water(Verhille, 2013). Drinking water supplies derived from surface waters tends to support higher numbers of heterotrophic bacteria than those derived from groundwater sources. This is due to the differences in concentrations of assimilable carbon associated with each type of source. It is, therefore, not the absolute number of bacteria count enumerated from a supply that are of importance, but whether, over time, there are significant changes or long-term trends in those numbers. (WHO, 2003; Environment Agency, 2002 ;Verhille, 2013).

7. Limitations of microbial indicators

None of the microbial indicators currently in use for routine monitoring of drinking water quality is perfect because no single indicator represent all potential pathogens that can be present in the water. For many pathogens such as viruses and protozoan parasite,

reliable indicators are not available. Even if there were, there is no absolute correlation between the number of indicator organisms and the actual presence, or number of enteric pathogen or the risk of illness occurring (Payment and Locas, 2011). In order to respond to evolving challenges posed by water microbiology, researchers continue to search for the perfect suite of microbial indicators. The major drawback associated with microbial indicators is the length of time needed to culture the microorganisms. Depending on the method used it, takes 24 to 48h for laboratory results to come back, which means the water has already been consumed before the operator knows it is safe to drink. Another disadvantage of microbial test is related to cost of analysis compared with the cost of physical and chemical tests performed on site. However, the absence of microbial indicators does not guarantee that water is safe to drink (Environment Agency, 2002; Verhille, 2013).

2.7.2. Physical indicators of Drinking Water Quality

Taste, colour, odour, turbidity are all physical parameters that can affect the aesthetics;which determine whether people will use a source of water for drinking purposes or not.

1. Colour

The color of water can be an indicator of the presence of organic compounds (humic andfulvic acids), iron and other metals. It can sometimes also be an indicator of industrial effluents. There is no health standard set for the color of drinking water. Typically, water is considered to beacceptable to drink by people when the color is below 15 TCU (true colour units). It is often dependent on the personal tastes of people in the region and on what is available (Meghan, 2001).

2. Taste and Odour

Taste and odor of water can also be indicators of contamination. They can originate from natural organic processes, treatment processes, such as chlorination, they can be the result of hazardous contamination, or they can develop during storage and distribution. There is no specific health guideline set for taste and odor of drinking water and it is dependent upon the range of acceptability for taste and odor of the consumer (Meghan, 2001; WHO, 2003).

3. Turbidity

Turbidity is the amount of particulate matter suspended in water. It measures the scattering effect that suspended solids have on light. It is widely agreed that the higher the intensity of scattered light, the higher the turbidity. Clay, salt, finely divided organisms make water opaque. Different particles have significantly different effects on perceived turbidity. The particulate matter suspended making drinking water turbid could either be organic or inorganic, or both. The turbidity of water interferes with disinfection and could affect the colour, taste or even the odour of water (Meghan, 2001; WHO, 2003).

2.7.3. Chemical Indicatorsof Drinking Water Quality

1. pH

pH is a measure of the acidity or alkalinity of water. It is measured using a pH meter/scale. The pH scale commonly ranges from 0 - 14. Pure water is said to be neutral, with a pH of 7. Water with a pH below 7.0 is considered acidic while water with pH greater than 7.0 is considered basic or alkaline. One of the main objectives in controlling

the pH is to minimize corrosion and incrustation in the distribution system. pH levels below 7 may cause severe corrosion of metals in the distribution level while levels above 8 causes a progressive decrease in the efficiency of the chlorine disinfection process. The acceptable pH from drinking water ranges from 6.5-8.5 (Gordel andJadhav, 2013).

2. Conductivity

Conductivity is the ability of water to carry electrical charges. It indicates the presence of ions in the water. Conductivity relates to the amount of dissolved substances in water, but it, however, does not give an indication of which mineral is present. Changes in conductivity over time may indicate changing water quality. With regards to acceptability results, there is no health standard.

Conductivity source may be natural or human-made dissolved substances. The presence of inorganic compounds makes water exhibit high conductivity (Ntengwe, 2006).

3. Total dissolved Solids (TDS)

Total dissolved Solids (TDS) comprise inorganic salts (principally calcium, magnesium, potassium, sodium, bicarbonates, chlorides and sulphates) and small amount of organic matter that are dissolve in water. TDS can affect taste of drinking water. Water becomes increasingly unpalatable and objectionable at level greater than 1000 – 1200mg/1 which is the recommended value of TDS in drinking water. There is no health based proposed value for TDS (Ntengwe, 2006).

4. Alkalinity

Alkalinity is the sum total of components in the water that tends to elevate the pH to the alkaline side of neutrality. It is measured by titration with standardized acid to a pH value of 4.5 and is expressed commonly as milligrams per liter as calcium carbonate

(mg/L as $CaCo_3$). Alkalinity is a measure of the buffering capacity (ability to resist changes in pH) of the water, and since pH has a direct effect on organisms as well as an indirect effect on the toxicity of certain other pollutants in the water that increase alkalinity are carbonates, bicarbonates, phosphates and hydroxides. Limestone bedrock and thick deposits of glacial till are good sources of carbonate buffering (Gordel and Jadhav, 2013).

2.8 WATER BORNE PATHOGENS

2.8.1 Bacteria pathogens

1. *E. coli* O157:H7

E. coli O157:H7 is an enteropathogenic strain *of E. coli*. The organism was first identified as a possible human pathogen in 1975 in a California patient with bloody diarrhea and was first associated with a food borne (ground beef) outbreak of disease in 1982 (Doyle *et al.,* 2006). It has been estimated that *E. coli* O157:H7 causes about 73, 500 cases of illness and 60 deaths annually in the U.S. Contaminated water have been reported as a source of human infection. This includes drinking water sources contaminated with animal faeces and also contaminated lake and pool water used for swimming and playing. Fruits and vegetables have also been cited as vehicles for human infection with *E. coli* O157:H7. They have presumably been exposed to untreated manure in the environment or else foods have been washed or irrigated with contaminated water (Jones and Roworth, 2001).

2. *Vibrio cholerae*

*Vibrio cholera,*the causative agent of cholera is endemic in many parts of the world, especially in countries that lack clean water supplies and adequate public health facilities (Mandomando*et al*., 2007). *Vibrio cholera* is strictly a waterborne pathogen. Among

about 200 identified serogroups only two are known to cause epidemic cholera: O1 and O139 (Teh*et al*., 2011). Contaminated drinking water, cooked food, and raw or undercooked seafood served as vehicles of transmission in Malaysia (Lim, 2001).

3. *Campylobacter species*

Campylobacter are slender, spirally curved, motile, Gram-negative bacteria. They are strictly microaerophilic, non-saccharolytic and oxidase positive. There are 16 species and six subspecies assigned to the genus *Campylobacter*, of which *C.jejuni* is the most frequently reported organism associated with human disease. Approximately, 5% - 14% of all diarrhoea worldwide is believed to be caused by *Campylobacter* (WHO, 2004). *Campylobacter* have frequently been isolated from surface water, such as streams, rivers, and lakes, due to discharges from freshwater treatment plants, runoff from pasture after rain, and direct contamination by wild life. They have occasionally been isolated from ground water. Several studies reported the persistence of *Campylobacter*spp in water in a viable but no-cultivable from (VBNC) which is thought to be capable of causing infection (Talibart*et al.*, 2000).

4. *Helicobacter pylori*

*Helicobacter pylori*is a gran-negative microaerophilic rod found in the human gastric mucosa and is associated with different digestive diseases such as peptic ulcer, gastritis, and mucosa-associated lymphoid tissue lymphoma (Ahmed *et al*., 2013). Although the epidemiology of *H. pylori* is still unclear, studies on the seroprevalence suggest that drinking water might play some role in infection with *H. pylori* (Mckeown*et al*., 1999; Ahmed *et al*., 2013). The organism cannot multiply in watebr, however, several studies have shown its presence in biofilms in water distribution systems (Bunn *et al*., 2002; Azevedo *et al*.,2003; Watson *et al*., 2004)

5. *Salmonella species*

Salmonella are the cause of two enteric diseases called salmonellosis: enteric fever (typhoid), resulting from bacteria invasion of the bloodstream, and acute gastroenteritis, resulting from a foodborne infection/intoxication (Radhakrishna*et al.,* 2003). *Salmonella* species are often detected in sewage, river and inrrigation water, fresh water, marine coastal water and ground water (Ho and Tam, 2000: Fewtrell *et al.,* 1994: Baudart, 2000: Catalao *et al.*,2000: Gannon *et al.*,2004). One study reported the isolation of *Salmonella typhimurium* from drinking bottled water (Radhakrishna *et al.,* 2003).

2.8.2. Protozoan Pathogens

1. Cryptosporidium

Cryptosporidium is now one of the most commonly identified intestinal pathogens throughout the world (WHO, 2004).

Cryptosporidium is an obligate, intercellular, coccidian parasite with a complex life cycle including sexual and asexual replication, which infect the gastrointestinal tract of animals and humans. Thick-walled spherical oocysts with a diameter of 4-6µm are shed in faeces. The oocyst containing four sporozoitesis the environmentally resistant transmission stage of the parasite. The whole life cycle of *Cryptosporidium* can be completed within a single host.

*Cryptosporidium*oocysts are ubiquitous in surface water worldwide (WHO, 2002). The sources of environmental contamination can be both human and sewage and animals. Groundwater that mix with surface water or other sources of contamination (e.g. surface run-off) may contain low level of *Cryptosporidium*. They also have been detected in treated water samples.

2. *Giardia*

Giardia is a ubiquitous parasite affecting human and a wide range of domestic and wild mammals. *Giardia lamblia* is the most common human intestinal parasite worldwide (Marshall *et al.,* 1997). *Giardia* has a relatively simple life cycle consisting of a flagellate trophozoite that multiplies in the gastrointestinal tract, an infective thick-walled cyst that is shed intermittently but in large numbers in faeces. The cyst is the infective form, which is responsible for the transmission of the parasite and the infection of humans and animals. *Giardia lamblia* cysts are elliptical, 8-12um long and 7-10um wide. The cyst wall is 0.3-0.5 um thick and has a fibrillose structure (WHO, 2004). Like *Cryptosporidium*oocysts, *Giardia* cysts are ubiquitous in surface waters worldwide. Reported concentrations are generally in the range of 0.01-100 per liter (WHO, 2002). The source of contamination can be human, sewage and animals.

3. *Entamoebahistolytica*

Entamoebahistolytica is a pseudopob forming non- flagellate protozoan parasite. Infections with *E. histoloytica* occur worldwide, and it has been suggested that 12% of the world's population is infected with this organism. About 10% of those infected every year have clinical symptom; 80 - 98% of these patients have symptoms related to the intestinal mucosa and in the remaining 2 - 20%, the amoebae invade beyond the intestinal mucosa. Once the infection is cleared, recurrence of invasive colitis or amoebic abcess is usual (Marshall *et al*., 1997). With the exception of malaria causing plasmodia and schistosomes, E. histolytica causes more deaths than any other parasite. Groups of high risk for amoebiasis in developed countries include travelers, immigrants, migrants' workers, immunocompromised individuals, institutionalized individual, and sexually male homosexuals (Marshall *et al*., 1997).

Transmission by water is common in developing countries, where much of the water supply for drinking is untreated and focally contaminated. The use of human faeces as fertilizer is also an important source of infection (Marshall *et al.*, 1997)

2.8.3. Viral pathogens

1. Human adenoviruses

Human adenoviruses are ubiquitous and can be found in environments where contamination by human faeces and sewage has occurred. Using molecular techniques, adenoviruses have been frequently found in treated and untreated waters. Although the potential health risks constituted by adenoviruses in water sources and supplies are widely recognized (American Water Works Association 1997; WHO 2002), no food or waterborne outbreak has ever been reported (American Water Works Association, 1997; WHO, 2002). The only waterborne outbreak that was reported in literature was due to inadequately chlorinated swimming pools. These outbreaks are generally associated with pharyngoconjuctivitis (Harley *et al.*,2001).

2. Enterovirus

The genus enterovirus is a member of the Picarnaviridae family. Enteroviruses are one of the most common causes of human infections, however most infections are unapparent and lead to non-specific febrile illness referred to as “summer flu”. Some serotypes, however, may cause serious clinical syndromes such as poliomyelitis, encephalitis, meningitis, myocarditis and pericarditis (WHO 2004). Enteroviruses are widely present in water. Numerous studies have documented the presence of enteroviruses in raw water (Pusch *et al.*, 2005: Jiang and Chu, 2004), groundwater (Fout*et al.*,2003; Abbaszadegan *et al.*, 1999), wasteswater (Payment 1981), recreational water (Denis-Mize and Fout, 2004) as well as in treated drinking water (Ehlers *et al.*, 2005). Despite the wide

occurrence of enteroviruses in the water environment, few water-related outbreaks have been reported. The limited knowledge of the role of waterborne transmission could be related to a number of factors, including the wide range of clinical symptoms, frequent asymptomatic infections, the diversity of serotypes and the dominance of person to person spread (WHO, 2004).

3. Hepatitis A Virus

Hepatitis A virus is classified under the genus Hapatovirus within the Picornaviridae family.

Hepatitis A is an acute, usually self-limiting infection of the liver caused by Hepatitis A Virus (HAV). Symptoms typically include fever, malaise, anorexia, nausea and abdominal discomfort, followed by dark urine and jaundice. An estimated 1.5 million clinical cases of hepatitis A occur every year. The prevalence of clinical disease has typical geographically based characteristics (Grabow, 2002). In countries where inadequate sanitary conditions prevail, the virus persists in the environment and almost 100% of the population acquire infection in childhood. In developed, industrialized countries HAV has ceased to circulate in the environment and the general population. Here, infections predominantly occur in adults travelling to endemic areas or exposed to infected individuals or members of high risk groups (e.g. children in day care centres, IV drug users) (Grabow, 2002). Waterborne outbreaks have often been associated with water supplies that complied with generally acceptable guidelines for indicators and treatment procedures (Grabow, 2002). Using molecule biology techniques, HAV has been found in environmental water(Grabow 2002), sewage and wastewater (Jothikumar and Cliver, 1998: Espigares and Garcia, 1999) and in drinking water (Jothikumar *et al.*,2000).

4. Hepatitis E Virus

Hepatitis E Virus (HEV) was previously classified into the family Caliciviridae. However, it is now reclassified into the Hepatitis E-like viruses as an unassigned genus (Berke, 2000). These are indications of antigenic variation, and possible even differences in serotypes of the virus (WHO 2004). The disease caused by HEV is called Hepatitis E. Hepatitis caused by HEV is clinically indistinguishable from hepatitis A disease. The risk factors for HEV infection are related to poor sanitation. Large outbreaks associated with sewage-contaminated drinking water have been reported in Asia, Africa, the Middle East, and North America (Mexico) (Mast and Krawczynski 1996).

5. Rotavirus

Rotaviruses are classified with the Reoviridae family. Seven serological groups have been identified, three of which (groups A, B, and C) infect humans with group A being the most important human pathogen, whereas others infect a wide spectrum of animals (Cukor and Blacklow, 1984) (Jiang and Chu, 2004). Human rotaviruses (HRVs) are the most common cause of severe diarrhea among children, worldwide (Cukor and Blacklow, 1984). It is estimated that rotavirus is responsible for the deaths of approximately 500, 000 children each year, over 80% of them in developing countries (Ehlers *et al.,* 2005). In developed countries death are relatively scarce, but rotavirus gastroenteritis is the most frequent reason for admission of young children to hospital.

Foodborne and waterborne spread is probably a significant route of transmission in developing countries, but in developed countries reports are rare. Several waterborne outbreaks of rotavirus have been reported (Sutmoller*et al*., 1982; Grimwood*et al.,* 1983; Ijaz*et al.,* 1985).All the outbreaks of rotavirus were associated with a direct faecal contamination of the water supply, or improper treatment of water.

2.9. WATER TREATMENT

Water treatment is the process of converting raw water from surface or sub-surface source into a potable form that is suitable for drinking and other domestic uses (Ojo*et al.,* 2012). It also involves the removal of pathogenic organisms and toxic substances (Oluwande, 1983). The convection methods by which water is made potable are namely; aeration, coagulation, flocculation, sedimentation, filtration and other means of disinfection which make use of physical processes to achieve their objectives.

2.9.1 Aeration

In Aeration, water is brought in contact with air in order to increase their oxygen content to facilitate precipitation and result in the removal of iron and manganese in their ferric and manganese forms, and organic compounds. Aeration reduces the carbon dioxide content of water and thus decreasing the solubilization tendencies of water, which causes corrosion and leaching of plumbing materials into water. Ground high is iron and manganese benefit from aeration (Ojo*et al.,* 2012).

2.9.2. Coagulation and Flocculation

This is the addition of Alum (Al_2 $(SO_4)_{3.}$ $14H_2O$, thus forming colloids with size similar to those of bacteria (Ojo*et al.,* 2012). Coagulation ensures the gathering together of small size particles into bigger ones with higher setting velocity or sedimentation. The sludge formed can then be disposed. Coagulation reduces load on filters, thereby reducing costs through the extension of the life of the filter.

2.9.3. Sedimentation

This is known as clarification and is the unit process where particles heavier than the liquid they are in are removed by gravitational settling. Sedimentation affects the chemical quality of water, through the settling of complexes formed between heavy metals and flocs(Ojo*et al.,* 2012).

2.9.4. Filtration

Filtrationis commonly used after sedimentation to further reduce turbidity and remove pathogens. Filtration is a physical process which involves passing water through filter media.

Some filters are also designed to grow a biological layer that consumes pathogens and improves the removal efficiency. Sand and ceramic are the most common filter media, although cloth and membranes can also be used. (Frik, 2006).There are various types of filters that are used by households around the world, these include:

1. Strainy through a cloth
2. Biosand filter
3. Ceramic pot filter
4. Ceramic candle filter
5. Membrane filter.

2.9.5. Disinfection

The last step in household water treatment is to remove or kill any remaining pathogens through disinfection. The most common methods used by households around the world to disinfect their drinking water are:

1. Chlorine disinfection
2. Solar disinfection (SODIS)
3. Solar pasteurization
4. Ultraviolet (UV) disinfection
5. Boiling

When water has high levels of turbidity, pathogens hide behind the suspended particles and is difficult to kill using chemical, SODIS and UV disinfection. Reducing turbidity by sedimentation and filtration will improve the effectiveness of these disinfection methods (Arun, 2015)

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2.9.6. Distillation

Distillation is another method of using the sun's energy to treat drinking water. It is the process of evaporating water into vapour, and then capturing and cooling the vapour so it condenses, back into a liquid. Any contaminants in the water are left behind when the water is evaporated (Frik, 2006).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Study Area

The study area was Zaria. Zaria is a major city in Kaduna State in Northern Nigeria and is home to the popular Ahmadu Bello University. It was one of the original seven Hausa city-states, formerly known as Zazzau. It occupies an area of about 300km^2 at latitude 11^0 07' 51" N and longitude 7^0 43'43"E with a population of 408,198 according to the 2006 census. The study area was divided into three sampling locations namely;

a) Samaru
b) Sabon-gari
c) Tudun Wada

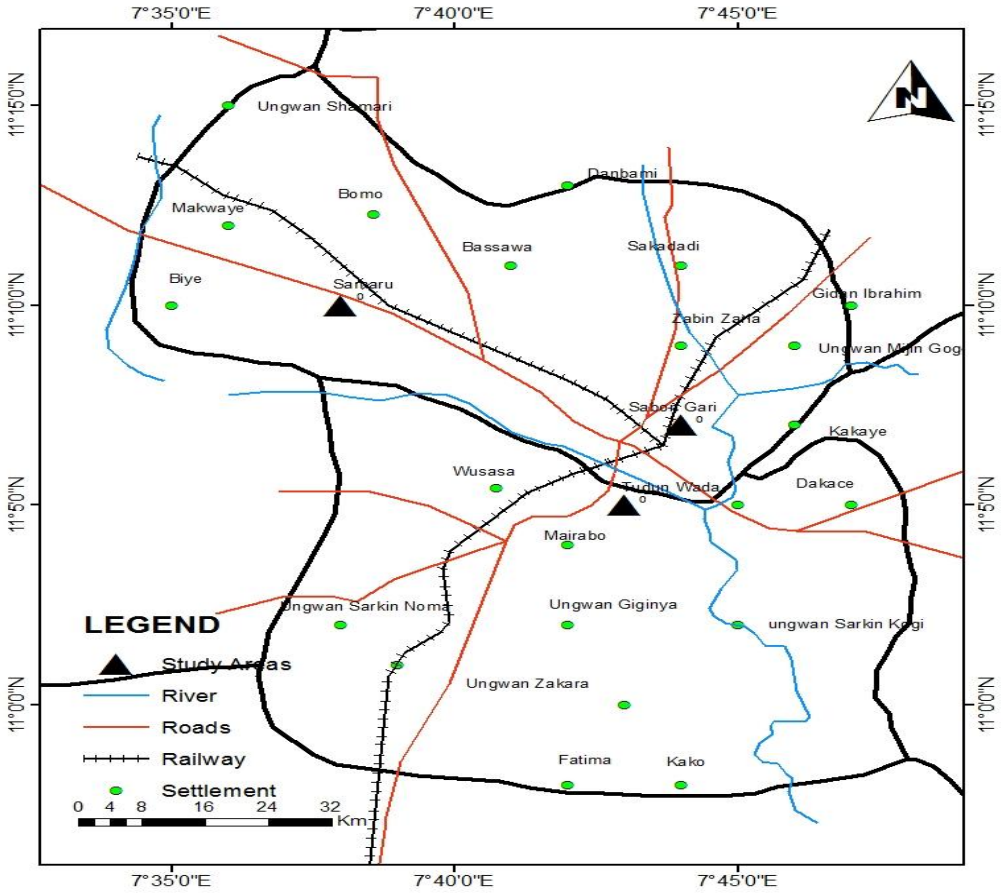


Figure 3.1Map of Zaria showing the study areas.

Source: Adapted from the administrative map of Kaduna state.

3.2. Samplesize Determination

The sample size was calculated using a prevalence of 9.3% of *E. coli* isolated from drinking water in Taraba(Umaru*et al.*,2015).

$$N = \frac{Z^2 pq}{I^2}$$

Where;

Z = 1.96 for 95% confidence interval

P = prevalence of 9.3% for *E. coli*

q = 1-p

I = allowable error (5%)

N = number of samples

$$N = \frac{1.96^2 \text{ x } 0.093\text{x}0.907}{0.5^2} = 129.6171$$

N≅130

3.3. Collection of Samples

Water samples were collected according to the method prescribed by America Public Health Association (APHA) (1992). The study area was divided into three sampling locations namely; Samaru, Sabon-gari and Tudun Wada. A total of 142 wells (53, 44 and 45 wells from Samaru, Sabon-gari and Tudun Wada respectively from both lined wells and unlined wells) were sampled for a period of six months encompassing both rainy and dry seasons (July to September for rainy season and November to January for dry season).

Table 3.1Distribution of samples across the sampling locations.

Sampling locations	Number of lined wells	Number of unlined wells
Samaru	30	23
Sabon-gari	20	24
Tudun Wada	12	27
Total	**68**	**74**

Well water samples from the three different locations were collected in clean 100ml capacity bottles and transported to the Department of Chemistry, ABU, Zaria for physicochemical analysis. But temperature and pH were taken at the point of sampling.

Similarly, water samples were collected using 10litres graduated Gerry cans and 100ml sterile capacity bottles and transported to the Department of Microbiology, ABU, Zaria for examination of parasites and bacteria respectively. The Gerry cans were disinfected using sodium hypochloride as the cysts of *Entamoebahistolytica* are resistant to chlorination. All samples were collected during the early hours of the day (between 8am and 10am) and analyzed not later than 6 hours from the time of collection (APHA, 1992)

3.4. Physico-Chemical Analyses

The major water quality parameters assayed in this study were temperature, pH, electrical conductivity (EC) and total dissolved solids (TDS).

3.4.1. Temperature Measurement

Temperature of each sample was measured with a mercury-filled Celsius thermometer (HANA Model) with a scale marked for every 0.1^0C with markings etched on the capillary glass.

3.4.1. pH Measurement

The pH was measured using a digital pH meter. The meter was switched on and allowed to warm for 15minutes. The electrode attached to the meter was introduced into the water sample and measurement was taken after a stable reading was obtained (APHA, 1992).

3.4.2. Conductivity Measurement

Conductivity of the water sample was measured using a digital conductivity meter (HANA Model). The meter was switched on and allowed to warm for 15minutes; this was then standardized with 0.01M KCl. The electrode was immersed into the water sample and conductivity reading was recorded (APHA, 1992).

3.4.3. Determination of total Dissolved Solids

The programme menu of conductivity meter used in 3.4.2 above was switched to total dissolved solids and the result of a total dissolved solids was displayed and reading from the screen was recorded.

3.5. Parasitological Analyses

Membrane filtration technique was used to filter the sampled volume (10L) through 0.45µm membrane filter paper at a flow rate of 3litresper minute (Medema, 1999; Bishop and Inabo, 2015). Particulates retained on the membrane filter were washed in 50mls of distilled water. The parasite cysts were then concentrated by centrifugation at 300Rev/min for 2minutes (Bishop and Inabo, 2015). The supernatant was discarded and a wet mount preparation of the residue was made and examined under the microscope using x10 and x40objectives. A drop of Lugol's iodine was used to create a good background to view the cysts. The parasite cysts were identified using colour atlas of parasitology by (Parija and Prabhakar, 1995; Sullivan, 2000).

3.6. Bacteriological Analysis

3.6.1. Standard Plate Count

One hundred millilitres of the water sample was filtered through 0.45µm sterile membrane filter paper. The filter was then placed on pre-sterilized and solidified Standard plate count agar (SPCA) and incubated at 37^0C. Colonies were counted and expressed as colony forming units per millilitre (cfu/ml) after 24h of incubation (APHA, 2005).

3.6.2. Coliforms Counts

3.6.2.1. Total coliforms and *E. coli* counts

One hundred millilitre of the water sample was filtered through 0.45µm membrane sterile filter paper. The filter paper was then placed on pre-sterilized and solidified Membrane Lactose Glucoronide agar (mLGA) plate and incubated at 30^0C for 4h for high recovery and at 37^0C for 20h. The result was read within 15mins after removal from the incubator because the yellow coloration of the colonies changes on cooling and standing. Green colonies were regarded as presumptive*E. coli* while yellow colonies were regarded as other coliforms. The total coliform counts were counts of both the green and yellow colonies and expressed as cfu/100ml (APHA, 2005).

3.6.2.2. Thermotolerant Coliforms

One hundred (100ml) of the water sample was filtered through 0.45µm membrane sterile filter paper. The filter was then placed on pre-sterilized and solidified Membrane Lactose Glucoronide agar (mLGA) plate and incubated at 30^0C for 4h for high recovery and at 45^0C for 20h. Green colonies were regarded as presumptive *E. coli* while the green plus the yellow colonies were counted as thermotolerant coliforms and expressed as cfu/100ml (APHA, 2005).

3.6.3. Biochemical Characterization of the Bacteria Isolates

After enumeration of total bacterial count, total and thermotolerant coliforms and *E. coli* counts, 2 colonies were randomly picked from countable plates of mLGA that were incubated at 45^0C. The colonies were Gram stained and Gram negative isolates were transferred into nutrient agar slants and stored at 4^0C forbiochemical characterization. The following tests were carried out on the isolates;

3.6.3.1. Motility test

Motility medium was prepared according to manufacturer's instruction and dispensed into bijou bottles. Using a sterile needle, a colony of 24h culture of the test organism was picked and stabbed into the motility medium to within 1cm of the bottom of the tube. The medium was then incubated at 37^0C for 24h and was observed for evidence of growth (Vashist*et al*., 2013).

3.6.3.2. Citrate utilization test

Simmon citrate agar was prepared according to manufactuter's instruction and dispensed into bijou bottles and sterilized at 121^0C for 15mins. The tubes were inoculated with 24h culture of the test organism and incubated at 37^0C for 24h. The tubes were then observed for colour change from green to blue (Vashist*et al*., 2013).

3.6.3.3 Indole test

One colony of a 24hr culture of the test organism was inoculated into bijou bottles containing 3ml of sterile peptone water and incubated at 37^0C for 24h. 0.5ml Kovac's reagent was then added and examined for red colour in the surface within few minutes (Cheesbrough, 2006).

3.6.3.4 Urease test

Urea agar was prepared according to the manufacturer's instruction and dispensed into bottles and sterilized. Glucose and phenol red were added to the medium and steamed for 1h. A filtered sterilized urea solution was added and mixed properly and dispensed into sterile bottles. Twenty four (24h) growth culture of the test organisms were inoculated into the bottles and incubated at 37^0C for 24h, then observed for pink colour in the medium (Vashist*et al*., 2013).

3.6.3.5. Methyl red and Voges-Proskauer (MR-VP) tests

A tube was inoculated with a single colony from a 24h culture of the test organism and incubated at 37^0C for 24h. after incubation, using a sterile pipette, two-1ml aliquots were removed and placed into two small tubes; one tube for methyl red test and the other for Voges-Proskauer test. 5 drops of methyl red were added to one tube and the result was read immediately. For the Voges-Proskauer test, 15 drops of Voges-Proskauer A reagent was added and mixed well. 5 drops of Voges-Proskauer B reagent was added to the tube and mixed well.

Methyl red; a red colour at the surface was considered a positive result while a negative test was indicated by a yellow colour at the surface.

Voges-Proskauer; a positive test was indicated by a pink-red colour developing within 5 minutes (Vashist*et al.*, 2013).

3.6.4. Characterization of the Bacteria isolates using microgen Test kit

The isolates were further characterized using microgenGnA ID system.

3.5.4.1 Preparation of suspension of the test organism

A single colony from a 24h pure culture of the test organism was emulsified in 3ml of 0.85% NaCl solution, making sure that the suspension was homogenous and without clumps of floating bacteria.

3.6.4.2 Inoculation of the organism and incubation

The back of the adhesive tape sealing the microwells test strips was peeled. One hundred millilitre of the suspension of the test organism was inoculated into each well with a sterile micro pipette. After inoculation, wells 1, 2, 3 and 9 were overlaid with 3drops of mineral oil. The top of the microwell test strip was sealed with the adhesive tape removed earlier and incubated at 37^0C for 24h.

3.6.4.3. Reading and Addition of Reagent

The wells were read after 24h of incubation. The proper reagents were added to the compartments accordingly as described in the chart. Using the identification colour chart, results were recorded in the result sheets. The substrate reactions were organized into triplets with each substrate assigned a numerical value. The sum of the positive reactions for each triplet formed a single digit of the octal code that was used to determine the identity of the isolate. The octal code was entered into the microgen identification software, which generated a report of five most likely organisms in the database. The software provided identification based on the probability, percentage probability and likelihood with an analysis of the quality differentiation. Potential organism was recorded based on the percentage probability.

3.6.5. Antibiotic susceptibility test

Antibiotic susceptibility test was performed using the disk diffusion method of Kirby-Bauer on Mueller-Hinton agar plates. Susceptibility was determined according to the Clinical and Laboratory Standard Institute (CLSI) 2015 guidelines. Isolates were subjected to susceptibility tests against 11 commonly used antibiotics namely; Sulfomethoxazole (25µg), Tetracycline (30 µg), Cefpoxodime (5 µg), Amoxicillin (3 µg), Azithromycin (15 µg), Nitrofurantoin (300 µg), Ciprofloxacin (5 µg), Ceftaxidime (30 µg), Pefloxacin (5 µg), Gentamicin (3 µg), and Chloramphenicol (30 µg).The bacterial suspensions (0.5 turbidity, McFarland standard, BioMerieux, Madrid, Spain) were seeded on Mueller-Hinton agar plates and the antibiotic-containing disks placed on the surface. All the plates were incubated at 37^0C for 24h. The diameters of zones of clearance or inhibition round the antiobiotcs were measured. Organisms were classified as sensitive (S), intermediately resistant (I) or resistant (R) to the antibiotics on the basis

of the size of the zone of growth inhibition according to the guidelines of the Clinical and Laboratory Standard Institute (CLSI), 2015.

CHAPTER FOUR

4.0. RESULTS

Physicochemical Parameters

The mean values of the physicochemical parameters in lined and unlined well water according to season and sampling locations are presented in Tables 4.1. The mean temperature ranges between 24.9^{0}C and 26.4^{0}C with a narrow range of 1.5. The pH values of the water samples had narrow range of between 6.9 and 8.5. The highest conductivity was observed in Tudun Wada during the rainy season with a value of 1201µS/cm while the lowest was recorded in Sabongari during the rainy season with a value of 786µS/cm. Total Dissolved Solid (TDS) also shows a narrow range of 146mg/l, with the lowest and highest values being 540mg/l from unlined wells located at Samaruand 646mg/l from lined wells located at Tudun Wada respectively. Highest and lowest values were all observed during the rainy season.

Table 4.2 shows the comparison of the mean values of the physicochemical parameters of the well water samples drawn from the three sampling locations. Electrical Conductivity was higher in Tudu Wada (1119µS/cm) compared to Samaru (847µS/cm) and Sabon-gari (797µS/cm), and the difference was statistically significant (P=0.03). There were no statistically significant differences with other parameters between the locations (P-value were all greater than 0.05).

Table 4.3 represents the comparison of the mean values of the physicochemical parameters of the well water samples drawn during the rainy and dry season. There were no significant differences between well water samples drawn during the rainy and dry season in all the parameters analysed (p-values were greater than 0.05 in all the parameters).

Table 4.1: Mean values of physicochemical parameters in lined and unlined well water according to season and sampling location (Mean ± standard error of mean)

Location		Temperature (^{0}C)		pH		Electrical Conductivity (µS/cm)		Total Dissolved Solids (mg/l)	
		Lined	Unlined	Lined	Unlined	Lined	Unlined	Lined	Unlined
T/W	R	25.7± 0.317	26.0± 0.392	7.1 ± 0.130	6.9± 0.174	1186± 72.59	1201± 87.50	646± 59.07	604± 74.45
	D	25.7± 0.189	25.8± 0.209	6.9± 0.851	6.8± 0.946	1023± 51.78	1066± 58.88	557± 34.08	580± 41.10
SM	R	26.1± 0.271	26.3± 0.403	6.9± 0.119	6.8± 0.153	835± 52.39	796± 77.95	551± 50.86	540± 78.14
	D	25.8± 0.194	26.0± 0.243	7.2± 0.085	7.0± 0.103	892± 42.06	863± 46.50	554± 38.01	576± 43.90
SG	R	25.4± 0.275	25.5± 0.388	7.1± 0.157	7.0± 0.181	799± 46.50	786± 63.42	616± 87.45	600± 87.45
	D	24.9± 0.577	26.4± 0.236	8.2± 1.142	8.5± 1.476	801± 51.90	803± 51.90	577± 48.74	576± 43.29

KEY; T/W-TudunWada, SM- Samaru, SG-Sabon-gari, R – Rainy season, D – Dry season

Table 4.2: Comparison of the mean values of the physicochemical parameters of the well water samples drawn from the three sampling locations.

Parameter/location	Samaru	Sabon-gari	Tudun Wada	P-value
Temperature (^{0}C)	26.1± 0.265	25.6± 0.390	25.8±0.184	0.10ns
pH	7.0±0.132	7.7±0.651	6.9±0.855	0.40ns
Electrical Conductivity (µS/cm)	847±31.00^b	797±44.75^b	1119±74.50^a	**0.03s**
Total Dissolved Solids (mg/l)	555±39.75	592± 59.75	596 ± 61.28	0.38ns

KEYS; ns – not significant, s – significant

Table 4.3: Comparison of the mean values of the physicochemical parameters of the well water samples drawn during the rainy and dry season

Parameters	Rainy season	Dry season	Level of significance (p-value)
Temperature (^{0}C)	25.83 ± 0.22	25.77 ± 0.07	0.81ns
pH	6.70 ± 0.06	7.43 ± 0.46	0.38ns
Electrical conductivity (µS/cm)	933 ± 31.60	908.00 ± 49.36	0.87ns
Total dissolved solids (mg/l)	593 ± 24.17	570 ± 33.34	0.40ns

KEY; ns – not significant

Table 4.4 shows the comparison of the mean values of the physicochemical parameters of the well water samples drawn from lined and unlined wells. There were no significant differences between wells with internal wall lining and wells without lining in all the parameters analyzed (p-values were greater than 0.05 in all the parameters).

Entamoebahistolytica

The results on the prevalence of *Entamoebahistolytica* in the well water samples during the rainy and dry season in the sampling locations are shown in Table 4.5. An overall prevalence rate of 27.5% was recorded in the sampling area (Zaria). The highest prevalence of 34.1% was recorded in Sabon-gari while the lowest prevalence of 24.4% was recorded in Tudun Wada. Prevalence of 33.3% was recorded during the rainy season and 21.4% during the dry season.

The percentage occurrence of *Entameobahistolytica* in well water samples drawn from lined and unlined wells in the Zaria are presented in Table 4.6. Prevalence of 33.8% was recorded in wells with internal wall lining and 21.6% in wells without lining.

The effect of internal wall lining on the occurrence of*Entamoebahistolytica* in the well water sampled from Zaria is presented in Table 4.7. There was a statistical difference between wells with internal wall lining and wells without internal wall lining ($P < 0.05$).

Table 4.4: comparison of the mean values of the physicochemical parameters of the well water samples drawn from lined and unlined wells

Parameters	Lined	Unlined	Level of significance (p-value) (ns)
Temperature (^{0}C)	25.24 ± 0.39	25.76 ± 0.16	0.20
pH	6.90 ± 0.09	7.63 ± 0.68	0.31
Electrical conductivity (µS/cm)	905.40 ± 37.68	905.40 ± 39.36	0.99
Total dissolved solids (mg/l)	565.34 ± 33.32	558.37 ± 29.75	0.88

KEY; ns – not significant

Table 4.5. Prevalence of *Entameobahistolytica* in the well water samples during the rainy and dry season in the sampling locations (Zaria).

Location	number of samples		Number of positive		Total (%)
	R	D	R (%)	D (%)	
Tudunwada	20	25	5(25.0)	6(24.0)	11(24.4)
Samaru	28	25	7(25.0)	6(24.0)	13(24.5)
Sabon-gari	24	20	12(50.0)	3(15.0)	15(34.1)
Total	72	70	24(33.3)	15(21.4)	39(27.5)

Key; R- Rainy season

D-Dry season

Table 4.6. The percentage occurrence of *Entameobahistolytica* in well water samples drawn from lined and unlined wells in the Zaria

Location	number of samples		Number(percentage positive)		Total (%)
	Linedunlined	linedunlined			
Tudunwada	18	27	7(38.9)	5(18.5)	12(26.7)
Samaru	30	23	9(30.0)	4(17.4)	13(24.5)
Sabon-gari	20	24	7(35.0)	7(29.2)	14(31.8)
Total	68	74	23(33.8)	16(21.6)	39(27.5)

Table 4.7: The effect of internal wall lining on the occurrence of*Entamoebahistolytica*in the well water sampled from Zaria.

Occurrence of Parasite	Lined	Unlined	Level of significance
	No (%)	No (%)	(P-value)
Absence	45 (66.2)	58(78.4)	
Presence	23(33.8)	16(21.6)	0.031^{s}

X^2= 16.77 df=1 p=0.031

KEY: s – highly significant, No - Number, (%)- Percentage

Bacteriological Analyses

Table 4.8 represents the result for total bacterial count of lined and unlined wells according to seasons and sampling locations. Bacterial counts ranges from $1.73 \times 10^3 \pm 2.73 \times 1\ 0^2$ - $2.02 \times 10^2 \pm 1.85 \times 10^2$ cfu/ml during the rainy season and $4.17 \times 10^2 \pm 3.07 \times 10^1$ - $1.39 \times 10^3 \pm 3.27 \times 10^2$cfu/ml during the dry season.

Coliforms and *E. coli* were present in all the samples with total coliform counts ranging from 1.06×10^3 - 2.12×10^4 cfu/100ml and *E. coli* counts ranging from 5.10×10^2 -5.0×10^4cfu/100ml (plates incubated at 37^0C). Counts were also higher during the rainy season in all the location compared to the dry season (Table 4.9).

Faecal coliforms and *E. coli* (plates incubated at 45^0C) counts were present in most of the samples with values ranging from $1.7x10^2 \pm 6.10x10^1$ - $6.16x10^3 \pm 1.6x10^3$(Table 4.10). Counts were also higher during the rainy season in all the location.

Table 4.11 is the percentage occurrence of faecal coliforms and *E. coli* in the well water samples. Faecal coliforms were higher in wells without lining (91.9%) compared to those with lining (91.2%) while *E. coli* counts were higher in wells with lining (77.9%) compared to those without lining (66.2%).

Table 4.12 shows the comparison of the mean values of the bacterial counts of the well water samples drawn from the three sampling locations.There were no statistical significant differences in all the bacterial counts between the locations (P-values were greater than 0.05).

Also, there was no significant difference in the bacterial counts between well water drawn during the rainy season and well water drawn during the dry season as shown in Table4.13 (p-values were all greater than 0.05).

Table 4.8: Mean total bacterial count (Standard Plate Count) of lined and unlinedwell according to season and sampling locations (mean ± standard error of mean).

Location		No of samples		Total bacterial count (cfu/100ml)	
		Lined	Unlined	Lined	Unlined
TudunwadaR		7	13	2.13x102 ± 4.01x10^2	1.96 x 10^3±1.98 x10^2
	D	11	14	4.03 x102 ± 4.9 x 10^1	4.27 x 10^2 ±3.97 x 10^1
Samaru	R	15	13	1.94 x 10^3 ± 4.60 x 10^2	4.04 x 10^2 ± 5.00 x10^1
	D	15	10	1.61 x 10^3 ± 5.27 x 10^2	1.07 x 10^3 ± 2.18 x10^2
Sabongari	R	10	14	1.44 x 10^3 ± 3.18 x 10^2	2.37 x 10^3 ± 3.5 x 10^2
	D	10	10	531 x 10^2 ± 5.7 x 10^1	3.54 x 10^2 ± 8.31 x10^1

Key; R- Rainy season, D- Dry season

Table 4.9: Mean value of total coliform and total *E. coli* counts of lined and unlined well water according to season and sampling locations (mean ± standard error of mean).

Location		Number of samples		Total coliform count (cfu/100ml)		*E.coli* count (cfu/100ml)	
		Lined	Unlined	Lined	Unlined	Lined	Unlined
Tudunwada	R	7	13	$1.56x10^4 \pm 2.94x10^3$	$2.00x10^4 \pm 4.22x10^3$	$1.05x10^4 \pm 2.31x10^3$	$1.60x10^4 \pm 4.20x10^3$
	D	11	14	$3.65x10^3 \pm 4.31x10^2$	$3.71x10^3 \pm 5.10x10^2$	$1.41x10^3 \pm 3.00x10^2$	$1.05x10^3 \pm 2.91x10^2$
Samaru	R	15	13	$2.2x10^4 \pm 4.21x10^3$	$3.65x10^3 \pm 4.32x10^2$	$9.92x10^3 \pm 3.01x10^3$	$1.41x10^3 \pm 3.00x10^2$
	D	15	10	$3.50x10^3 \pm 4.80x10^2$	$1.06x10^3 \pm 9.20x10^1$	$1.27x10^3 \pm 2.84x10^2$	$5.10x10^2 \pm 1.54x10^2$
Sabon-gari	R	10	14	$1.37x10^4 \pm 4.25x10^3$	$6.16x10^3 \pm 1.1x10^3$	$2.06x10^3 \pm 4.41x10^2$	$8.57x10^2 \pm 2.98x10^2$
	D	10	10	$2.29x10^3 \pm 4.69x10^2$	$2.97x10^3 \pm 7.23x10^2$	$3.50x10^2 \pm 1.19x10^2$	$1.09x10^3 \pm 6.09x10^2$

Key; R- Rainy season, D- Dry season

Table 4.10: Mean values of faecal coliform and *E. coli* counts of lined and unlined well water according to season and sampling locations. (Mean ± standard error of mean).

Location		Number of samples		Faecalcoliform count (cfu/100ml)		*E.coli* count (cfu/100ml)	
		Lined	Unlined	Lined	Unlined	Lined	Unlined
Tudun wada	R	7	13	$4.63x10^3 \pm 1.35x10^3$	$6.13x10^2 \pm 3.07x10^2$	$2.89x10^3 \pm 1.23x10^3$	$1.88x10^3 \pm 7.70x10^2$
	D	11	14	$6.41x10^2 \pm 2.05x10^2$	$3.71x10^3 \pm 5.10x10^2$	$6.10x10^2 \pm 1.19x10^2$	$4.79x10^2 \pm 1.20x10^2$
Samaru	R	15	13	$2.55x10^3 \pm 6.99x10^2$	$3.65x10^3 \pm 4.32x10^2$	$6.42x10^2 \pm 2.05x10^2$	$2.14x10^2 \pm 6.10x10^1$
	D	15	10	$1.05x10^3 \pm 1.66x10^2$	$1.06x10^3 \pm 9.92x10^1$	$3.42x10^2 \pm 9.00x10^1$	$1.72x10^2 \pm 6.10x10^1$
Sabon-gari	R	10	14	$2.26x10^3 \pm 4.02x10^2$	$6.16x10^3 \pm 1.61x10^3$	$1.38x10^3 \pm 3.45x10^2$	$4.30x10^2 \pm 1.91x10^2$
	D	10	10	$3.60x10^2 \pm 1.38x10^2$	$2.97x10^3 \pm 7.23x10^2$	$5.10x10^2 \pm 1.81x10^2$	$1.39x10^2 \pm 7.70x10^1$

Key; R- Rainy season, D- Dry season

Table 4.11: Percentage frequency of occurrence of faecal coliforms and *E. coli* in lined and unlined well water according to season and sample locations

Location		Faecal coliform (%)		*E. coli* (%)	
		Lined	Unlined	Lined	Unlined
Tudunwada	R	7(100)	11(86.6)	7(100)	8(61.5)
	D	11(100)	14(100)	8(72.7)	12(85.7)
Samaru	R	14(93.3)	10(76.9)	14(93.3)	9(69.2)
	D	15(100)	10(100)	13(86.7)	6(60)
Sabon-gari	R	9(90)	14(100)	7(70)	9(64.3)
	D	6(60)	9(90)	4(40)	5(50)
Total		62(91.2)	68(91.9)	53(77.9)	49(66.2)

Key; R- Rainy season, D- Dry season

Table 4.12: Comparison of the mean values of the bacterial counts of the well water samples drawn from the three sampling loations. (mean ± standard error of mean).

Bacteria counts (cfu/100ml)	Samaru	Sabon-gari	Tudun Wada	P-value
Total bacteria count	$1.2x10^{2}\pm 0.8x10^{1}$	$1.4x10^{3}\pm 1.2x10^{2}$	$7.7x10^{2}\pm 3.3x10^{2}$	0.43ns
Total coliform count	$2.1x10^{2}\pm 1.6x10^{1}$	$5.5x10^{3}\pm 3.2x10^{2}$	$6.8x10^{2}\pm 3.2x10^{2}$	0.53ns
E. coli count	$3.7x10^{3}\pm 2.0x10^{2}$	$1.1x10^{3}\pm 3.7x10^{2}$	$7.2x10^{3}\pm 6.0x10^{2}$	0.56s
Feacal coliform conut	$2.1x10^{3}\pm 1.0x10^{2}$	$2.9x10^{3}\pm 1.2x10^{3}$	$2.4x10^{3}\pm 2.2x10^{2}$	0.82ns

KEY; ns – not significant, s - significant

Table 4.13 Seasonal influence on the bacterial counts in the well water samples drawn from Zaria (mean ± standard error of mean).

Bacterial count (cfu/100ml)	Rainy season	Dry season	Level of significance (ns)
Total bacteria count	$1.4x10^3 \pm 2.6x10^2$	$9.5x10^3 \pm 8.6x10^2$	0.40
Total coliform count	$7.5x10^3 \pm 1.9x10^3$	$2.1x10^3 \pm 9.0x10^2$	0.07
E. coli count	$6.8x10^3 \pm 3.5x10^3$	$1.2x10^3 \pm 3.0x10^2$	0.10
Faecal coliform	$3.3x10^3 \pm 4.7x10^2$	$1.6x10^3 \pm 3.2x10^2$	0.14

KEY; ns – not statistically significant

Effect of lining on the bacterial counts in the well water is presented in Table 4.14. However there was no significant difference in the bacterial counts between wells with internal wall lining and wells without lining (p-values were all greater than 0.05).

Table 4.15 is the correlation between parasite and *E. coli* from the well water samples and it shows there was a weak positive correlation between parasite and *E. coli*.

Table 4.16 presents the percentage occurrence of the bacteria species isolated from the sampling location. A total of 168 organisms were isolated and 98 (69.0%) were isolated from wells with internal wall lining while 76 (53.5%) were isolated from wells without internal wall lining. *E. coli* was isolated more frequently (48.3%) compared to other species and was more frequent in wells without internal wall lining (50.0) compared to wells with internal wall lining (47.1%). Other species were more frequent in wells with internal wall lining.

The antibiotic susceptibility pattern of the bacteria species isolated from the well water samples are presented in table 4.17. All the isolates were susceptible to Nitrofurantoin and Chloramphenicol. High resistance was recorded with Amoxiciline (46.7%) and pefloxacin (51.1%).

Table 4.14 Effect of lining on the bacterial counts in the well water drawn from Zaria (mean ± standard error of mean).

Bacterial counts (cfu/100ml)	Lined	Unlined	P-value
Total coliform count	$9.9x.10^3 \pm 1.4x10^2$	$7.2x10^3 \pm 1.1x10^3$	0.18ns
Faecal coliform count	$4.1x10^3 \pm 8.5x10^2$	$3.9x10^3 . \pm 9.8x10^2$	0.15ns
E. coli count	$1.0x10^3 \pm 6.4x10^2$	$7.4x10^2 \pm 1.6x10^2$	0.13ns
Total bacterial count	$1.3x10^3 \pm 1.7x10^2$	$1.3x10^3 \pm 1.2x10^2$	0.90ns

KEY; ns – not statistically significant

Table 4.15; correlation between parasite and *E. coli* in the well water

	Parasite	Faecal
Parasite	1	0.37
Faecal E. coli	0.37	1

The correlation was positive but weak.

Table 4.16: Percentage occurrence of the bacteria species isolated from well water samples.

Organism	Number of isolates		Percentage occurrence		Total
	LinedUnlined		LinedUnlined		(%)
E. coli	32	37	47.1	50.0	69(48.6)
Klebsiella pneumonia	8	6	11.8	8.1	14(9.9)
Klebsiellaozanae	9	6	13.2	8.1	15(10.6)
Enterobacteragglomerans	8	8	11.8	10.8	16(11.3)
Enterobactergergoviae	10	5	14.7	6.8	15(10.6)
Enterobacteraerogenes	6	4	8.8	5.4	10(7.0)
Citrobacterfreundii	6	3	8.8	4.1	9(6.3)
Proteus mirabilis	4	0	5.9	0	4(2.8)
Serratialiquefaciens	7	7	10.3	9.5	14(9.9)
Acinetobacteriwoffi	2	0	2.9	0	1(1.4)
Total	98	76	69.0	53.5	168

KEY; N=Number, %=percentage occurrence

Table 4.17 Antibiotic susceptibility pattern (%) of the bacterial species isolated from the well water samples

Antibiotic (Concentration)	*E. coli* n=18			*Klebsiella* species n=10			*Enterobacter*species n=10			*S. liquefaciens* n=7			Total n=35		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
SXT(25µg)	16 (88.9)	0 (0)	2(11.1)	8(80)	1(10)	1(10)	10(00)	0(0)	0(0)	7(100)	0(0)	0(0)	41(91.1)	1(2.2)	3(6.7)
TE (30µg)	2(11.1)	8(44.1)	8(44.1)	5(50)	2(20)	3(30)	6(60)	3(30)	1(10)	4(57.1)	3(42.9)	0(0)	17(37.8)	16(35.6)	12(26.7)
CPD (10µg)	9(50)	8(44.1)	1(5.6)	8(80)	2(20)	0(0)	3(30)	1(10)	6(60)	6(85.7)	0(0)	1(14.3)	26(57.8)	11(24.4)	8(17.8)
AMC (3µg)	2(11.1)	7(38.9)	1(5.6)	0(0)	6(60)	4(40)	4(40)	4(40)	2(20)	1(14.3)	0(0)	6(85.7)	7(15.6)	17(37.8)	21(46.7)
AZM (15µg)	11(61.1)	-	7(38.9)	6(60)	-	4(40)	10(100)	-	0(0)	5(71	-	2(28.6)	32(71.1	-	13(28.9)
F (300µg)	18(100)	0(0)	0(0)	10(100)	0(0)	0(0)	10(100)	0(0)	0(0)	7(100)	0(0)	0(0)	45(1000	0(0)	0(0)
CIP (5µg)	15(83.3)	0(0)	3(16.7)	10(100)	0(0)	0(0)	10(100)	0(0)	0(0)	7(100)	0(0)	0(0)	42(93.3)	0(0)	3(6.7)
CAZ (30µg)	17(94.4)	0(0)	1(5.6)	10(100)	0(0)	0(0)	9(90)	1(10)	0(0)	6(85.7)	0(0)	1(14.3)	42(93.3)	1(2.2)	2(4.4)
PEF (5µg)	9(50)	-	9(50)	2(20)	-	8(80)	6(60)	-	4(40)	5(71.4)	-	2(28.6)	22(48.9)	-	23(51.1)
GN (3µg)	18(100)	0(0)	0(0)	10(100)	0(0)	0(0)	10(100)	0(0)	0(0)	7(100)	0(0)	0(0)	45(100	0(0)	0(00
C (30µg)	15(83.3)	2(11.1)	1(5.6)	10(100)	0(0)	0(0)	10(100)	0(0)	0(0)	7(100)	0(0)	0(0)	42(93.3)	2(4.4)	1(2.2)

Keys; Sulfomethoxazole-SXT, Tetracycline-TE, Cefpoxodime-CPD, Amoxicillin-AMC, Azithromycin-AZM, Nitrofurantoin-F, Ciprofloxacin-CIP, Ceftaxidime-CAZ, Pefloxacin-PEF, Gentamicin-GN, and Chloramphenicol-C.

CHAPTER FIVE

5.0. DISCUSSION

In this study, water samples were collected from 142 wells from three different locations in Zaria for physioco-chemical, parasitic and bacteriological analyses as discussed in the methodology.

Physico-chemical Parameters

The observed temperature falls within the normal temperature range of 20 - 32^0C (WHO, 2009) that allows the proliferation of most bacteria isolated from the samples. However there is no health impact regarding temperature values in water (Nigeria Industrial Standard (NIS), 2007)

The pH in the study area shows a narrow range of pH values from 6.9 - 8.5 which falls within the normal range of 6.5 - 8.5 (WHO, 2011). The pH of water is generally affected by the geology of the area and buffering capacity of water. Extreme pH values in water can affect the health of consumers of the water and its disinfection (Akaahan*et al*., 2010). Exposure to extreme pH values in water results in irritation to the eyes, skin and mucous membranes. Eye irritation and exacerbation of skin disorders have been associated with pH values greater than 11. Exposure to low pH values also results in similar effects. For effective disinfection with chlorine, the pH should preferably be less than 8 (WHO, 2003).

The electrical conductivity in the study areas had values beyond the acceptable limit of 180 - 500umS/cm (WHO, 2009). This may be due to the dissolution of some earth materials by infiltration. Total dissolved solids (TDS) also showed higher values above the desirable limit. TDS in water supplies can originate from natural sources, sewage, urban and industrial waste. Although there are no recent data on health effects associated

with the ingestion of TDS in water, however, high TDS in drinking water can affect the taste of the water (WHO, 2011).

Analysis of variance on the data obtained shows there were no significant differences in the physicochemical parameters of the water samples between the three locations. Similarly, the parameters were not affected by seasonal influence. There were also no significant differences in the physicochemical parameters of the water samples between wells with internal wall lining and wells without internal wall lining in the study areas as shown in the Table 4.4. This could mean that lining does not have a direct impact in the physicochemical quality of well water.

Parasite Contamination *(Entamoebahistolytica)*

Parasitic contamination of ground water has always been of great importance in public health especially in rural communities. In the present study, the prevalence of cysts of *Entamoebahistolytica* varies between location, and well lining. The prevalence rate was higher in Sabon- Gari. These could be because most of the wells were public wells, not properly covered or without cover at all which allows contaminated surface water to drain into the aquifer along the outer well lining (Drinking Water and Human Health, 2010). Also, because it is a high commercial area, during daily operation, reasonable quantities of different waste are dumped on fresh waterways consistently.This can lead to occasional minor flooding and/or contamination of river, lakes, hand dug wells and reservoirs used by people as source of household water (Chukwu, 2008). There was also higher prevalence rate during the rainy season may be because of high level of surface runoff and infiltration.

The overall contamination rate of 27.5% was recorded in the study area which was lower than that of 72.9% by (Bishop and Inabo, 2014) and higher than that of 19.9% recorded

by Yousefi*et al.*, (2009). Higher parasite prevalence (30.5%) was observed in wells with internal wall lining than in wells without internal wall lining (23.3%). Bishop and Inabo (2015) also reported higher prevalence of parasites in well with internal wall lining (48.7%) than in wells without internal wall lining (25.8%). The lining of the internal wall may provide structural attachment sites for parasites, their eggs and cysts. Also, some components of the materials used in lining may support growth of the parasite (Bishop and Inabo, 2015).

Bacteriological Analyses

Raw water may contain a wide variety of harmless heterotrophic and pathogenic microorganisms (Stainier*et al.*, 2007). Many bacteria provide an indication of faecal pollution in water (Verhille, 2013) and they were recovered from this study. This suggests possible contamination of well water with faecal matter.

The total bacterial count of the sample range from $1.73 \times 10^3 \pm 2.73 \times 1\ 0^2$ cfu/ml - $2.02 \times 10^2 \pm 1.85 \times 10^2$ cfu/ml during the rainy season and $4.17 \times 10^2 \pm 3.07 \times 10^1$ cfu/ml - $1.39 \times 10^3 \pm 3.27 \times 10^2$cfu/ml during the dry season (mean = standard error of mean). The standard plate count also called heterotrophic plate count (HPC) is used to estimate the total amount of bacteria in the water and indicates the overall status of the water (Aydin, 2007). HPC has no health effect, however, the lower the concentration of bacteria in the water the better maintained the water system. (U.S. EPA, 2016).

Total coliforms, thermotolerant coliforms, and E. coli were detected at 100, 91.5 and 71.8% in the well water samples respectively. Aydin(2007) reported values of 25, 17.5 and 15% for total coliforms, thermotolerant coliforms, and *E. coli* respectively. These values are lower than the values reported in this study. Total coliforms, thermotolerant coliforms, and *E. coli* are bacteria whose presence indicatesfaecal contamination from

human or animal sources. Pathogens from these sources in water cause diarrhoea, cramps, nausea, headaches and other symptoms. These pathogens may pose a special health risk for infants, young children and the immunocompromised (Aydin, 2007; U.S EPA, 2015).

According to Nigeria standard for drinking water quality, there should not be more than 10cfu/ml of total coliforms in water, and zero tolerance for thermotolerant coliforms and *E. coli* (NIS, 2007). The presence of significant amount of coliforms and *E. coli* in the water samples is indicative of inadequacy of the depths of the wells and/or a breach of the sanitary integrity of the wells. Therefore the wells may fail to meet the standard for safe drinking water.

Total coliform count, faecal coliform count and total bacterial count were higher in the wells with internal wall lining than wells without. However the level of significance is not statistically different (values were greater than p-value- 0.05; Table 4.10). This means that internal wall lining of the well may not have a direct contribution to the bacteriological quality of well water.

Furthermore, 168 faecal coliform organisms belonging to 7 Genera and 10 species of bacteria were confirmed with microgen test kits for the identification of enterobacteriacaea as seen in the Table 4.12.

E. coli was isolated more frequently from the water samples with the percentage occurrence of 48.6% (69 isolates). The presence of *E. coli* in drinking water sources often indicates recent faecal contamination. That means there is a higher risk that pathogens are present. *E. coli* is usually a normal flora of the gastrointestinal tract of humans and warm-blooded animals. However some strains can cause illness (Edema *et al.*, 2011). Waterborne transmission of pathogenic *E. coli* has been documented for

recreational water and contaminated drinking water (WHO, 2014). A well-documented report of outbreak of illness caused by *E. coli* O157:H7 occurred in Walkerton town in Ontario, Canada in May, 200 which led to 7 deaths and more than 2300 illness. (O'Connor, 2002). There are no indications that the response of enteropathogenic strains of *E. coli* to water treatment and disinfection differ from that of other *E. coli.* Hence, convectional testing of *E. coli* or thermotolerant coliforms provides an appropriate index for the enteropathogenic serotypes in drinking water (O'Connor, 2002).

Bacteria belonging to the genus *Enterobacter* were also isolated in high numbers. Three *Enterobacter* species isolated include; *Enterobacteragglomerans* (11.2%), *Enterobactergergoviae* (10.5%) and *Enterobacteraerogenes* (7.0%). The presence of *Enterobacter* in water sources may be indicative of faecal contamination. Other sources could be soil, polluted water and plants (Ihejirika*et al*., 2011; Esomonu*et al*., 2012). Although these organisms have been isolated in drinking water sources and have been found to colonize distribution systems, no outbreak of associated human disease have been conclusively reported. However, they have been associated in nosocomial infections and hospital acquired infections in humans (Davin-Regli*et al.*, 2008; Esomonu*et al*., 2012).

Two species of *Klebsiella* were isolated from the water samples; *Klebsiella pneumonia* (9.9%) and *Klebsiellaozanae* (10.5%). *Klebsiella* species are natural inhabitants of many fresh water environments and they may multiply to high numbers in water rich in nutrients. These organisms are also excreted in the faeces of many healthy humans and animals, and they are readily detected in the sewage polluted water. *Klebsiella* species isolated in drinking water sources are usually biofilm organisms and are unlikely to represent health risk (Ainsworth, 2004). However *Klebsiella pneumonia* accounts for a significant proportion of hospital acquired urinary tract infections, pneumonia,

septicemia and soft tissue infections (Kathleen and Talero, 2004; Mbah and Muhammed, 2015).

Nine species (6.3%) of *Citobacterfruundii* were isolated from the water samples. *Citrobacter*is a commensal bacteria found in the intestine of humans and almost every other environments including water, wastewater and soil. The strain *Citobacterfreundii* is often implicated in opportunistic infections mostly associated with abnormal inflammatory changes in the intestinal tract. It can also affect the billary, urinary and respiratory tracts, and can enter the blood stream of immunocompromised patients.

Four species of *Proteus mirabilis* (2.8%), 14 species of *SerratiaLiquefaciens* (9.9%), and 2 species of *Acinebacter*(1.4%) were isolated from the water samples. These organisms are naturally present in the environment and not regarded as pathogens, but can cause opportunistic infections when present in drinking water. These organisms acquire public health importance under certain conditions. Therefore their removal in water should be given moderate priority.

Antibiotic Susceptibility Test

In this study all isolated (45) were susceptible to Nitrofurantoin (300 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg) and Gentamicin (3 µg) except *E. coli* which showed resistance of 5.6 and 16.7% to chloramphenicol, ciproxacin respectively. High level of resistance was exhibited by *E. coli* as shown by its resistance to 9 different antibiotics out of 11 used in this study. Most of the isolate were resistant, some were moderately susceptible and very few susceptible as presented in table 4.11.

The high level of resistance among the *E. coli* isolates is of great concern as some of the isolates showed multiple drug resistance (resistance to more than 2 antibiotics). Amaya

et al., (2012)and Atif*et al.,*(2010) reported high resistance of*E. coli* isolated from water samples.

Waterborne outbreaks of enteric pathogens have been documented from a number of countries especially *E. coli* and the difficulties in the treatment of water associated gastrointenstinal diseases due to *E. coli* have been reported (Bartlett, 1996; Ogden *et al*., 2001; Atif*et al*., 2010). The problem is compounded by the continued emergence of antibiotic resistance to a growing number of antibiotics (Atif*et al*., 2010; Amaya *et al.*, 2012). Increase in antibiotic resistance is now a global problem. A high density population of patients in frequent contact with health care practitioners and the attended risk of cross infection contribute to the spread of resistant microbes in the environment. Since water is one of the components of the environment, and a usual habitat for microbes, the avaliabily of antibiotic resistance cannot be denied.

Over the past few decades, antimicrobials have become increasingly available for broad spectrum of pathogens. Due to the widespread use of these drugs, new forms of antibiotic resistance have emerged (Atif*et al*., 2010; Amaya *et al.*, 2012). Antibiotic resistance profile of clinical *E. coli* isolated from human, animals and some environmental have well been documented by various researchers. Opportunistic pathogens presenting broad-spectrum antibiotic resistance have emerged extensively in hospital environments, causing serious infection in immunocompromised patients. However, less attention is given to environmental strains.

The extent to which bacterial isolates are exposed to antibiotics before they are released into the environment could be responsible for the resistance of the isolates in this study. The correlation between antibiotic use and resistance of commensal bacteria is well

documented by Van den Boagaard and Stobberingh (2000) and by Nyamboyo*et al*., (2013)

CHAPTER SIX

6.0. CONCLUSION AND RECOMMENDATION

6.1. Conclusions

All the samples had temperature and pH within the range that allows proliferation of microorganisms in water. But most of the samples had Electrical Conductivity and Total Dissolved Solids exceeding the acceptable limit.

The presence of *Entemoebahistolytica* and significant counts of coliform bacteria and *E. coli* in hand dug well in some parts of Zaria communities is of great concern. This is indicative of improper well construction in terms of topography and distance from septic system and/or breach of the sanitary integrity of the wells. The study also revealed high resistance of bacterial isolates (especially *E. coli*)against commonly used antibiotics. The wells fail to meet the standard for safe water and are therefore not safe for domestic and especially drinking purposes.

6.2. Recommendation

1) Laws should be enforced to regulate or prohibit indiscriminate construction of hand-dug Wells.
2) There should be proper education of people in these communities on the importance of sanitation and hygiene and proper method of waste disposal, and the dangers of drinking contaminated water.
3) Household treatment of the water should be applied before use,either by boiling to kill bacteria, or by filtration to remove cysts of parasites, or by chemical disinfection such as chlorination, or combination of both.

4) Surveillance of antibiotic resistance should be carried out on waterborne pathogens and periodic monitoring of antibiotic susceptibility both in communities and hospitals should be done.

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Appendix A



A Well located in Samaru. The well is not covered and residents wash their cloths around the well environment which could be a source of contamination

APENDIX B



A poorly hygienic Well surrounding in Sobon-Gari. The fetcher is left on the ground and the rope soaked with dirty water from the surrounding. This could be a source of contamination of the water.

APPENDIX C



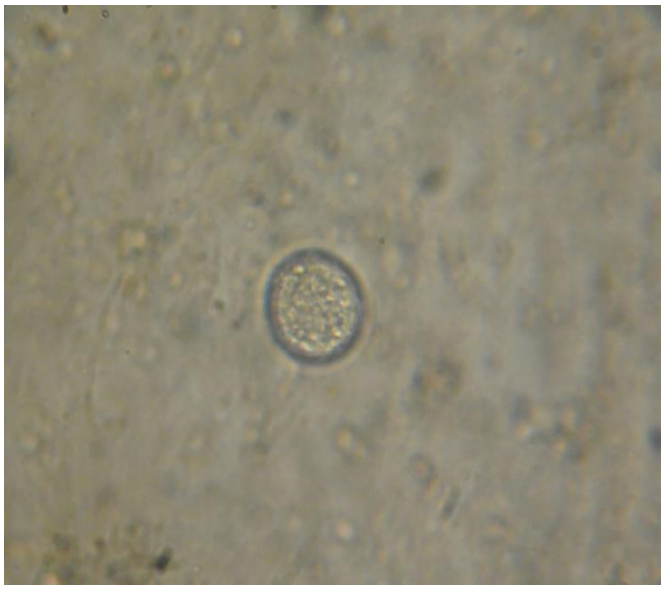
Grazing animals on the surrounding very close to a well in Tudun Wada. This could be a source of contamination of the water.

APENDIX D



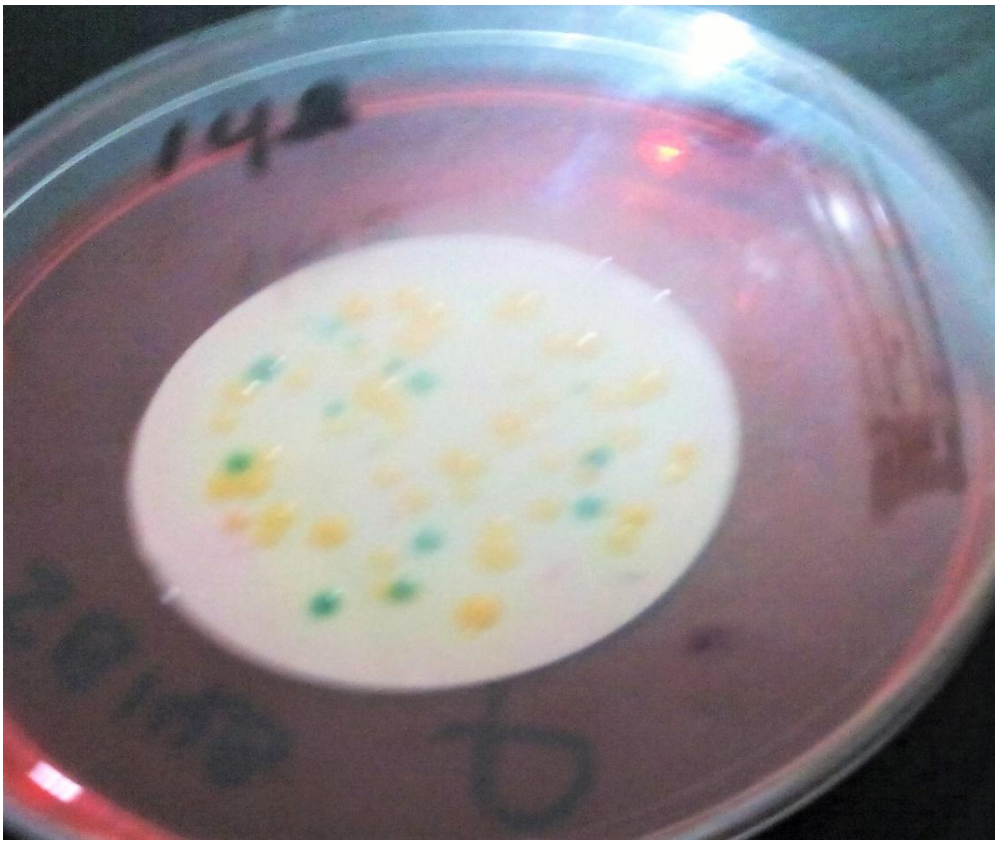
Multiple Membrane Filtration Unit used during Filtration of the water samples.

APENDIX E



A microscopic view of cyst of *Entamoebahistolytica* from the wet mount preparation of one of the samples

APENDIX F



Coliform bacteria growth on the membrane filter paper after 24 hours of incubation. The green colonies were *E. coli* while the yellow colonies were other coliform bacteria.

APENDIX G



MicrogenIdentificatio micro wells plate after culture and addition of reagent.