# FAECAL CONTAMINATION OF DRINKING WATER SOURCES IN CHANCHAGA LOCAL GOVERNMENT AREA, MINNA AND ITS PUBLIC HEALTH IMPLICATIONS

**BY**

**ENIOLORUNDA Michael Folorunsho**
**MTECH/SLS/2017/6803**

**DEPARTMENT OF MICROBIOLOGY,**
**FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA.**

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## ABSTRACT

Water is the most essential renewable natural resource needed by every living thing. Bacteriological and physicochemical studies of eighty (80) samples from four (4) sources of drinking water (tap, well, borehole and sachet water) from Chanchaga Local Government Area, Minna, Niger State, Nigeria was carried out. Membrane filtration technique, cultural and biochemical tests were used for bacteriological examination while the methods described by the American Public Health Association (APHA) were used for the physicochemical analysis. One- way analysis of variance (ANOVA) was used to analyze all the data. Enumeration of total coliforms, thermotolerant coliforms and *Escherichia coli* were used as indices for determining potability of the water samples while Singleplex polymerase chain reaction technique was used to investigate for specific virulent genes in pathogenic *Escherichia coli* strain. Results revealed that bacteriological counts of most samples were above the limits specified by World Health Organization and Nigerian Standards for Drinking Water Quality. Well water had total coliform counts ranging from 03-360 cfu/mL, sachet water had coliform counts that ranged from 01-15cfu/mL The total coliform counts from borehole and tap water ranged from 03-26 and 03-107cfu/mL respectively. The thermotolerant coliforms identified were *Escherichia coli* and *Klebsiella pneumoniae.* The mean pH, total dissolved solid, turbidity and total suspended solid of the well water showed significant difference ($p<0.05$) between sachet water and borehole water. Identification of the virulent gene using Singleplex PCR technique revealed the presence of eaeA gene from pathogenic *Escherichia coli* isolated from the drinking water sample. The presence of faecal coliforms in the drinking water samples in the study area is a cause for public health concerns and that indicated that the government needs to redouble her effort and intensify the present reforms on water and sanitation. However, regular surveillance of drinking water sources should help prevent contamination of water.

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# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background to the Study

The most essential renewable natural resource needed by every living thing is water (Bala *et al*., 2019). It is used for drinking, bathing, food production or recreational purposes (Eboh *et al.,* 2017). Having been adjudged to be man's most used and consumed substance (Ibahdode *et al*., 2017), it is therefore not an exaggeration to say that water is life (Adeleye *et al*., 2014). Water is important for the sustenance of life, and sufficient provisions should be made available to consumers (Owolabi *et al.,* 2014). Martin (2001) reported that humans are two-third water and we require water to live. However, in spite of its importance in sustenance of live and livelihood, it is the major cause of mortality because of limitations in access and quality (Gimba, 2011). As reported by Policy and Practice of Water Supply in Nigeria (2007), poor quality drinking water is responsible for spread of deadly diseases such as cholera, typhoid, dysentery, shigellosis, hepatitis A, poliomyelitis and *Escherichia coli* diarrhea. As a result, it is of importance to evaluate the potability of water before using it for domestic purposes like drinking and cooking.

The human right to water and sanitation encapsulates the importance of water to human health and wellbeing as it entitles everyone to sufficient, safe, acceptable physically, accessible and affordable water for personal and domestic uses (Bain *et al*., 2014). This was affirmed by the United Nations General Assembly and Human Rights Council in 2010

(United Nations, 2010). The World Health Organization (WHO, 2006) defined safe drinking water as water that does not present any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages. Safe drinking water must be aesthetically acceptable and does not contain pathogenic agents and dangerous chemical substances (Park, 2005; WHO, 2006; Nigerian Standards for Drinking Water Quality, 2007).

Safe drinking water, as a basic necessity as it is, is still a luxury for many poor developing countries of the world today (WHO 2006; United Nations International Children Emergency Fund, 2006). They estimated that over a billion people lack access to safe drinking water of which 80 % of this population live in these three regions: Sub-Saharan Africa, Eastern Asia and Southern Asia (Akanwa and Okonkwo, 2015). In another report by the WHO (2014), it is estimated that globally, about 1.8 million people die from diarrheal diseases annually, many of which have been linked to be acquired from the consumption of contaminated water (Nwabor *et al*., 2016). The United Nations International Children Emergency Fund (2010) reported that 884 million people in the world use unimproved drinking water source. In a similar report by United Nations Department of Economic and Social Affairs (2009), the worldwide estimate for people without access to safe drinking water is nearly 900 million. According to WHO/UNICEF (2000), about 2.6 billion, almost half the population of the developing world lack access to adequate sanitation. Over 80 % of people without safe drinking water and 70 % of people without sanitation live in developing countries, Nigeria inclusive (Nwabor *et al*., 2016). Nigeria as a nation is in no way disassociated from the challenges of safe drinking water supply. As the most populated country on the continent, Nigeria will be central as to whether or not Africa reaches the Millennium

Development Goals (MDG) (Ajibade *et al*., 2015). Nigeria, the giant of Africa in terms of population and oil economy has lots to achieve in order to reach these targets, meanwhile it has a rapid population growth.

Nigeria represents the eight most populous nations in the world with a total population of 180 million people out of which less than 30 % of the population have access to potable water and safe source of water supply (Aper, 2011). In a report, only 48 % of Nigerians in 2004 had access to improved safe drinking water (WHO, 2004a). Over the years, as a measure for water provision, residents have resorted to the use of boreholes, streams and patronized water vendors, who get their water from unimproved sources as proved by various researches that these sources are unsafe for drinking (Akanwa *et al*., 2011; Onwuemesi *et al*., 2013). The democratic government had to cope with extreme poverty, low human development, a history of corruption and decentralization of responsibility for water and sanitation from central to local governments (Ajibade *et al*., 2015). These constraints partially explain why the government has not been successful in fulfilling its responsibility to provide safe water supply to her citizens up till now. As a result of this, Nigeria is one of the countries in the world that has unsafe water supplies and most of her citizens will usually contract a waterborne illness (Adeyinka *et al.*, 2014). In Nigeria, 315,000 under 5 children die annually due to diarrhea which is 23 times more than under 14 years mortality in Europe as a result of same (Gimba, 2011).

The Nigeria Ministry of Health's data rate diarrhea second after malaria as a disease of high prevalence, it accounts for 16 % of under 5 mortality (UNICEF, 2007). The assessment of drinking water quality comes with a rigorous task that entails assessing the physical, chemical and bacteriological parameters of water. A myriad of chemical substance,

bacteriological agents and physical properties of water needs to be considered in order to ascertain the safety of water for consumption. The greatest risk, however to human health especially in developing countries is from faecal contamination of water sources (WHO, 2006).

The source of water contamination responsible for the spread of infectious disease is undoubtedly faeces (Adewale and Vincent, 2018). Faecal contamination of drinking water is a major health problem, which accounts for many cases of diarrhea mainly in infants (Ahmed *et al*., 2016). It is no news that waterborne diseases are a significant public health issues and many originates from contact with water contaminated with human or animal faecal material. There are numerous types of bacteria, virus and protozoa that can be transmitted through contaminated water with numerous outbreaks, which have been reported (Ahmed *et al*., 2016). In water, the detection and enumeration of all pathogenic microorganisms potentially present is impossible due to the large diversity of the pathogens, the low abundance of each species and the absence of standardized and low cost methods for the detection of each of them. Thus, for routine monitoring, faecal indicator bacteria (FIB) are usually enumerated to evaluate the level of microbial water contamination (Nouho *et al*., 2011). The detection of *Salmonella* sp, *Shigella* sp or *Clostridium perfringens* from water is sufficient evidence that the water is not potable, unless treated (Olajubu *et al*., 2014). Across the globe, these group of microorganisms are accepted as useful indicators of microbiological water quality, since they indicate a high and close relationship with health hazards associated with the water use, mainly for gastrointestinal symptoms and they are always present in feces of warm- blooded animals (Layton *et al*., 2006; Olajubu *et al*., 2014).

The outbreak of waterborne diseases in North Central States of Nigeria are reportedly caused by drinking water containing infectious viruses or bacteria, which often come from faecal contamination (Bademosi, 2018). The production of unhygienic packaged sachet water has become a cause for concern and menace in North Central States, Nigeria, especially when they are produced under unhygienic conditions (Bademosi, 2018), bearing in mind the significance of safe drinking water to the health. The 2016/2017 report, which is the fifth round of the Multiple Indicator Cluster Survey (MICS) series, revealed that an overwhelming majority representing 90.8 percent of households in Nigeria drink water contaminated by faeces and other impure substances like *E. coli* (Bademosi, 2018). Poor sanitary quality of drinking water and unsafe drinking water or lack of clean water supply has been reported in some regions in North Central State, Nigeria due to faecal contamination (Adabara *et al*., 2011; Bala *et al*., 2016; Oyedum *et al*., 2016; Abdulrahman *et al.,* 2018).The sources of water contamination include the sewage, industrial and trade wastes, agricultural pollutants like fertilizer and radioactive substances. In most developing countries, much of the water available for drinking is not only short in supply, but also unsafe for drinking due to contamination mostly with human and animal faeces as a result of poor sanitation, i.e, lack of improved excreta and solid waste disposal (Gimba, 2011). The World Health Organization (2011a) has estimated that up to 85 % of all diseases occur as a result of microbially contaminated water, improper or inadequate sanitation and hygiene. According to WHO/UNICEF, 2.6 billion, more than 40 % of the world population do not use toilet, but defecate in open or unsanitary place (WHO, 2016). The microbiological guidelines for drinking water (WHO, 2004b) stated coliform bacteria or faecal indicator bacteria must not be detected in 100 mL samples of water for the water to be considered

safe; their detection in water indicates pathogenic bacterial contamination (WHO, 1993; WHO, 1997; WHO/UNICEF, 2010; WHO, 2011b; Chalchisas *et al*. 2017).

## 1.2 Statement of the Research Problem

The World Health Organization (2005) estimated that globally, about 1.8 million people die from diarrheal diseases annually, many of which have been linked to diseases acquired from the consumption of contaminated waters. UNICEF (2010) reported that 884 million people in the world use unimproved drinking water source, and estimates that in 2015, 672 million people will still use an unimproved drinking water source. In another report, United Nations Department of Economic and Social Affairs (UNDESA, 2009) put the worldwide estimate for people without access to safe water at nearly 900 million. According to WHO/UNICEF (2000), about 2.6 billion, almost half the population of the developing world do not have access to adequate sanitation. In Nigeria, a vast majority of people living along the course of water bodies still source and drink from rivers, streams and other water bodies without any form of treatment. These natural waters contain a myriad of microbial species, many of which have not been cultured, much less identified (Nwabor *et al*., 2016). The number of organisms present varies considerably between different water types. Polluted surface waters can contain a large variety of pathogenic microorganisms including viruses, bacteria and protozoa which can come from nonpoint sources or point sources like municipal wastewater treatment plants and drainages from areas where livestock are handled (William *et al*., 2012).

Faecal contamination of water is globally recognized as one of the leading causes of waterborne diseases. In Nigeria, cases of water-related diseases abound. The most common

waterborne diseases in Nigeria include diarrhea, cholera, dracunculiasis, hepatitis and typhoid (Adeyinka *et al*., 2014). Cases of water-borne diseases linked to contaminations of drinking water with pathogens have been reported in several towns (Nwabor *et al*., 2016). Globally, death rate due to diarrhea (which has being rated as a disease of high prevalence) as a result of consumption of contaminated water is alarming. In Nigeria, for example, about 315,000 children under age of 5 years die annually due to diarrhea. This indicates that Nigeria is not on the path to achieving Millennium Development Goals except it redoubles her effort and intensifies the present reforms on water and sanitation.

### 1.3 Justification for the Study

Water is one of the commonest routes of transmitting disease (Cabral, 2010). According to the WHO (2018) fact sheet on potable water, contaminated drinking water is estimated to cause 502,000 diarrhoeal deaths each year. In Africa, records have it that every child have five episodes of diarrhea yearly and that 800,000 children die each year from diarrhea and dehydration (Raji and Ibrahim, 2011). In Nigeria, about 150,000 children reportedly die of diarrhea-related diseases annually for drinking unsafe water, while a large sum of rural dwellers still lacks access to potable water (Bademosi, 2018).About 51 % of Nigeria's 165 million population resides in rural or remote areas (Ohunakin *et al.*, 2013) and only 47 % of this rural populace have access to improved water sources (Onabolu *et al.*, 2011). Out of the 49% that live in urban and peri-urban areas, only 72 % have access to improved water sources (Onabolu *et al*., 2011). Nigeria faces disease epidemics as 63 million lack access to safe water (Ogundipe *et al*., 2017). This depicts the need for improve and safe water supply devoid of faecal contamination in Nigeria.

The presence of faecal contamination in drinking water is an indicator that a potential health risk exists for individuals exposed to this water. Some waterborne pathogenic diseases include the typhoid fever, viral and bacterial gastroenteritis and hepatitis A. Consequently, consumers of such water are exposed to series of health risks. However, these risks are most serious in the developing world or countries like Nigeria where 99.8 % of the annual 1.7 million deaths relating to unsafe water supply, sanitation, and hygiene occur (WHO, 2002a; Akor, 2017; WHO, 2018; UNICEF Nigeria, 2018; WHO, 2019). Various studies have revealed the presence faecal contaminants in drinking water sources in the region and outbreak of waterborne diseases (Gimba, 2011; Kuta *et al*., 2014; Oyedum *et al*., 2016). *Escherichia coli* have been isolated and implicated as a source of water contamination in the region by various researchers, but characterization of *E. coli* into different pathotypes have been ignored. This study is focused on the use of membrane filtration method for isolation of coliform organisms in drinking water sources and using Singleplex polymerase chain reaction technique to characterize thermotolerant coliforms into various pathotypes and also to evaluate their public health implications to consumers in the region.

## 1.4 Aim and Objectives of the Study

### 1.4.1 Aim of the study

Aim of the study was to examine faecal contamination of drinking water sources in Chanchaga local government area of Niger State, Nigeria and its public health implications.

### 1.4.2 The objectives of the study

The objectives of the study were to:

i. isolate and identify total and thermotolerant coliforms in drinking water sources

ii. determine the physico-chemical properties of the drinking water sources

iii. characterize thermotolerant coliforms

iv. ascertain the public health implications of drinking water sources in this region

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Properties of Water

Water is the most essential chemical substance for survival of all forms of life. It is one of the very few substances that can exist in all three states on earth: solid water as ice, liquid form called water and gaseous form known as vapor (Gimba, 2011). Water in its natural form has many unique properties. It is clear, transparent, tasteless and odorless fluid. In small quantity, it is colourless but appears pale blue through a deep column (Aliyu, 2000). It freezes at 0 $^{o}$C and boils at 100 $^{o}$C under standard atmospheric pressure of 760 mmHg. Water can dissolve many substances, giving it different taste and odor. Water is generally referred to as universal solvent because many substances dissolve in it.

### 2.2 Water Classification Based on Sources

The sources of water can be grouped into two general types namely: Ground water sources: mainly wells and surface water sources such as rivers, lakes and streams (Komolafe *et al.*, 2013).

#### 2.2.1 Ground water

Groundwater is the largest source of fresh water available on earth (Naggar, 2005).Water wells are hydraulic structures used for supplying water from the groundwater storage. Ground water comes from some deep ground from water that may have falling as rain for years. Soil and rock layer naturally sieve the ground water to the highest level of cleanliness,

such water may form springs, or may be drawn out from boreholes or wells. Ground water is generally of very high quality but the water typically is rich in dissolved solids, carbonates and sulfates of calcium and magnesium (Komolafe *et al.,* 2013).

#### 2.2.1.1 *Borehole water*

Borehole water is the water that comes from the part of sub-surface water, which is found in the part of the ground that is fully saturated and flow into a well under force. It is the source of drinking water for human population. The depth borehole drilled should be beneath the sea level and should be supply with pumping machine (NAFDAC, 2002).

### 2.2.2 Surface water

Surface water is found on top of river, upland lake that is seen by human and may be encircling by a shielding area to restrict the chances of contamination. Bacteria and pathogen levels are usually low, but some bacteria, protozoa or algae will be present (Komolafe *et al.,* 2013).

## 2.3 Safe Drinking Water

Although often perceived to be pretty ordinary, water is the most remarkable substance (Martin, 2001). It covers approximately 70 % of the earth and remaining amount is found in the environment. Out of this, only 2 % of the world's water is drinkable (Arun *et al.,* 2017). Access to safe drinking water (potable water) is a basic human need, it is essential to health and a component of effective policy for health protection (WHO, 2017). Approximately 780 million people in the world do not have access to safe drinking water (UNICEF, 2012). Safe and sufficient drinking water supply is a major fundamental need for survival of humans

(Gayeon, 2012). The WHO (2017) in its report defined safe drinking water as water that does not present any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages. A safely managed drinking water service is one which is located on premises, available when needed and free from contamination (WHO and UNICEF, 2017). However, inadequate drinking water supply and issues of quality is a major concern, particularly in developing countries (WHO, 2002b). In many countries, ingestion of unsafe water, lack of access to sanitation and limited availability of water for hygiene ranks the third out of top twenty significant health risk factors (Gayeon, 2012). For example, 90 % of deaths among children under the age of 5 years old were attributed to contaminated drinking water in 42 developing countries (i.e, 10.8 million children deaths worldwide in 2001). Poor water quality combined with inadequate sanitation and poor hygiene accounted for 88 % of diarrheal diseases, which is the primary cause of these preventable childhood deaths (Black *et al*., 2003).

### 2.3.1 Improved and unimproved sources of drinking water

The Joint Monitoring Programme (JMP) for Water Supply and Sanitation of UNICEF and WHO in the year 2002 coined the terms 'Improved Water Source' and 'Unimproved Water Source'. It defined improved water source as piped water on premises, i.e, piped household water connection located inside the users dwelling and other improved sources like protected dug wells, protected springs, public taps or stand pipes (Hannah and Max, 2018). While these sources are likely to provide safe and adequate water as they may prevent contact with human excreta, for example, this is not always the case (Hannah and Max, 2018). Bain *et al*. (2014) reported that approximately 25 % of improved sources contained faecal contamination. Unimproved sources include unprotected dug well, unprotected

spring, surface water (river, stream, lake, pond, canal, dam), tanker truck water, vendor-provided water (WHO and UNICEF, 2012). These sources are not separated from human contact through unimproved sanitation facilities such as pit latrines without slabs or platforms, open defecation in fields, forests, bushes, water bodies or other open spaces.

### 2.3.2 Potable water accessibility in developing countries and Nigeria

In Africa, nearly 40 % of the population lacks access to improved water and sanitation, 19 % in Asia share similar fate and 52 % lacks improved sanitation. Other regions of the world have higher rates of access, but many millions remain without access in Latin America and the Caribbean (WHO and UNICEF, 2009). The WHO (2004) reported that 83 % of the world population had access to drinking water from improved sources. This seemingly high global statistics hid a critical situation in some developing countries. In sub-Saharan Africa and Oceania, only 54 % and 50 % of their populations respectively are served with improved sources of drinking water. Meanwhile, at the same period, over 90 % of the population in the Caribbean, Northern Africa and Western Asia had access to water from improved sources.

In Nigeria, the situation is not different from her counterparts in the sub-Saharan African regions. For instance, the situation among the low income groups that constitute about 70% of the country's over 180 million people is critical (Nwaka, 2005). With an urban population of about 60 million and an urbanization rate of 5.5 %, conservative estimates have indicated that only about 49.2 % of the urban poor in Nigeria have access to safe drinking water (WHO, 2003). Between 1990 and 2004, the percentage of Nigerians with access to improved drinking water dropped from 49 % to 48 % (WHO, 2006; UNICEF, 2006). It was

observed that the percentage of urban population having access to safe drinking water in the country declined from 81 % in 2000 to 73.4 % in 2006 (WHO, 2006; UNICEF, 2006; National Bureau of Statistics, 2006). Similarly, the proportion of the population with access to sanitation within the same period also dropped from 85 % to 77 % but only 7 % of the population have their houses connected to tap- borne water supply and at the same period, 30 % of Nigerian rural population have access to improved drinking water sources and 2 % of the rural household are connected to tap borne water supply (WHO, 2008a).

### 2.4 Faecal Contamination of Water

One major global problem is faecal contamination of water (Soller *et al*., 2010). All faeces contain varying levels of pathogenic bacteria such as *Escherichia coli*, *Lactobacillus*, *Clostridium*, *Streptococcus* and sometimes *Salmonella* or *Campylobacter* (Sinead, 2015). The adverse effect water contamination by faecal matter can have on human health via drinking and bathing water and the impact the faecally contaminated water has on the quality of local environment is a major concern (Allevi *et al*., 2013; Frey *et al*., 2013; Marti *et al*., 2013; Walters *et al*., 2013). Water containing pathogenic bacteria such as *E. coli* can cause diarrheal disease, which is responsible for 1.2 million deaths annually (Austin *et al*., 2012). Gastroenteritis is another prevalent illness associated with drinking water that is from faecally contaminated water sources (Soller *et al*., 2010). For example, there was an outbreak of gastroenteritis in Nokia, Finland after the drinking water supply was contaminated with sewage effluent. Approximately 6,500 people took ill after coming into contact with the infected water (Laine *et al*., 2011). Individuals who bathe or partake in water related recreational activities such as surfing or swimming in water that is contaminated with faeces are at a high risk of contracting illnesses such as gastroenteritis,

skin irritation, respiratory disease, eye infection and ear, nose and throat infections (Tseng and Jiang, 2012).

### 2.4.1 Point and diffuse sources of faecal contamination

Faecal contamination of water can arise from two sources namely; point sources and diffuse sources (Sinead, 2015). A point source is a single, easily identifiable point of faecal pollution such as a wastewater treatment plant or industrial effluent discharge (Converse *et al*., 2009). The diffused sources are dispersed in an irregular manner with multiple potential inputs of faecal contaminants. This can originate from agriculture where there is run off from farmyard areas, seepage from manure or slurry incorrectly stored manure, or washing from the milking of dairy farms (Vinten *et al*., 2008; Kay *et al*., 2010). Human settlement can also be a contributing factor to diffuse pollution via defective septic tank wastewater treatments (Arnscheidt *et al*., 2007). Domestic and wild animals can also add to the problems of diffuse faecal pollution (Ahmed *et al.,* 2008; Converse *et al*., 2009). This mixture of faecal material can pose a human health risk due to pathogenic bacteria, zoonotic waterborne pathogen, viruses or protozoa parasites (Baldursson and Karanis, 2011; Austin *et al*., 2012; Mattioli *et al*., 2012).

## 2.5 Faecal Indicator Organisms (FIO)

Faecal indicator organisms can be used as an index of faecal contamination in water and they may suggest the presence of pathogens (Traister and Anisfeld, 2006; Paruch and Mæhlum, 2012). Monitoring FIO is easier and more effective than monitoring pathogens in water (Edberg *et al*., 2000; Harwood *et al*., 2013). This is because there are a large variety of pathogens , such as bacteria, viruses, zoonotic waterborne pathogens and protozoa that can

affect human health if they gain access to water through faecal contamination (Austin *et al.*, 2012; Baldursson and Karanis, 2011; Mattioli *et al*., 2012). They can be present in low concentration making their identification a challenge (Harwood *et al*., 2013). Furthermore, the methods used to detect pathogens can be difficult to utilize, expensive, labor-intensive and may not always give accurate results (Edberg *et al.*, 2000). Research began to find a suitable FIO to access possible faecal contamination in water in 1890s (Edberg *et al.*, 2000). In order to achieve this, various factors were considered; the type of FIO that is suitable for tracking faecal pollution, how specific this FIO is, the relationship between water pollution and hydrology, and the methods used to detect the bacterium and associated problems and solutions.

### 2.5.1 Characteristics of faecal indicator organisms

A candidate FIO must meet the following criteria; It should not be pathogenic, it must be universally inhabitant in the intestine of warm-blooded animals, it should be easily detected by simple and cost effective methods, it should have prominent association with pathogens, it should not be able to multiply outside the host, it should be resistant to various environmental stresses, its concentration must be greater in value than pathogens (Ahmed *et al*., 2008; Paruch and Mæhlum, 2012).

## 2.6 Indicators and Drinking Water Regulations

Basically, there are two main methods to identify microbially contaminated water sources (Samantha, 2012). The first is to test for pathogens. Direct testing means that many individual tests have to be run, as each screen test for only one unique type of pathogen. Although a more accurate method, this method can be a cumbersome process involving

complicated, time consuming and often expensive procedure, as there are a high number of pathogens that have been identified as harmful.

Alternatively, there is the option to use an indicator organism, or non pathogenic bacteria, as a proxy for harmful bacteria as they are present in the same environments. Indicator organisms are determined primarily based on their presence in the human gut and their inability to exist outside of that environment for extended period of time. Thus, the presence of an indicator organism in water suggests the presence of faecal contamination and potentially of pathogens (Samantha, 2012).

### 2.7 Indicators Examination Methods

When checking for the presence of an indicator organism in a sample, there are three methods researchers commonly employ. Each of these methods can be used to check for the presence of various indicators and are used in almost all commercially available water testing products.

#### 2.7.1 Presence – absence (P/A)

This method is the simplest method of testing because it is not a quantitative assessment of the contamination level. Rather by adding a water sample to a selective media, a user is able to determine whether or not contamination is present in the sample after 24-48 hours of incubation. If the result is positive however, this method is followed by a more rigorous enumerative method (Samantha, 2012).

### 2.7.2 Membrane filtration technique

This technique was introduced in the late 1950s as an alternative to the Most Probable Number (MPN) procedure for microbiological analysis of water samples. The membrane filtration technique allows for the enumeration of the number of coliforms in a sample by passing a given volume of water sample through a small (0.45 micrometer) membrane filter. The filter is then placed in a dish containing growth medium and incubated for 24 hours. The exact incubation period and temperature will vary based on the type of bacterial contamination being tested. Coliforms and *E. coli* can both be cultured with this method depending on the presence of the appropriate sugar dyes in the broth (Samantha, 2012). This method offers the advantage of isolating discrete colonies of bacteria whereas the MPN procedure only indicates the presence or absence of an approximate number of organisms (indicated by turbidity in test tubes) and it also allows for sampling of large volume of water sample as much as 500 mL, and provides absence or presence information within 24 hours.

### 2.7.3 Most probable number (MPN)

This method makes use of statistical trends to infer the exact level of contamination based on a series of P/A tests. A typical example requires 3-5 test tubes containing various dilution of the sample water mixed with a broth to be incubated and then examined for the presence of gas, indicating positive coliform growth. With the aid of the MPN table, the total number of colony forming units (cfu) per 100mL can be determined. In most cases, the testing stops at this point, however to be thorough, a confirmatory test should also be completed. A confirmatory test consists of culturing some of the positive presumptive tests with an appropriate media and noting the subsequent growth of colonies (Samantha, 2012).

## 2.8 Coliform Bacteria as Indicator Organisms

Coliforms, also known as “indicator organisms” refer to a wide range of bacteria that can be found throughout the environment, i.e, in soil, water surfaces, vegetations as well as on the skin or intestinal tract of warm blooded organisms such as man. Although, some are capable of causing diseases that can be either mild or life threatening, most of them are harmless. Regardless, detection of coliform indicates the presence of potential pathogen not only in water, but also in foods and drinks (milk, etc). This group of bacteria is thus important because they help raise awareness and determine the source of the bacteria. This group of indicator organisms is divided into three main groups; total coliforms, faecal coliform and *E. coli*.

### 2.8.1 Total coliforms

The coliform group consists of Gram-negative, non-spore forming, rod shaped bacteria. This group has been trusted as the most reliable indicator for drinking water in industrialized nations. This group is largely composed of harmless, closely related bacteria that can be found in human and animal wastes, water, vegetation and soil where they inhabit freely. They ferment lactose within 24- 48 hours when incubated at 35 $^{o}$C, a feature, which helps in identifying them among other bacteria. While they are generally harmless, their presence in drinking water or water source that supply drinking water is important because they are indicative of possible contamination. Coliforms are the broadest category of organisms used as an indicator, meaning that a variety of species are used to identify the potential presence of contamination. Often the presence of total coliforms simply indicates that, further, more specific testing is required. The species included in the coliform group include, but not

limited to; *Escherichia*, *Citrobacter*, *Klebsiella*, *Enterobacter cloacae* and *Citrobacter freundii* (Gerba, 2000).

### 2.8.2 Thermotolerant (Faecal) coliforms

Thermotolerant coliforms are a subset of the total coliform group. The coliform species that are considered to be part of this subset are only those that have the ability to ferment lactose and grow at a temperature of 44.5 °C. Often, the term 'thermotolerant' is used interchangeably with 'faecal', incorrectly combining temperature and origin classifications. Given that a number of natural environments exists that maintain temperatures as high as those found in the human gut, it is important to use the terminology correctly as to not confuse the implications of an indicator, thus the term "thermotolerant coliform" is therefore more correct and is becoming more commonly used. Nonetheless, the presence of thermotolerant coliforms nearly always indicates faecal contamination (WHO, 1996). This group of bacteria comprises the bacterial genus *Escherichia*, and to a lesser extent *Klebsiella*, *Enterobacter* and *Citrobacter* species all fall into the sub-category of thermotolerant bacteria (Gerba, 2000). Among these organisms, only *E. coli* is specifically of faecal origin. However, concentrations of thermotolerant coliforms are usually directly related to that of *E. coli* and thus can be used as a surrogate test for *E. coli* (Chian, 2001). The WHO drinking water guideline states that zero thermotolerant coliform or *E. coli* may be found per 100 mL of drinking water.

### 2.8.3 Faecal coliforms

Faecal coliforms are a more defined subset within the thermotolerant coliform group. Many of these organisms are physiologically similar to their parents set, however their origin is

known to be the gut of human or other warm-blooded animal species (Samantha, 2012). When they are outside the host's body, these organisms cannot live for long because their survival is largely dependent on the host. The faecal coliforms are composed of both pathogenic and non-pathogenic bacteria. As such, their detection in a sample of drinking water is an indication that the water is contaminated with sewage or human and other warm blooded animal faeces.

### 2.8.4 *Escherichia coli*

*Escherichia coli* is a specific subset of the thermotolerant coliform bacteria which possess the enzyme β- galactosidase and β-glucoronidase that hydrolyzes 4-methyl-umbelliferyl-β-D-glucuronide (MUG). They are found abundantly in human faeces (as much as $10^9$ per gram of fresh faeces) and warm- blooded animals (Chian, 2001). Sewage, treated effluents, all natural waters and soils that are subject to recent faecal contamination from humans or wild animals will contain *E. coli.* Usually, *E. coli* cannot multiply in any natural environment and they are thus used as specific indicators for faecal contamination (WHO, 1996). There are many strains of *E. coli*, only a small fraction of which cause disease. Most commonly is strain 0157:H7 that is implicated for severe cases of breaches in public health, however, the presence of any strain of *E. coli* is likely indicative of faecal contamination of water source and further testing is required (Samantha, 2012). A pictorial representation of the relationship between these groups of coliform is presented in Figure 2.1.

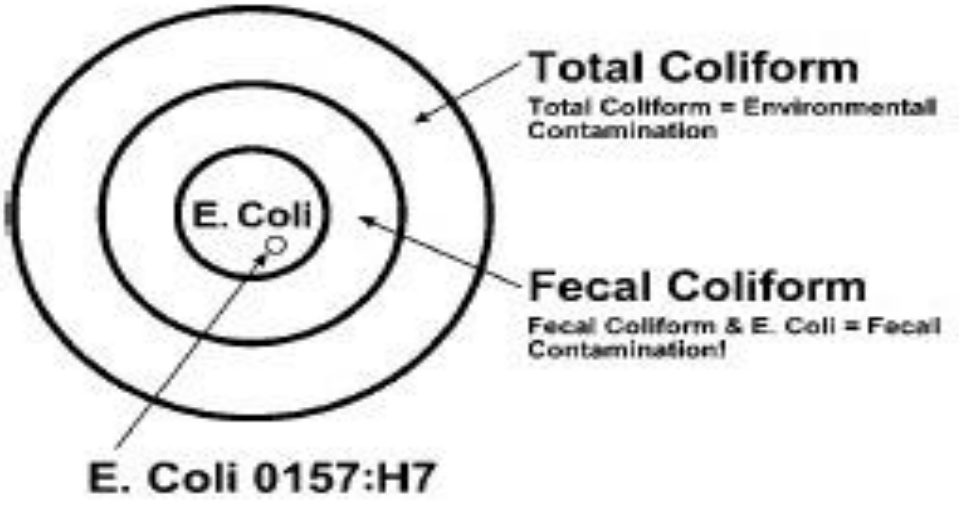


**Figure 2.1.Relationship between total coliforms, faecal coliforms and *E. coli***

Source: (Samantha, 2012).

## 2.9 Public Health Implications of Faecal Contaminated Drinking Water Sources

The human health depends on reliable access to safe drinking water (Jimmy *et al*., 2012). However, if water is faecally polluted, it spread diseases in consumers to a great number of people (Nakade, 2013). Worldwide, contamination of drinking water poses a major health treat, for examples, the World Health Organization estimated in 2000 assessment that there are four billion cases of diarrhea yearly in addition to millions to other cases of illness associated with lack of access of clean water (WHO, 2000). It is well established that infectious diseases are transmitted primarily through water supplies contaminated with human and animal excreta majorly faeces (Deepesh *et al*., 2013). Faecal-oral diseases account for the dominant health outcome of the unsafe water, sanitation and hygiene (WASH) risk factors (Annette Pruss-Ustun *et al*., 2010), and the fact that faecal-oral pathogens can be spread through water route is well established (Andersson and Bohan,

2001). In general terms, the greatest microbial risks in drinking water are associated with ingestion of drinking water that is contaminated with human or animal faeces (WHO, 2008b).

Disease-causing organisms (pathogens) transmitted through drinking water are predominantly of faecal origin and therefore known as enteric pathogens (Ashbolt, 2014). These pathogens in human or animal wastes get into drinking water sources in diverse of ways. Ground water maybe contaminated through failure of an on-site sewage disposal system e.g. septic system which causes direct infiltration to groundwater, or contamination of shallow well from an offal or dairy-shed waste pit, especially during a drought when groundwater level is low. The surface water may also be contaminated through runoff of animal wastes to surface water from pastures, discharge of untreated or improperly treated sewage to rivers and reservoirs, over application of dairy effluents onto pastures, causing contaminated water to run off to surface water and/or infiltrate to groundwater.

Waterborne disease outbreak is said to happen if two or more persons experience a similar illness after consumption or use of water intended for drinking and epidemiological evidence showed the water as the source of illness. It has been estimated that yearly, nearly a million people die due to waterborne diseases, around 37.7 million are affected by waterborne diseases, 1.5 million children are estimated to die of diarrhea alone, and 73 million working days are lost due to waterborne disease annually (Pal *et al*., 2018). In low and middle in-come countries, diarrhea-related diseases are the tenth leading cause of death (Murray and Schaller, 2010). Pal *et al*. (2018) also reported that unsafe water and poor sanitation cause more than 500,000 infant deaths each year in the pacific region of Asia. Approximately, 11 % of child deaths worldwide are attributed to a diarrheal disease and of

these cases, 88 % are caused by unsafe water or improper sanitation. Thus, the quality of drinking water cannot be overlooked when assessing the role of water in public health. The most common diseases that can be transmitted through contaminated water are diarrheal diseases such as Bacillary dysentery, Typhoid, Paratyphoid, Cholera, Salmonellosis, Colibacillosis, Amoebiasis, Giardiasis and Cryptosporidiosis (Skider *et al*., 2013).

Diarrhea alone kills more children than malaria and tuberculosis together worldwide (Pal *et al*., 2018). Acute microbial diarrheal diseases are a major public health problem in developing countries and the category of people affected by diarrheal diseases are those with the lowest financial resources and poorest hygienic facilities (Seas *et al*., 2000). However, microbial waterborne disease also affects developed countries, in the USA for example, it has been estimated that yearly, 560,000 people suffer from severe waterborne diseases, and 7.1 million suffer from a mild to moderate infections, this resulting to 12,000 deaths yearly (Medema *et al*., 2003).Therefore, Water quality is a critical issue to ensure public health. Everyday activity of human is closely associated with water, thus, providing safe drinking water is one of the important public health priorities.

Diarrheal diseases such as *E. coli* infection, gardiasis and typhoid are very common diseases that happen due to using unsafe water. Diverse groups of individuals with low or compromised immunity are more susceptible to waterborne diarrheal diseases (Medema *et al*., 2003). For example, children and infants, due to the improper developed immune system are more vulnerable to pathogen related to water borne disease and also cancer patients, HIV/AIDS patients, transplant patients. The World Health Organization recommends that water with any amount of faecal coliforms should not be consumed (Ayeni, 2014). The coliforms or Enterococci are usually faecal indicators that indicates human or animal wastes

in water and these organisms causes cramps, nausea, diarrhea, headache, hemolytic uremic syndrome, serious kidney disease with potential lifelong complications (Hrudey and Walker, 2005). Bacteria such as *Vibrio cholerae*, *Salmonella typhi* and several species of *shigella* normally cause serious disease like cholera, typhoid fever and bacillary dysentery respectively.

Enteric viruses, such as hepatitis A, Polio viruses, Norwalk virus, Rotavirus, Echoviruses and Coxsackie viruses are excreted in the feces of infected individual as they harbor them in their intestine and these may contaminate water intended for drinking. Polyoma virus and cytomegalovirus which are excreted in urine can also be spread through water (Cannon *et al*., 2011). These enteric viruses that are transmitted through water infect the gastrointestinal or respiratory tracts causing diarrhea, fever, hepatitis, paralysis and meningitis in humans (Medema *et al*., 2003). Rotavirus is the leading cause of severe acute diarrhea in children under the age of five, resulting in over half a million deaths annually worldwide (WHO, 2008b). Viral infections can cause great morbidity in the elderly, immunocompromised individual and pregnant women (Gall *et al*., 2015).

### 2.9.1 Public health implication of *Klebsiella pneumoniae* in drinking water

*Klebsiella pneumoniae* is Gram negative, non motile, it is usually encapsulated rod shaped bacteria. It belongs to the family Enterobacteriaceae. Members of the Enterobacteriaceae family are generally facultative anaerobic, and range from 0.3 to 1.0 mm in width and 0.6 to 6.0 mm in length. The genus consists of 77 capsular antigens (K antigens), leading to different serogroups (Deepesh *et al*., 2013).

*Klebsiella* is ubiquitous and occur predominantly in the tropical and subtropical regions, including forest environments, vegetation soil, water and mucosal membrane of host species (Janda and Abbott, 2006) and they may multiply greatly waters rich in nutrients such as pulp mill waters, textile finishing plants and sugar cane processing operations. In drinking water distribution systems, they are known to colonize washers in taps. The organism can grow in water distribution systems and are also excreted in the feces of many healthy humans and animals, they are readily detected in sewage-polluted water (WHO, 2007). *Klebsiella pneumoniae* occurs in the nasopharynx and intestinal tracts of humans as a saprophyte and it is one of the leading causes of community acquired pneumonia (Deepesh *et al*., 2013). Furthermore, it has been implicated to cause primary liver abscess and of microbial fascial space infections in diabetic patients (Janda and Abbott, 2006). Some reports have it that *Klebsiella* spp. is responsible for 16 to 43 % of central nervous system (CNS) infections and brain abscesses (Podschun *et al*., 2001). Deepesh *et al*. (2013) also reported the virulence of environmental strains of *K. pneumoniae* as equally virulent as clinical strains.

*Klebsiella* can cause nosocomial infections, and contaminated water and aerosols may be a potential source of the organism in hospital environment and other health care facilities. It has been reported that 7 to 14 % of all cases of nosocomial pneumonia, 4 to 15 % of septicemia, 6 to 17 % of urinary infections, 3 to 20 % of neonatal septicemias and 4 to 17 % of intensive care unit (ICU) infections and 2 to 4 % of wound infections are *Klebsiella* spp. related (Deepesh *et al*., 2013). *Klebsiella* spp can as well cause bacteremia and hepatic infections and have been isolated from a number of unusual infections like endocarditis, peritonitis, acute cholecytitis, crepitant myonecrosis, pyomyositis, fascial space infections of the head and neck and septic arthritis. They are also important opportunistic pathogens

especially among the immunocompromised. Its pathogenicity factors include adhesions, siderophores, capsular polysaccharides (CPLs), cell surface lipopolysaccharides (LPSs) and toxins that plays specific roles in the pathogenesis of these species (Janda and Abott, 2006).

Cells of *Klebsiella* spp. may adhere and attack upper respiratory tract epithelial cells, cells in gastrointestinal tract, endothelial cells, or uroepithelial cells then colonization of mucosal membranes. Some common underlying conditions include alcoholism, diabetes mellitus, chronic liver disease (cirrhosis), chronic renal failure, cancer, transplants, burns, use of catheters (Janda and Abott, 2006).

### 2.9.2 Public health implications and of *Escherichia coli* in drinking waters

The microbiological quality control of water for human consumption requires a simple and reliable assessment of the presence of pathogens (Cabral, 2010). One of the most abundant bacteria associated with the sanitary risk of water is *Escherichia coli* (Carrillo-Gomez, 2019). *Escherichia coli* also known as *E. coli* is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (Rock and Rivera, 2014). They are about 2.0 micrometers (µm) long and 0.25-1.0µm in diameter with a cell volume of 0.6- 0.7µm. *Escherichia coli* does not survive well outside of the intestinal tracts and hence its presence in environmental samples, food or water usually indicates recent faecal contamination of poor sanitation practices (Odonkor and Ampofo, 2013). During rainfall, snow melts or other types of precipitation, *E. coli* may be washed into creeks, rivers, streams and groundwater and when these water are used as source of drinking water untreated, *E. coli* may end up in drinking water (Saati and Faidah, 2013).

The presence of *E. coli* in water does not indicate directly that pathogenic microorganisms are in the sample, but it does indicate that there is a great risk of the presence of other faecal-borne bacteria and viruses, many of which are pathogenic, like the *Salmonella* spp or hepatitis A virus (Brussow *et al*., 2004). Hence, *E. coli* is widely used as an indicator organism to identify water sample that may contain unacceptable levels of faecal contamination (Odonkor and Ampofo 2013), and it has become a quality parameter for the creation of regulations or standards where the maximum admissible limits of this bacterial indicator are established (Carrillo-Gomez, 2019).

Although, most *E. coli* strains are harmless as they are part of the normal flora of the guts, and can benefit their hosts by producing vitamin K2 or by preventing the establishment of pathogenic bacteria within the intestine (Odonkor and Ampofo, 2013), certain strains are pathogenic and cause diseases such as watery diarrhea, bloody diarrhea, urinary tract infection, meningitis and sepsis which can lead to death (Cho *et al*., 2018). This pathogen has been responsible for waterborne outbreaks in human through contaminated drinking water both in developing countries and industrialized countries (Probert *et al*., 2017). Environmental water sources are liable to contamination by this pathogen from both humans and animals. Possible human sources include wastewater discharge, sewage leaks and failing septic tanks as well as municipal, residential, medical and industrial waste facilities. Animal sources include runoff from animal farms, land application of animal manure, pet wastes from parks and wildlife. Since surface waters are often used for recreational and drinking purposes, the presence of pathogenic *E. coli* in water ways may increase the likelihood of human infections after exposure to these water sources (Cho *et al*., 2018).

## 2.10 Water and Water-borne Diseases

Countries all over the world are concerned with the effects of unclean drinking water because water-borne diseases are a major cause of morbidity and mortality (Nwabor *et al*., 2016). Water-borne diseases are typically caused by enteric pathogens, which are mainly excreted in feces by infected individuals, and ingested by others in the form of faecally contaminated water or food (Chian, 2001). Clean drinking water is vital for overall health and plays a substantial role in infants and child health and survival (Anderson *et al*., 2002). The WHO (2005) estimated that globally, about 1.8 million people die from diarrheal diseases annually, many of which have been linked to consumption of contaminated waters and seafood. Throughout the less developed part of the world, the proportion of households that use unclean drinking water source has declined, but it is extremely unlikely that all households will have a clean drinking water source in the foreseeable future (Nwabor *et al*., 2016). In Nigeria, a vast majority of people living along the course of water bodies still source and drink from rivers, streams and other water bodies irrespective of the state of these water bodies without any form of treatment (Nwabor *et al*., 2016). Polluted surface waters can contain large varieties of pathogenic microorganisms and these organisms are often faecal in origin, which may be from point sources or non point sources such as domestic and wild animal's defecation, malfunctioning sewage and septic systems, storm water drainage and urban runoff (Chigor *et al.,* 2012). Contamination of water by faecal matter is globally recognized as one of the leading causes of waterborne diseases. The potential of drinking water to transport microbial pathogens to great numbers of people thereby causing subsequent illness is well documented in countries at all levels of economic development (Nwabor *et al*., 2016). For example, the outbreak of Cryptosporidiosis of 1993

in Milwaukee, Wisconsin, in the United States where it was estimated that about 400, 000 individuals suffered from gastrointestinal symptoms due to *Cryptosporidium*(Nwabor *et al*., 2016). A more recent outbreak involving *Escherichia coli* 0157:H7, the most serious of which occurred in Walkerton, Ontario Canada in the spring of 2000 resulted in six deaths and over 2,300 cases. The number of outbreaks reported throughout the world demonstrated that transmission of pathogens by drinking water remains a significant cause of illness (Nwabor *et al*., 2016).

In Africa, it has been estimated that every child has five (5) episodes of diarrhea per year and that 800,000 children die each year from diarrhea and dehydration (Raji and Ibrahim, 2011). According to Wittenberg (1998), infective diarrhea is predominantly a disease of poverty, overcrowding and environmental contamination.

In Nigeria, cases of water-related diseases abound. Common examples include cholera, hepatitis and typhoid (Adeyinka *et al*., 2014). Several cases of water-borne diseases linked to intake of contaminated drinking water with pathogens have been reported in several towns (Ibrahim *et al*., 2000; Adekunle *et al*., 2007; Biu *et al*., 2009). The role of water as a vehicle for the transmission of all manners of water related illness is no longer a subject for debate; this fact is contained in ancient histories and books. Poor sanitary quality of drinking water and unsafe drinking water or lack of clean water supply has been reported in some regions in North-Central States, Nigeria due to faecal contamination (Bala, 2006; Adabara *et al*., 2011; Bala *et al*., 2016; Oyedum *et al*., 2016; Abdulrahman *et al.,* 2018). Water-borne disease outbreak as a result of consumption of contaminated drinking water has been documented. For example, the recent cholera outbreak in Nigeria began in Borno State in August 2017 with a combined total of 5,365 cases and a final death toll of 61 people. The

outbreak was caused by poor sanitary conditions and the lack of clean water or ingestion of contaminated water with faecal matter (UNICEF Nigeria, 2018). Twenty States reported outbreaks of cholera across Nigeria with Bauchi, Zamfara, Borno, and Katsina States accounting for 74 % of the cumulative cases (WHO, 2019). Kuta *et al*. (2014) reported that a total of 179 people suffered from diarrhea, 195 people from typhoid fever, and 12 from streptococci and others from varying diseases as a result of faecal contamination of drinking water in Lapai Local Government Area of Niger State in 2008. (Gimba, 2011) noted that the water for drinking in Bosso town did not conform to WHO standards for potable water. Oyedum *et al*. (2016) also reported the presence of faecal contaminants in water sources in Bosso town, Niger State. Table 2.1 shows some of the diseases related to water and sanitation, which are endemic in sub Saharan Africa as well as their route of infection.

**Table 2.1: Diseases Related to Water and Sanitation Endemic in Sub-Saharan Africa**

| Group | Disease | Route of leaving host | Route of infection |
|---|---|---|---|
| Disease, which are | Cholera | Faeces | Oral |
| often water borne | Typhoid | Faeces/urine | Oral |
| | Infectious hepatitis | Faeces | Oral |
| | Giardiasis | Faeces | Oral |
| | Amoebiasis | Faeces | Oral |
| | Dracunculiasis | Cutaneous | Oral |
| Disease, which are | Bacillary dysentery | Faeces | Oral |
| often associated with poor | Scabies | Cutaneous | Oral |
| hygiene | Lice and typhus | Bite | Bite |
| | Amoebiasis | Faeces | Oral |

| | | | |
|---|---|---|---|
| | Paratyphoid fever | Faeces | Oral |
| | Trachoma | Cutaneous | Cutaneous |
| Diseases, which are often | Ascariasis | Faecal | Oral |
| related to inadequate | Trichuriasis | Faecal | Oral |
| sanitation | Hookworm | Faecal | Oral |

Source: Nwabor *et al*.(2016).

### 2.11 Quality of Drinking Water and Drinking Water Quality Guideline

Water quality refers to suitability of water for certain purpose. Hence, quality of drinking water is the suitability of water for drinking and domestic uses including personal hygiene. A good safe drinking water is that, which is aesthetically acceptable and does not contain pathogenic agents and dangerous chemical substances (WHO, 2009). The consequences of contaminated water are so all pervasive that they defy effort to draw up global balance sheet. The implication of dirty water includes not only loss of lives but also of socioeconomic momentum. For example, World Bank estimated that Peru's loss due to reduction in agriculture exports and tourism was about $100,000 in the first 10 weeks after cholera outbreak in January 1991 (John, 1993). Due to the importance of water for sustenance of life and livelihood, the World Health Organization published the first guideline for drinking water quality and subsequent editions were published in order to update previous publications (WHO, 2009). It is a United Nation's document for member states to either adopt or adapt in their own countries based on their local situations (Gimba, 2011). Assessing quality of drinking water is a rigorous task which entails assessing the physical, chemical and bacteriological parameters of water. However, bacteriological analysis and *E.*

*coli* count is the most appropriate indicator of faecal pollution in assessing quality of drinking water (Gimba, 2011). In 2005, the National Council on Water Resources (NCWR) recognized the need to urgently establish acceptable Nigerian Standards for Drinking Water Quality (NSDWQ) because it was observed that the Nigerian Industrial Standards for Potable Water developed by the Standard Organization of Nigeria (SON) and the National Guidelines and Standards for Water Quality in Nigeria developed by the Federal Ministry of Environment did not receive a wide acceptance by all stakeholders in the country. In accordance to the above mentioned, drinking water must meet the minimum requirements set out by NSDWQ as stated in Tables 2.2 and 2.3.

**Table 2.2**: **Permissible Limits for Inorganic Constituents in Drinking Water**

| Parameter | Unit | Maximum limit |
|---|---|---|
| pH | - | 6.5 – 8.5 |
| Total dissolved solids | mg/L | 500 |
| Hardness ($CaCO_3$) | mg/L | 150 |
| Conductivity | μS/cm | 1000 |
| Free residual chlorine | mg/L | 0.2- 0.25 |
| Turbidity | NTU | 5 |
| Total suspended solid | mg/L | 5 |

Source: Nigerian Standards for Drinking Water Quality (2007).

**Table 2.3: Maximum Permissible Microbiological Limits**

| Parameter | Unit | Maximum Limit | Health Impact |
|---|---|---|---|
| Total coliform count | cfu/mL | 10 | Indication of faecal contamination |
| Thermotolerant Coliform or *E. coli* | cfu/100mL | 0 | Urinary tract infection, bacteremia, diarrhea, acute renal failure, haemolytic |
| Faecal *Streptococci* | cfu/100mL | 0 | Indication of recent faecal contamination |
| *Clostridium perfringens* spores | cfu/100mL | 0 | Index of intermittent faecal contamination |

Source: Nigerian Standards for Drinking Water Quality (2007).

## 2.12 Physicochemical Parameters of Drinking Water

### 2.12.1 pH

pH is classed as one of the most important water quality parameters. Its measurements relates to the acidity or alkalinity of the water. A sample is considered to be acidic if the pH is below 7.0. Meanwhile, it is alkaline if the pH is higher than 7.0. Acidic water can lead to corrosion of metal pipes and plumbing system. Meanwhile, at alkaline pH, there is a progressive decrease in the efficiency of the chlorine disinfection process (Rahmanian *et al*., 2015). The normal pH range mentioned in WHO and NSDWQ guidelines is between 6.5 and 8.5 (NSDWQ, 2007).

### 2.12.2 Electrical conductivity

Electrical conductivity is the ability of any medium to carry an electric current. The presence of dissolved solids such as calcium, chloride and magnesium in water samples carries electric current through water (Rahmanian *et al*., 2015). The maximum allowable level of conductivity in drinking water is 1000μS/cm (NSDWQ, 2007).

### 2.12.3 Turbidity

Turbidity is the cloudiness of water as a result of variety of particles and is another key parameter in drinking water analysis. This also relates to the content of disease-causing organisms in water, which may come from soil runoff (Rahmanian *et al*., 2015). The standard recommended maximum turbidity limit set by WHO and NSDWQ for drinking water is 5 Nephelometric turbidity units (NTU) (NSDWQ, 2007).

### 2.12.4 Total suspended solids (TSS)

Total suspended solid is the dry weight of suspended particles that are not dissolved in a sample of water that can be trapped by filter that is analyzed using a filtration apparatus. These particles are larger than 2 microns and are a significant factor in observing water clarity. The maximum TSS limited recommended by NSDWQ is 25mg/L (NSDWQ, 2007).

### 2.12.5 Total dissolved solids (TDS)

Total dissolved solids are the inorganic salts (principally calcium, magnesium, potassium, sodium, bicarbonates, chlorides and sulphates) and small amounts of organic matter that are present as solution in water (Rahmanian *et al*., 2015). TDS can affect the taste of drinking

water. The set standard value for TDS by NDWQS is 1000mg/L (NSDWQ, 2007). Water becomes increasingly unpalatable and objectionable at level greater than 1000-1200mg/L.

### 2.12.6 Residual chlorine

Water treated by chlorination usually contains some amount of chlorine which provides a margin of safety against subsequent microbial contamination such as may occur during storage and distribution. The minimum recommended concentration of free chlorine (residual chlorine) is 0.5mg/L (Gimba, 2011).

### 2.12.7 Water hardness

Small water supplies using groundwater often encounter significant levels of hardness, but some larger surface water supplies also have the same issue. Water hardness is caused by the presence of calcium and magnesium ions in water. Calcium concentrations up to and exceeding 100mg/L are common in natural sources of water, especially groundwater. Magnesium is present in natural groundwater usually at lower concentrations, hence calcium based hardness usually predominates (WHO, 2011b). The exposure to hard water has been suggested to be a risk factor that could exacerbate eczema (Langan, 2009). A suggested explanation relative to hard water is that increased soap usage in hard water results in metal or soap salt residues on the skin or clothes that are not easily rinsed off and that lead to contact irritation (Thomas and Sach, 2000).

### 2.12.8 Temperature

Temperature refers to degree of hotness or coldness and it can be measured in degree Celsius. It is important because of its influence on water chemistry. The rate of chemical

reaction generally increases at higher temperature. Groundwater with higher temperature can dissolve more minerals from the rocks and thus higher electric conductivity. Temperature exerts a major influence on biological activities and growth. Turbidity and color are indirectly related to temperature as temperature affects coagulation. The efficiency of coagulation is dependent on temperature and the optimum pH of coagulation decreases as temperature increases (Kale, 2016).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Description of the Study Area

Chanchaga Local Government is one of the twenty-five (25) Local Government Areas in Niger State with its headquarters in Minna, the state capital. It lies between latitude 9°35'00" to 9°41'00" and longitude 6°25'00" to 6°37'00". It covers an area of 72 km² with total population of 201,429 people (Sule *et al*., 2014). It is made up of 11 wards; Limawa 'a', Limawa 'b', Makera, Minna central, Minna south, Nasarawa 'a', Nasarawa 'b', Nasarawa 'c', Sabon Gari, Tudun Wada north and Tudun Wada south. The relief of the study area is relatively flat and rocky at the river channel drained by River Chanchaga. Chanchaga Local Government Area falls within the Guinea Savanna Belt. It is covered with grassland, which is basically used for agricultural purposes. The area is also characterized by two climatic seasons, each season lasting for about six months. Chanchaga has total annual rainfall of between 1200mm in the North to 1600mm in the South. The dry season, which is characterized by the N-E trade wind begins in November and usually ends in March. The mean maximum temperature remains high throughout the year, hovering about 32 °C, particularly between March and June, while the lowest temperature occurs usually between the months of December and January during the harmattan period (Akande *et al*., 2016). Sources of drinking water in the study area included well water, tap water, household stored borehole, water vendors (*mai ruwa*) and sachet water. Figure 3.1 is a map of Nigeria showing Niger State, Figure 3.2 is a map of Niger State showing the twenty-five (25) Local Governments areas while Figure 3.3 shows the map of Chanchaga Local Government Area (indicating sampling points).

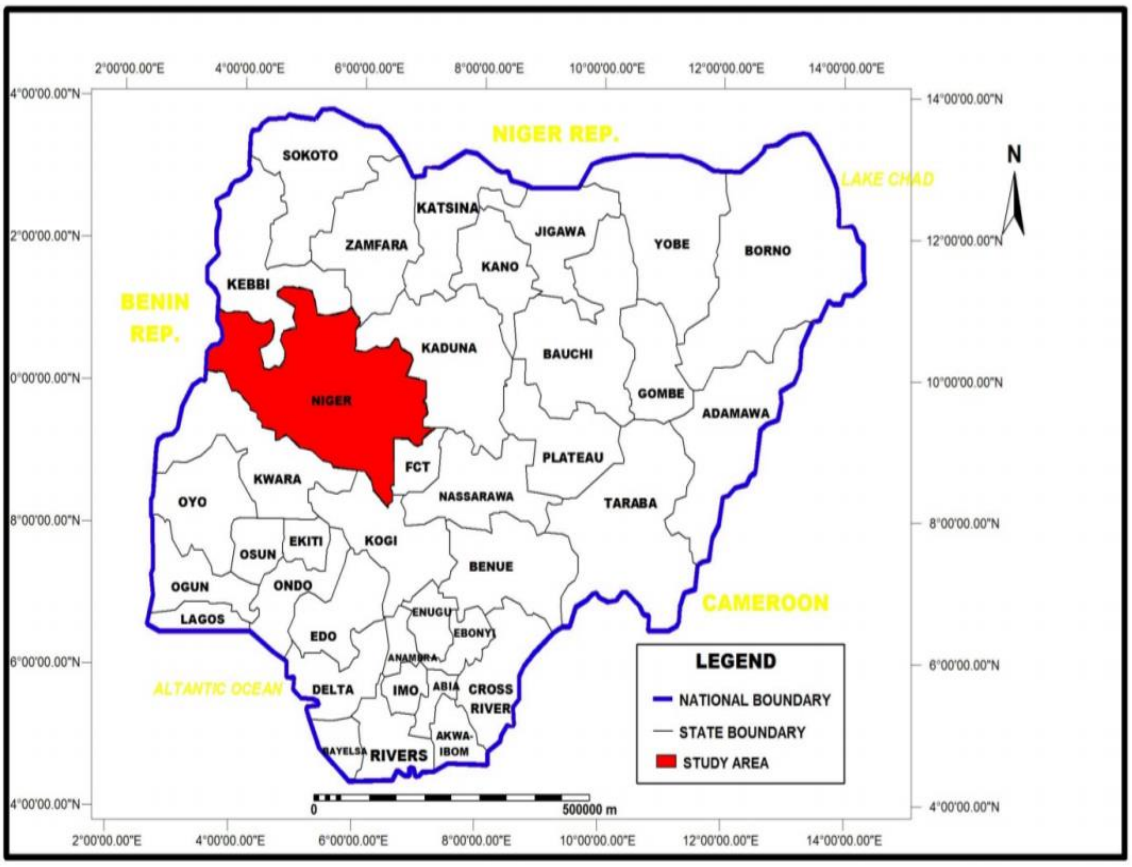


**Figure 3.1: Map of Nigeria indicating the location of Niger State in Nigeria**

**Source: Department of Urban and Regional Planning, FUT Minna**.

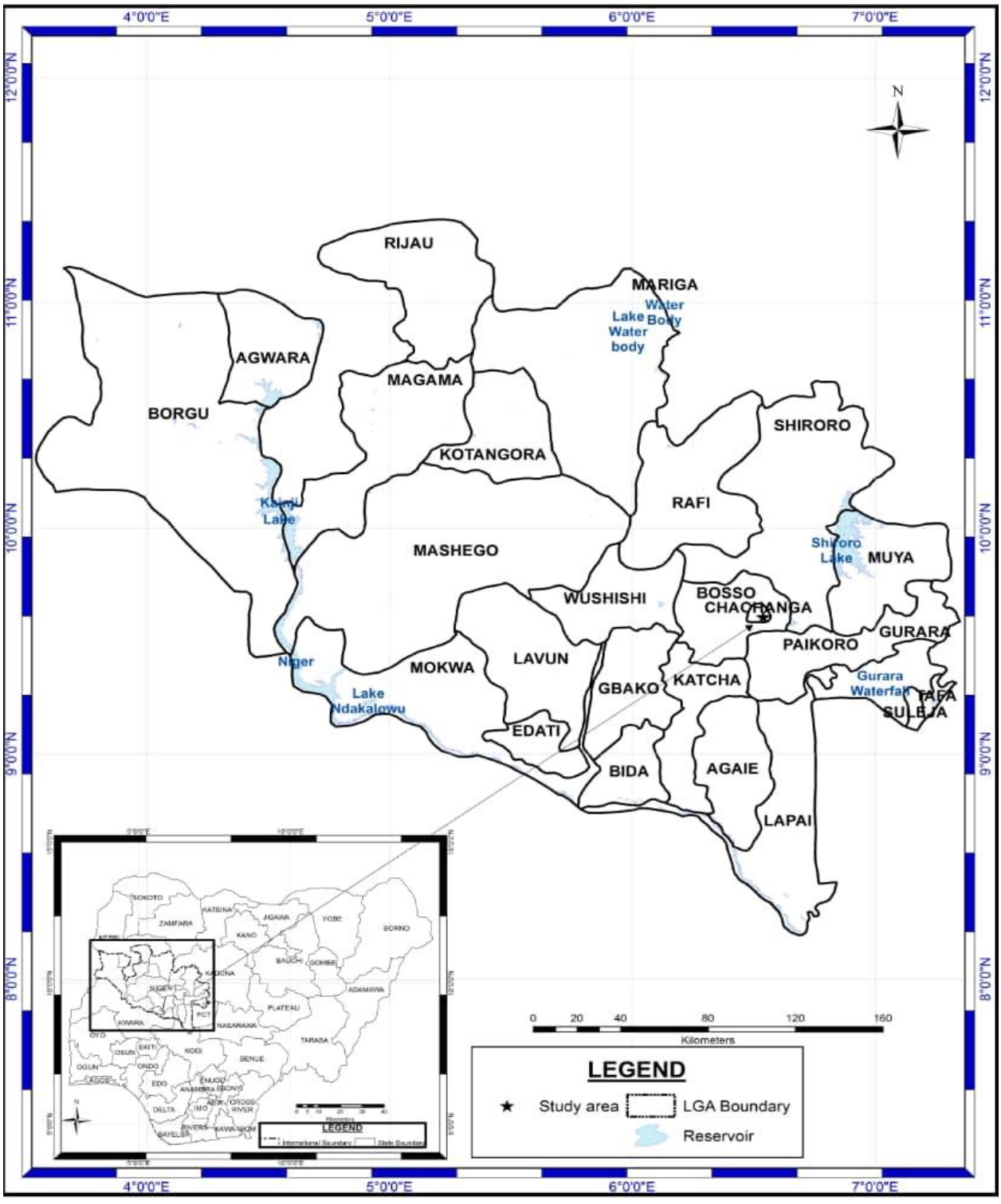


**Figure 3.2 Map of Niger State showing Chanchaga Local Government Area**

**Source: Department of Geography, FUT Minna**.

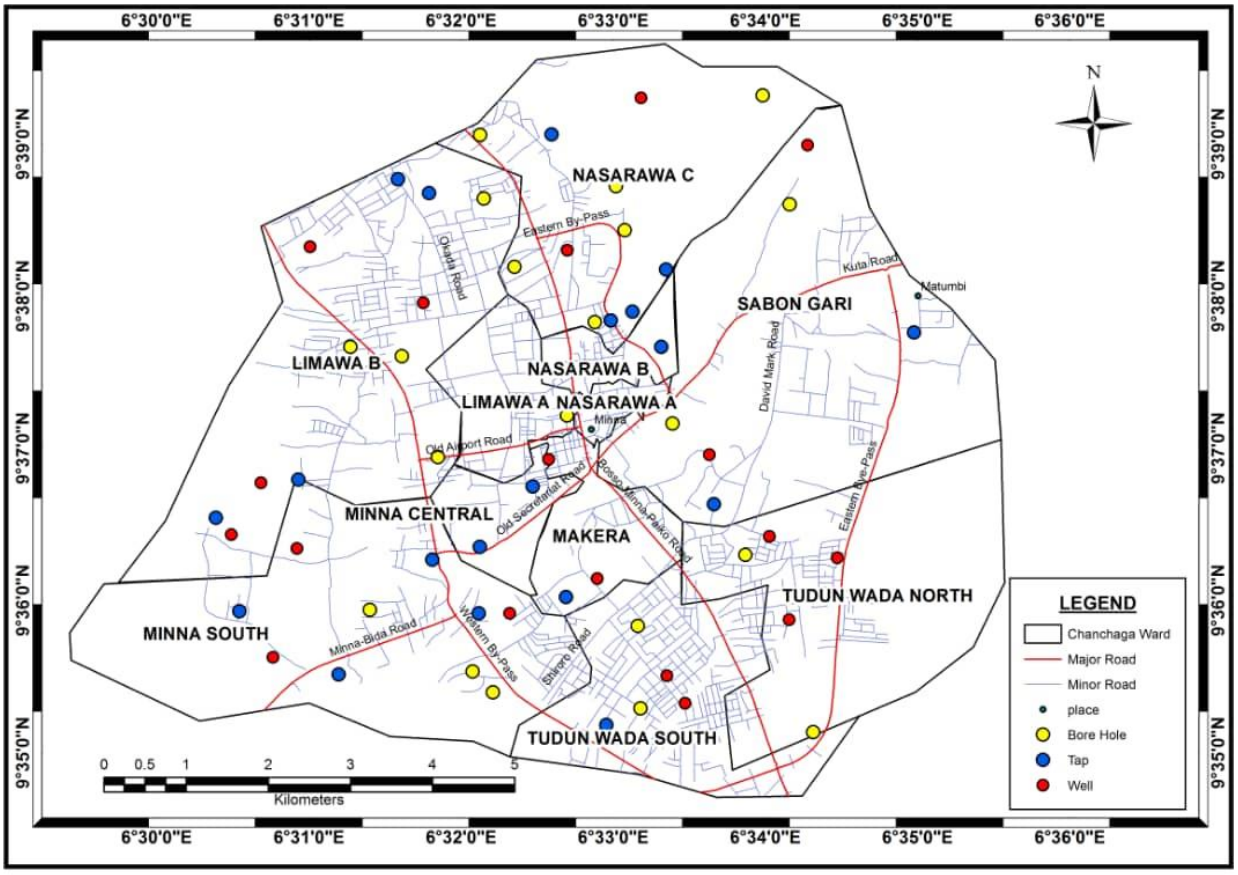


**Fig 3.3 Map of Study Area Showing Sampling Points**

**Source: Department of Geography, FUT Minna**.

### 3.2 Study Design

The study design is a descriptive study.

### 3.3 Ethical Consideration

In collection of the water samples, the nature of the study was explained to respondents and verbal consent was obtained and the brand names for all the water sachet water samples in this study were excluded

### 3.4Determination of Sample Size

Based on convenience, a total of 80 samples were collected and analyzed in the study area. They are 20 wells, 20 public taps/pipe borne water, 20 household water samples and 20 different brands of sachet water.

### 3.5 Administration of Questionnaire

The sample outline consists of all sources of drinking water at different locations within the study area. 106 household (10 households in 10 ward and 6 households in Limawa A) heads of families were interviewed voluntarily between August 4$^{th}$ and September 2$^{nd}$2019 to determine the sources of their drinking water, to know if they were satisfied with the water qualities and also to know their mode of water treatment (where applicable).

### 3.6 Sampling

Drinking water sources that were sampled included: Tap water, well water, packaged sachet water and household stored water. The selection of the regions where the drinking water sources were sampled was based on the availability of the different sources, the frequency of their use and being depended upon by the larger populations of the people that resides there.

### 3.7 Sample Collection and Processing

Water samples were collected aseptically using 250mL sterile sampling containers. In collecting water sample from the borehole and tap, the mouth and the outer parts of the borehole taps were sterilized with the flame of a cigarette lighter, it was allowed to cool by running the water for a minute before collection. At the point of collection, the containers

were rinsed three times with the borehole and tap water sample prior to collection(WHO, 1998; Bala, 2006; Kihupi *et al*., 2016). All of the collected water samples were immediately transported to the Microbiology Laboratory of Federal University of Technology, Minna and stored in the refrigerator at 4 °C in the laboratory prior to analysis to avoid microbial action affecting their concentration. Water source sampling from wells involved drawing water using a bucket and taking 250mL into a sterile container (Bala, 2006; Too *et al*., 2016). This was considered to be more representative of what is actually being consumed by the household. The bottles were properly corked and transported to the Microbiology laboratory of the Federal University of Technology, Minna, for analysis. Sachet water samples were purchased from roadside vendors and transported to the Laboratory in the Department of Microbiology, Federal University of Technology, Minna, for immediate analysis.

### 3.8 Bacteriological Water Quality Determination

#### 3.8.1 Enumeration of microorganisms

##### 3.8.1.1 *Enumeration of total coliforms*

The method of membrane filtration as described by Noble *et al*. (2003) was used. A vacuum pump machine (VE 115D, Zenokoon, USA) was used. This apparatus was made up of a filter cup, filtering head, filtering membrane, clamp and a conical flask. Sterilization of the membrane filter apparatus was done by wrapping with aluminum foil and kept in the oven at 100 °C for an hour before each use. The vacuum pump machine was connected to a membrane filter apparatus and connected to a power source. The membrane filters had a known uniform porosity of predetermined size (0.45µm) small enough to trap microorganisms. Using membrane filtration technique, sample was passed through the

membrane using a filter funnel and vacuum system. Organisms in the sample were concentrated on the filter paper. The membrane filter with its trapped bacteria was placed in a specific plate containing a pad saturated with the appropriate medium (Membrane Lauryl sulphate broth was used in this research) using sterile forceps. Plates were incubated at 35 °C for 24 hours. Discrete colonies formed on membrane filter were counted and recorded. The results were recorded and expressed in cfu/100mL.Population of total coliforms in each water sample was enumerated using Membrane Lauryl sulphate broth as described by Brenner *et al*. (1993). A sterile tong was used to introduce the sterile membrane filter paper of 0.45µm pore size on the filtering head and 100mL from each water sample was poured into the calibrated filter cup, after filtration, the filter paper was removed and introduced into sterile plate containing filter pad and already prepared Membrane Lauryl sulphate broth near flame, plates were incubated for 24 hours at 35 °C and afterwards filter papers which retained organisms present in water samples were examined for visible growths and colony count (Brenner *et al*., 1993).

#### 3.8.1.2 *Enumeration of thermotolerant coliforms*

Pour plate method was employed for isolation of thermotolerant coliforms from water samples as described by Tamagnini and Gonzalez (2001). One (1) mL of water was transferred using a sterile pipette on to sterile plates, molten cool Eosin Methylene Blue agar (EMB) was poured into the Petri dish containing water sample, it was gently swirled together and plates were incubated at 44.5 °C for 48 hours after which plates were observed for growth while biochemical and morphological characterization were carried out for identification.

#### 3.8.1.3 *Isolation of pathogenic E. coli*

Isolates from Eosin Methylene blue agar were inoculated on a sterile MacConkey Sorbitol Agar (MSA), which is a selective medium for the growth and isolation of pathogenic *E. coli*. Plates were incubated at 44.5 ºC for 48 hours after which the plates were observed for visible growth.

### 3.9 Identification of Isolates

#### 3.9.1 Morphological and biochemical identification the of isolates

#### 3.9.1.1 *Gram's staining*

A drop of water was added on clean grease free slide. A sterile wire loop was used to pick a distinct colony to make a thin smear, air dried and heat fixed by passing through flame for about four times. The slide was placed on a staining rack, two drops of crystal violet were added and allowed to stand for 60 seconds and then rinsed with distilled water. Two drops of Grams iodine were added for 60 seconds and rinsed with distilled water. The slide was decolorized with alcohol for 60 seconds and rinsed with distilled water. Two drops of safranin was added and allowed to stand for 2 minute, rinsed with distilled water and air dried. The stained slide was observed under the microscope using x 100 objective (Fawole and Oso, 2007).

#### 3.9.1.2 *Triple sugar iron test*

To each 10 mL of peptone water in a test tube, 1.5g of each of the sugars (glucose, sucrose and lactose) was dissolved and 3 drops of 0.01% phenol red were added. Durham tubes were

introduced in inverted position in the test tubes and autoclave at 121°C for 15 minutes. Pure bacterial culture isolates were inoculated and incubated at 37 °C for 24 hours. The test tubes were observed for colour change and gas production. Colour change from pink to yellow indicated acid production while a black portion indicated the production of hydrogen sulphide (Cheesebrough, 2006).

#### 3.9.1.3 *Oxidase test*

Filter paper was put on a clean Petri dish to which two drops of oxidase reagent were dropped on the filter paper. The test isolate was smeared on the filter paper. Colour change from blue to purple indicated a positive result while no colour change indicated a negative result (Cheesebrough, 2006).

#### 3.9.1.4 *Starch hydrolysis test*

Each bacterial isolate was inoculated on nutrient agar containing soluble starch by streaking once and the plates were incubated at 37 $^{0}$C for 24 hours. After incubation, the plates were flooded with iodine solution. Positive test was indicated by colourless areas around the colony growth while negative test retained the black colouration around the colony, which did not utilize the starch, thus did not show any clear zone around the bacterial colony (Fawole and Oso, 2007).

#### 3.9.1.5 *Indole test*

Peptone water was inoculated with the test isolate in a test tube and incubated at 37 °C for 24 hours, after which 0.5 mL of Kovac's reagent was added and shaken gently. A positive test was indicated by red colour in reagent layer above the broth within one minute while in

a negative test, the indole reagent retained its original yellowish brown colour (Fawole and Oso, 2007).

#### 3.9.1.6 *Methyl-Red- Voges-Proskaner test (MR-VP)*

Eight grams (8g) of MR-VP broth was weighed on a weighing balance and transferred into different test tubes and autoclaved at 121 $^{0}$C for 15 minutes. Bacterial isolates were then inoculated into the test tubes and incubated at 37 $^{0}$C for 48 hours. Methyl red indicator (α-naphthol solution) and 40 % KOH solution were added to the test tubes containing the isolates to distinguish coliform bacteria. Bright red colour indicated a positive result while yellow indicted a negative test (Cheesebrough, 2006).

#### 3.9.1.7 *Citrate test*

Simmon citrate agar was prepared according to manufacturer's instruction and cooled in slant position. The organisms were inoculated and incubated for 35 ºC for 7 days. Colour change from green to blue indicated a positive result, while no colour change indicated negative result (Cheesebrough, 2006).

#### 3.9.1.8 *Urease test*

Christensen's urease agar slant was prepared, urea salt was added and the test isolate was inoculated and incubated at 37 ºC for 24 hours. Colour change from yellow to pink indicated a positive test while no colour change indicated a negative result (Fawole and Oso, 2007).

#### 3.9.1.9 *Catalase test*

Single drop of hydrogen peroxide (3%) was added on a glass slide. Test organism was picked and emulsified with the hydrogen peroxide using a sterile wire loop. Immediate bubbles showed a positive result while no bubbles indicated a negative result (Fawole and Oso, 2007).

### 3.10 Physicochemical Analysis of Water Samples

#### 3.10.1 Determination of pH

The pH of the water samples was analyzed using the Wagtech pH meter (Orion Versa Star Pro VSTAR-pH Japan). Ten (10) mL of each of the samples was poured into a sterile beaker. The anode of the pH meter was standardized using distilled water and the anode of the pH meter was dipped into it and readings were taken and recorded (American Public Health Association, 1995).

#### 3.10.2 Determination of turbidity

A two part calibrated turbidity tube was used, with calibrations from 5-25 turbidity units. The joined tubes were held over a white paper, while slowly pouring the water sample into the tube until the black cross at the bottom was no longer visible. At this point the reading was taken from the side of the tubes as the turbidity and value of the water sample was recorded (APHA, 1995).

### 3.10.3 Determination of total hardness

Total hardness was analyzed by titration of 50 mL water sample against standard Ethylenediamine tetraacetic acid (EDTA). EDTA was added in drops at pH 10 using Erichrome black T indicator until the colour changed into purple and the hardness was calculated by multiplying the average number of drops of EDTA (titre value) used for the sample by the calibration factor of 20 (APHA, 1995).

### 3.10.4 Determination of electrical conductivity

One hundred (100) mL volume of distilled water and 100 mL of water sample were poured into two separate beakers. The conductivity meter was switched on and its sensor rod dipped into the beaker containing distilled water to standardize it and then it will be dipped into the second beaker containing water sample and readings were taken and reported in microSiemens/cL (APHA, 1995).

### 3.10.5 Determination of total suspended solid (TSS)

This parameter was determined by pouring 100 mL of water sample through a pre-weighted filter (glass fibre) of specific pore size. The filter was weighed again after the drying process that removed all the water from the filter. The gain in weight was a dry weight measure of the particulates present in the water samples. Results were reported in milligrams of solids per liter of water (mg/L) (APHA, 1995).

### 3.10.6 Determination of temperature

Temperature of all water samples was determined using mercury-in-glass thermometer. The thermometer was dipped into each sample and readings were taken after 2 minutes. The mercury level was regarded as the temperature of the sample and the results were expressed in degree Celsius (Umar *et al*., 2017).

### 3.10.7 Determination of total dissolved solids

A clean and dry evaporating dish was weighed. One hundred (100) mL of sample was filtered through a filter paper and the filtrate was taken in an evaporating dish. The sample was evaporated on hot water bath. When the whole water was evaporated, the weight of the evaporating dish and the weight of the evaporating dish after cooling in a desiccator were noted and the difference was calculated using Equation 1. The results were expressed as mg/L

$$\text{TDS}\ (mg/L) = \frac{A - B}{V} X100 \quad (1)$$

Where TDS = total dissolved solid, A= final weight of evaporating dish (g), B = initial weight of evaporating dish (g), and V= volume of sample taken (mL) (APHA, 1995).

### 3.10.8 Determination of residual chlorine

Residual chlorine of the water samples was determined by taking 25 mL of samples and N,N-diethyl-p-phenylenediamine(DPD) total chlorine was added. Using 0.00564 N ferrous ethylene-diammonium sulphate (FES) cartridge, it was titrated against 0.00564N FES to a

colorless end point. Residual Chlorine was calculated using Equation 2 below as described by APHA (1995);

$$\mathrm{RC} = \frac{Digital\ reading}{V}\frac{mg}{L} \qquad (2)$$

### 3.11 Identification and Characterization of pathogenic *Escherichia coli* strain using Singleplex Polymerase Chain Reaction

#### 3.11.1 DNA extraction

Deoxyribonucleic acid (DNA) was extracted using the protocols of Trindade *et al*. (2007). Single colonies grown on medium were transferred into 1.5 mL of liquid medium and cultures were grown on a shaker for 48 hours at 28 ºC. Following this, cultures were centrifuged at 4600g for 5 minutes. The resulting pellets were resuspended in 520 µL of TE buffer (10 mMTris-HCl, 1mM EDTA (pH 8.0). Fifteen microliters of 20 % SDS and 3 µL of Proteinase K (20 mg/mL) were then added. The mixture was incubated for 1 hour at 37 ºC. Then 100 µL of 5 M NaCl and 80 µL of a 10 % CTAB solution in 0.7 M NaCl were added and votexed. The suspension was incubated for 10 minutes at 65 ºC and kept on ice for 15 minutes. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min and centrifugation at 7200 g for 20 minutes. The aqueous phase was then transferred to a new tube and isopropanol (1: 0.6) was added and DNA precipitated at –20 ºC for 16 hours. DNA was collected by centrifugation at 13000 g for 10 minutes, washed with 500 µL of 70 % ethanol, air-dried at ambient temperature for approximately three hours and finally dissolved in 50 µL of TE buffer.

### 3.11.2 Polymerase chain reaction

Polymerase chain reaction (PCR) sequencing preparation cocktail consisted of 10 µL of 5x GoTaq colourless reaction, 3 µL of 25mM $MgCl_2$, 1 µL of 10 mM of dNTPs mix, 1 µL of 10 pmol each 27F 5′- AGA GTT TGA TCM TGG CTC AG- 3′and - 1525R, 5′- AAGGAGGTGATCCAGCC-3′ primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µL with sterile distilled water 8µL DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94 °C for 5 minutes, followed by a 30 cycles consisting of 94 °C for 30 seconds, 50 °C for 60seconds and 72°C for 1 minute 30 seconds, and a final termination at 72 °C for 10 minutes, and chill at 4 °C (Frank *et al*., 2008).

### 3.11.3 Integrity testing using agarose gel electrophoresis

The integrity of the amplified 1.5Mb gene fragment was checked on a 1 % Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5 % agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60 °C and stained with 3µl of 0.5 g/mL ethidium bromide (which absorbedinvisible UV light and transmitted the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 l) of 10X blue gel loading dye (which gave colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µL of each PCR

product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel.

### 3.11.4 Purification of amplified product

Subsequent to gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. A 7.6 µL of Na acetate 3M and 240 µL of 95 % ethanol were added to each about 40µL PCR amplified product in a new sterile 1.5 µL tube eppendorf, it was mixed thoroughly by vortexing and kept at -20 °C for at least 30 minutes. Centrifugation for 10 minutes at 13000 g was done at 4 °C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µL of 70 % ethanol and mixed then centrifuged for 15 minutes at 7500 g and 4 °C. All supernatant (inverted tube on trash) were removed and tubes were inverted on paper tissue and allowed to dry in the fume hood at room temperature for 15 minutes then it was resuspended with 20 µL of sterile distilled water and kept in -20 °C prior to sequencing. The purified fragment was checked on a 1.5 % Agarose gel ran on a voltage of 110V for one hour as previous, to confirm the presence of the purified product and quantified using a nanodrop of model 2000 from thermo scientific.

### 3.11.5 Sequencing analysis

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer (Applied Biosystems, using manufacturers' manual while the sequencing kit used was that

of BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis

### 3.11.6 Molecular analysis of virulence-coding genes

Molecular investigation of virulence-coding gene in the *E. coli* was by simple PCR on the extracted DNA using ESBL-coding regions specific primers. Reaction cocktail used for all PCR per primer set included (Reagent Volume µL) - 5X PCR SYBR green buffer (2.5), $MgCl_2$ (0.75), 10pM DNTP (0.25), 10pM of each forward and backwards primer (0.25), 8000U of taq DNA polymerase (0.06) and made up to 10.5 with sterile distilled water to which 2 µL template was added. Buffer control was also added to eliminate any probability of false amplification. Polymerase chain reaction was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) using the appropriate profile as designed for each primer pair.

## 3.12 Data analysis

Means, standard error of mean (SEM), minimum and maximum values and multiple comparisons were calculated using SPSS (version 16.0 for windows). One way ANOVA was used to test for the significance between the various sources of drinking water.

# CHAPTER FOUR

## 4.0 RESULTS AND DISCUSSION

### 4.1 Results

#### 4.1.1 Percentage use of different water sources in the study area

Eighty (80) water samples from four different sources (20 wells, 20 taps, 20 boreholes, 20 sachet waters) were analyzed for total coliforms, thermotolerant coliforms (*E. coli*), pH, total suspended solid, total dissolved solid, temperature, turbidity, total hardness, residual chlorine and electrical conductivity. The sample frame consisted of all sources of drinking water at different locations within the study area. A survey was carried out to determine the sources of drinking water in the area, a total of 106 household (10 households in 10 ward and 6 households in Limawa A) heads were interviewed voluntarily between August 4$^{th}$ and September 2$^{nd}$, 2019 to ascertain the sources of their drinking water. Results from the findings revealed that 14 households (13.21%) use well as sole source of drinking water, 18 households (16.98%) derive their drinking water from pipe borne taps, 10 households (9.43%) from household stored water, 44 households (41.50%) from sachet water while 20 households (18.87%) uses more than one source of drinking water. Due to language barrier, information from four (4) households was inaccessible. Questionnaires were administered by interviewers (research assistants) in English or Pidgin. However, Hausa language was used when necessary. Representation of the numbers (in percentage) of respondents that used various drinking water sources in the study area is presented in Figure 4.1

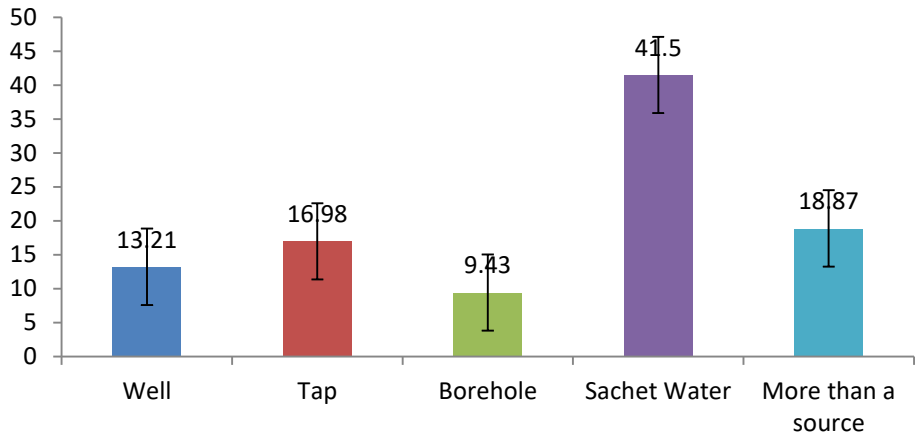


**Figure4.1: Percentage Use of Sampled Drinking Water Sources by Residents in Study Area**

### 4.1.2 Mean total coliform counts in water samples

The results for the mean total coliforms in all sampled drinking water revealed that samples had coliform counts above the stipulated drinking water guideline. Well water had the highest mean coliform number with 95cfu/mL, while sachet water samples had the least value at 8.56cfu/mL. Tap water samples also had high mean coliform count of 26.95cfu/mL while borehole water samples had mean count of 13cfu/mL. Well water showed significant difference from three other drinking water sources. Table 4.1 shows the results for the mean total coliform counts of drinking water samples.

**Table 4.1: Mean Total Coliform Counts of Drinking Water Samples According to Water Sources**

| Drinking water source | (cfu/100mL) |
|---|---|
| Well | 95± 20.46[a] |
| Borehole | 13± 1.422[b] |
| Tap | 26.95±5.56[b] |
| Sachet water | 8.65± 2.074[b] |

Each value is expressed as Mean ±Standard error. Values with the same superscript across the column are considered not significantly different ($p < 0.05$).

### 4.1.3 Thermotolerant coliform counts in samples drinking water sources

Results from thermotolerant coliform enumeration revealed that, of all the drinking water samples enumerated, only twelve (12) sampled had thermotolerant coliform. Well water samples had highest occurrence with seven (7) samples. Three (3) tap water samples, one borehole and one sachet water had thermotolerant coliform. Well 6 had the highest number of thermotolerant coliform while tap 6, well 13 and sachet water 14 had 3 counts respectively. Result for thermotolerant coliform counts is presented in Table 4.2.

**Table 4.2: Thermotolerant Coliform (*E. coli*) Counts in Sampled Drinking Water Sources**

| Drinking Water Source | Count cfu/mL |
|---|---|
| Tap 3 | 04 |
| Tap 5 | 06 |
| Tap 6 | 03 |
| Well 1 | 11 |
| Well 2 | 08 |
| Well 5 | 06 |
| Well 6 | 12 |
| Well 11 | 13 |
| Well 13 | 03 |
| Well 16 | 04 |
| Borehole 11 | 04 |
| Sachet water 14 | 03 |

### 4.1.3 Physicochemical properties of water samples in the study area

Eight physicochemical properties were analyzed for all the water samples. The highest mean value for pH was recorded for sachet water samples with value at 8.09 while well water samples had the lowest value at 7.12. There was no significant difference between the mean values for pH of borehole, sachet and tap waters, and all samples were within the permissible limits by drinking water standard guidelines. Mean values for temperature revealed that all water samples fell within the ambient required standard for drinking water. Borehole water had the highest mean value for temperature at 24.85 ℃ while 24.45 ℃ was

the lowest recorded mean value for sachet water samples and the result revealed that there was no significant difference between the mean temperature values for all drinking water sources. Results for electrical conductivity values showed that well water had the highest value at 901µS/cm, which is above the standard by World Health Organization, and it showed a significant difference with all other drinking water sampled. Sachet water had the least mean value at 180µS/cm. Sachet water had the least mean values for total dissolved solid at 37 mg/L, followed by tap water with mean value of 38 mg/L. the highest values for mean TDS was recorded in well water samples at 591 mg/L, followed by borehole water at 258mg/L. Having a mean value of 430 mg/L, well water had the highest total suspended solid, followed by tap water with value at 338 mg/L. There was significant difference between borehole water and well water values. Results for water hardness showed significant difference among all drinking water samples with borehole water having the highest mean value at 221.92 mg/L, while sachet water had the least mean value at 104.33 mg/L and it is the only water source that conformed to the drinking water guidelines. Tap water samples and well water samples did not conform to standard guidelines for drinking water for turbidity, both having values of 6.90 NTU and 7.60 NTU respectively. Sachet water and borehole water showed significant difference for water hardness. The results for mean values of residual chlorine revealed that there was significant difference between the sachet water samples and tap water samples. 1.53 mg/L was recorded for sachet waters while tap water had mean value of 2.41 mg/L. Both borehole water and well water had no values for residual chlorine because they were not chlorinated as a means of treatment. The results for mean physicochemical properties of drinking water sources are presented in Table 4.3.

**Table 4.3: Mean Results for Physicochemical Parameters of Drinking Water Samples**

| Samples | pH | Temp (°C) | Conductivity (µS/cm) | TDS (mg/L) | TSS (mg/L) | Hardness (mg/L) | Turbidity (NTU) | Residual chlorine (mg/L) |
|---|---|---|---|---|---|---|---|---|
| **BOREHOLE WATER** | 7.91±0.119$^{b}$ (7.11-8.65) | 24.85±0.13$^{a}$ (24-26) | 392.55±99.66$^{a}$ (1.00-1700) | 258±69.26$^{b}$ (0.64-1088) | 84±4.39$^{b}$ (38-114) | 221.92±28.92$^{c}$ (60-550) | 4.25±0.43$^{b}$ (1-9) | _ |
| **WELL WATER** | 7.12±0.106$^{a}$ (6.47-8.01) | 24.50±0.14$^{a}$ (24-26) | 901±105.14$^{b}$ (250-1800) | 591±69.24$^{c}$ (160-1152) | 430±47.67$^{c}$ (112-804) | 158.57±12.31$^{b}$ (80-256) | 7.60±0.21$^{c}$ (5-9) | _ |
| **SACHET WATER** | 8.09±0.078$^{b}$ (7.04-8.40) | 24.45±0.14$^{a}$ (24-26) | 180.50±38.34$^{a}$ (10-700) | 37±4.63$^{a}$ (0.72-68.43) | 15.30±0.65$^{a}$ (10.2-21.9) | 104.33±5.54$^{a}$ (51-145) | 2.55±0.25$^{a}$ (2-6) | 1.53±0.25$^{a}$ (0.32-4,25) |
| **TAP WATER** | 7.92±0.089$^{b}$ (7.08-8.45) | 24.50±0.16$^{a}$ (24-26) | 304.50±26.42$^{a}$ (110-540) | 38±4.53$^{a}$ (0.73-58.43) | 338.23±10.54$^{a}$ (208.2-394.6) | 184.49±16.49$^{bc}$ (114-335) | 6.90±0.26$^{c}$ (5-9) | 2.41±0.13$^{b}$ (0.71-3.55) |
| ***NSDWQ** | 6.5-8.5 | Ambient | 1000 | 1000 | 25 | 150 | 5.0 | _ |
| ****WHO** | 6.5-8.5 | 40 | 400 | 1000 | _ | _ | 5.0 | 5 |

Superscripts with the same letters in each column are not significantly different ($p>0.05$)

**World Health Organization (2011). *Nigerian Standards for Drinking Water Quality (2015).

### 4.1.0 Cultural, morphological and biochemical characteristics of the bacterial isolates

The summary of biochemical characteristics of bacteria isolated from different water samples is presented in Table 4.4. Isolate 1 appeared smooth, circular, convex and yellow in colour. It was negative to Gram's stain, Urease test but positive for indole. It was identified as *Escherichia coli*. While isolate 2 appeared convex and circular in shape. It was negative for Gram's test but positive for urease. It was identified as *Klebsiella pneumonia*

**Table 4.4: Cultural, Morphological and Biochemical Characteristics of the Bacterial Isolates**

| **Characteristics of Isolate** | **Isolate 1** | **Isolate 2** |
|---|---|---|
| Cultural characteristics | Smooth yellowish circular colonies with convex shape on Membrane Lauryl Sulphate broth. | Reddish, convex and circular in shape on membrane Lauryl Sulphate broth. |
| | It has green metallic sheen on Eosin Methylene Blue (EMB). | It has pink coloration on Eosin Methylene Blue |
| Biochemical tests | | |
| Gram Stain | - | - |
| Catalase Test | + | + |
| Urease | - | + |
| Methyl Red Test | + | - |
| Voges Proskauer (VP) | - | + |
| Indole Test | + | - |
| Starch hydrolysis | - | - |
| Gas Production Test | + | + |
| Oxidase Test | - | - |

| | | |
|---|---|---|
| Citrate Utilization Test | - | + |
| Lactose Utilization Test | + | + |
| Sucrose Utilization Test | + | + |
| Glucose Utilization Test | + | + |
| Probable Organism | *Escherichia coli* | *Klebsiella pneumoniae* |

KEY:

- = Negative Reaction

+ = Positive Reaction

**4.2 Results of Molecular Identification**

Gel electrophoresis indicating a positive amplification of the 16S region of the bacterial isolates using 16S ribosomal universal primer showed that the isolates have a band size of approximately 1500bp as seen in Plates I and III.

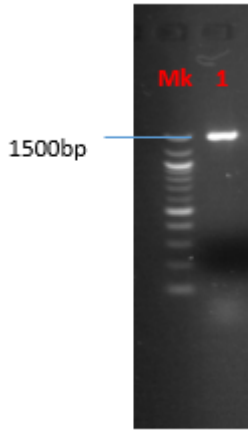


Plate I: Agarose gel electrophoresis of the 16S rRNA of the bacteria isolate indicating approximately 1500bp.

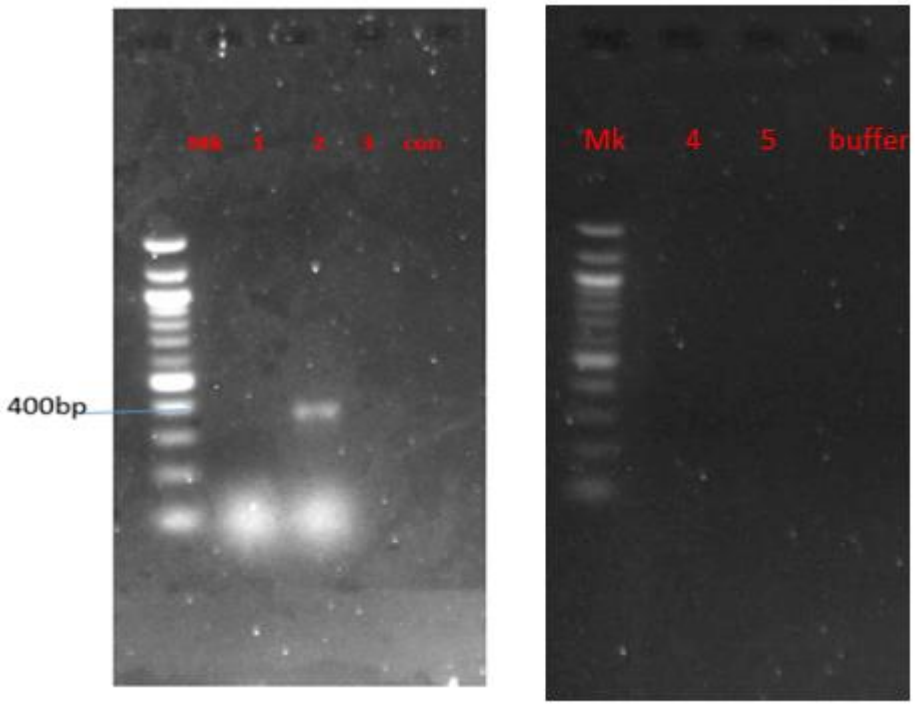


Plate II : Agarose gel electrophoresis of the polymerase chain reaction products of virulence gene in *E. coli* amplified with (1) aggR (2) eaeA (3)Stx-1 (4) Elt (5)Est specific primers. (Band sizes approximately 254bp, 384bp, 610bp, 450 and 128 respectively). Gel image indicates the presence of eaeA gene only.

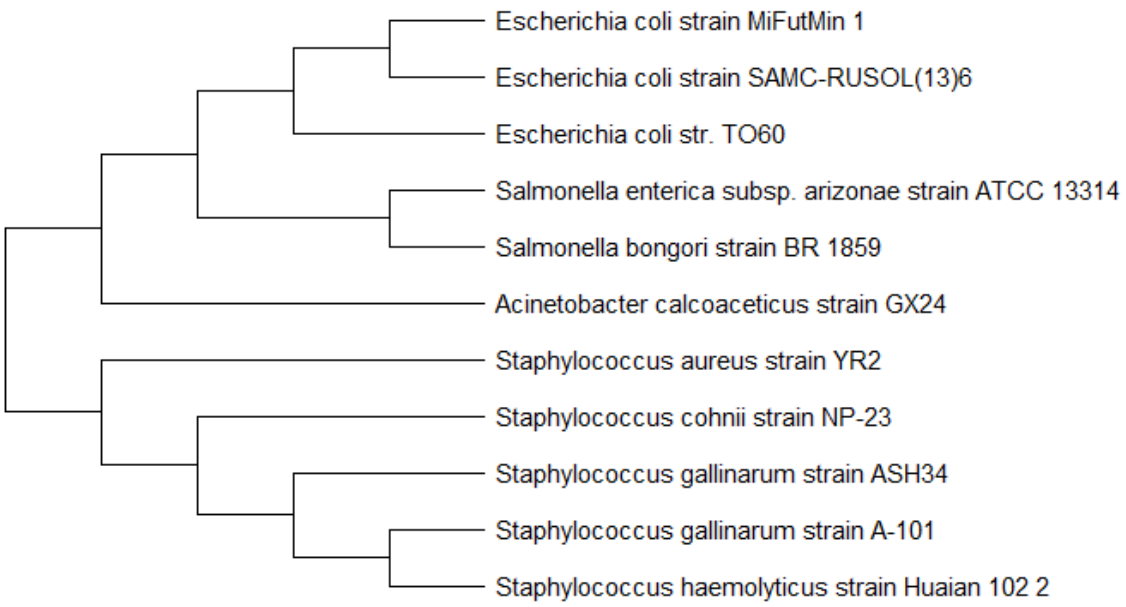


Figure 4.2: Phylogenic tree of *Escherichia coli*MiFutMin_1 (MN712503).

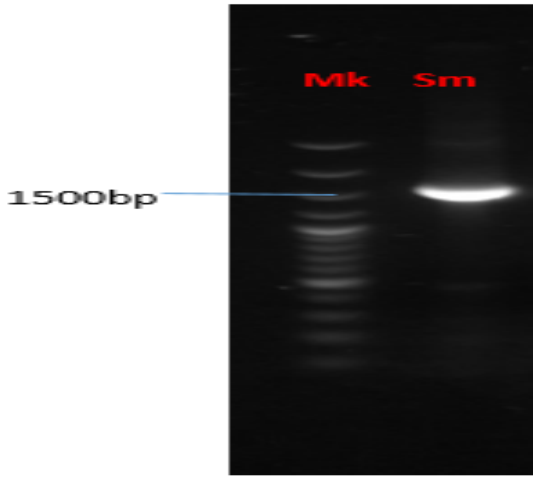


Plate III: Agarose gel electrophoresis of the 16S rRNA of the bacteria isolate indicating approximately 1500bp.

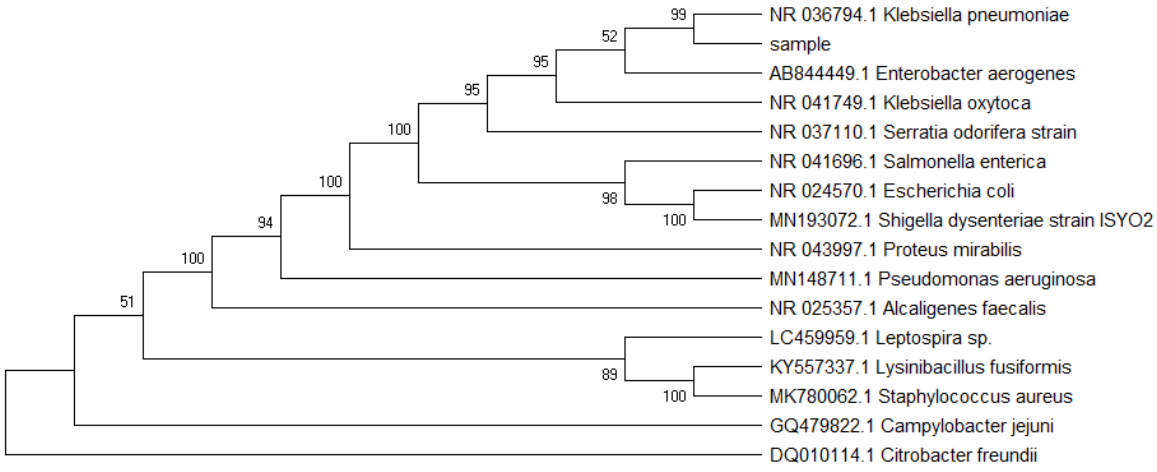


Figure 4.3: Phylogenic tree of *K. pneumoniae* strain MiFutMin_1 (MT128987).

Sample ID 99.4% identical to *Klebsiella pneumoniae* strain GXNN3 16S ribosomal RNA gene, partial sequence

## 4.3 Discussion

Results from this study showed that most of the water samples analyzed contained coliform bacteria exceeding stipulated standards except for few samples. Twenty nine (29) samples had total coliform counts below permissible limits as required of potable water source. The values of bacteria count ranged from 01 cfu/100mL to 306 cfu/100mL, with well water having the highest number of total coliforms/100mL water sample. Well water sample had coliform counts as high as 306 cfu/100mL which is above permissible standards. This result agrees with the work of Egbe *et al*. (2013) who reported high number of coliform bacteria from well water samples examined in Fulani settlements in Gidan Kwano, Minna, Niger State. Egbe *et al*. (2013) further reported that forty (40) percent of the sampled well water was contaminated with *Klebsiella pneumoniae*, which was also isolated in most water samples examined in this study. Similarly, this report is in agreement with the report of Bala (2006) that recorded the presence of coliform organisms (mostly *E. coli* and *Klebsiella pneumoniae*) in well water samples from Jimata, Yola, Adamawa State. This result further agrees with the results of Amadi *et al*. (2014), who reported the presence of a high number of total coliform bacteria in sampled wells in Edati, Niger State. This may be due to absence of proper covering for most wells, which hence, allowed external contamination of the water samples. According to this result, it is pertinent to state that even the slightest trace amount of total coliform most likely confirms the dreaded reality of faecal contamination of water samples, not bearing in mind that the WHO designated a total coliform count range of 1-10 cfu/100mL as a low risk contamination. Sachet water samples analyzed in this study had coliform counts ranging from 01-40 cfu/mL. This indicated that all sachet water sampled had coliform bacteria, and hence most likely faecal contamination. A similar result was

reported by Daniel and Daodu (2016) who recorded high coliform presence in sachet waters analyzed in Ugbor, Benin city Nigeria. A similar result from Ugochukwu *et al*. (2015) agrees with results of this study as they recorded a high level of total coliforms in sachet water samples in Samaru-Zaria, Kaduna State. Various studies carried out on sachet water from different cities in Nigeria have revealed the lack of purity of such sachet waters (Afiukwa *et al*., 2010; Edema *et al*., 2011; Akpoborie and Ehwarimo, 2012; Onilude *et al*., 2013). The result from this study also agrees with the report of Omalu *et al*. (2012) that reported a high load of coliform bacteria like *Escherichia coli*, *Klebsiella Pneumoniae* in sachet water analyzed in Minna. A similar study conducted by Kalpana *et al.* (2011) is in agreement with this present study. Results from borehole water sampled shows that borehole water had coliform counts ranging from 03-26 cfu/mL. This is in agreement with the result of Bala (2006) who reported that presence of coliform bacteria in all borehole water analyzed. Bala (2006) noted that the borehole water was fit for consumption because coliform counts fall within the limits permissible by World Health Organization. In this study however, only nine (9) samples fall within the permissible limit for potable water and others failed to meet the required standard for drinking water quality. This may be as a result of poor fortification of the boreholes in the study area and also seepage of contaminated surface water into the aquifer. This study reported a coliform count ranging from 03 -107 cfu/100 mL for sampled tap water. From this study, five (5) tap water sources conformed to the drinking water standard regulations while fifteen sampled taps fell short of the permissible limit. Comparison of this study with a similar study conducted in Enugu State Nigeria by Ohanu *et al.* (2012) is not encouraging as the result agrees with this study where all tap water sampled had coliforms ranging from 188 - > 1000 cfu/100 mL in tap water,

thus exceeding the permissible limit by drinking water standard regulations. This may largely be due to the failure of treatment process form treatment plants, poor sanitation of pipes used to convey water samples as seen in plates VII or due to the lack of booster stations where water sample is supposed to be re-chlorinated in this study area.

The mean pH values of water samples ranged from 6.47 – 8.65. Most water samples had pH slightly above 7.0, which suggests a tendency for slight alkalinity of water samples, however, all the water samples were between the range stipulated by NSDWQ and WHO (6.5 – 8.5) except for a borehole sample, which had 8.65. This slight deviation may be due to the presence of greater number of organic matter or alkaline substances. Results from this work revealed that pH of sampled sachet water ranged from 7.04 -8.40. A more acidic pH was reported by Omalu *et al*. (2012), with range of 7.11-7.57 for pH of sachet water samples. The electrical conductivity of the water samples analyzed in this study ranged from 1.00µm-1800µm. Well water samples had high results for electrical conductivity with results ranging from 330- > 1700µm. A similar result was record by Fawole *et al*. (2017) who reported a high value for EC (>700) in well water samples, a report which is in contrast to the results of Esiegbe *et al*.(2018) who reported a low EC for hand dug well water samples. The variation in these results may be as a result of different physical features of well sampled, e.g., presence of protective covering and concrete internal ringing that can prevent the well water from external particles. This study shows that sampled borehole water has EC range from 01µm to >1600µm. This result is in agreement with that of Isa *et al*. (2013) who recorded EC as high as 747µm and 1092µm in sampled borehole water. A total of 30 water samples analyzed in this study had electrical conductivity values above the WHO standard of 400.00µm for potable water. However, most of the samples analyzed were

in line with the NSDWQ (2007) guideline of a maximum EC of 1000µm. On the other hand, all sachet water sampled complied with the stipulated standard for drinking water guideline as results ranged from 1-100µm. Samples with values of electrical conductivity higher than the standard could be as a result of an excessive concentration of dissolved ionic solids within the water samples. It should be noted that pure water is naturally a poor conductor of electricity and heat. The temperature of all water samples ranged from 24°C – 26°C. This result corresponds to that of Esiegbe *et al*. (2018) who reported similar result from studied tap, hand dug well and borehole water samples. Turbidity, which is the measure of clarity of water samples, ranged from 1NTU to 9NTU. Forty (40) water samples, most of the well water samples had turbidity level above the stipulated standard of 5.00 NTU for potable water. Only a sample of sachet water failed to meet the standard limit for turbidity in drinking water with turbidity of 6.0 NTU. More often than not, turbidity is brought about by the presence of colloidal matter and/or suspended particles in water. Results from the value of the total suspended solids (TSS) which are particles that are larger than 2 microns found in water column, revealed that most of the water samples analyzed in this study fell short of the required standard value as set by NSDWQ of 25mg/L. The values of TSS from samples ranged from 10.20 mg/L to 804 mg/L. the result showed that all sachet water conformed with the set standard for drinking water guideline with range from 10.20mg/L to 20.09mg/L. The high value of TSS in majority of water samples may be attributed to the presence of high inorganic materials in water samples. However, organic materials from decomposing materials could also have contributed to the TSS concentrations in the water samples. The results for total dissolved solids (TDS) in water samples range from 0.64mg/L to 1152mg/L. TDS are a leading cause of turbidity in drinking water. TDS measures the total organic and

inorganic materials present in water samples. These solids are primarily minerals, salts and organic matters that can be a general indicator of water quality. Eight (8) samples from the study area failed to meet the NSDWQ and WHO stipulated maximum of 1000 mg/L TDS for potable water.

Similarly, from the results, the values for total hardness of water ranged from 51mg/L to 550 mg/L. Thirty-three (33) samples had values above stipulated standard of 150mg/L. Hard water requires more soap to produce lather and it often produces noticeable deposit of precipitate in containers. From the results of residual chlorine, the water samples had a range between 0.32mg/L to 4.25 mg/L. Of all the 40 samples tested for residual chlorine, 22 samples (14 sachet water and 8 tap water samples) had residual chlorine value below the stipulated minimum of 2mg/L for potable water. Chlorine in drinking water is used as disinfectant to kill microorganisms, however if in excess in drinking water, it can confer unpleasant taste to water and can dissuade people from using the supply.

Twelve (12) samples (3 taps, 7 wells, 1 borehole and 1sachet water) had thermotolerant coliforms present in them after incubation at 44.5 $^{o}$C. This clearly does not conform to the guideline standards for drinking water sources as stated by WHO and NSDWQ that stipulated 0 cfu/100mL for *E. coli* and other thermotolerant coliforms and this pose a great risk to public health of consumers as this greatly indicates the likelihood of the presence of other pathogenic organisms that are of faecal origin both from humans and animals. The works of Daniel and Daodu (2016); Bamigboye *et al.* (2018) is in agreement with the results of this study. The presence of faecal coliform especially in borehole water calls for serious concern. This is because groundwater is thought to be more potable and less prone to contamination (Isa *et al.*, 2013). The continuous infiltration of faecal matter into the

underground aquifers as a result of open defecation over a long period of time may be responsible for this. Also its vulnerability to contamination could be due to poor construction, proximity to toilet facilities and various human activities surrounding it. Results from biochemical characterization, morphological features and molecular identification were used to identify the isolates from samples as *Klebsiella pneumoniae* and *Escherichia coli*. Not all *E. coli* strains are pathogenic, as it is often used as an index pointing to the fact that a sample is likely to contain other pathogenic organisms that are of faecal origin. Despite that, there are increasing evidences that *E .coli* strains originating from human and animal feces contains several virulent genes, but only a few studies have determined whether *E. coli* strains isolated from water contains virulent genes and are potentially pathogenic. This study further investigated the presence of some specific virulent genes using Singleplex polymerase chain reaction; *aggR*, *eaeA*,*stx*1, *Elt* and *est* genes in the isolated organism. Result of PCR revealed the presence of *eaeA* genes only. The *eaeA*, which codes for intimin protein is necessary for intimate attachment to host epithelial cells in EPEC pathotypes and Donnenberg *et al*.(1993) in a research to understand the role of eaeA gene in experimental Enteropathogenic *E. coli* infection came to a summary that it is responsible for the full virulence of the pathotype. EPEC was the foremost strain of *E. coli* incriminated as the cause of outbreaks of infantile diarrhea in the 1940s and 1950s. For reasons yet unknown, EPEC strains are no longer important causative agents of infantile diarrhea in developed countries, but it remains a major cause of infantile diarrhea in the developing countries. The hallmark of EPEC infections is the attaching and effacing (A/E) histopathology which can be observed on epithelial cells at ultra structural levels. However, Boerlin *et al*.(1999) noted that the presence of a single or multiple virulent genes in an *E.*

*coli* strain does not necessarily indicate that a strain is pathogenic unless that strain has the appropriate combination of virulent genes to cause disease in host.

# CHAPTER FIVE

## 5.0 CONCLUSION AND RECOMMENDATIONS

### 5.1 Conclusion

Two species of total coliform group identified as *Escherichia coli* and *Klebsiella pneumonia* were isolated from all drinking water sources examined while thermotolerant coliform (*E. coli*) was isolated from twelve (12) drinking water samples in Chanchaga local government area. Results from the physicochemical analysis of water sampled revealed that well water did not comply with the stipulated values for drinking water guidelines while sachet water is in conformity with permissible limits for potable water as stated by regulatory bodies. Results from total suspended solids showed a significant difference at $p< 0.05$ between borehole and tap water. The eaeA gene, which is solely responsible for the virulence in enteropathogenic *E. coli* (EPEC) strain, was identified using Singleplex Polymerase Chain Reaction technique. The detection of virulent gene responsible for infantile diarrhea in an isolate from drinking water sample is a great public health concern.

### 5.2 Recommendations

Based on the results from this research, the following recommendations are made:

i. Booster stations should be made available to allow for re-chlorination of tap water before getting to final consumers

ii. Private home owners and estate developers should adhere to the standard practice of giving allowance of at least 30meters between septic tanks and boreholes/hand dug wells as against what is shown in plate V as seen in one of sample areas and private home owners

should not position their septic tanks/soak away pits near perimeter fence demarcating their premises from their proximal neighbors. This is to prevent the rampant cases of underground water movement of biodegraded liquid like faecal waste form septic tank and contaminated waste water from their soak-away pits to their neighbors' borehole water source caused by the unprofessional placement of borehole/hand dug well and septic tank/soak away pit in a common plane of the underground fluid flow direction and gradient.

iii. Further research should be carried out to identify the sources of faecal pollution whether from humans or animals using microbial source tracking (MST) technique. This will aid to understand the source of the faecal pollutant and also to put in place preventive measures against future contamination from such source.

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## APPENDIX 1


**Plate IV: Setup of a membrane filter apparatus for isolation of total coliforms from water samples**

## APPENDIX 2

**Plate V: A well that serves as a source of drinking water in Chanchaga Local Government**

## APPENDIX 3

**Plate VI: Pipe borne water in Chanchaga Local Government**

## APPENDIX 4

**Plate VII: Water pipes in drainage system in Chanchaga Local Government.**

## APPENDIX 5

**Plate VIII: A well close to a septic tank in Chanchaga Local Government**

## APPENDIX 6

**Total coliform counts (cfu/100mL) of water samples in study area**

| Well | Count | Borehole | Count | Tap | Count | Sachet water | Count |
|---|---|---|---|---|---|---|---|
| W1 | 250 | BH1 | 15 | T1 | 40 | SW1 | 15 |
| W2 | 170 | BH2 | 18 | T2 | 37 | SW2 | 13 |
| W3 | 180 | BH3 | 16 | T3 | 56 | SW3 | 08 |
| W4 | 210 | BH4 | 15 | T4 | 50 | SW4 | 03 |
| W5 | 209 | BH5 | 12 | T5 | 52 | SW5 | 06 |
| W6 | 306 | BH6 | 20 | T6 | 107 | SW6 | 40 |
| W7 | 110 | BH7 | 24 | T7 | 25 | SW7 | 02 |
| W8 | 40 | BH8 | 26 | T8 | 28 | SW8 | 04 |
| W9 | 70 | BH9 | 11 | T9 | 19 | SW9 | 03 |
| W10 | 30 | BH10 | 19 | T10 | 14 | SW10 | 11 |
| W11 | 12 | BH11 | 03 | T11 | 23 | SW11 | 02 |
| W12 | 08 | BH12 | 08 | T12 | 11 | SW12 | 09 |
| W13 | 28 | BH13 | 09 | T13 | 06 | SW13 | 18 |
| W14 | 20 | BH14 | 17 | T14 | 11 | SW14 | 15 |
| W15 | 03 | BH15 | 10 | T15 | 12 | SW15 | 15 |
| W16 | 50 | BH16 | 10 | T16 | 18 | SW16 | 03 |
| W17 | 55 | BH17 | 10 | T17 | 10 | SW17 | 02 |
| W18 | 65 | BH18 | 06 | T18 | 10 | SW18 | 01 |
| W19 | 36 | BH19 | 05 | T19 | 07 | SW19 | 01 |
| W20 | 47 | BH20 | 06 | T20 | 03 | SW20 | 02 |

## APPENDIX 7

**Mean values for physicochemical properties of well water samples in study area**

| Samples | pH | Temp (°C) | EC (µs/Mol) | TSS (mg/L) | TDS (mg/L) | Total Hardness (mg/L) | Turbidity (NTU) |
|---|---|---|---|---|---|---|---|
| W1 | 6.57 | 24 | 710 | 362.11 | 438.11 | 145.00 | 7.0 |
| W2 | 6.68 | 25 | 500 | 485.20 | 320.04 | 140.00 | 7.0 |
| W3 | 6.94 | 24 | 330 | 385.11 | 438.11 | 90.00 | 7.0 |
| W4 | 7.02 | 24 | 250 | 635.28 | 160.09 | 90.00 | 9.0 |
| W5 | 6.92 | 24 | 300 | 245.82 | 192.01 | 80.09 | 7.0 |
| W6 | 7.39 | 26 | 1800 | 528.21 | 1152.01 | 250.00 | 8.0 |
| W7 | 7.81 | 24 | 1710 | 681.80 | 1094.40 | 240.99 | 8.0 |
| W8 | 7.20 | 25 | 1630 | 421.26 | 1043.20 | 255.62 | 8.0 |
| W9 | 8.01 | 25 | 800 | 731.25 | 512.00 | 165.09 | 9.0 |
| W10 | 7.20 | 25 | 1100 | 225.21 | 704.05 | 135.40 | 5.0 |
| W11 | 6.91 | 24 | 890 | 720.24 | 603.00 | 169.03 | 8.0 |
| W12 | 7.24 | 24 | 930 | 626.01 | 819.00 | 171.23 | 8.0 |
| W13 | 7.26 | 24 | 1100 | 211.09 | 712.00 | 200.17 | 7.0 |
| W14 | 6.63 | 26 | 1540 | 119.09 | 1026.40 | 231.30 | 9.0 |
| W15 | 6.70 | 24 | 410 | 219.09 | 284.10 | 129.52 | 7.0 |
| W16 | 6.47 | 24 | 650 | 410.00 | 312.07 | 192.00 | 8.0 |
| W17 | 6.65 | 25 | 720 | 440.00 | 411.13 | 140.32 | 7.0 |
| W18 | 7.88 | 25 | 700 | 432.00 | 360.22 | 120.09 | 7.0 |
| W19 | 7.90 | 24 | 900 | 560.07 | 804.16 | 136.07 | 8.0 |
| W20 | 6.95 | 24 | 1050 | 700.00 | 112.30 | 90.00 | 8.0 |

## APPENDIX 8

**Mean values for physicochemical properties of borehole water samples from study area**

| Samples | pH | Temp (°C) | EC (µs/Mol) | TSS (mg/L) | TDS (mg/L) | Total Hardness (mg/L) | Turbidity (NTU) |
|---|---|---|---|---|---|---|---|
| BH1 | 7.13 | 25 | 140 | 105.00 | 890.29 | 125.06 | 1.0 |
| BH2 | 7.61 | 25 | 520 | 114.30 | 341.82 | 265.93 | 4.0 |
| BH3 | 7.53 | 25 | 110 | 93.21 | 74.81 | 135.00 | 2.0 |
| BH4 | 7.56 | 26 | 120 | 61.40 | 75.24 | 130.00 | 4.0 |
| BH5 | 7.67 | 25 | 100 | 75.83 | 60.88 | 105.32 | 5.0 |
| BH6 | 8.60 | 25 | 1100 | 63.25 | 703.21 | 500.06 | 6.0 |
| BH7 | 8.65 | 24 | 1 | 38.41 | 0.64 | 60.38 | 3.0 |
| BH8 | 8.40 | 25 | 790 | 101.83 | 508.21 | 370.11 | 5.0 |
| BH9 | 8.60 | 24 | 1700 | 93.21 | 1088.00 | 550.04 | 5.0 |
| BH10 | 8.45 | 25 | 580 | 84.75 | 362.80 | 345.26 | 9.0 |
| BH11 | 8.43 | 25 | 200 | 80.21 | 83.05 | 185.20 | 2.0 |
| BH12 | 8.47 | 25 | 260 | 81.06 | 83.44 | 192.64 | 5.0 |
| BH13 | 7.54 | 25 | 410 | 112.08 | 101.09 | 235.11 | 5.0 |
| BH14 | 7.59 | 26 | 130 | 61.44 | 78.20 | 138.75 | 4.0 |
| BH15 | 7.66 | 25 | 210 | 80.90 | 83.00 | 183.50 | 4.0 |
| BH16 | 7.60 | 24 | 260 | 81.11 | 83.44 | 192.00 | 3.0 |
| BH17 | 7.11 | 25 | 100 | 75.70 | 60.29 | 135.33 | 6.0 |
| BH18 | 7.38 | 25 | 500 | 114.36 | 311.08 | 262.04 | 6.0 |
| BH19 | 8.57 | 24 | 200 | 80.40 | 83.00 | 185.44 | 1.0 |
| BH20 | 7.67 | 24 | 150 | 76.53 | 79.46 | 141.23 | 5.0 |

## APPENDIX 9

**Mean values for physicochemical properties of tap water samples from study area**

| Sample | pH | Temp. (°C) | EC (µs/Mol) | TSS (mg/L) | TDS (mg/L) | Total hardness (mg/L) | Turbidity (NTU) | Residual chlorine (mg/L) |
|---|---|---|---|---|---|---|---|---|
| T1 | 7.90 | 25 | 110 | 309.81 | 70.40 | 135.00 | 6.0 | 2.13 |
| T2 | 7.95 | 24 | 210 | 382.61 | 134.63 | 195.21 | 6.0 | 2.13 |
| T3 | 8.30 | 24 | 540 | 394.62 | 341.60 | 335.26 | 8.0 | 0.71 |
| T4 | 7.86 | 24 | 1100 | 529.40 | 704.13 | 408.11 | 6.0 | 1.13 |
| T5 | 7.91 | 24 | 370 | 310.00 | 144.08 | 196.70 | 6.0 | 2.24 |
| T6 | 7.92 | 25 | 250 | 390.73 | 139.20 | 192.82 | 7.0 | 2.45 |
| T7 | 7.85 | 25 | 550 | 401.88 | 389.09 | 310.26 | 7.0 | 0.73 |
| T8 | 7.64 | 24 | 160 | 205.00 | 1171.10 | 137.00 | 5.0 | 0.06 |
| T9 | 7.87 | 24 | 170 | 324.25 | 70.80 | 135.21 | 5.0 | 2.86 |
| T10 | 8.40 | 24 | 240 | 390.00 | 138.64 | 189.77 | 5.0 | 2.11 |
| T11 | 8.32 | 24 | 210 | 388.46 | 137.11 | 192.02 | 6.0 | 3.50 |
| T12 | 8.44 | 24 | 320 | 396.23 | 140.33 | 197.41 | 6.0 | 3.60 |
| T13 | 8.53 | 25 | 1410 | 340.19 | 1143.18 | 411.96 | 6.0 | 0.08 |
| T14 | 7.95 | 24 | 600 | 125.00 | 56.51 | 128.91 | 7.0 | 2.77 |
| T15 | 8.30 | 24 | 300 | 118.36 | 52.65 | 128.11 | 7.0 | 3.00 |
| T16 | 7.90 | 24 | 140 | 160.93 | 72.74 | 133.67 | 7.0 | 2.08 |
| T17 | 7.56 | 25 | 200 | 118.00 | 52.10 | 126.77 | 6.0 | 3.13 |
| T18 | 7.01 | 25 | 460 | 306.62 | 274.34 | 200.08 | 5.0 | 0.73 |
| T19 | 7.11 | 24 | 930 | 1001.11 | 609.11 | 358.51 | 6.0 | 0.19 |
| T20 | 7.63 | 25 | 1200 | 670.01 | 1120.03 | 408.94 | 6.0 | 0.16 |

## APPENDIX 10

**Mean values for physicochemical properties of sachet water samples in study area**

| Sample | pH | Temp. (°C) | EC (µs/Mol) | TSS (mg/L) | TDS (mg/L) | Total hardness (mg/L) | Turbidity (NTU) | Residual chlorine (mg/L) |
|---|---|---|---|---|---|---|---|---|
| Sw1 | 8.21 | 25 | 1 | 15.35 | 0.72 | 67.04 | 4.0 | 0.71 |
| Sw2 | 8.20 | 26 | 40 | 14.90 | 25.60 | 145.28 | 2.0 | 0.71 |
| Sw3 | 8.30 | 25 | 90 | 10.20 | 51.25 | 130.11 | 2.0 | 2.84 |
| Sw4 | 8.36 | 24 | 80 | 18.35 | 58.30 | 100.63 | 4.0 | 4.25 |
| Sw5 | 8.09 | 25 | 100 | 15.55 | 65.21 | 135.20 | 6.0 | 1.38 |
| Sw6 | 8.32 | 25 | 50 | 16.20 | 32.80 | 50.90 | 2.0 | 0.35 |
| Sw7 | 8.27 | 25 | 80 | 21.85 | 51.20 | 90.30 | 4.0 | 0.35 |
| Sw8 | 8.34 | 24 | 30 | 15.43 | 25.40 | 100.32 | 2.0 | 0.45 |
| Sw9 | 8.06 | 24 | 20 | 14.79 | 23.06 | 128.74 | 2.0 | 0.71 |
| Sw10 | 8.24 | 24 | 2 | 18.01 | 0.86 | 120.66 | 2.0 | 0.71 |
| Sw11 | 8.23 | 25 | 20 | 11.74 | 23.56 | 98.76 | 3.0 | 2.14 |
| Sw12 | 8.09 | 24 | 50 | 15.56 | 31.94 | 90.00 | 2.0 | 2.36 |
| Sw13 | 7.53 | 25 | 30 | 11.45 | 25.38 | 115.04 | 2.0 | 3.25 |
| Sw14 | 7.04 | 24 | 30 | 12.62 | 25.36 | 123.60 | 2.0 | 2.84 |
| Sw15 | 7.89 | 24 | 70 | 20.09 | 53.10 | 120.70 | 2.0 | 1.79 |
| Sw16 | 8.11 | 24 | 10 | 13.44 | 20.76 | 96.44 | 2.0 | 0.65 |
| Sw17 | 8.40 | 24 | 100 | 17.09 | 63.06 | 93.27 | 2.0 | 1.63 |
| Sw18 | 8.30 | 24 | 120 | 12.08 | 68.43 | 124.73 | 2.0 | 1.66 |
| Sw19 | 8.38 | 24 | 100 | 14.36 | 63.10 | 74.74 | 2.0 | 1.43 |
| Sw20 | 7.52 | 24 | 60 | 16.00 | 32.64 | 80.09 | 2.0 | 0.32 |

## APPENDIX 11

**Identity of specific virulent genes in pathogenic *E. coli* using singleplex polymerase chain reaction**

| Target gene | primer | Primer sequences 5′-3′ | Procedure | Reference |
|---|---|---|---|---|
| *aggr* | aggRks 1 | GTATACACAAAAGAAGGAAGC | An initial denaturating of 5 min at 94 °C then 35 cycles at 94 °C for 30 sec, 60 °C for 30 sec and 72 °C for 60 sec and terminate at 72 °C for 10min | Ratchtrachenchai *et al.* (1997) |
| | aggRks 2 | ACAGAATCGTCGTCAGCATCAGC | | |
| *Stx*-1 | Stx-1F | ACACTGGATGATCTCAGTGG | An initial denaturing for 5 min at 94 °C, then 35 cycles of 94 °C for 30 sec , 62 °C for 45 sec and 72 °C for 60 sec and terminate at 72 °C for 10 min | Paton and Paton (1998) |
| | Stx-1R | CTGAATCCCCCTCCATTATG | | Paton and Paton (1998) |
| *eaeA* | Eae-F | GACCCGGCACAAGCATAAGC | An initial denaturing for 5 min at 94 °C, then 35 cycles of 94 °C for 30 sec, 63 °C for 30 sec, 72 °C for 60 sec and terminate at 72 °C for 10min | |
| | Eae-R | CCACCTGCAGCAACAAGAGG | | |
| *Elt* | Elt-F | GGCGACAGATTATACCGTGC | An initial denaturing for 5 min at 94 | Stacy-Phipps *et al* (1995) |
| | Elt-R | CGGTCTCTATTCCCTGTT | | |

| | | | | |
|---|---|---|---|---|
| | | | ºC, then 35 cycles at 94 ºC for 30 sec, 64 ºC for 30 sec, 72ºC for 60 sec and terminate at 72 ºC for 10 min | |
| *Est* | Est-F | CCTTTCGCTCAGGATGCTAAAC | An initial denaturing for 5 min at 94, then 35 cycles of 94 for 30 sec, 63 for 30 sec, 72 for 60 sec and terminate at 72 for 10 min | Chandran and Mazumder (2014) |
| | Est-R | CAGTAATTGCTACTATTCATGCTTTCAG | | |

## APPENDIX 12

**Gene sequencing for identification of *Escherichia coli* MN712503**

ACTTACGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGG
TAACAGGAAGCAGCTTGCTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGT
CTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACC
GCATAACGTCGCAAGCACAAAGAGGGGGACCTTAGGGCCTCTTGCCATCGGAT
GTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGAT
CCCTAGCTGGTCTGAGAGGATGACCAGCAACACTGGAACTGAGACACGGTCCA
GACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGA
TGCAGCCATGCAGCGTGTATGAAGAAGGCCTTAGGGTTGTAAAGTACTTTCAGC
GGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCCCAGAAG
AAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCG
TTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATG
TGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTC
TCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGG
AGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGC
GAAAGCGGGGGGGACAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAAC
GATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAAATAACGCGTT
AAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGAC
GGGGGCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAA
CCTTACCTGGTCTTGACATCCACGGAAGTTCAGAGATGAGAATGTGCCTTCGGG
AACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGG
GTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCGGCCGG
GAATCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAA
GTCATCATGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAA
GAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGG
ATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATC
AGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCA
TGGGAGTGGGTTGCAAAAGAAGTAGGTACATTCT

## APPENDIX 13

**Gene sequencing for identification of *Klebsiella pneumoniae* MT128987**

GAGGCGCGGCAGGCCTAACAAATTTCAAGTCGAGCGGTAGCACAGAGAGCTTG
CTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATG
GAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACC
AAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAGC
TAGTAGGTGGGGTAACGGCTCACCTAGGCGACGACCCCTAGCTGGTCTGAGAGG
ATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGC
AGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGT
GAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGATGAGG
TTAATAACCTCATCGATTGACGTTACCCTGCAGAAGAAGCACCGGCTAACTCCG
TGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGG
CGTAAAGCGCACGCAGGCGGTTcCGTCAAGTCGGATGTGAAATCCCCGGGCTCA
ACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAG
AATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCG
AAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCA
AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGATTTGGAGGT
TGTGCCCTTGAGGGTGGTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGAG
TACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACAT
CCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGC
TGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAACTCAAAGGAGACT
GCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTT
ACGACCAGGGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCGACCTC
GCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTGCAA
CTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTG
AATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGC
AAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCAT
GACTGGGGTGAAGTCGTAACATCATT

## APPENDIX 14

**National Center for Biotechnology (NCBI) blast result**

| Sample I.D | Most likely organism | Max score | Total score | Query cover | E. value | Percentage identification | Accession |
|---|---|---|---|---|---|---|---|
| Isolate 1 | *Escherichia coli*strain SG4 16S ribosomal RNA gene partial sequence | 2549 | 2549 | 99% | 0 | 99.08% | MN318323.1 |
| Isolate 2 | *Klebsiella pneumoniae* strain GXNN3 16S ribosomal RNA gene partial sequence | 2658 | 2658 | 99% | 0 | 99.45% | KU936064.1 |

**Blast Result for Isolated Microorganisms**

| Sample I.D | Organism | Strain | Accession |
|---|---|---|---|
| Isolate 1 | *Escherichia coli* | MiFutMin1 | MN712503 |
| Isolate 2 | *Klebsiella pneumoniae* | MiFutMin_1 | MT128987 |

## APPENDIX 15

### Media used for isolation and enumeration of isolates

Media used were Membrane Lauryl sulphate (MLS) broth, Eosin Methylene blue agar (EMB), MacConkey sorbitol agar, Nutrient agar and Nutrient broth. They were prepared according to manufacturer's instruction and then sterilized using autoclave at 121 ºC for 15 minutes. Membrane Lauryl sulphate (MLS) broth was used to determine the total coliform count using the membrane filtration technique, plates were incubated at 35 ºC for 24 hours. Eosin Methylene blue agar was used to determine the thermotolerant coliforms load by pour plate method incubating plates at 44.5 ºC for 48 hours. MacConkey sorbitol agar was used for the isolation of pathogenic *E. coli* strains associated with diarrhea, Nutrient agar was prepared in a slant bottle for pure stock culture in order to carry out biochemical characterization and nutrient broth was used to preserve the isolates before molecular characterization.

**APPENDIX 16**

# Questionnaire for Samples Collection

Kindly feel free to complete the form appropriately as all information given will be treated discretely.

**Section 1: Personal profile**

i. Respondent

□ Male

□ Female

ii. Age _____

iii. House address _____

iv. Educational Qualification _____

**Section 2: Drinking water source(s) and quality**

i. What is/are the source (s) of drinking water in your household?

□ Tap

□ Sachet water

□ Well

□ Borehole

□ Two or more of the above

□ Others (specify) _____

ii. What is the commonest source of drinking water in your neighborhood?

_____

iii. If answer to ii above is tap, how regular is water supply?

iv. What is your perception to the source of drinking water in your household? Kindly tick appropriately.

a. Taste : Tasteless

Has taste

b. Color : Colorless

Has color

c. Smell : odorless

Has odor.

**Section 3: Sanitary conditions**

If the answer to question 1 in section 2 is well, kindly fill the appropriate answers to the questions below:

a. Distance from septic tank _____

b. Is the well covered _____

c. Is there a permanent fetcher attached to the well _____

d. Is the well ringed (concrete lining) _____

e. Do you treat water before drinking _____

f. If yes above, which method

□ Boiling

□ Alum

□ Filtration

□ Others (specify) _____

g. Any straying dogs around the premises of drinking water source? _____

**Section 4: Awareness about waterborne diseases**

a. Are you aware that contaminated water can transmit of disease? _____

b. Has any member of your household suffered from waterborne diseases in recent time? _____