SCREENING FOR ANTIBIOTIC RESISTANCE GENES IN *ESCHERICHIA COLI* AND *SALMONELLA ENTERICA* ISOLATED FROM DRINKING WATER FROM PARTS OF KADUNA STATE, NIGERIA

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT FOR THE AWARD OF A DOCTOR OF PHILOSOPHY DEGREE IN MICROBIOLOGY

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FEBRUARY, 2017

DECLARATION

I declare that the work in this thesis entitled "**SCREENING FOR ANTIBIOTIC RESISTANCE GENES IN *ESCHERICHIA COLI* AND *SALMONELLA ENTERICA* ISOLATED FROM DRINKING WATER FROM PARTS OF KADUNA STATE, NIGERIA"** was performed by me in the Department of Microbiology, Ahmadu Bello University, Zaria, under the supervision of Professors J.B. Ameh, I.O. Abdullahi and C.M.Z. Whong.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

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_____	_____	_____
Name of Student	Signature	Date

CERTIFICATION

This thesis entitled SCREENING FOR ANTIBIOTIC RESISTANCE GENES IN *ESCHERICHIA COLI* AND *SALMONELLA ENTERICA* ISOLATED FROM DRINKING WATER FROM PARTS OF KADUNA STATE, NIGERIA by Olajoke Mofoluke OLUKOSI meets the regulations governing the award of the degree of Doctor of Philosophy of the Ahmadu Bello University, and is approved for its' contribution to knowledge and literary presentation.

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ABBREVIATIONS

ACRONYM	MEANING
bla	Beta-lactamase
BLAST	Basic Local Alignment Search Tool
BOD	Biological Oxygen Demand
CDC	Centres for Disease Control
cfu	Colony forming units
DO	Dissolved Oxygen
FDA	Food and Drug Administration
h	Hour
LGA	Local Government Area
m	minute
MDR	Multidrug Resistance
NAFDAC	National Agency for Food and Drug Administration and Control
NCBI	National Centre for Biotechnology Information
rRNA	Ribosomal Ribonucleic acid
SON	Standard Organization of Nigeria
stx	Shiga toxin gene
TEM	Temoniera
UNICEF	United Nations Children Educational Fund
WHO	World Health Organization

ABSTRACT

This study investigated drinking water from some parts of Kaduna state, Nigeria for multi-drug resistant *Escherichia coli* and *Salmonella enterica* (serovars Typhi and Typhimurium). The physicochemical and bacteriological qualities of the water samples were also investigated. This was done by determining some parameters such as the BOD, DO, conductivity, turbidity and also determining the presence and level of some heavy metals such as copper, chromium and iron. The total and faecal coliform counts were determined, together with the presence of *Escherichia coli* (O157:H7 and non-O157) and *Salmonella enterica.* A total of five hundred water sources (borehole, tap, well, stream and packaged water) were sampled from six local government areas in Kaduna state between March 2014 and February 2015, which included the wet and dry seasons (246 and 254 samples respectively). The mean conductivity and total dissolved solids content of the borehole samples were significantly higher than all other samples, while tap water had the least content. Furthermore, the mean values of the samples collected in the wet and dry seasons showed that the values of the nitrate, pH and Lead were significantly higher during the wet season ($P<0.05$).Packaged and borehole water were observed to be of relatively better physicochemical quality than water from other sources. Standard procedures were used for the identification of the target organisms and for estimating the total and faecal coliform counts of the water samples. The faecal coliform counts were significantly higher during the wet season as compared to the dry season. A total of 178 isolates were characterized biochemically, of which 73(46%) and 12(2.4%) of them were *Escherichia coli* and *Salmonella enterica* respectively. Six of the *E. coli* isolates were confirmed serologically to belong to the O157:H7 group by testing with a latex agglutination kit, while the *Salmonella enterica* were confirmed to belong to the Typhi and Typhimurium serovars. Other bacteria detected included *Proteus mirabilis, Hafnia alvei, Enterobacter aerogenes, Citrobacter freundii and Klebsiella pneumoniae*. Antibiotic susceptibility tests to ten commonly used antibiotics were carried out using the Kirby-Bauer method. The highest resistance observed by *E.coli* and *Salmonella enterica* was to Tetracycline (56.1 and 77.8% respectively). Forty (97.6%) of the *E. coli* isolates were susceptible to Chloramphenicol and Gentamicin, while all the 9 *Salmonella enterica* isolates (100%) were susceptible to Amoxycillin, Amoycillin-clavulanic acid, Ampicillin, and Gentamicin. One isolate was resistant to six of the antibiotics, while three were resistant to seven antibiotics. These multidrug resistant isolates were gotten from various water sources. Eight of them were isolated from borehole water, six from packaged water and one from stream water. Polymerase Chain Reaction was used to assay for the presence of some virulence genes, antibiotic resistance genes and 16S rRNA genes. Some of the isolates produced amplicons for the following genes: stx2, invA, eaeA, hlyA, sul1, bla_{TEM}, tetA, tetB and 16S rRNA. However, none of the isolates tested produced amplicons for the stx1, spvA and cmlA genes. The 16S rRNA genes were further subjected to sequencing, and the sequences were analysed using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website, thereby confirming their identities. Other strains of *E. coli* were identified after sequencing such as *E. coli* O104:H4 and *E. coli* IAI39. The *Salmonella enterica* isolates were confirmed to belong to the Typhi and Typhimurium serovars. Bottled water proved to be of better bacteriological quality as compared to other water sources. These results show that many of the water samples had been contaminated by faecal sources, and

The presence of multi-drug resistant strains also indicates a great risk to consumers especially those who drink these waters without any further treatment.

CHAPTER ONE

INTRODUCTION

1.1 General Overview

Water is essential for human existence, and its importance for individual health and the well-being of a nation cannot be underestimated. Notwithstanding, many people in developing countries do not have access to safe and clean drinking water, or to adequate amounts of water for basic hygiene (World Health Organization (WHO) and United Nations Children's Fund (UNICEF), 2010). It is needless to say that the quality of water is of vital importance to public health. Efficient surveillance and check strategies are important for executing a high quality management of this resource (Abdullah and Youssuf, 2011). However, poor water quality continues to be a leading cause of health problems especially in developing countries where it is estimated that about 3.4 million deaths occur every year especially among children (UNICEF, 2008).

Millions of people are exposed to dangerous levels of biological contaminants and chemical pollutants in their drinking water due to inadequate management of urban, industrial or agricultural wastewater. In addition, dangerously high concentrations of chemical hazards, such as arsenic and fluoride, originating from natural sources affect millions and cause conditions such as cancer and fluorosis (WHO, 2013). Most bacterial pathogens potentially transmitted by water infect the gastrointestinal tract and are excreted in the faeces of infected humans and other animals, and they may find their way via sewage into sources of drinking water (Brock and Madigan, 1999; WHO, 2011).

Due to the inadequacies and lack of pipe-borne water, most of the populations have to resort to drinking water from wells and streams especially in the rural and suburban communities. These water sources are largely untreated and might harbour waterborne and vector-borne diseases such as cholera, typhoid fever, diarrhoea, hepatitis and guinea worm infection (Adekunle *et al.*, 2004; Fenwick, 2006). These diseases are caused by pathogenic bacteria, viruses, protozoa and other microbes which are shed in human faeces and pollute water supplies, which people utilize for drinking and washing purposes (Oyedeji *et al*., 2011).

Another alternative source of drinking water being used is the sachet or packaged water, commonly referred to as "pure water". Sachet or packaged water is any water that is in a sealed plastic and distributed or offered for sale and is intended for human consumption (Food and Drug Administration, 1995). Potential health problems may exist due to the microbial content of sachet water since water is one of the vehicles for the transmission of pathogenic organisms (Tagoe *et al*., 2011). In Nigeria particularly, there is an astronomical increase in the consumption of packaged water especially bottled and sachet drinking water. The increased demand for these drinking water products is attributed largely to factors such as inadequate or non-availability of reliable, safe municipal water in urban areas, the impression that high quality natural spring water and drinking water offer a healthy, refreshing and great tasting alternative to high-calorie soft drinks and ordinary tap water; and convenience, which has made the products meet the requirements of any lifestyle when needed (Oyedeji *et al*., 2011).

Members of the faecal coliform group especially *E. coli* are used as indicators of possible sewage contamination because they are commonly found in human and animal faeces. Other microbial indicators of possible faecal contamination are faecal enterococci, especially *Enterococcus faecalis* and *Clostridium perfringens* spores (WHO, 2013). Contamination by human or animal excreta is the most common reason for water to be considered unsafe for drinking because of the high probability of presence of pathogenic organisms. Exposure to contaminated water through ingestion such as drinking water, recreation or irrigation is a significant mode of transmission of gastrointestinal tract infection (Umoh *et al*., 2006).

Detection of pathogens such as *Salmonella enterica*, *Escherichia coli* (*E. coli*), *Campylobacter*, *Shigella* species and *Vibrio cholerae* from water sources, connotes a serious public health risk to consumers, which is further exacerbated by the widely reported cases of resistance of enteric bacterial pathogens to several antibiotics (Ash *et al*., 2002; Okeke *et al*., 2007). Antimicrobial resistance plays an important role in formulation of health policies, and involves an increasing number of bacteria and resistance mechanisms (Titilawo *et al.*, 2015). It has been observed that the highest occurence of antimicrobial resistant bacteria occur in countries where antibiotics are used extensively for prevention and treatment of microbial infections in humans as well as animals (Junco-Diaz *et al.*, 2006).

Antimicrobial resistant bacteria including multidrug resistant *Escherichia coli* are now an emerging threat to humans and even animals in developing and developed countries.Globally, antimicrobial resistant bacteria and their associated determinants have

become an important environmental contamination issue which is currently receiving an increased attention (Sapkota *et al.,* 2007). There is therefore need of adequate information on antimicrobial resistant pathogens and their genetic determinants in water sources, to serve as a basis for proper selection of the best treatment in case of any outbreak (Titilawo *et al.,* 2015).

1.2 Statement of the Research Problem

Potable water that is fit for consumption deserves special attention because of its implication in affecting public health and quality of life (WHO and UNICEF, 2013). It has been estimated that 80% of ill health in developing countries are water and sanitation related (Admassu *et al*., 2004). Isolation of pathogens such as *E. coli* O157:H7 and *Salmonella enterica* from water sources connotes a serious public health risk to consumers due to the risk of transmission of gastrointestinal illness (Umoh *et al*., 2006; WHO, 2011). Several waterborne outbreaks which have been linked to multidrug resistant enteric bacteria have been reported worldwide and the high prevalence of multidrug resistance indicates a serious need for antibiotic surveillance and planning of effective interventions to reduce multidrug resistance in such pathogens (Olayinka *et al.,* 2004).

There has been recorded increase of antimicrobial drug resistance among members of the Enterobacteriaceae in several countries including Nigeria (Doughari *et al.,* 2009). The evolutionary prowess of microorganisms presents serious challenges to successfully stopping the development of antimicrobial resistance (Stephan and Mathew, 2005). The spread of antibiotic resistance determinants into the environment through water is a possibility because when water is contaminated with feacal materials which contain

antibiotic resistant bacteria, they could serve as a pool or reservoir for genetic elements that could be transferred to other bacteria, thus increasing the problem of antibiotic resistance (Titilawo *et al.,* 2015).

1.3 Justification of the Study

There is limited information regarding the genotypic characteristics of antibiotic resistance in *Escherichia coli* and *Salmonella* isolates recovered from drinking water in Kaduna State, despite the fact that antibiotic resistance has been reported in bacteria isolated from drinking water sources (Olukosi *et al*., 2008; Garba *et al*., 2009; Chigor *et al.,* 2010). Emphasis is usually laid on drug resistance in clinical isolates even though there have been reports of higher incidence of multidrug resistant *E. coli* amongst aquatic isolates as compared to clinical isolates (Chigor *et al.,* 2010), suggesting that drinking water might also be a major reservoir of antibiotic resistance in the study area.

Monitoring of drinking water for pathogenic bacteria and resistance to antimicrobials is therefore of great importance (Doughari *et al.,* 2010). It is in respect of this, that this study was carried out involving drinking water sources from parts of Kaduna State with the following aim and objectives.

1.3 Aim of Study

The aim of this study was to screen multidrug resistant *Escherichia coli* and *Salmonella enterica* isolated from drinking water sources available to the public in some parts of Kaduna State, Nigeria for the presence of some antibiotic resistance genes.

1.5 Specific Objectives

The specific objectives of this research were to:

1. determine the physicochemical quality of the water samples
2. determine the bacteriological quality of the water samples and characterize *Escherichia coli* (O157:H7 and non-O157) and *Salmonella enterica* in the water sources.
3. determine the antibiotic susceptibility patterns of the isolates to commonly used antibiotics and screen for the presence of multiple antibiotic resistant strains.
4. screen the *Escherichia coli* O157:H7 and non- O157:H7 for the possession of some virulence genes (Stx1, Stx2, eaeA and hlyA) and the *Salmonella enterica* isolated for the carriage of the virulence genes (invA and spvA) using the polymerase chain reaction (PCR) technique.
5. screen the multidrug resistant isolates for some antibiotic resistance genes (tetA, tetB, sul1, and bla_{TEM}).
6. confirm the identities of the isolates using 16S rRNA sequencing analysis and Basic Local Alignment Search Tool (BLAST), and subsequently construct phylogenetic trees.

CHAPTER TWO

LITERATURE REVIEW

2.1 Water Pollution

Water is absolutely essential for all forms of life. It is a basic necessity, therefore it is important that it is handled properly, otherwise it could serve as a medium for transmission of organisms which cause epidemic and communicable diseases (Akande *et al.*, 2011). Water provides essential elements, but when polluted it may become an undesirable substance dangerous to human health (Karavoltsos *et al*., 2008). Indeed the microbial quality of potable water should not exceed limits specified in the water quality guidelines (APHA, 2011). Unfortunately however, the drinking water in several rural communities in Nigeria has been reported to be of poor microbial quality, and is therefore unsafe and unacceptable for human consumption (Ibrahim and Ajibade, 2012).

Pollution is caused when a change in the physical, chemical or biological condition in the environment harmfully affects quality of human life, other animals and plants (Esemikose and Akoji, 2014). Agricultural practices, industrialization and discharge of sewage into water bodies are among the factors responsible for water pollution. Pollution easily arises when population growth outmatches availability of potable water due to inadequate urban planning processes (Oluyemi *et al.*, 2010).

The quality of water, whether used for drinking, domestic purposes, food processing or recreational purposes has an important impact on health. Water of poor quality can cause disease outbreaks and it can contribute to background rates of diseases manifesting themselves on different time scales (WHO, 2013).

In humans, water helps maintain the moisture of internal organs of the body, maintains the volume and consistency of fluids such as blood and lymph, regulates body temperature, eliminates poisons or toxins from the body through urine, sweat and breathing, and is essential for regulating the normal structure and functions of the skin. The body looses about four litres of water every day; therefore it is necessary to replenish this volume by drinking at least the equivalent amount of quality water every day (Odonkor and Ampofo, 2013).

Water pollution has been suggested to be the leading cause of deaths and diseases worldwide. Biological pollutants in drinking water cause the most widespread diseases. They include bacteria, fungi, viruses, actinomycetes, algae and protozoa. Bacteria transmitted by drinking water usually grow in the intestinal tracts of animals and humans. They are excreted in faeces of infected persons and may find their way through sewage into sources of drinking water (Akande *et al.*, 2011). Contaminated water could therefore serve as a medium for transmission of communicable diseases such as diarrhoea, cholera, dysentery, typhoid and guinea worm infection. For children under five, this burden is greater than the combined burden of HIV/AIDS and malaria (Liu *et al.*, 2012).

Millions of people are exposed to dangerous levels of biological contaminants and chemical pollutants in their drinking water due to inadequate management of urban, industrial and agricultural waste water. In addition, dangerously high concentrations of chemical hazards such as arsenic and fluoride, originating from natural sources affect millions and leads to conditions such as cancer and fluorosis (Flanagan *et al*., 2012). Although consumption of contaminated water represents the greatest risk, other routes of transmission can also lead to

disease and contribute to the disease burden. While water can be a very significant source of infectious organisms, many of the diseases that may be waterborne may also be transmitted by other routes, including person-to-person contact, droplets and aerosols and food intake (WHO, 2011). The disease burden attributable to bathing water exposures is significant, largely due to the high exposed population at recreational beaches world-wide (Shuval, 2003).

The common sources of drinking water in developing countries like Nigeria include the streams, rivers, well and boreholes which are mostly untreated and associated with various health risks (Okonko *et al*., 2008a, b). The quality of water influences the health status of any populace; hence, analyses of physical, biological and chemical properties including trace element contents are very important for public health studies (Shalom *et al*., 2011).

2.2 Challenges of Unhygienic Environments

Most developing countries are characterized by poor and unhygienic conditions. The majority of the poor people live in slums where unhygienic practices such as disposal of filled septic tanks directly into gutters, throwing of faeces wrapped in polythene bags directly into streams, rivers or the nearby bush or burying the solid faecal matter in soil are carried out and the people may be ignorant or care less because they are pre-occupied with the struggles of day to day survival. Most urban centers are also characterized by huge refuse dumps in which faeces, left-over and decaying foods, animal carcasses and rotting vegetables are left indefinitely. Such practices and environments facilitate the rapid spread of gastrointestinal pathogens among the population (Doughari *et al.,* 2010). Food and water

bodies easily gets contaminated due to runoffs or floods thus serving as ready sources of human infection.

Outbreaks of diarrhoea and cholera have been reported especially among school children and refugee camps in various developing countries such as Nigeria, Rwanda, Congo, Zimbabwe, Sudan, Afghanistan, Chile and Brazil (Field, 2003; Thapar and Sanderson, 2004). Urban populations in developing countries are characterized by high densities of people, poor housing, inadequate sanitation and solid waste removal, and unsafe drinking water among others. Thus, more people are forced to share the same toilets or spaces in rented apartments or slums. In such conditions, diarrhoeal and other bacterial infections are much more easily transmitted. With the prevalence in abundance of predisposing factors such as poor hygiene, poverty, inadequate medical care and potable water, Africa is at serious risk of bacterial infections (Doughari *et al.,* 2010).

2.3 Drinking Water Availability

Potable water of good quality is in increasing demand in developing countries, which are characterized by deteriorating environments (Odonkor and Ampofo, 2013). Contaminated or polluted water may contain injurious chemicals or microbial waterborne pathogens. Waterborne pathogens represent a serious and growing hazard, and infectious diseases continue to affect populations throughout the world. Other factors like the aging of water treatment infrastructures, the increasing occurrence or perhaps, the increasing recognition and detection of organisms resistant to conventional disinfection treatments also increases the indices of these infections (Doughari *et al.,* 2010).

An increasing number of countries can be considered water stressed. Of all environmental questions, those related to water are perhaps the most far reaching in their long-term consequences and the most difficult to tackle from the scientific and medical point of view. In many parts of the world there is a widespread scarcity, gradual destruction and increasing pollution of fresh water sources. Many nations face growing problems associated with guaranteeing an adequate drinking water supply and in the developing world, one in every three persons lacks safe drinking water and sanitation (Doughari *et al.,* 2010).

By 2025, half of the world population will be living in water stressed areas (WHO, 2011). In the developing countries, the population of people in the urban cities alone stands at 1.9 billion and this figure is expected to increase to 3.3 billion by 2030 (Hunter *et al.,* 2003). In addition, most of the world's 6.9 million displaced people and refugees reside in refugee camps or temporary shelters in developing countries. These environments are often over crowded, and are characterized by poor sanitation practices, inadequate potable water and health care services, and also have inadequate barriers to vectors and animals that may carry infectious diseases. Person-to-person contact is usually increased, thus diarrhoeal diseases are more common, with dire effects (Houmsou *et al.,* 2010).

Due to the inadequacies and lack of pipe-borne water, most of the populations have to resort to drinking water from wells and streams especially in the rural and suburban communities (Shittu *et al.,* 2008). These water sources are largely untreated and might harbour waterborne and vector-borne diseases such as cholera, typhoid fever, diarrhoea, hepatitis and guinea worm infection (Rahman *et al*., 2001; Adekunle *et al.*, 2004; Fenwick, 2006). These diseases are caused by pathogenic bacteria, viruses, protozoa and other

microbes which are shed in human faeces and pollute water supplies which people utilize for drinking and washing purposes (Oyedeji *et al*., 2011).

In Nigeria, there has been a massive rise in the consumption of packaged water especially bottled and sachet drinking water. The increased demand for these drinking water products is largely due to factors such as inadequate or non-availability of reliable, safe pipe-borne water, the impression that high quality natural spring water offers a healthy, refreshing and great tasting alternative to high-calorie soft drinks and ordinary tap water; and convenience, which has made the products meet the requirements of any lifestyle when needed (Oyedeji *et al*., 2011). The sachet drinking water was introduced into the Nigerian market as a cheaper means of accessing safe drinking water than bottled water (Ogundipe, 2008). It also acts as an improvement over the former types of drinking water packaged for sale to consumers in hand-filled, hand-tied polythene bags. While some bottled and sachet water manufacturers make use of sophisticated techniques such as ozonization and reverse osmosis during their production processes, many just boil well water and filter with unsterilized filteration equipment (Oyedeji *et al*., 2011).

Bottled water is water that is sealed in bottles or other containers with no added ingredients except that it may contain safe and suitable fluorides intended for human consumption. Small scale entrepreneurs introduced small nylon sachets which are electrically heated and sealed at both ends to the market and is popularly called ‘pure water’. It is mostly patronized by members of the low socio-economic class (Narasimhan and Himabindu, 2010). Most bottled water manufacturers in Nigeria also engage in sachet water packaging and obtain their raw water mostly from boreholes, municipal piped water or well water.

Adherence to production and analytical standards are doubtful as most of the factories are observed to lack the appropriate technology for achieving these (Oyedeji *et al*., 2011).

Surveillance carried out in Nigeria by the National Food and Drug Administration and control (NAFDAC) between 2004 and 2005 revealed that some producers of packaged water indulge in sharp practices such as packaging of untreated water, production under unhygienic conditions, illegal production of unregistered products in unapproved premises, use of non-food grade sachets and release of packaged water for distribution and sale without date marking (Edema *et al.*, 2011). Several studies on the microbial quality of bottled and sachet water have reported violations of international quality standards. A study on different brands of bottled water in Trinidad showed that 18 out of the 344 samples checked revealed the presence of total coliforms. While five of the samples were contaminated with *Escherichia coli*, colonies of *Enterococcus faecalis* were occasionally detected in the samples (Bharath *et al*., 2003). The quality monitoring of sachet water in Nigeria has also been documented. A study in Enugu showed that there was evidence of coliform contamination in both tap and sachet water (Ohanu *et al.*, 2012). Studies in Kebbi State and other parts of Nigeria showed similar results (Adekunle *et al*., 2004; Onifade and Ilori, 2008; Dada, 2009; Kalpana *et al.*, 2011).

Potential health problems may exist due to the microbial content of sachet water since water is one of the vehicles for the transmission of pathogenic organisms (Tagoe *et al*., 2011). Continuous increase in the sale and indiscriminate consumption of packaged water in Nigeria is of public health significance (Ogundipe, 2008). It has been widely observed that the advent of “pure water” has significantly increased the cases of Salmonellosis and

typhoid fever (Oluyege, 2014). In addition to natural contamination, the product can also deteriorate before it reaches the consumer (Da Silva, 2007).

2.4 Drinking Water Standards

The Nigerian Standard for Drinking Water Quality (NSDWQ, 2007) contains mandatory limits concerning constituents and contaminants of water that are known to be hazardous to health and also those that raise complaints from consumers. The standard includes a set of procedures and good practices required to meet the mandatory limits. Since water quality issues are health related issues, the Federal Ministry of Health, collaborating with the Standards Organization of Nigeria (SON) which is the only body responsible for developing National Standards in Nigeria, and working through a technical committee of key stakeholders developed this Standard. The selection of parameters and the determination of maximum allowable limits have been conducted taking into consideration the WHO guideline for drinking water quality (SON, 2007).

In Nigeria, the National Agency for Food and Drug Administration and Control (NAFDAC) is the parastatal under the Federal Ministry of Health charged with the responsibility of regulating and controlling imported and locally processed foods and water products (Omotayo and Denloye, 2002). To ensure strict adherence to international standards, NAFDAC's regulation for bottled and sachet-packed water in Nigeria has been put at the standards established by the World Health Organization (WHO). According to these standards, potable water for human consumption must be free of microbial indicators

of faecal contamination and coliform count per 100 ml of drinking water must be zero (Pierre, 1999; WHO, 2012).

In the United States of America, the safe drinking water act was enacted by congress on December 16, 1974 and was amended in 1986 and 1996. The act authorizes the United States Environmental Protection Agency (USEPA) to set standards called the National Primary Drinking Water Regulations (NPDWRS). There are two categories of drinking water regulations namely the primary or maximum contaminant level, and the secondary contaminant level (Sloat and Ziel, 1992).The primary contaminant level gives the highest concentration of a particular contaminant allowed in drinking water. The maximum contaminant level goal for coliforms in potable water is zero per 100ml of water. The secondary contaminant standard regulates contaminants that cause offensive taste, odour, and colour, corosivity, foaming and staining. Both these secondary and primary standards serve as a guide to ensure safe drinking water.

In many countries water is processed to meet the bacteriological standard by chlorination. Chlorinated water should contain no total or faecal coliform or *E. coli* per 100 ml of water, while unchlorinated water should contain no faecal coliforms (WHO, 1996). Chlorination is usually insufficient in dealing with waterborne viruses and parasites (Gale, 2001). The presence of indicator organisms in excess of the standards indicates faecal pollution and the possible presence of pathogen.

2.4.1 Physical and chemical indicators of drinking water quality

The potability of water does not only depend on its bacteriological quality, but also on its physical and chemical qualities and the content of toxic substances in it. The health concerns associated with chemical constituents of drinking water differ from those associated with microbial contamination and arise primarily from the ability of chemical constituents to cause adverse health effects after prolonged periods of exposure. There are few chemical constituents of water like high levels nitrate, sulphate, fluoride and high or low pH, etc that can lead to health problems resulting from a single exposure, except through massive accidental contamination of a drinking water supply. Moreover, it has been shown that whenever such incidents occur, the water usually becomes unpalatable for drinking due to unacceptable odour, appearance and taste (Esemikose and Akoji, 2014). Most chemicals arising in drinking water are of health concern only after years of exposure. The principal exception to this is nitrate. Typically, changes in water quality occur progressively, except for those substances that are discharged or leach intermittently to flowing surface waters or groundwater supplies from sources like contaminated landfill sites.

The chemical contaminants in water are of various categories:

1. Naturally occurring chemicals. For example: bromide, chloride, hardness, hydrogen sulphide, Molybdenum, sulphate
2. Chemicals from industrial sources and human dwellings. For example: Arsenic, Boron, Chromium, Selenium
3. Chemicals from agricultural activities. For example pesticides and ammonia

4. Chemicals used in water treatment or from materials in contact with drinking water. For example: chlorine and chlorination by-products, lead and copper.

Some of the physical and chemical parameters which are of importance in drinking water will be discussed briefly.

2.4.1.1 Total dissolved solids (TDS)

Total dissolved solids refer to the inorganic salts (mainly calcium, magnesium, potassium, sodium bicarbonates, chlorides and sulphates) and small amounts of organic matter that are dissolved in water. TDS in drinking water originates from natural sources, sewage, urban runoff and industrial wastewater. Concentrations of TDS in water vary considerably in different geological regions due to differences in the solubilities of minerals.

Reliable data on possible health effects associated with the ingestion of TDS in drinking water are not available, and no health-based guideline value is proposed. However, the presence of high levels of TDS in drinking water may be objectionable to those who consume it (WHO, 2003). The palatability of water with a TDS level of less than about 600 mg/l is generally considered to be good. High amounts of TDS may result in adverse taste effects as drinking water becomes significantly unpalatable at TDS levels higher than 1000 mg/l. The presence of high levels of TDS may also be objectionable to consumers, because it causes excessive scaling in water pipes, heaters, boilers and household appliances which leads to their deterioration. However, water with extremely low concentration of TDS may also be unacceptable because it tastes flat and insipid. It is recommended that waters containing more than 500 mg/l of dissolved solids not be used if other less mineralized supplies are available (IWI, 2014).

2.4.1.2 Electrical conductivity

Conductivity measures the electrical conductance in water. This is an indication of the quantity of dissolved inorganic acids, bases and salts in the water. Ions from the dissolved solids in water have a direct effect on the ability of that water to conduct an electrical current, which can be measured using a conductivity meter. Ideally, drinking water should have an electrical conductivity ranging from 20 - 60 µmHos/cm. When correlated with laboratory TDS measurements, electrical conductivity can provide an accurate estimate of the TDS concentration (IWI, 2014).

2.4.1.3 Colour

Potable water should ideally have no visible colour. Colour in drinking water is usually due to the presence of coloured organic matter associated with the humus fraction of soil. Colour is also strongly influenced by the presence of iron and other metals, which could be present either as natural impurities or as products of corrosion. It may also result from the contamination of the water source with industrial effluents and may be the first indication of a dangerous situation. Levels of colour below 15 TCU are often acceptable to consumers. High colour from natural organic carbon (e.g. humics) could also indicate a high probability of the production of by-products from disinfection processes. No health-based guideline value is proposed for colour in drinking-water (IWI, 2014).

2.4.1.4 Temperature

Water that is cool is generally more palatable than warm water. High temperature in water enhances the growth of microorganisms and may increase problems related to taste, odour, colour and corrosion. Water temperature is important because it affects the rates of

biological and chemical processes. Temperature is measured in degrees Fahrenheit or degrees Celsius. The optimal health of aquatic organisms from microbes to fish depends on temperature. If temperatures are outside the optimal range for a prolonged period, organisms would get stressed and can die (IWI, 2014).

As temperature rises, the dissolved oxygen content of water reduces. Water temperature is affected by other factors such as environmental conditions, season, removal of vegetation, building dams on rivers, discharging cooling water, discharging storm water, and groundwater influx (IWI, 2014).

2.4.1.5 Turbidity

Turbidity, like transparency, is a measure of water clarity (how far light can travel through water). The more particles suspended in a sample of water, the more difficult it is for light to travel through it and the higher the water's turbidity. Turbidity in water is as a result of suspended particles or colloidal matter that obstructs light transmission through the water. It may be caused by inorganic or organic matter or a combination of the two. Microorganisms are usually attached to particulates; therefore the filtration of water will significantly reduce its microbial content. Turbidity in some groundwater sources is a consequence of inert clay or chalk particles or the precipitation of non-soluble reduced iron and other oxides when water is pumped from anaerobic waters (IWI, 2014).

Turbidity can affect the efficiency of disinfection by providing protection for organisms. Therefore, much of water treatment is directed at removal of particulate matter before disinfection. Turbidity in drinking water is usually unacceptable to consumers. Turbidity is

measured by an instrument called a nephelometer and is reported in nephelometric units (NTUs).

2.4.1.6 pH

pH describes the acidity or alkalinity of water and represents the balance between hydrogen ions (H+) and hydroxide ions (OH) in water. The value for pH is expressed on a scale ranging from 0 to 14. Solutions with more H+ than OH^- ions have a pH value lower than 7 and are said to be acidic. On the other hand, solutions with pH values higher than 7 have more OH^- than H+ ions and are said to be basic, or alkaline. If the pH value is 7, the solution is said to be neutral (an equal number of H+ and OH^- ions) and is neither acidic nor alkaline (IWI, 2014).

2.4.1.7 Dissolved oxygen (DO)

Dissolved oxygen (DO) refers to the amount of oxygen dissolved in water. Waters of consistently high dissolved oxygen are usually considered healthy and stable aquatic ecosystems capable of supporting many different kinds of aquatic organisms. The dissolved oxygen content of water is influenced by the quantity of organic matter in it, its source, temperature, treatment and chemical or biological processes taking place in the distribution system. Depletion of dissolved oxygen in water supplies can encourage the microbial reduction of nitrate to nitrite and sulphate to sulphide. It can also cause an increase in the concentration of ferrous iron in solution, with subsequent discoloration at the tap when the water is aerated.

No health-based guideline value is recommended. However, very high levels of dissolved oxygen may worsen corrosion of metal pipes. DO is usually expressed as a concentration of oxygen in a volume of water (milligrams of oxygen per litre of water, or mg/l) (IWI, 2014).

2.4.1.8 Nitrate (NO_3) and Nitrite (NO_2)

Nitrite and nitrate are sources of nitrogen, an important nutrient for plants and algae. As ammonia is broken down by bacterial action, nitrite is formed and is then converted to the more stable, much less toxic nitrate through a process called nitrification.

The typically low natural levels of nitrate in surface water can be supplemented with nitrate from human sources. Nitrate from the fertilizer not taken up by crops in fields and grass in lawns can enter water bodies in runoff. Nitrate can also enter water bodies from wastewater discharge or runoff from feedlots.

As early as 1940, it was recognized that consuming waters with high nitrate levels contributed to methemoglobinemia ("blue baby" syndrome). This condition, usually in infants, impairs the ability of blood to carry oxygen. The United States Environmental Protection Agency (USEPA) National Primary Drinking Water Standards require that nitrate nitrogen not exceed 10 mg/L in public water supplies. Nitrite, much more toxic than nitrate, is regulated at a level not to exceed 1.0 mg/L in public water supplies (IWI, 2014).

2.4.2 Heavy metals in drinking water

Heavy metals can enter a water supply through industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers,

and also into groundwater. Heavy elements are metallic elements with high atomic weight which is at least five times greater than that of water (Butu and Bichi, 2013). They include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), chromium (Cr), copper (Cu), iron (Fe) and the platinum group elements (Dorherty *et al.*, 2012). Heavy metals could be dangerous because they tend to bioaccumulate.

Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. They are non-biodegradable and could be persistent environmental contaminants which may be deposited in water bodies. Some heavy metals are highly toxic and need to be monitored continuously because they could result in illness and death of living organisms (Ayotunde *et al.*, 2011).

2.4.2.1 Copper

Dissolved copper can sometimes give drinking water a light blue or blue green colour and also give it an unpleasant bitter taste. Copper in a drinking-water supply usually arises from the corrosive action of water leaching copper from copper pipes in buildings. Copper is also used in making fungicides, algicides, and insecticides, wood preservatives and so on (WHO, 2004). High levels of dissolved oxygen have been shown to accelerate copper corrosion in some cases. Concentrations can vary significantly with the period of time the water has been standing in contact with the pipes.

At lower doses, copper ions can cause symptoms of typical food poisoning such as headache, nausea and diarrhoea. Long term exposure could result in impaired liver functioning (WHO, 2004).

2.4.2.2 Iron

All natural water contains dissolved iron in trace amounts. Iron is present in all rocks, soil and sand, usually in the form of ferrous iron. Water which contains iron on exposure to air becomes reddish brown due to the formation of ferric hydroxide, which could give an astringent taste to the water (Ocheri, 2010; Trivedi *et al.,* 2010). Consumption of water with high iron content over a long period might lead to liver disease. High concentration of iron in groundwater is widespread (Ocheri, 2010), and may also lead to stain in plumbing fixtures, laundry and cooking materials (Butu and Bichi, 2013). The colour that excessive iron gives water may lead to consumers rejecting such water. Although this has no direct health significance, problems arise if communities decide not to use this water and return to old polluted sources (Ocheri, 2010).

2.4.2.3 Chromium

Chromium is one of the trace elements that occur as an abundant element in the earth crust. It is an odorless and tasteless metallic element that is found naturally in rocks, plants, soil, volcanic dust, and animals (USEPA, 2015). Its compounds are found in the environment due to erosion of chromium containing rocks and from other man-made soures such as its wide range use in metal plating, pipes (Oluyemi *et al.,* 2010). Excessive exposure to Chromium is suspected to be carcinogenic because it bioaccumulates (SON, 2007).

Chromium-3 is an essential human dietary element which occurs in many vegetables, fruits, meats, grains, and yeast. Chromium-6 occurs naturally in the environment from the erosion of natural chromium deposits. It can also be produced by industrial processes. There are established findings of chromium being released to the environment by leakage, poor storage, or inadequate industrial waste disposal practices (USEPA, 2015). In some epidemiological studies, it has been established that there is a link between occupational exposure to chromium-6 compounds and mortality due to lung cancer (WHO, 2003).

2.4.2.4 Lead

Lead is the one of the most commonly occurring heavy elements, accounting for 13 mg/kg of Earth's crust. It is used in the production of lead acid batteries, solder, alloys, cable sheathing, pigments, rust inhibitors, ammunition, glazes and plastic stabilizers (WHO, 2011). Tetraethyl and tetramethyl lead are important because of their extensive use as antiknock compounds in petrol. Lead can enter drinking water when service pipes that contain lead are corroded, especially where the water has high acidity or low mineral content that corrodes pipes and fixtures (USEPA, 2016). Polyvinyl chloride (PVC) pipes also contain lead compounds that can be leached from them and result in high lead concentrations in drinking water. A number of factors affect the amount of Lead that is dissolved from plumbing systems including the presence of chloride and dissolved oxygen, pH, temperature, water softness and standing time of the water, soft, acidic water being the most plumb solvent (WHO, 2011).

The level of lead in drinking-water may be reduced by corrosion control measures such as the addition of lime and the adjustment of the pH in the distribution system. Infants,

children aged 6 years and below, pregnant women and foetuses are those who are more at risk of lead poisoning. Its effects on the central nervous system can be particularly serious. Signs of acute intoxication include dullness, restlessness, irritability, poor attention span, headaches, muscle tremor, abdominal cramps, kidney damage, hallucinations, loss of memory and encephalopathy (WHO, 2011). Other health effects include increased risk of hypertension, depressed sperm counts, preterm delivery in pregnant women and malformations of foetuses (USEPA, 2016).

2.5 Bacteriological Indicators of Water Pollution

The source of water contamination responsible for the spread of infectious diseases is almost invariably faeces. Faecal contamination of water is established by the isolation of an organism that occurs only in faeces, never free-living in nature. There are several such organisms like *Echerichia coli*, *Clostridium perfringens* and *Streptococcus faecalis*. The finding of *E. coli* or *Clostridium perfringens* and *S. faecalis* is sufficient evidence that the water in question is not safe, since enteric pathogens may be presumed present (Ohanu *et al.*, 2012).

Faecal coliform counts and *Escherichia coli* detection in particular, remains the major and most reliable tool in the assessment of the health risks posed by pathogens in water (Byamukama *et al*., 2000). Monitoring the microbiological quality of drinking water relies largely on examination of indicator bacteria such as coliforms, *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa*.

Escherichia coli belongs to the faecal coliform group and is a more specific indicator of faecal pollution than other faecal coliforms. Two key factors have led to the trend toward the use of *E. coli* as the preferred indicator for the detection of faecal contamination, not only in drinking water, but also in other matrices as well: first, the finding that some faecal coliforms were non-faecal in origin and second, the development of improved testing methods for *E. coli* (Nataro and Kaper, 2004).

Faecal coliforms are increasingly being referred to as thermotolerant coliforms. This, combined with improved detection methods for *E. coli*, has started a trend toward the use of *E. coli* in place of thermotolerant coliforms as a more reliable indicator of faecal pollution in drinking water. At present, *E. coli* appears to provide the best bacterial indication of faecal contamination in drinking water. This is based on the prevalence of thermotolerant (faecal) coliforms in temperate environments as compared to the rare incidence of *E. coli*, the prevalence of *E. coli* in human and animal faeces as compared to other thermotolerant coliforms, and the availability of affordable, fast, sensitive, specific and easier to perform detection methods for *E. coli* (Prescott *et al*., 1999).

In recent years, it has been recognized that certain criteria should exist before an indicator organism can be reliable in predicting a health risk. These criteria are as follows:

i. The indicator organism should be suitable for the analysis of all types of water: river, ground water, impounded water, recreation, estuary, seas and waste waters.
ii. The indicator bacteria should be present whenever enteric pathogens are present.
iii. The indicator bacteria should survive longer than the hardiest enteric pathogens.

iv. The indicator bacteria should not reproduce in the contaminated water and produce an inflated value.

v. The testing method to detect the indicator should be easy to perform.

vi. The indicator should be harmless to humans.

vii. The assay for the indicator should have great specificity.

viii. The level of the indicator bacteria in the contaminated water should have some direct relationship to the degree of faecal pollution (Prescott *et al.,* 1999).

2.5.1 Faecal coliforms

Faecal coliforms are a group of bacteria, which are natural inhabitants of the gut of humans and other warm-blooded animals. *Escherichia coli (E. coli)* is a member of faecal coliforms that contaminate the drinking water from human and animal faecal waste (Patoli *et al.*, 2010). The presence of faecal coliform bacteria in drinking water is strong evidence of contamination by faecal matter. This is a significant health problem, as faecal coliforms frequently co-exist with human pathogens that are the causative agents of diseases such as cholera, typhoid fever, hepatitis, dysentery. Unlike faecal coliforms, these disease-causing organisms do not survive long enough outside the body to be detected (Sloat and Ziel, 1992).

Faecal coliforms have the ability to ferment lactose at 44 – 45°C. This means that unlike other coliforms they are thermotolerant. Comprising the genus *Escherichia,* and to a lesser extent, *Klebsiella, Enterobacter* and *Citrobacter,* coliform organisms are part of the family *Enterobacteriaceae* (WHO, 1996). Other coliforms have been shown to grow in non-

animal environments such as the soil, plant surfaces and even industrial effluents (Hobson and Poole, 1998).

2.5.2 *Escherichia coli*

In 1885, Theodore Escherich described an organism which he isolated from faeces of new born babies. For many years, the organism bore the generic name *Bacterium coli* but now it is recognized as *Escherichia coli (E. coli)* (Papa, 1982). *Escherichia coli* as a genus belongs to the Family *Enterobacteriaceae*, classified according to the recommendations of the *Enterobacteriaceae* sub-committee of the Nomenclature committee of the International Association of Microbiological Societies whose reports appear in the International Bulletin of Bacteriological Nomenclature and Taxonomy. As a result of the meeting held in Rio de Janeiro by the above sub- committee, and as described in their third report, they agreed on the following definition of *Escherichia.*

The *Escherichia* group consists of motile and non- motile bacteria that conform to the family *Enterobacteriaceae* and have the following biochemical characteristics: lactose fermentation (positive), indole (positive),Voges – Proskauer (negative), citrate utilization (negative), acid and gas from glucose (positive), and urease production (negative). Members of the *Escherichia* group can be killed by exposure to a temperature of 55°C for an hour or a temperature of 60°C for 15 – 20 minutes (Papa, 1982). All *Enterobacteriaceae* are rod – shaped, non – spore forming and Gram negative. They are able to grow in the presence of bile salts or other surface – active agents and are also cytochrome–oxidase

negative. Coliforms are distinguished by their ability to ferment lactose at either 35°C or 37°C with the production of acid, gas and aldehyde within 24 to 48 hours.

Most *E. coli* strains are normal flora of the gastrointestinal tract of humans and animals. However, there are some strains that have acquired virulence factors and have become pathogenic. These pathogenic *E. coli* can be categorized based on serogroups, pathogenicity mechanisms, clinical symptoms, or virulence factors (Lim *et al.,*2010). It is a Gram negative bacillus that lives in the intestinal tract of virtually everyone and usually become opportunistic when it gains access to certain parts of the body such as urinary tract, bloodstream and wounds, where it can spread causing various infections. *Escherichia coli* may also cause infections in the biliary tract and abdominal cavity (peritonitis), septicaemia, neonatal meningitis, infantile gastroenteritis, tourist diarrhoea, and hemorrhagic diarrhoea (Prescott *et al*., 1999).

Some serotypes produce shiga toxin and have been implicated amongst other enteric bacteria in many water and food contaminations. An *E. coli* infection may arise due to environmental exposure. Infections with this type of bacteria pose a serious threat to public health with outbreaks arising from food and water that has been contaminated with human or animal faeces or sewage. Exposure may also occur during hospitalization, resulting in pneumonia in immunocompromised patients or those on a ventilator. *Escherichia coli* is one of many species of bacteria living in the small intestines of mammals, known as gut

flora. When located in the large intestine, it assists with waste processing, vitamin K production and food absorption (Feng *et al.*, 2002).

Escherichia coli has been reported to be the leading cause of diarrhoea diseases in addition to pathogens such as *Salmonella, Shigella, Yersinia, Vibrio, Campylobacter species, Entamoeba histolytica,* and *Giardia lamblia* in developing countries (Prescott *et al*., 1999). *Escherichia coli* strains causing diarrhoea worldwide, mostly in infants include; enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EaggEC) and enteroadherent *E. coli* (EAEC) or diffusely adhering *E. coli* (DAEC) and enterohaemorrhagic *E. coli* (EHEC) (Paton and Paton, 1998a; Lisa and Rodgers, 1999; Burton and Paul, 2000; Prescott *et al*., 1999). Faecal oral and food borne transmission of *E. coli* have been well documented (Nataro and Kaper, 1998; Poppe *et al*., 2005).

2.5.2.1 Enteropathogenic E. coli (EPEC)

EPEC was the first pathotype of *E. coli* to be described. It was associated with large outbreaks of diarrhoea in children in the United Kingdom. This pathotype of *E. coli* remains a major cause of infant diarrhoea in developing countries (Kaper *et al.,* 2004).

The EPEC strains of *E. coli* attach to the brush border of intestinal cells and cause a specific type of cell damage called effacing lesions. Effacing lesions or attaching effacing lesions represent destruction of the brush border microvilli adjacent to adhering bacteria (Trabulsi *et al.,* 2002). The cell destruction leads to subsequent diarrhoea. It is now known that EPEC is an important cause of diarrhoea in children residing in developing countries. Twelve

EPEC serogroups have been recognized including O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158 (Prescott *et al.*, 1999).

2.5.2.2 Enterotoxigenic E. coli (ETEC)

ETEC is a strain of *E. coli* that produces one or both of two distinct enterotoxins which are responsible for diarrhoea and distinguished by their heat-stability. These toxins are heat-stable enterotoxin and heat-labile enterotoxin. ETEC causes watery diarrhoea, which can range from mild, self-limiting disease to severe purging disease. The organism is an important cause of childhood diarrhoea in the developing world and is the main cause of diarrhoea in travellers to developing countries (Kaper *et al.*, 2004). ETEC colonizes the surface of the small bowel mucosa and elaborates enterotoxins, which give rise to intestinal secretion.The gene for the toxins is usually plasmid-borne. The first toxin binds to a glycoprotein receptor that is coupled to guanylate cyclase on the surface of intestinal epithelial cells. Activation of guanylate cyclase stimulates the production of cyclic guanosine monophosphate (cGMP) which leads to the secretion of electrolytes and water into the lumen of the small intestine and is manifested by the watery diarrhoea characteristic of ETEC.

The second toxin which is the heat-labile toxin binds to specific gangliosides on the epithelial cells and activates membrane-bound adenylate cyclase, which leads to the increased production of cyclic adenosine monophosphate (cAMP) through the same method as that of the cholera toxin. This also results in the hypersecretion of electrolytes and water into the intestinal lumen (Prescott *et al.*, 1999). Quite a number of strains are members of

this group including O6, O8, O20, O25, O27, O63, O78, O85, O128, and O159 (Schultz *et al.*, 2000).

2.5.2.3 Enteroinvasive E. coli (EIEC)

EIEC are biochemically, genetically and pathogenically closely related to *Shigella* spp. Numerous studies have shown that *Shigella* and *E. coli* are taxonomically indistinguishable at the species level but, owing to the clinical significance of *Shigella*, a nomenclature distinction is still maintained (Kaper *et al.*, 2004). EIEC might cause an invasive inflammatory colitis, and occasionally dysentery, but in most cases EIEC elicits watery diarrhoea that is indistinguishable from that due to infection by other *E. coli* pathogens. EIEC are distinguished from *Shigella* by a few minor biochemical tests, but they share essential virulence factors. The duplex real-time PCR assay, which is simple, rapid, reliable and specific, can be used for differentiation of *Shigella* spp. from *E. coli* and in particular EIEC (Ud-din and Wahid, 2014).

EIEC was first reported as 'paracolon bacillus' in 1944, but it was later designated as *E. coli* O124. EIEC is associated with specific *E. coli* O-serotypes: O28ac:NM, O29:NM, O112ac:NM, O121:NM, O124:NM, O124:H30, O135:NM, O136:NM, O143:NM, O144:NM, O152:NM, O159:H2, O159:NM, O164:NM, O167:H4, O167:H5, O167:NM and O173:NM (Ud-din and Wahid, 2014).

2.5.2.4 Enteroaggregative E. coli (EaggEC)

EAEC strains resemble ETEC strains in that they bind to the small intestinal cells and are not invasive. They differ from ETEC strains basically in the sense that they do not adhere

uniformly over the surface of the intestinal mucosa but tend to clump in small aggregates. EAEC strains produce a heat-stable like toxin and haemolysin-like toxin (Kaper *et al.*, 2004). This strain has been associated with diarrhoea in children living in developed and developing countries. The basic strategy of this group of *E. coli* infection involves colonization of the intestinal mucosa, probably majorly that of the colon, followed by secretion of enterotoxins and cytotoxins. Studies on human intestinal explants indicate that EAEC induces mild, but significant mucosal damage (Kaper *et al.,* 2004).

2.5.2.5 Diffusely adherent E. coli (DAEC)

DAEC are defined by the presence of a characteristic, diffuse pattern of adherence to HEp-2 cell monolayers. DAEC have been implicated as a cause of diarrhoea in several studies, particularly in children over 1 year in age (Kaper *et al.*, 2004). DAEC strains adhere over the entire surface of epithelial cells and usually cause disease in immunocompromised or malnourished children. Diffuse adherence occurs when bacterial cells adhere to the entire surface of the mucosal host cells without the formation of discrete micro-colonies (Scaletsky *et al.,* 2002). It has been suggested that DAEC may have an as yet undefined virulence factor. Serogroups include O15 and O158.

2.5.2.6 Enterohaemorrhagic E. coli (EHEC)

They are also known as verocytotoxigenic *E. coli* (VTEC) and are also called Shiga toxin producing *E. coli* (STEC) (Calderwood *et al.*, 1996). They were first recognized as a cause of human disease in 1982. EHEC causes bloody diarrhoea (haemorrhagic colitis), non-bloody diarrhoea and haemolytic uremic syndrome (HUS) (Kaper *et al.,* 2004). These

strains carry the genetic determinants for attaching effacing lesions and shiga-like toxin production. These attaching effacing lesions cause haemorrhagic colitis with severe abdominal pains and cramps followed by bloody diarrhoea. It is believed that some shiga-like toxins I and II kill vascular endothelial cells. The major reservoir of this group of *E. coli* is the bovine intestinal tract (Kaper *et al.,* 2004). A major form of EHEC is *E. coli* O157:H7. Other strains are O113:H21, O26:H4, O113:H4, O26:H11, O118:H16 and O146:H21 (Bettelheim and Beutin, 2003).

2.6 *Escherichia coli* O157: H7 (*E. coli* O157: H7)

Of the hundreds of strains of *E. coli*, *E. coli* O157:H7 is an emerging cause of food borne and waterborne illnesses. Although most strains of *E. coli* are harmless and are normal flora of healthy humans and animals, this particular strain produces a powerful toxin and can cause severe illness. *Escherichia coli* O157:H7 was recognized as a cause of haemorrhagic colitis in the 1980s. This is a diarrhoeal illness characterized by severe cramps, abdominal pain, initially watery diarrhoea followed by grossly bloody diarrhoea, and little or no fever (Kaper *et al.,* 2004). Since the etiologic role of this rare serotype of *E. coli* was first reported during the study of two outbreaks that occurred in the United States in 1982, infections due to the organism has been reported (LeJejune *et al.,* 2001).

Escherichia coli O157:H7 was identified for the first time in 1975 (Horrigan *et al.,* 2002). But it was not until 1982 that its aetiology as an enteric pathogen was established. This was when an outbreak was traced to contaminated hamburgers from a fast food outlet (Prescott *et al.,* 1999). Since then, most infections have been linked to eating undercooked ground

beef. However, some have been also linked to drinking contaminated water and swimming in contaminated pools (Ackman *et al.,* 1997).

2.6.1 Epidemiology of *E. coli* O157:H7

The organism was first identified as a cause of illness in 1982 and infections have since been reported with increasing frequency. It is now of public health importance as it is readily isolated from human and animal waste that pollute the environment (Smith et al., 2003). The incidence of food and water contamination therefore poses serious health hazard to the people especially infants and the elderly. The consumption of contaminated food especially meat dishes had been found to be responsible for illness and cattle as major reservoirs of the organism Most disease outbreaks have been traced to foods of bovine origin, like beef and raw milk, that become contaminated with bovine faeces at slaughter houses or dairy farms (Sharma *et al*., 1999; Jalil *et al*., 2011).

The epidemiological significance of virulent *E. coli* is well known and of public health concern. The known sources of infection with *E. coli* O157 include consumption of sprouts, lettuce, spinach, salami, unpasteurized milk and juice, and by swimming in or drinking sewage-contaminated water. Bacteria in loose stool of infected persons can be passed from one person to another if hygiene or hand washing habits are inadequate (CDC, 2006). Waterborne infection by *E. coli* O157 and other enterohaemorrhagic strains of *E. coli* is far less common than infection by *Campylobacter*, but the symptoms of infection are more severe, including haemolytic uraemic syndrome and death. Severity of the illness varies

considerably; it can be fatal, particularly in young children, the elderly or the immunocompromised, but is more often mild (CDC, 2006).

An outbreak occurred in Germany in 2011, and spread to many countries of the European Union (Dadie *et al.,* 2014). There have been reports of outbreaks in the United States of America almost every year in recent times, associated with vegatables, salads and ground beef (CDC, 2016). The infective dose can be very low, even fewer than 100 organisms (WHO, 2011).

Although reported outbreaks in Africa have been few to date, available information indicates that the pathogen has a wide geographical distribution. Since 1992, *E. coli* O157 diarrhoeal illness had been reported in multiple locations (Mashood *et al.,* 2006). In South Africa, the first reported case of haemorrhagic colitis was by Browning *et al.* (1990). EHEC had also been reported from during a large outbreak that resulted in 40,912 patients of less than five years of age making hospital visits for diarrhoea-related illnesses (Effler *et al.,* 1992). In Uganda, a study was carried out on 237 diarrhoeic infants. The reports demonstrated that EHEC were not frequent in urban centres in Uganda, but domestic cattle were identified as an important natural reservoir for this organism (Arimis *et al.,* 2000; Kaddu- Mulindw *et al.,* 2001).

In Nigeria, Ogunsanya *et al.* (1994) reported a 3% prevalence of *E. coli* O157:H7 from 215 children under 5 years of age with diarrhoea that were seen at paediatric clinics in Lagos, Nigeria. Akinyemi *et al.* (1998) reported a prevalence of 8.4% for infections caused by *E. coli* O157:H7. Okeke *et al.* (2000) carried out a study in a small town and rural health care

centre in South- western Nigeria and the results show that 1.2% of the strains were EHEC. Olorunshola *et al.* (2000) examined the prevalence of non-sorbitol fermenting *E. coli* O157:H7 in 100 patients with diarrhoea in Lagos, Nigeria. The detection rate was 6%. Also, a 17% prevalence of *E. coli* O157:H7 was reported in healthy animals in Lagos, Nigeria (Smith *et al.,* 2003). A study in Kaduna state by Tijjani *et al.* (2006) reported an isolation rate of 33.3% of *E. coli* O157 in wells and waste water from abattoirs, markets and households. Agbobgu *et al.* (2006) and Olukosi *et al.* (2008) also reported an isolation rate of 2.2% of the organism in surface waters and well water respectively in Zaria, Kaduna state. Isibor *et al*. (2013) reported a prevalence of 2.7% in Edo state, while a prevalence of 4.3% was reported in South-West Nigeria (Ademokoya *et al*., 2013).

Escherichia coli O157:H7 has also been reported in other African countries such as Democratic Republic of Congo (Germanii *et al.,*1998a), Cameroon (Germanii *et al.,*1998b), Tanzania (Hayghaimo *et al.,* 2001), Egypt (Abdul-Raouf *et al.,* 1996) and South Africa (Abong'o and Momba, 2008).

2.6.2 Infections caused by *E. coli* O157:H7

Escherichia coli O157:H7 infection often causes severe bloody diarrhoea and abdominal cramps. Sometimes the infection causes non-bloody diarrhoea or no symptoms. Usually little or no fever is present, and the illness resolves in 5 to 10 days. In some persons, particularly children under 5 years of age and the elderly, the infection can also cause a complication called haemolytic uraemic syndrome (HUS), in which the red blood cells are destroyed and the kidneys fail. About 8% of persons whose diarrhoeal illness is severe enough that they seek medical care develop this complication (Kaper *et al*., 2004).

2.6.3 Mode of spread

Outbreaks have been associated with swimming in a crowded lake (Ackman *et al*., 1997), contaminated drinking water (CDC, 1999; Olsen *et al*., 2002) as well as surface water (Effler et *al*., 2001). Other modes of transmission include human-to-human contact, contact with inanimate surfaces and with animals or their faeces (Payment and Riley, 2002; Pruss *et al*., 2003). *Escherichia coli* O157:H7 has been reported to be acid tolerant, therefore many cells are able to survive the low pH of the human stomach to cause an infection. (Buchanan and Doyle, 1997).

E. coli O157:H7 comes from human and animal wastes. During rainfalls or other types of precipitations, the organism may be washed into creeks, rivers, streams, lakes and ground water including well water. When these waters are used as source of drinking water without adequate treatment, the organism may end up being ingested and causing disease (Tijjani *et al.,* 2006).

Direct contact with animals and person-to-person transmission has also been documented. Children shed the bacterium for one or two weeks after infection (Olson, 2000; Cornick and Helgerson, 2004). The environment of domestic animals may also be an important reservoir for *E. coli* O157:H7 and pose a continued risk for human exposure. Visitors, particularly young children at open farms, petting zoos and agricultural fairs can become exposed by direct contact or contact with contaminated bedding, hair coats, farm structures, water or soil (Davis *et al.,* 2005).

The general method of disposing wastes apart from using them as fertilizers in farmlands is simply dumping them together with other garbage on land or gullies and other depressions

or even carelessly anywhere (Olawoye, 2005). This method of disposal poses a potential environmental and public health problems which impact negatively on the public through surface and ground water quality of urban areas (Tijjani *et al.,* 2006). Faecal- oral, foodborne and waterborne transmission of *E. coli* O157:H7 has been well documented (Nataro and Kaper, 1998; Poppe *et al.,* 2005). It has also been shown that Shiga toxin-producing E. coli (STEC), specifically O157:H7, can be found in filth flies on cattle farms, in house flies, can grow on wounded fruit and be transmitted to and by fruit flies (Alam and Zurek, 2004; Franiczek *et al.,* 2006).

2.6.4 Virulence factors of *E. coli* O157:H7

2.6.4.1 Shiga toxin gene (Stx1 and Stx2)

Many strains of *Escherichia coli* produce a variety of potent toxins, including Shiga-like toxins (Stx). These strains are often referred to as verotoxin-producing *E. coli* due to the effect these toxins have on Vero cells in culture. Such toxin-producing isolates have been identified as a cause of serious human gastrointestinal disease worldwide, often with severe complicating problems that include bloody diarrhoea, haemorrhagic colitis (HC), and the life-threatening condition haemolytic-uraemic syndrome (HUS). The ability to produce one or more Shiga toxins (Stx1 and Stx2), also called Verotoxins (VT1 and VT2), is an important virulence characteristic of *E. coli* O157:H7 (Bettelheim and Beutin, 2003).

Stx1 is genetically and immunologically related to shiga toxin (Stx) which is produced by *Shigella dysenteriae* type 1 strains (Faruque, 2002). All these toxins bind to the same receptor, globotriaosyceramide (Keusch *et al.,* 1998). Five subtypes of Stx2 have been

identified, they include Stx2, Stx2c, Stx2d, Stx2e, and Stx2f (Wang *et al.,* 2002). Stx2c was formerly subdivided into Stx2-Va and Stx2-Vb (Wang *et al.*, 2002).

The genes coding for vero toxins in *E. coli* O157:H7 are carried on lysogenic phages, which are mobile genetic elements (Duffy, 2003). This is of concern because these genes may move through the animal population or environment within vegetative cells, or by the transduction of verotoxin - encoding phages among existing *E. coli* populations. Studies have shown that verotoxigenic phages capable of infecting *E. coli* O157:H7 can survive long enough in sewage and water, suggesting that they may represent a potential source of virulence genes (Muniesa and Jofre, 1998; Muniesa *et al.*, 1999). Stx is produced in the colon and travels through the bloodstream to the kidney, where it damages renal endothelial cells and occludes the microvasculature through a combination of direct toxicity and induction of local cytokine and chemokine production, resulting in inflammation of the kdney.

This damage can lead to HUS, which is characterized by haemolytic anaemia, thrombocytopaenia and potentially fatal acute renal failure. Stx also induces apoptosis in intestinal epithelial cells. Stx was first purified from *Shigella dysenteriae*, and HUS can also result from infection with this species, although not with other *Shigella* species or EIEC, which do not produce Stx. Stx also mediates local damage in the colon, which results in bloody diarrhoea, haemorrhagic colitis, necrosis and intestinal perforation (Kaper *et al*., 2004).

2.6.4.2 Intimin gene (eaeA)

The adhesion protein called intimin (a 95kDa outer membrane protein encoded by the eaeA gene) is responsible for the intimate adherence between bacteria and enterocyte membranes (Mead and Griffin, 1998). Intimin, the product of the *eaeA* gene involved in the bacterial attaching and effacing adherence phenomenon and clustered in a pathogenicity island termed the locus for enterocyte effacement (Kaper *et al*., 2004).

2.6.4.3 Haemolysin gene (hlyA)

The haemolysin gene is encoded by the HlyABCD gene cluster. The haemolysin is produced widely by STEC strains named EHEC-Hly. It participates in inflammatory process of epithelial cells or amplifies effects of LPS and STX (Mazaheri *et al.*, 2005). The gene targets erythrocytes and leukocytes, leading to cell lysis (Kaper et al., 2004).

2.6.5 Diagnosis of infections caused by *E. coli* O157:H7

Infection with *E. coli* O157:H7 is diagnosed by detecting the bacterium in the stool. Many laboratories do not routinely test for *E. coli* O157:H7 in diarrhogenic stool, (Raji *et al.*, 2008) so it is important to request that the stool specimen be tested on sorbitol – MacConkey (SMAC) agar for this organism. It is advisable that all persons who suddenly have diarrhoea with blood should get their stool tested for *E. coli* O157:H7.

Polymerase chain reactions (PCR) has become a very rapid and reliable method for the molecular biology-based diagnosis of a variety of microorganisms (including *E. coli* O157:H7) from microbial cultures and directly from clinical samples (Holland *et al*., 2000).

PCR assays are usually targetted at detecting the shiga toxins, the intimin protein and the plasmid-encoded enterohaemolysin (Jalil *et al.*, 2011).

2.6.6 Treatment of *E. coli* O157:H7 infections

Most people that get infected recover without antibiotics or other specific treatment within 5-10 days. Antibiotics are ideally not usually used to treat this infection. There is no evidence that antibiotics improve the course of disease, and it is thought that treatment with some antibiotics could lead to kidney complications (Tarr, 1995).

2.6.7 Vaccine

Escherichia coli vaccines were under development for many years (Girard *et al.,* 2006). In 2006, a vaccine that produced an immune response against the *E. coli* O157:H7 O-specific polysaccharide conjugated to recombinant exotoxin A of *Pseudomonas aeruginosa* (O157-Repa) was observed to be safe and immunogenic in children 2-5 years old and adults (Ahmed *et al.,* 2006). A vaccine has been developed for cattle which immunizes them against the bacterial's cel wall proteins which act as a receptor for intestinal cells (Pearson, 2007; BCRC, 2016).

2.6.8 Survival of *E. coli* O157:H7 in the environment

Escherichia coli O157:H7 has been reported to survive for long periods in water by being changed into a viable but non-culturable state for up to two (2) months (Wang and Doyle, 1998; Artz and Killham, 2002; Avery *et al.,* 2008). It also survives and multiplies inside an environmental protozoon (*Acanthamoeba polyphaga*) which occurs mainly in soils and

effluents (Dixon, 2002). This protozoon could therefore be a vehicle for the dissemination of *E. coli* O157:H7.

The fate of *E. coli* O157:H7 after excretion is important in determining whether it is introduced into water, food supply or is transmitted to human or other animals. It has been shown to survive in temperatures as low as 8ºC, in environments with high levels of nitrates, nitrites and salts, and at pH less than 4.0 (Zhao *et al.*, 2003). *Escherichia coli* has been isolated from the edges of hot springs (Science Daily, 2001). It is a frequent member of multispecies biofilms. Some strains have pili and can accept and transfer plasmids (rings of DNA), including those that code for multidrug resistance from and to other bacteria of the same and different species (Franiczek *et al.*, 2006).

The organism has also been observed to survive for a long periods in water, especially at cold temperatures. Water trough sediments contaminated with bovine faeces can serve as a long-term (longer than 8 months) reservoir of *E. coli* O157:H7 and the surviving bacteria in contaminated troughs can serve as source of infection (Lim *et al*., 2010).

2.7 *Salmonella* species

The genus Salmonella was named after Daniel Elmer Salmon, an American veterinary pathologist. While Theobald Smith was the actual discoverer of the type bacterium ("*Salmonella enterica*" var. Choleraesuis) in 1885, Dr. Salmon was the administrator of the USDA research program, and thus the organism was named after him. Smith and Salmon had been searching for the cause of common hog cholera and proposed this organism as the causal agent (Pui *et al*., 2011).

Later research, however, showed that this organism (now known as *Salmonella enterica*) rarely causes enteric symptoms in pigs, and was thus not the agent they were seeking (which was eventually shown to be a virus). However, related bacteria in the genus *Salmonella* were eventually shown to cause other important infectious diseases. Organisms belonging to this group are Gram negative, facultatively anaerobic, motile, non-spore forming rods. They ferment glucose and mannose without producing gas but do not ferment lactose or sucrose. The genus currently contains just two species, *Salmonella enterica* (including six subspecies) and *Salmonella bongori*. Most of the *Salmonella* isolates from cases of human infection belong to *Salmonella enterica* subspecies *enterica*. The genus is also further subdivided into approximately 2,500 serovars (or serotypes), characterised on the basis of their somatic (O) and flagellar (H) antigens.

Salmonellae are non-fastidious organisms. As such, they can multiply under various environmental conditions outside living hosts. They can survive in 0.4 - 4% of Sodium Chloride, though they do not require it for growth. Most *Salmonella* serotypes grow at temperature range of 5 - 47°C with optimum temperature of 35 - 37°C but some can grow at temperature as low as 2 - 4°C or even as high as 54°C (Gray and Fedorka-Cray, 2002). They are very sensitive to heat and often killed at temperature of 70°C or above. Salmonellae grow in a pH range of 4 - 9 with the optimum between 6.5 and 7.5. They require high water activity (a_w) between 0.99 and 0.94 yet can survive in environments with low a_w such as on dried foods (Bhunia, 2008).

Salmonella enterica is a ubiquitous pathogen which can infect many host species and cause different diseases (Lavigne and Blanc-Potard, 2008). Their hosts range from domestic animals to food animals and humans, and disease symptoms can range from self-limiting

gastroenteritis to invasive systemic infections with have a high mortality rate (Lavigne and Blanc-Potard, 2008). Some serovars of S. *enterica* can survive in a wide variety of hosts and cause different diseases such as *Salmonella typhimurium*, which causes no symptoms in adult poultry, can cause gastroenteritis in humans, or cause highly invasive systemic enteric fever in mice (Chan *et al*., 2003; Lavigne and Blanc-Potard, 2008). Other serovars are host-specific like *Salmonella* Typhi which is the causative agent of typhoid fever.

The organism is an obligate human pathogen and is endemic in some parts of the world like Africa. (Bhan *et al*., 2005). Most other serovars of *Salmonella* usually cause only gastroenteritis in humans while some other serovars can potentially cause both gastroenteritis and invasive infections in humans (Stoycheva and Murdjeva, 2006).

They are often pathogenic to humans or animals when ingested. Humans acquire the bacteria from contaminated foods such as beef products, poultry, fish, eggs and egg products and also through contaminated water (Pescott *et al*., 1999). *Salmonella* Typhi has also been implicated in large outbreaks of waterborne typhoid (WHO, 2011). Until recently, individual serovars were referred to as if they were species, for example *Salmonella typhimurium*. However the current convention is to refer to this serovar as *Salmonella enterica* subsp. *enterica* serovar Typhimurium. However, it is customary to shorten this to *Salmonella* Typhimurium.

Individual serovars can be further characterised (typed) by a number of methods, including phage typing and antibiotic resistance profiles. The most severe form of *Salmonella* infection is typhoid fever caused by serovars adapted to a human host, such as *S.* Typhi and *S.* Paratyphi. But infection by non-typhoid salmonellae is much more common and

usually causes gastroenteritis, with symptoms including diarrhoea, abdominal pain, and nausea and vomiting lasting from 1-7 days (Le Hello *et al.,* 2011).

Healthy adults rarely suffer other symptoms and the mortality rate is less than 1%, but children, the elderly and the immunocompromised may develop much more severe infections, such as septicaemia. The infective dose can be quite low (10-100 cells) in vulnerable individuals or when contaminated food with a high fat content, like chocolate or cheese, is consumed (Le Hello *et al.,* 2011). However, the infectious dose *Salmonella* depends upon the serovar, and growth condition and host susceptibility (Pui *et al.*, 2011). On the other hand, host factors controlling susceptibility to infection include the condition of the intestinal tract, age and underlying illnesses or immune deficiencies.

2.7.1 Epidemiology of *Salmonella enterica*

The World Health Organization (WHO) has estimated that about 16-17 million cases occur every year, resulting in about 600,000 deaths. The mortality rates differ from region to region, but can be as high as 5-7% despite the use of appropriate antibiotic treatment. On the other hand, non-typhoidal cases account for 1.3 billion cases with 3 million deaths (Pui *et al*., 2011).

Food and waterborne outbreaks caused by *Salmonella* represent a major public health problem worldwide (Pires *et al.,* 2012). Tagoe *et al*. (2011) and Adesiyun *et al*. (1983) reported an isolation rate of 5.15% and 10% for *Salmonella enterica* when working with sachet water in Cape coast metropolis of Ghana and Katsina State, Nigeria respectively. In

another study carried out in Katsina state, Nigeria, children had the highest isolation rate of *Salmonella* Typhi. This was linked to the fact that 80% of them drank water from non-potable sources. Apart from contaminated water, faecal-oral route of transmission may also be a reason for the preponderance of salmonellosis in children (Abdullahi *et al*., 2014). A study carried out in Kaduna state also reported a 34.5% prevalence of *Salmonella* infections in stool samples collected from hospitals across the state (Matthew *et al.,* 2015).

Typhoidal salmonellosis and non-typhoidal salmonellosis are major health problems in developing countries like Nigeria. The problems, according to many findings are associated with unhygienic processing and preparing of foods, substandard water supply, inadequate sanitary measures and emergence of multidrug resistance in some *Salmonella* strains (Abdullahi *et al*., 2014).

2.7.2 Infections caused by *Salmonella enterica*

Typhoid and Paratyphoid fevers are caused by *Salmonella typhi* and *S. paratyphi* A, B (*S. schottmuelleri*) and C *(S. Hirschfield*). Typhoid fever is much more severe and has claimed more lives, while paratyphoid fever has milder symptoms and lower mortality rate. Both serotypes are solely human pathogens (Pui *et al.,* 2011). About 10% of infected individuals might have a relapse, die or encounter serious complications such as typhoid encephalopathy, gastrointestinal bleeding and intestinal perforation. Relapse is the most common occurrence probably due to persisting organisms within the reticuloendothelial system. Typhoid encephalopathy, often accompanied by shock, is usually fatal. Slight gastrointestinal bleeding can be resolved without blood transfusion but in 1 - 2% of cases can be fatal if a large vessel is involved. Intestinal perforation may present with abdominal

pain, rising pulse and falling blood pressure in sick people. Hence, it is very serious in 1 to 3% of hospitalized patients (Parry, 2006).

Typhoid and paratyphoid B occur worldwide, while paratyphi A is common in the East, and C is rare. The diseases are prevalent in countries with low standard of sanitation one local study reported up to 5% incidence in children (Mukhtar *et al*., 2001). The clinical presentation is marked after an incubation period of about two weeks with onset of headache (10 days for typhoid), myalgia and tiredness, cough and fever with about 0.5^0C rise per day to reach up to 40^0C in the first week with accompanying fever.

Non-typhoidal salmonellosis has also been associated with about 150 serotypes especially *Salmonella* Typhimurium and *Salmonella* Enteritidis. Infection always occurs via ingestion of water or food contaminated with animal waste rather than human waste (Pui *et al.*, 2011). The emergence of multidrug-resistant *S.* Typhimurium DT104 has been associated with outbreaks related to beef contamination and resulted in hospitalization rates twice than that of other foodborne salmonellosis (Gray and Fedorka-Cray, 2002; Yousef and Carlstrom, 2003). Bacteraemia is also a complication of non-typhoidal salmonellosis.

2.7.3 Mode of spread of *Salmonella enterica* infections

Salmonella spp have been widely isolated from raw meats, poultry and poultry products, milk and milk products, water, food and the environment (Hendriksen *et al.*, 2008; Khan *et al.*,2009; Abakpa *et al*., 2013; Firouzi *et al.*, 2013; Raufu *et al.*,2014 and Smith *et al.*, 2015). In addition outbreaks have also been associated with poor cooking, reheating of

foods, and improper handling of food (Smith *et al.*, 2015). Lack of hygiene, inadequate hand washing and possession of pet reptiles or amphibians are also factors that encourage the spread of this organism (WHO, 2016).

Salmonellosis can also be spread by chronic carriers especially those who work in food-related outfits. Up to 10% of untreated convalescent typhoid cases will excrete *S.* Typhi in faeces for 1-3 months and between 1-4% become chronic carriers excreting the microorganism for more than one year (Parry, 2006; Pui *et al.*, 2011) *Salmonella* contamination of fresh produce could occur as a result of the entry of *Salmonella* through scar tissues, entrapment during embryogenesis of produce, natural uptake through root systems and transfer onto edible plant tissues during slicing (Pui *et al.*, 2011).

2.7.4 Virulence factors of *Salmonella enterica*

Salmonella enterica possess various virulence genes that code for products that help the organism express its virulence in its host.They include inv, sef and pef which are responsile for the adhesion and invasion of the organism in its host, spv gene which is responsible for systemic disease state in the host and stn gene which codes for enterotoxins production. Others are the sop and pip genes which are associated with the actual expressions of the host pathogenic process (Murugkar *et al*., 2003; Das *et al.*, 2012).

2.7.5 Diagnosis of salmonellosis

Typically, diarrhoea and vomiting are indicative of gastroenteritis, and there are no specific diagnostic tests required for most patients with the condition. The Widal test has been used for many years to diagnose typhoid fever. It measures agglutinating antibodies against *S.* Typhi's O and H antigens. However, its major challenge is that it cross-reacts with some other bacteria of the same genus (Aziz and Haque, 2012).

The isolation of *Salmonella* Typhi from the bone marrow is now the gold standard method for confirming a case of typhoid fever; however, this method is extremely painful, time-consuming and requires equipment and trained laboratory personnel which may not be available in the primary healthcare facilities in the developing world (Ezeigbo *et al.,* 2015). It is usually only done if other tests have presented no conclusive results. Blood culture, a more practical but less sensitive alternative is often used. This has its own lapses as it takes 2-3 days opposed to the Widal test. As a result, diagnosis may appear delayed or overlooked and patients without typhoid may receive unnecessary and inappropriate antimicrobial treatment. Additional tests used to diagnose typhoid fever include enzyme-linked immunosorbent assay (ELISA) and fluorescent antibody test.

2.7.6 Treatment of *Salmonella enterica* infections

Antibiotics such as amynoglycosides, β-lactam penicillins and fluoroquinolones have been widely used for treatment of the disease in animals and other bacterial diseases in humans However; the efficacy of these drugs has been threatened due to development of resistance. **(**Parry and Threlfall, 2008; Salihu *et al.,* 2014). Other treatment steps aimed at managing

symptoms include drinking fluids and eating a healthy diet. Some strains become resistant to antibiotics after long-term use, and antibiotics have known side effects.

However, in children and pregnant women, treatment is usually limited to β-lactams because fluorquinolone interferes with cartilage. Therefore resistance to β-lactams is a considerable concern in *Salmonella enterica* (Parry and Threlfall, 2008). In cases where *Salmonella enterica* is resistant to first line treatment, alternative drugs will be used such as aminoglycosides, or folic acid pathway inhibitors like sulfisoxazole or sulfamethoxazole with or without trimethoprim.

2.8 Antibiotics

An antibiotic is a microbial product or its derivative that are able to kill or inhibit the activity of other organisms (Black, 2005). Some antimicrobial agents are also synthesized by chemically modifying a substance that has been produced by a microorganism. Others such as sulphonamides are completely synthetic (Presott *et al.*, 2000).

The discovery and development of antibiotics rank among the greatest achievements of medicine (Rogers *et al.,* 2012). Antimicrobial chemotherapy has played a vital role in the treatment of human infectious diseases in the 20th century. Since the discovery of penicillin in the 1920s, literally hundreds of antimicrobial agents have been developed or synthesized, and dozens of these are currently available for clinical use. An antibiotic must be selectively toxic. It should kill or inhibit the microbial pathogen, while causing as little damage as possible to the host cell. Broad spectrum antibacterials are active against both

Gram-positive and Gram-negative organisms. Examples include: tetracyclines, phenicols, fluoroquinolones, third generation and fourth generation cephalosporins.

Narrow spectrum antibacterials have limited activity and are primarily only useful against particular species of microorganisms. For example, glycopeptides and bacitracin are only effective against Gram-positive bacteria, whereas polymixins are usually only effective against Gram negative bacteria. Aminoglycosides and sulfonamides are only effective against aerobic organisms, while nitroimidazoles are generally only effective against anaerobes. The frontline antibiotics recommended for the treatment of waterborne bacterial illnesses include chloramphenicol, ciprofloxacin, sulphamethoxazole-trimethoprim and ampicillin (WHO, 2001).

There are five major ways in which antibiotics act against microorganisms. They include: inhibition of cell wall synthesis, inhibition of protein synthesis, nucleic acid synthesis inhibition, cell membrane disruption and metabolic antagonism (Prescott *et al.,* 1999; Hooper, 2001; Liwa and Jaka, 2015).

2.8.1 Antibiotics that inhibit synthesis of cell wall

The cell wall is an essential microbial structure responsible for the cell shape. Furthermore, it prevents cell lysis due the high cytoplasmic osmotic pressure and allows the anchoring of membrane components and extracellular proteins, such as adhesins (Guilhelmelli *et al*., 2013). Synthesis of the bacterial cell wall is perhaps, the most exploited target area for development of antibiotics (Liwa and Jaka, 2015). The components of the cell wall synthesis machinery are appealing antimicrobial targets because they are not present in the

human cell, thereby providing intrinsic target selectivity. The most commonly used antimicrobial agents that inhibit cell wall biosynthesis include β -lactam antibiotics such as penicillins, cephalosporins and glycopeptides (Kotra and Mobashery, 1998).

The β -lactams contain a β-lactam ring which attaches to enzymes (transpeptidase) that cross-link peptidoglycans. These transpeptidase inhibitors thus block the conversion of immature peptidoglycan to the mature form; therefore these enzymes are often termed penicillin-binding proteins (PBPs). They are able to do this owing to the stereo-chemical similarity of the β-lactam moiety with the D-alanine–D-alanine substrate. In the presence of the drug, the transpeptidases form a lethal covalent penicilloyl enzyme complex that serves to block the normal transpeptidation reaction. This results in weakly cross-linked peptidoglycan, which makes the growing bacteria highly susceptible to cell lysis and death (Liwa and Jaka, 2015).

2.8.2 Inhibition of protein synthesis

Several groups of antimicrobial agents act by inhibiting bacterial protein synthesis (ribosome function). These include aminoglycosides, macrolides, tetracyclines, lincosamides, ketolides, streptogramins, oxazolidinones and chloramphenicol (McKee *et al*., 2006). Microbial protein synthesis is directed by ribosomes together with cytoplasmic factors, which transiently bind to particles during the initiation phase, elongation phase and termination phases. Microbial ribosomes contain 70S particles comprising two subunits, of 50S and 30S, which join at the initiation step of protein synthesis and separate at the termination step.

Antimicrobial agents block various stages in bacterial protein synthesis by interfering with the function of either the cytoplasmic factors or the ribosomes. Inhibitors that bind to the 30S ribosomal subunit interfere primarily with initiation, although some also interfere with elongation. Inhibitors that bind to the 50S ribosomal subunit or to the elongation factors, which are transiently linked to ribosomes at certain steps of the cycle, interfere with steps involved in the elongation process (Liwa and Jaka, 2015).

Aminoglycosides act by binding to specific ribosomal subunits. The aminoglycoside-type drugs can combine with other binding sites on 30S ribosomes and they kill bacteria by inducing the formation of aberrant, non-functional complexes as well as by causing misreading. At lower and less toxic doses, aminoglycosides tend to be bacteriostatic (Black, 2005).

Other agents that bind to 30S ribosomes are the tetracyclines. However, tetracycline binding is transient, so they are bacteriostatic. Nonetheless, they inhibit a wide variety of bacteria, chlamydias and mycoplasmas and are extremely useful agents. There are three important classes of drugs that inhibit the 50S ribosomal subunit. Chloramphenicol is a bacteriostatic agent that inhibits both Gram-positive and Gram-negative bacteria. It inhibits peptide bond formation by binding to a peptidyltransferase enzyme on the 50S ribosome. Macrolides are large lactone ring compounds that bind to 50S ribosomes and appear to impair a peptidyltransferase reaction or translocation, or both. The most important macrolide is erythromycin, which inhibits Gram-positive species and a few Gram-negative species such as *Haemophilus, Mycoplasma, Chlamydia,* and *Legionella* (Liwa and Jaka, 2015).

2.8.3 Nucleic acid synthesis inhibition

2.8.3.1 Inhibitors of DNA topoisomerases

DNA synthesis, mRNA transcription and cell division require the modulation of chromosomal supercoiling through topoisomerase-catalyzed strand breakage and rejoining reactions (Kohanski *et al*., 2010). DNA topoisomerase enzymes are divided into two types, I and II, depending on whether they catalyze reactions involving the transient breakage of one (type I) or both (type II) strands of DNA. Topoisomerases control the topological state of DNA in cells and are essemtial for the essential processes of protein translation and cell replication. DNA gyrase, a type II DNA topoisomerase, is the enzyme that negatively supercoils DNA in the presence of (Adenosine triphosphate) ATP. Topo I is unique in its architecture and function from gyrase and topoisomerase IV and as such represents an attractive target for the discovery of new antibacterial chemotypes (Ehmann and Lahiri, 2014).

As a result, the enzyme is vital for almost all cellular processes that involve duplex DNA, namely replication, recombination and transcription. It is exclusive to the prokaryotic kingdom and is essentially crucial for the survival of the organism. Thus, DNA gyrase remains an ideal and attractive target for antibacterial drugs (Liwa and Jaka, 2015). Quinolones are the most successful antimicrobial agents that target DNA gyrase. They inhibit reactions of DNA gyrase such as supercoiling and relaxations that require DNA breakage and reunion. Specifically they interfere with the breakage-reunion reaction of DNA gyrase by interacting with subunit A (GyrA). The first-generation quinolones, nalidixic acid and oxolinic acid have relatively weak antimicrobial activity. However, the synthesis of fluoroquinolones and their improvement over several generations has led to a

range of potent antimicrobial agents such as norfloxacin and ciprofloxacin (second generation), levofloxacin (third generation) and moxifloxacin and gemifloxacin (fourth generation) (Collin *et al*., 2011).

2.8.3.2 *Inhibitors of microbial RNA synthesis*

Rifamycins inhibit DNA-dependent transcription by binding with high affinity to the β-subunit (encoded by rpoB) of a DNA-bound and actively transcribing RNA polymerase. The β-subunit is located in the channel that is formed by the RNA polymerase-DNA complex, from which the newly synthesized RNA strand emerges. Their activity is attributed to the ability of the drug molecule to inhibit nascent RNA strand initialization (Liwa and Jaka, 2015).

2.8.4 Cell membrane disruption

Biologic membranes are composed basically of lipids, proteins and lipoproteins. The cytoplasmic membrane acts as a diffusion barrier for water, ions, nutrients and transport systems. A number of antimicrobial agents can cause disorganization of the membrane. These agents can be divided into cationic, anionic, and neutral agents. The best known compounds are polymyxin B and colistemethate (polymyxin E) (Liwa and Jaka, 2015). Polymyxins are obtained from the soil bacterium *Bacillus polymyxa.* They have limited spectra of antimicrobial activity because the cytoplasmic membrane forms an effective barrier to many antimicrobial agents. The mode of action of some antimicrobial agents may lie in their ability to increase the permeability of the membrane, facilitating their entry and that of other compounds.

Cationic antibacterial agents, such as polymyxin B have been reported to increase the permeability of the outer membrane to lysozyme and hydrophobic compounds. The initial action of these antimicrobial agents is to disrupt outer membrane structure, thereby permitting their entry into the cell leading to the inhibition of specific metabolic processes (Liwa and Jaka, 2015).

2.8.5 Metabolic antagonism

Trimethoprim and sulfonamides interfere with folic acid metabolism in the microbial cell by competitively blocking the biosynthesis of tetrahydrofolate, which acts as a carrier of one-carbon fragments and is necessary for the ultimate synthesis of DNA, RNA and bacterial cell wall proteins. Unlike mammals, bacteria and protozoan parasites usually lack a transport system to take up preformed folic acid from their environment. Most of these organisms must synthesize folic acid, although some are capable of using exogenous thymidine, circumventing the need for folic acid metabolism (Liwa and Jaka, 2015). Sulfonamides have been characterized as broad-spectrum antibiotics with a bacteriostatic mode of action based on inhibition of folic acid metabolism. They competitively inhibit the conversion of pteridine and p-aminobenzoic acid (PABA) to dihydrofolic acid by the enzyme pteridine synthetase. Sulfonamides have a greater affinity than PABA for pteridine synthetase. Trimethoprim has a tremendous affinity for bacterial dihydrofolate reductase (10,000 to 100,000 times higher than for the mammalian enzyme); when bound to this enzyme, it inhibits the synthesis of tetrahydrofolate (Liwa and Jaka, 2015).

2.9 Antibiotic Resistance

Antibiotic resistance is the ability of bacteria to resist the effects of an antibiotic. Antibiotic resistance occurs when bacteria change in a way that reduces the effectiveness of drugs, chemicals, or other agents designed to cure or prevent infections. The bacteria survive and continue to multiply, causing more harm (WHO, 2016).

Although the introduction and use of antibacterial agents played an important role in treating bacterial infections, the emergence and increase in the number of antibiotic resistant clinical isolates, together with the low rate of development and introduction of new antimicrobials, have been a constant threat to human health. These resistant bacteria are frequently resistant to at least one of the conventional antibiotics, leading to significant increases in morbidity and mortality (Fernandez and Hancock, 2012). Patients infected with drug resistant microbial pathogens are likely to require hospitalization and to have a prolonged hospital stay. Resistance also compels the need for more toxic or more expensive alternative antimicrobial agents (WHO, 2015).

Resistant bacteria are able to withstand attack by antibacterial drugs so that standard treatments become ineffective and infections persist, increasing the risk of spread to others (WHO, 2015). This rising antimicrobial resistance has been accompanied by a decline in new antibiotic discovery over the last few decades, which now poses a serious threat to public health (Kovac *et al.*, 2015). In Europe it has been estimated that 25,000 patients die annually due to incurable infections with multidrug-resistant (MDR) bacteria, and that the

healthcare system is burdened with at least an additional 1.5 billion Euros each year because of the prolonged treatments that are needed for infections with MDR bacteria.

In Nigeria, several researchers have published works on the presence of drug resistance however, there exist some challenges which include lack of a comprehensive policy and plan to address antibiotic resistance, weak medicines regulatory capacity and circulation of substandard and counterfeit antibiotics, lack of antibiotic resistance surveillance strategies, weak laboratory capacity on antibiotic resistance testing and reporting, lack of essential laboratory reagents and consumables and limited quality assurance and control protocols (Nasir *et al.*, 2015).

Drug resistance is favoured by the extensive use of antibacterial drugs in our communities. The overuse and misuse of these drugs have led to the death of sensitive strains leaving resistant ones to survive, multiply and infect new hosts (Yusha'u *et al*., 2008). The situation in developing countries is serious for the following reasons. Antibiotics are obtainable outside recognized treatment centres, and are usually taken without medical authorization or supervision which could result in inappropriate use. The drugs could be taken at wrong dosages and for an insufficient length of time (Mukhtar *et al*., 2001). Guidelines regarding the selection of drugs and information about drugs resistance are not perfectly communicable, especially in rural areas.

It should be noted that *Enterobacteriaceae* that were previously sensitive to antibiotics, could still be introduced into environmental waters by wild animals and even human rural dwellers that were not necessary exposed to drugs (Hilde and Hening, 1994). But the

resistance factors are retransferred to them as they come across the resistant ones in such water. This is epidemiologically very important to note (Mukhtar, 2010).

Antibiotic resistant bacteria had previously been isolated from surface waters such as streams (McArthur and Tuckfield, 2000; Ash *et al*., 2002; Roe *et al*., 2003; Ikpeme *et al*., 2011), underground water sources such as wells and boreholes (Oyetayo *et al*., 2007; Olukosi *et al*., 2008 Akande *et al*., 2011; Oyedeji *et al*., 2011) and also from tap and satchet water (Garba *et al*., 2009; Tagoe *et al*., 2011).

2.10 Mechanisms of Drug Resistance in Bacteria

2.10.1 Drug resistance by target site modification

An interaction between an antimicrobial agent and a target molecule is very specific so even small changes in a target molecule can influence antibiotic binding to a target (Giedraitien *et al.*, 2011). Antimicrobial agents act on targets that are present in microbial cells but differ sufficiently from those present in mammalian cells to allow for selective inhibition of the bacterial counterparts. Because of the vital cellular functions of the target sites, the bacteria cannot do without them entirely. However, microbes acquire some mutational changes in the target sites that reduce susceptibility to inhibition whilst retaining their cellular function (Lambert, 2005). This strategy has been observed in bacteria such as Staphylococci, Enterococci and *Mycobacterium* spp.

2.10.1.1 Alterations of penicillin binding proteins (PBPs)

This is often observed in *Streptococcus pneumonia* which causes resistance to β-lactam antibiotics. The altered PBPs are generated by recombinational events between the PBP genes of *S. pneumoniae* and related PBP genes from closely related streptococcal species acquired by transformation of this naturally competent organism (Liwa and Jaka, 2015). It has also been observed in other bacteria like *Neisseria gonorrheae,* methicillin-resistant *Staphylococcus aureus* and *Listeria monocytogenes* (WHO, 2016).

2.10.1.2 Modification of peptidoglycan precursors

The cause of resistance to the glycopeptide antibacterial agents in *Enterococcus faecalis* and *Enterococcus faecium* is the acquisition of one of two related gene clusters, known as VanA and VanB. These gene clusters encode enzymes that produce a modified peptidoglycan precursor terminating in D-Alanyl-D-Lactate (D-Ala-D-Lac) instead of D-Ala-D-Ala. The glycopeptides bind with much lower affinity to D-Ala-D-Lac than to D-Ala-D-Ala. The low affinity binding of glycopeptides to D-Ala-D-Lac results from an altered pattern of intermolecular hydrogen bonding responsible for the normally high affinity binding between drug and its substrate (Miller *et al.*, 2014; Liwa and Jaka, 2015).

2.10.1.3 Modification by ribosomal methylation

This mechanism for drug resistance has become common in the *Enterobacteriaceae* including *Escherichia coli.* The mechanisms of resistance include inactivation of aminoglycosides by production of aminoglycoside-modifying enzymes and protection of 16S rRNA within the 30S ribosome subunit by production of 16S rRNA methylase. The

latter mechanism results in high-level resistance to multiple aminoglycosides. It represents an efficient means to avoid inhibition of their own protein synthesis and is prevalent among aminoglycoside-producing actinomycetes (Doi and Arakawa, 2007). So far, ribosomal methylation remains the most widespread mode of resistance to macrolides and lincosamides. In pathogenic organisms, proteins dimethylate a single adenine in nascent 23S rRNA, which is part of the large (50S) ribosomal subunit. As a consequence of methylation, binding of erythromycin to its target is impaired. The overlapping binding sites of macrolides, lincosamides and streptogramins B in 23S rRNA, account for cross-resistance to the 3 classes of drugs. A wide range of microbial pathogens that are targets for macrolides and lincosamides, including gram-positive species, spirochetes and anaerobes, express Erm methylases (Liwa and Jaka, 2015).

2.10.1.4 Interference with DNA synthesis

This mechanism of resistance involves modification of two enzymes: DNA gyrase (also known as topoisomerase II) (genes gyrA and gyrB) and topoisomerase IV (parC and parE) (Liwa and Jaka, 2015). Mutations in genes gyrA and parC are followed by replication failure, and then quinolones/fluoroquinolones cannot bind. The most common mutation in *E. coli* gyrA causes a reduced drug affinity for modified-DNA complex, and minimum inhibitory concentration (MIC) is higher. Quinolones (ciprofloxacin) bind to DNA gyrase A subunit. Usually resistance to quinolones is associated with mutations in chromosomes, but plasmid-mediated and point mutation-related (in genes gyrA and parC) resistance to quinolones have been reported as well (Giedraitien *et al*., 2011).

2.10.2 Inactivation of antibiotics

Inherent to this mechanism of resistance, and unlike other mechanisms, inactivation of antimicrobial agents confers resistance to structurally related drugs. The major enzymes that inactivate antimicrobial agents include β -lactamases, aminoglycoside-modifying enzymes and chloramphenicol acetyltransferases (Giedraitien *et al*., 2011).

2.10.2.1 Modification of antimicrobials by hydrolysis

There are about 300 known β-lactamases as they are broadly prevalent enzymes. The most clinically important resistance are produced by gram negative organisms (Wickens and Hade, 2005) and are coded on chromosomes and plasmids. Genes that encode β- lactamases are transferred by transposons but also they may be found in the composition of integrons (Jacoby and Munoz-Price, 2005). β - lactamases hydrolyze nearly all β-lactams that have ester and amide bond, e.g., penicillins, cephalosporins, monobactams, and carbapenems (Liwa and Jaka, 2015). Extended spectrum β –lactamases (ESBLs) genes such as *tem, shv, oxa, per, veb-1, bes-1, ges, ibc, sfo* and *ctx* mainly are encoded in large plasmids.

ESBL are resistant to penicillins (except temocillin), third-generation oxyiminocephalosporins (e.g., ceftazidime, cefotoxime, ceftriaxone), aztreonam (a monobactam), cefamandole (a second generation cephalosporin), and cefoperazone (a third generation cephalosporin), but they are sensitive to methoxycephalosporins, e.g., cephamycins and carbapenems, and can be inhibited by inhibitors of β-lactamases, e.g., clavulanic acid, sulbactam, or tazobactam (Thomson and Bonomo, 2005). Strains producing ESBL are commonly resistant to quinolones but their resistance depends not on multiple resistance plasmids but on mutations in gyrA and parC genes. Such strains are

found among *E. coli, K. pneumoniae,* and *P. mirabilis*. Hydrolysis of antimicrobials can be carried out by other enzymes, such as esterases (Dzidic *et al.,* 2008).

2.10.2.2 Drug inactivation by group transfer

The group of enzymes inactivating aminoglycosides, chloramphenicol, streptogramin, macrolides or rifampicin is called transferases. Inactivation is effected by binding adenylyl, phosphoryl, or acetyl groups to the periphery of the drug molecule. These modifications are achieved in the process of transport across the cytoplasmic membrane (Dzidic *et al.,* 2008). Aminoglycosides are neutralized by specific enzymes: phosphoryltransferases (APHs), nucleotidyltransferases or adenylyltransferases (ANTs), and acetyltransferases (AACs). These aminoglycoside modifying enzymes (AMEs) reduce affinity of a modified molecule, impede binding to the 30S ribosomal subunit and provide extended spectrum resistance to aminoglycosides and fluoroquinolones (Strateva and Yornadov, 2009). Gram-positive and gram-negative bacteria and some *H. influenzae* strains that are resistant to chloramphenicol have an enzyme chloramphenicol transacetylase that acetylates hydroxyl groups of chloramphenicol. Modified chloramphenicol is unable to bind to a ribosomal 50S subunit properly (Liwa and Jaka, 2015).

2.10.2.3 Drug inactivation by redox process

Oxidation and reduction reactions are less made use of by pathogenic bacteria as a resistance mechanism against drugs. *Streptomyces virginiae* produces type A antibiotic virginiamycin M1 and protects itself from its own antimicrobial agents by substituting a ketone group to an alcohol residue at position 16. Also, tetracycline can also be oxidized by the flavin-dependent monoxygenase TetX enzyme (Dzidic *et al.,* 2008; Liwa and Jaka,

2015). This enzyme transfers a single hydroxyl group to tetracycline at position 11a, giving rise to a structure that is less able to sequester Magnesium (Mg+) ions which are critical for binding of tetracycline to its bacterial target. TetX is present on a transposon, and this mechanism has been found to be responsible for bacterial resistance to a third generation tetracycline, tigecycline (Kumar and Varela, 2013).

2.10.3 Ribosome protection

Certain bacteria have developed resistance mechanisms that protect the antimicrobial target. For example, in the case of bacterial protein synthesis inhibitors, such as tetracycline, the bacteria have the ability to produce ribosome protection proteins that bind to the ribosomal target thus preventing the binding of tetracycline to the ribosome (Kumar and Varela, 2013). Such ribosome protected bacteria will be able to grow in the presence of tetracycline as protein synthesis will be possible. Disease-causing bacteria harboring such ribosome protection mechanisms have been demonstrated to be clinically important (Roberts, 2005).

2.10.4 Reduced membrane permeability

Drug molecules to a cell can be transferred by the following mechanisms: diffusion through porins, diffusion through the bi-layer, and by self-promoted uptake. Acquired resistance to all antimicrobial classes in *Pseudomonas aeruginosa* is due to low outer membrane permeability. Small hydrophilic molecules (β-lactams and quinolones) can cross the outer membrane only through porins. Aminoglycosides and colistin cannot be transferred to the cell through porins; therefore, self-promoted uptake to the cell is initiated

by binding to lipopolysaccharides of the outer side of the outer membrane (Liwa and Jaka, 2015).

A drug resistant phenotype of a bacterium may be due to the inability of the antimicrobial agent to gain entry into the cell where the drug targets are located (Kumar and Varela, 2013). One mechanism that results in reduced drug permeability in bacteria is the cell wall's lipopolysaccharide (LPS), which consists of lipid A, a core consisting of polysaccharide and O-antigen (Delcour, 2009).

Another mechanism that confers reduced permeability involves the porin channels that reside in the outer membrane and allow small molecular weight molecules, such as antimicrobial agents, to gain cellular entry (Kumar and Varela, 2013). Drug resistant bacteria alter the expression of these outer membrane proteins such that they fail to integrate into the outer membrane or are functionally defective, thus preventing the entry of growth-inhibitory molecules (Pages *et al.,* 2008). Clinically important bacterial pathogens like *Serratia marcescens, Enterobacter cloacae* and *Salmonella enterica* have utilized this reduced drug uptake system to resist important antimicrobial agents, such as the beta-lactams, fluoroquinolones, aminoglycosides, and chloramphenicol (Page, 2012).

2.10.5 Active drug efflux

Active efflux of drugs from the cell is one of the common mechanisms of antimicrobials resistance in bacteria. Efflux pumps are energy-dependent transporters that extrude toxic compounds, including antimicrobials, being one of the major mechanisms by which microbial pathogens resist to different classes of antimicrobial agents (Guilhelmelli *et al.*, 2013). The efflux systems are composed of three protein components, an energy-dependent

pump located in the cytoplasmic membrane, an outer membrane porin and a linker protein which couples the two membrane components together. Efflux pumps can be specific to antimicrobials or may be capable to pump a wide range of unrelated agents e.g. macrolides, tetracyclines and fluoroquinolones and thus significantly contribute to multidrug resistance (MDR) (Giedraitien *et al*., 2011).

Controlling microbial resistance to antimicrobial agents requires a multifaceted approach. Essential components of the approach must include reducing inappropriate prescribing, not only for humans but also animals, reducing transmission of resistant organisms through enhanced infection control and environmental hygiene, and identifying trends in resistance through surveillance. This approach fits neatly within the classic bug-drug-host paradigm. The overuse of antibiotics is considered the main factor in the emergence and dissemination of antimicrobial resistance. Many factors lead to inappropriate antimicrobial prescribing practices, including patient expectations and demands, desire of the physician to give the best possible treatment regardless of cost or subsequent effects, failure to consider alternative treatments, inappropriate use of diagnostic laboratory studies, inadequacy of the physician's knowledge and management of patients with infectious diseases, medico-legal considerations and the belief that the newer and broadspectrum agents represent the most effective treatment

The increased use of antibiotics in health care, as well as in agriculture and animal husbandry, is in turn contributing to the growing problem of antibiotic resistant bacteria. Products such as disinfectants, sterilants, and heavy metals used in industry and in household products are, along with antibiotics, creating a selective pressure in the

environment that leads to the mutations in microorganisms that will allow them better to survive and multiply (Baquero *et al*., 2008).

In an environment with multiple stresses, for example antibiotics and heavy metals, it would be more ecologically favourable, in terms of survival, for a bacterium to acquire resistance to both stresses. If the resistance is plasmid mediated, those bacteria with clustered resistance genes are more likely to simultaneously pass on those genes to other bacteria, and those bacteria would then have a better chance at survival. In such a situation, one may suggest an association with antibiotic resistance and metal tolerance (Baquero *et al*., 2008).

2.11 Multidrug Resistance (MDR)

Drug multi-resistance is the resistance of an organism to three or more antibiotics (Abdullah and Youssuf, 2011). The European Centre for disease prevention and control states that for *Enterobacteriaceae,* the isolate should be non-susceptible to at least 1 agent in ≥ 3 antimicrobial categories for it to be classified as MDR. Pathogenic bacteria with resistance to an antibiotic used to treat the infection caused by that organism are an obvious concern. Of equal importance but less obvious concern is the significance of the resistant environmental bacteria which could be non-pathogenic. This is because of the ability of environmental bacteria to transfer antibiotic resistance genes to human pathogens (Oyedeji *et al*., 2011).

Genes that are responsible for acquired antibiotic resistance are often carried on genetic elements that can easily be transferred among bacteria with the aid of plasmids, bacteriophages or transposons (Roy, 1999; Levy and Marshall, 2004). In many cases, these genetic elements carry more than one antibiotic resistance gene, thus transferring multiple antibiotic resistances to other organisms. Dissemination of antibiotic resistance genes by horizontal gene transfer has led to the rapid emergence of multidrug resistance (MDR) among bacteria. Infections caused by MDR Gram-negative bacteria are now recognized as important causes of morbidity and mortality among hospitalized patients (Paterson & Bonomo, 2005; Walsh *et al*., 2005). The family *Enterobacteriaceae*, for example, contains genera that inhabit the intestinal tracts of humans, which are frequently exposed to various antibiotics, frequently causing higher potential for the dissemination of antibiotic resistance genes (Driscoll *et al*., 2007).

Multiple antibiotics resistance (MAR) index is a tool that reveals the spread of bacteria resistance in a given population. A MAR index greater than 0.2 implies that the strains of such bacteria originate from an environment where several antibiotics are used. It also shows that the environment where such bacteria were isolated were high risk contamination sites (Chitanand *et al.*, 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study area was Kaduna State which is in the centre of Northern Nigeria and is about 960km from the Atlantic Ocean. It is located on a latitude of 11° 12` N and a longitude of 07° 37`E (Ukegbu, 2005).

The population of Kaduna State is 6,066,562 (National Population Commission, 2006). It is characterized by a tropical climate with two main seasons; a wet season of approximately 210 days (May to October), with a mean annual rainfall of 1092.8mm, and the dry season, which spans between November and April (Ukegbu, 2005).

The State is made up of twenty-three (23) Local Government Areas (LGA) and three senatorial districts namely the Kaduna North, Kaduna central and Kaduna south senatorial zones. Two LGAs were selected from each senatorial zone for this research. They are: Jema'a and Kagarko LGAs for Kaduna south, Kajuru and Kaduna North LGAs for the Kaduna central senatorial district and Ikara and Sabon-gari LGAs for Kaduna North senatorial district.

3.2 Study Design

This study was a cross-sectional study.

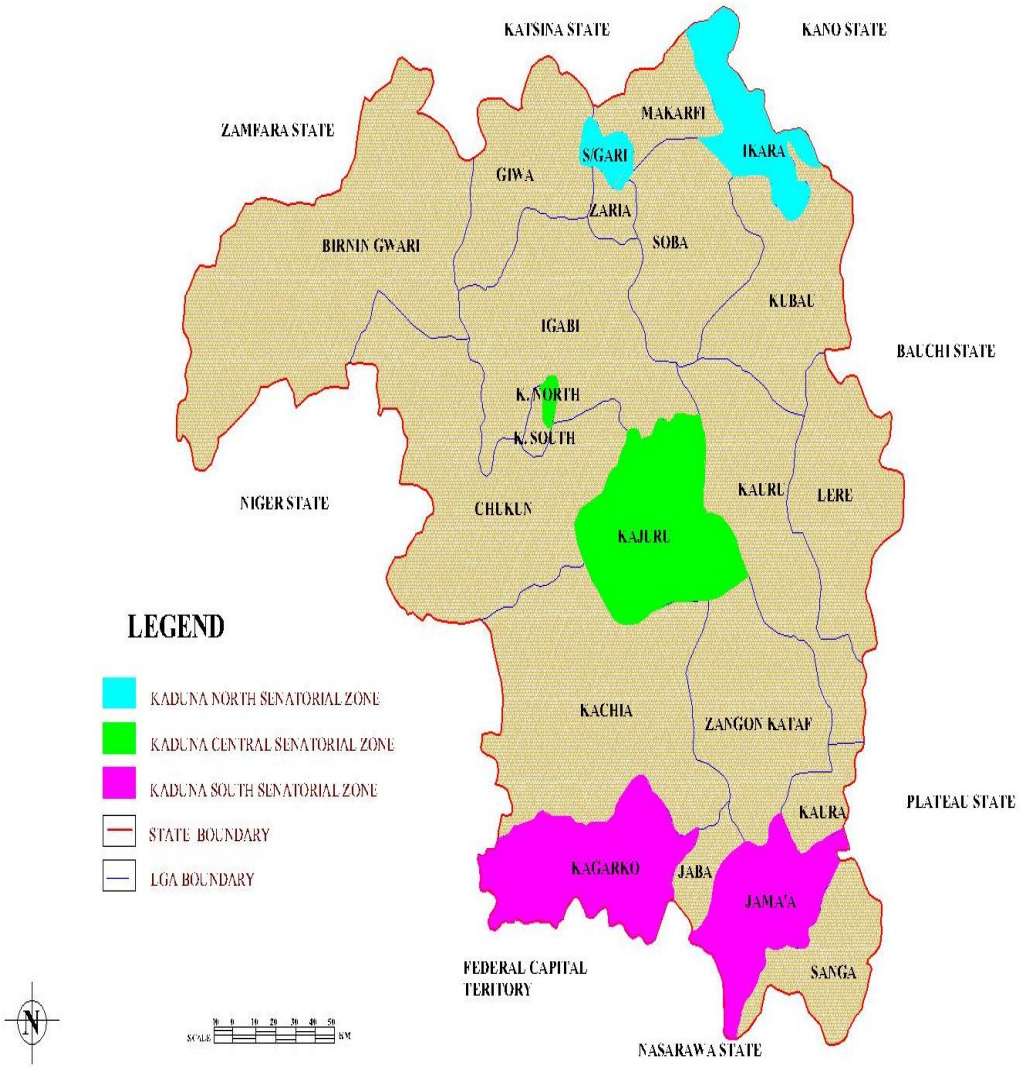


Fig 3.1: Administrative map of Kaduna State showing the study areas (Source: Adapted from administrative map of Nigeria, 2010)

3.3 Sampling Methodology

3.3.1 Sample size

This was calculated using the isolation rate of 2.2% as given by Chigor *et al*. (2010).

$$n = \frac{z^2 pq}{l^2}$$

Where,

Z = 1.96 for 95% confidence interval

P = Prevalence rate (2.2%)

q = 1 – P

l = allowable error (5%)

n = number of samples

$$= \frac{(1.96)^2 \times 0.022 \times 0.978}{(0.05)^2}$$

$$= 33.06$$

Approximately 33 samples.

For the purpose of this study, five hundred (500) samples were collected from the six local governments in Kaduna state that were sampled.

3.3.2 Sampling plan

The water samples were collected from the headquarters of each Local Government that was selected. Public boreholes, taps and wells and streams were selected randomly

(according to availability) and different brands of sachet water were purchased from vendors in the study areas. A total of 500 samples were collected from the six local governments depending on their availability. These samples were collected across the year spanning the wet and dry seasons (March 2014-Febraury 2015). During the sampling period, pipeborne water was not available in Jema'a, Kagarko and some parts of Ikara LGAs, and no stream was sampled in Kajuru LGA. The sample distribution is as shown in the table 3.1.

3.3.3 Collection of samples

3.3.3.1 Well water

A Total of 211 (two hundred and eleven) wells were sampled across the six LGAs as indicated in table 3.1. One litre of well water samples were collected in sterile 1000ml capacity bottles. A weight was attached to the bottles and it was lowered in to draw water out of the well. The bottles were filled with the water and capped carefully, labelled with sample code numbers and transported to the Bacteriology/Parasitology laboratory of the Department of Microbiology, Ahmadu Bello University (A.B.U.) Zaria in an ice cold box and analysed within six hours(h) (APHA, 2011).

3.3.3.2 Borehole water

One hundred and thirty (130) boreholes were sampled from the six LGAs. The tap orifices were flamed using a gas lighter and allowed to run for three minutes, after which samples were collected in sterile one litre capacity containers, labelled appropriately, and transported to the Bacteriology/ Parasitology laboratory in ice cold box for analysis within six hours (APHA, 2011).

Table 3.1: Distribution of samples from various Local Government Areas (LGAs)

LGA	Borehole	Streams	Taps	Wells	Packaged water
Sabon-gari(88)	20	3	11	26	28
Ikara(80)	24	3	5	29	19
Kad. North(83)	19	3	20	22	19
Kajuru(82)	31	0	0	48	3
Jema'a(87)	16	3	24	38	6
Kagarko(80)	20	3	0	48	9
Total (500)	**130**	**15**	**60**	**211**	**84**

3.3.3.3 Pipe borne water

Tap water (pipeborne water) was not available in Kajuru and Kagarko LGAs during the sampling period. Sixty (60) samples were collected across the six LGAs as indicated in table 3.1. The tap orifices were flamed using a gas lighter and allowed to run for three minutes, after which 1litre of samples were collected in sterile one litre capacity containers, labelled appropriately, and transported to the Bacteriology/ Parasitology laboratory in ice cold boxes for analysis within 6h. One (1) millilitre of a 3% solution of Sodium thiosulphate solution ($Na_2S_2O_3$) was added to the bottles that were used for tap water collection to neutralize residual chlorine (APHA, 2011).

3.3.3.4 Packaged water

Eighty-four packaged water (78 sachets and 6 bottled water) samples were purchased from the selling points, placed in an insulated cold box, transported to the laboratory and analysed within six hours (APHA, 2011). The brands available in each location were purchased.

3.3.3.5 Stream water

Fifteen streams were sampled from the LGAs except Kajuru LGA. The water samples were collected by dipping the sample bottles into 2m depth of the water body. The bottles were immediately capped and labelled, after which they were transported to the laboratory and analysed within six hours (APHA, 2011).

3.4 Sample Analysis

3.4.1 Physicochemical and heavy metal analysis of water samples

Immediately after collection, the water samples collected were taken to the Department of Water Resources and Environmental Engineering, Ahmadu Bello University, Zaria for analysis of some physicochemical parameters such as biological oxygen demand (BOD_5), dissolved oxygen, Total dissolved solids, Nitrate, Electrical conductivity and pH. The procedures are as follows:

3.4.1.1 Biological Oxygen Demand (BOD_5) and Dissolved Oxygen (DO)

These were determined by the Azide-Winkler method. Samples were collected in two 300ml BOD bottles per sample, to which 2ml of manganese sulphate solution was added. Two millilitres of alkali-iodide-azide was added below the surface of the liquid. The bottles were stoppered to exclude air bubbles and mixed by inverting it several times. A reddish-brown precipitate developed. When the precipitate settled leaving a clear supernatant, the bottle was shaken again and allowed to settle. When the settling had produced about 100ml of clear supernatant, 2ml of concentrated sulphuric acid was added, after which a yellow colour developed. The bottles were re-stoppered and mixed by gentle inversion. One of the bottles was incubated for five days at 20°C in an incubator (Gallenkamp).

From the second bottle, 200ml was taken for titration with 0.025M sodium thiosulphate solution and titration continued to a pale straw colour. Two millilitres (2ml) starch solution was added and titration continued till the first disappearance of a blue colour. Titration was

done for 200ml of solution from the first bottle after the 5day-incubation period was completed. The results were recorded in mg/l (Oluyemi *et al.*,2010; APHA,2011).

3.4.1.2 Electrical Conductivity and Total Dissolved Solids

These parameters were measured using an instrument (Hanna, HI 98360) designed specifically for measuring some parameters by following the manufacturer's instructions. The probe of the instrument was inserted into the sample and the instrument gave the readings for the two parameters automatically. Electrical conductivity was recorded in µs/cm while the Total dissolved solids was recorded in mg/l (APHA, 2011).

3.4.1.3 Nitrate

A nitrate instrument (Hanna HI3874) was used to take the measurement of nitrate in the samples, following the manufacturer's instructions. The results were recorded in mg/l (APHA, 2011).

3.4.1.4 pH

This was measured using an electronic meter model pHs.25. The machine was standardized using buffers with pH 4.0, 7.0 and 9.2. The samples were shaken thoroughly and then a small volume dispensed into a small container and the machine's probe and the pH reading was taken (Oluyemi *et al.*,2010).

3.4.1.5 Heavy metal analysis

The samples that were tested for heavy metals were acidified with 5ml of concentrated sulphuric acid after collection. The presence and levels of the following heavy metals were

determined: Iron (Fe), Chromium (Cr) and Lead (Pb) using the fast sequential atomic absorbance spectrophotometer (AAS) machine (Varian AA24OFS) at the Multi-user Laboratory, Department of Chemistry, Ahmadu Bello University, Zaria. The machine made use of absorption spectrometry to assess the concentration of an analyte in the sample. It used standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration.

Firstly, the correct wavelengths of the various elements were chosen, and a known standard curve of the elements was run. The absorbance values of the chemical elements present in the samples were determined. Using the standard absorbance of the various elements, the absorbance from the various heavy elements as contained in the samples was converted to parts per million (ppm) values as their levels of concentration. This was repeated three times for every element in every sample and the mean concentration was taken as the actual level of concentration of the heavy element in ppm (Ibrahim and Ajibade, 2012; Butu and Bichi, 2013).

3.4.2 Bacteriological analyses

3.4.2.1 Total and faecal coliform counts

Serial dilutions of the samples were carried out using tubes containing 9mls of sterile buffered peptone water. The dilutions were carried out from the 10^{-1} to the 10^{-4} dilutions. From selected dilutions, 0.1ml was spread aseptically onto duplicate plates of Eosin methylene blue agar (EMB) following standard procedures. The plates were incubated at 37 and 44°C for 24h for total and faecal coliforms respectively. Typical lactose fermenting

colonies (colonies with greenish sheen, and those with dark centers) were counted and the value multiplied by the dilution factor to get the correct colony forming units per ml for total coliforms and faecal coliforms. Characteristic colonies which appeared as green/black with metallic sheen on EMB agar were counted as faecal coliforms. Typical colonies were inoculated into Brilliant green lactose bile broth in test tubes containing inverted Durham`s tubes and were incubated at 44°C for 24h for confirmatory tests. Gas and acid production confirmed the presence of faecal coliform (Ogunshe *et al*., 2006; Romsics *et al.,* 2013; Oluyege, *et al*., 2014).

3.4.2.2 Isolation of Escherichia coli O157:H7

Escherichia coli O157:H7 was isolated from the water samples using the method of LeJejune *et al* (2001). Twenty (20) millilitres of each water sample were inoculated into duplicate flasks containing 20ml of sterile double strength Tryptone Soy Broth. The tubes were incubated at 44°C for 24h. After this time, a loopful of the enrichment culture was streaked on plates of EMB agar and incubated at 44°C for 24h. Mixed cultures were re-streaked for purity on plates of sterile EMB agar and then colonies that were observed to be shiny green with dark centres were transferred to nutrient agar slants and Gram stained before storage for further identification.

3.4.2.3 Isolation of Salmonella enterica

Ten (10) milliliters of each water sample was inoculated into 10ml of double strength Selenite F broth and incubated at 37^{o}C for 18h. After this period, a loopful of the

enrichment culture which showed growth (turbidity and reddish colouration) was streaked on selective plating medium, Salmonella-Shigella (SS) agar, and further incubated at 37^{o}C for 18h (Harvey and Price, 1979; Abakpa *et al.,* 2011). Suspected *Salmonella enterica* colonies appeared colourless with or without dark centres. The isolates were purified by re-streaking to obtain pure cultures and then they were Gram stained and stored on nutrient agar slants for further identification.

3.5 Gram Staining of Isolates

Freshly grown cultures on nutrient agar of isolates of the organisms were picked with a heat-flamed and cooled wire loop. The organism was emulsified in a drop of sterile distilled water on a clean slide to make a smear after which it was air-dried and heat fixed. Crystal violet was applied on the smear for one minute after which it was rinsed off with tap water and then Lugol's iodine was applied which served as a mordant. After 30 seconds(s), it was rinsed off and then about 7 drops of acetone was applied which served as a decolourizer. The slide was rinsed with tap water and then safranine was applied as a counter-stain for one minute. Organisms that were Gram negative appeared pink when they were viewed under the light microscope at the x100 magnification with oil immersion (Cheesebrough, 2006).

3.6 Biochemical Characterization of the Isolates

The presumptive *E. coli* and *Salmonella enterica* isolates were subjected to a number of conventional biochemical tests to further identify them, before they were tested with the Microgen GN A kit. These include:

3.6.1 Indole test

This test detected the ability of an organism to produce indole from tryptophan (an amino acid). Each organism that was tested was grown in 5mls of sterile peptone water and incubated at 37°C for 24h. After this, 3 drops of Kovac's reagent was added. A positive reaction was indicated by the development of a red colour in the reagent layer above the broth in one minute. This indicated that the organism elaborated the enzyme tryptophanase (Cheesebrough, 2006).

3.6.2 Methyl red- Voges Proskaeur (MRVP) test

The isolated colonies were inoculated into 5mls of the MRVP broth and incubated at 37°C for 24h. After incubation, 1ml of the broth was transferred to a sterile serological tube and then 3 drops of methyl red was added. An immediate red coloration indicated a positive result for the methyl red test.

To the rest of the broth, 0.6ml of ethanolic solution of 5% α- naphthol and 0.2ml of 40% potassium hydroxide were added sequentially. The tube was shaken vigorously and then kept in a sloppy position with its cover loosened. The development of a red colour within an hour was considered as a positive result for the Voges-Proskaeur test (Cheesbrough, 2006).

3.6.3 Citrate utilisation test

Small quantities of well-isolated colonies of the organisms to be tested were inoculated on the surface of a sterile Simmon's citrate agar slants using a sterile wire loop. The tubes were incubated at 37°C for 24h. The growth of the organism accompanied by a change in the colour of the medium from green to blue indicated that the organism was able to utilize citrate as a sole source of carbon (Cheesbrough, 2006).

3.6.4 Motility test

Using a sterile straight wire, small quantities of the isolated colonies was stabbed into tubes containing sterile motility media. The stabbing was done to be 1cm short of the bottom of the tube. The tubes were then incubated at 37°C for 24h. The spread of growth through the semi solid medium characterized by cloudiness through the medium indicated that the organism was motile. On the other hand, the confinement of the growth to the line of stabbing and clarity of the remaining part of the media showed that the organism was non–motile (Cheesbrough, 2006) (Appendix II).

3.6.5 Urease test

Well isolated colonies of pure culture of the isolates were inoculated on sterile urea agar slants. They were incubated at 37°C for 24h after which they were observed. The production of a deep pink colour indicated a positive result while the retention of the yellow colour of the medium indicated a negative result (Cheesbrough, 2006).

3.6.6 Triple sugar iron test (TSI)

A pure culture of a freshly grown isolate of the organism to be tested was picked with a sterile wire and the TSI medium was stabbed just short of the bottom of the tube and then

streaked on the surface under aseptic conditions. The tubes were capped loosely and then incubated at 37°C for 24h after which the results were read. The medium contained lactose, sucrose and glucose in the concentration of 10:10:1. If only glucose is fermented, the acid produced is only enough to turn the butt yellow but the slant will remain red. A large amount of acid turned both butt and slant yellow, thus indicating the ability of the culture to ferment either lactose or sucrose. The medium also contained iron in the form of ferrous sulphate which is an indicator of hydrogen sulphide (H_2S) formation and phenol red is a dye which is an indicator of acidification. It also contained peptone which acted as source of nitrogen, which when utilized led to the production of ammonia (Cheesbrough, 2006). *E. coli* produced an acid slant/acid butt with gas production (A/Ag), while *Salmonella enteica* produced an alkaline slant/acid butt with the production of Hydrogen sulphide gas (K/AH_2S) (Appendix II).

3.6.7 Identification of organisms using Microgen *Enterobacteriaceae* Gram negative-A (GN-A) ID kit.

The Microgen *Enterobacteriaceae* GN A ID kit was used for the biochemical characterization of the organisms. This test kit consisted of a plastic strip with twelve (12) microtubes containing dehydrated substances that could detect certain biochemical characteristics. The test substrates were inoculated with a pure culture of three colonies of the organism evenly suspended in sterile physiological saline. After incubation for 24h, the 12 test results were converted to a 4-digit octal code which was used to confirm the identity using Microgen ID computer software, version 1.2.5.26 (Appendix I).

3.7 Identification of *E. coli* O157:H7

The isolates that were confirmed to be *E. coli* on the basis of their Gram reaction and biochemical profiles) were streaked on cefuxime-tellurite Sorbitol MacConkey agar and incubated at 37°C for 18 hours. Those that were sorbitol negative (colourless colonies) were tested with the *E. coli* O157:H7 latex agglutination kit (Microgen, England) for confirmation of *E. coli* O157:H7. Those that did not show agglutination with the kit were recorded as non-O157 *E. coli* (Renter *et al.,* 2003).

3.8 Identification of *Salmonella enterica*

The isolates that were identified to be *Salmonella enterica* were tested further with the *Salmonella* latex agglutination kit (Microgen, England) by following the manufacturer's instructions. The isolates that showed agglutination reaction with the test reagent were confirmed to be *Salmonella enterica.*

3.9 Antibiotic Susceptibility Testing of the Isolates

The susceptibility testing was carried out using Mueller Hinton agar (Oxoid, UK). The organisms were tested *in vitro* for susceptibility to the following commonly used antibiotics: (i)Ampicillin(10µg), (ii)Gentamicin (10µg), (iii)Amoxycillin(10µg), (iv)Tetracycline(30µg), (v)Ciprofloxacin(5µg), (vi)Augmentin (30µg), (vii)Cefotaxime(30µg), (viii)Nalidixic acid(30µg), (ix)Chloramphenicol(30µg) and (x)Sulphamethoxazole-trimethoprim (25µg).

Using a sterile wire loop, three well isolated colonies that had been freshly grown on nutrient agar, were picked and emulsified in sterile normal saline. The prepared turbidity was matched with a turbidity standard (0.5 McFarland, approximately 1.5 x 10^8 cfu/ml) to have an equivalent suspension. A sterile swab was used to inoculate the suspension by streaking on the prepared and dried sterile Mueller- Hinton agar plate evenly after which it was allowed to stand for 3 minutes (m). Sterile forceps were then used to place the antimicrobial discs on the inoculated plates. Within 30m of applying the disc, the plates were incubated at 37°C for 24h. By using a meter rule on the underside of plate, the diameter of each zone of inhibition was measured in millimetres (CLSI, 2014).

Zone diameter for standards were compared with Clinical Laboratory Standards Institute published limits as indicated in Appendix III. Each isolate was classified as; sensitive (S), intermediate (I) and resistant(R). Organisms that were observed to be resistant to at least three different antibiotics were classified as being multidrug resistant (Eziekel *et al.,* 2011).

3.10 Molecular Analyses

3.10.1 Extraction of genomic DNA

A single colony of pure culture of each isolate selected for the polymerase chain reactions (PCR) were inoculated into 10ml of Luria-Bertani (LB) broth medium and incubated at 37°C overnight. The overnight culture was streaked on nutrient agar to obtain pure colonies. The colonies were picked into Eppendorf tubes and the cells were lysed in 400 microlitres (µl) of lysis buffer. Exactly 40µl of proteinase was added to 200µl of the isolates and mixed. The tubes were incubated at 65°C for 10m. After this, 400µl of phenol

chloroform was added and the tubes were vortexed to mix. They were centrifuged at 13,000 rpm for 10m, after which the upper layer was pippetted into freshly labelled tubes. To the new tubes, 400µl of chloroform was added and the tubes were vortexed and centrifuged again.

To the upper layer, 1ml of 100% ethanol and 40µl of 3M sodium acetate were added and the tubes were stored at -20°C overnight. After the incubation period, the tubes were centrifuged at -4°C for 1h after which the ethanol was discarded. Then, 400µl of 70% ethanol was added and the tubes were centrifuged at 13,000rpm for 10m. The ethanol was then discarded and then the DNA was air-dried. Then, 50µl of DNase-free water was added and the DNA was stored at -20°C for further use (Oswald, 2016).

3.10.2 Primer design

The oligonucleotide primer sequences used in this study were obtained from various studies of researchers as indicated in table 3.2. They were designed by Inqaba Biotech, South Africa, except the 16S rRNA primer which was manufactured by Integrated DNA Technologies, USA. They were all diluted following the manufacturer's instructions as indicated in Appendix IV to produce working solutions for the PCR (Moyo *et al*., 2007; Bitrus *et al*., 2011).

3.10.3 Polymerase chain reaction for the detection of virulence markers stx1, stx2, hlyA, and eaeA genes in the *E. coli* isolates

Specific primers sets were used to detect Stx1, Stx2, hlyA and eaeA genes in selected *E. coli* isolates in separate PCR reactions. The PCR reaction mixture of 10µl contained 5µl of master mix (Promega, USA), 1µl of nuclease free water, 3µl of template DNA and 0.5µl of

each primer mix. A tube containing all the above except the DNA template was also included to serve as a negative control.

PCR amplification included the initial denaturation at 94°C for 5m. This was followed by denaturation at 94°C for 20s; annealing was done at the respective temperature for each primer (60.4, 61.0, 61.59 and 61.53°C) for 30s and 36cycles; and extension at 72°C for 45s. The final extension was at 72°C for 5m and then a hold temperature at 4°C. Five (5) µl of the PCR product was electrophoresed in 2% agarose gel (Bioline) containing 5µl of 10 mg/ml ethidium bromide at 100V for 45m. A 1kb plus DNA marker was used as molecular size marker. DNA amplifications were examined under ultraviolet (U.V) transilluminator and results documented (Paton and Paton, 1998b; Moyo *et al*., 2007; Bitrus *et al*., 2011; Ding *et al.*, 2011; Jalil *et al*., 2011).

Table 3.2: List of primers and primer sequences used in this study

Primer	Primer sequence (5□-3□)	Size(bp)	Tm(°C)	Reference
hlyA	GCA TCA TCA AGC GTA CGT TCC AAT GAG CCA AGC TGG TTA AGC T	534	61.5	Paton and Paton, (1998b)
eaeA	TGA TAA GCT GCA GTC GAA TCC CTG AAC CAG ATC GTA ACG GC	229	61.5	Moyo *et al.*, (2007)
invA	ACA GTG CTC GTT ACG ACC TGA AT AGA CGA CTG GTA CTG ATC TAT	224	61.0	Das *et al.*, (2012)
spvA	GTC AGA CCC GTA AAC AGT GCA CGC AGA GTA CCC GCA	604	61.0	Del Cerro *et al.*, (2003)
Stx1	ACA CTG GAT GAT CTC AGT GG CTG AAT CCC CCT CCA TTA TG	614	60.4	Jalil *et al.*,(2011)
Stx2	ATGAAGTGTATATTATTTAAATGGGTA C TCACAGATAAAACACTCTCCAGG	1181	64.0	Ding et al., (2011)
bla_{TEM}	GCG GAA CCC CTA TTTG ACC AAT GCT TAA TCA GTG AG	964	60.4	Olesen *et al.*, (2004)
tetA	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	577	56.0	Lapierre *et al.*, (2006)
tetB	CTC AGT ATT CCA AGC CTT TG ACT CCC CTG AGC TTG AGG GC	415	56.5	Hendriksen *et al.*,(2008)
Sul1	TGA GAT CAG ACG TAT TGC GC TTG AAG GTT CGA CAG CAC GT	406	64.5	Hendriksen *et al.*,(2008)
16SrRNA	AGA GTT TGA TCA TGG CTC AG AAG GAG GTC ATC CAA CCG CCA	1500	56.0	IDT, USA

KEY: Tm- annealing temperature; hlyA- Haemolysin A gene; eaeA- Intimin gene; stx1 and stx2- shiga toxin 1 and 2 genes; bla_{TEM}- beta lactamase temoniera gene; tetA and tetB- Tetracycline resistance genes A and B; sul1- sulphonamide resistance gene 1; 16S rRNA- 16S ribosomal RNA gene.

3.10.4 Polymerase chain reaction for the detection of the invA and SpvA genes in *Salmonella enterica* isolates

Specific primers sets were used to detect invA and spvA genes in selected *Salmonella enterica* isolates in separate PCR reactions. The PCR reaction mixtures of 10 µl contained 5µl of master mix (Promega, USA), 1µl of nuclease free water, 3µl of template DNA and 0.5µl of each primer mix. A tube containing all the above except the DNA template was also included to serve as a negative control.

PCR amplification included the initial denaturation at 94°C for 5m. This was followed by denaturation at 94°C for 20 seconds; annealing was done at the 61°C for each primer for 30s and 36 cycles; and extension at 72°C for 45s. The final extension was at 72°C for 5m and then a hold temperature at 4°C. Five microlitres of the PCR product was electrophoresed in 2% agarose gel (Bioline) containing 5µl of 10 mg/ml ethidium bromide at 100V for 45m. A 1kb plus DNA marker was used as molecular size marker. DNA amplifications were examined under U.V transilluminator and results documented (Del Cerro *et al.,* 2003; Das *et al*., 2012).

3.10.5 Polymerase chain reaction for the detection of drug resistance genes tetA, tetB, sul1 and blaTEM genes in *Salmonella enterica* and *E. coli* isolates

Specific primers sets were used to detect tetA, tetB, sul1, blaTEM and cmlA genes in selected *Salmonella enterica* and *E. coli* isolates in separate PCR reactions. The PCR reaction mixtures of 10µl contained 5µl of master mix (Promega, USA), 1µl of nuclease

free water, 3µl of template DNA and 0.5µl of each primer mix. A tube containing all the above except the DNA template was also included to serve as a negative control.

PCR amplification included the initial denaturation at 94°C for 5m. This was followed by denaturation at 94°C for 20s; annealing was done at the respective annealing temperatures for each primer (65.6, 62.5, 60.4, 56.5 and 64.5°C) for 30s and 36 cycles; and extension at 72°C for 45s. The final extension was at 72°C for 5m and then a hold temperature at 4°C. Five microlitres of the PCR product was electrophoresed in 2% agarose gel (Bioline) containing 5µl of 10 mg/ml ethidium bromide at 100V for 45m. A 1kb plus DNA marker (Invitrogen) was used as molecular size marker. DNA amplifications were examined under U.V transilluminator and results documented (Olesen *et al*., 2004; Lapierre *et al*., 2006; Hendriksen *et al*., 2008).

3.10.6 16S rRNA Gene polymerase chain reaction and Sequencing

Escherichia coli and *Salmonella enterica* isolates were selected for PCR using the universal 16SrRNA primer (IDT, USA). The genomic DNA which had been extracted was used (3µl each in separate Eppendorf PCR tubes), together with 5µl of Mastermix (Promega, USA), 0.5µL of the forward and reverse primers , and 1µl of nuclease free water were all pipette into separately labelled tubes.

The PCR procedure consisted of initial denaturation at 94°C for 5 minutes, followed by denaturation at 94°C for 20s and 36 cycles of annealing at 56°C for 30s, extension at 72°C for 45s, and final extension at 72°C for 5s, and hold temperature of 4°C. The PCR product

was run on gel electrophoresis stained with ethidium bromide. This was done to ensure that the 16SrRNA gene was really amplified.

After this, the PCR product was purified by adding two volumes of 50µl of absolute ethanol after which the tubes were left to stand for 15m at room temperature. Afterwards, the tubes were centrifuged at 10,000rpm for 15m and the supernatants were discarded. After this, two 50µl volumes of 70% ethanol were added, and the tubes were spun for another 15m at 10,000rpm. The supernatants were discarded and then the tubes air-dried. The PCR products were re-constituted with 12µl of sterile distilled water and 3µl of it was run on 1.5% agarose gel electrophoresis to check for the amplicons at around 1500bp.

Sequencing was carried out with a sequencing machine (Applied Biosystems, HITACHI 3130 x 1 Genetic Analyzer). The sequences were analyzed with the Finch TV and BIOEDIT (version 7.2.5.0) software after which the Basic local alignment search tool (BLAST) was carried out on the National Centre for Biotechnology Information (NCBI) website (www.ncbi.nlm.nih.gov) to identify the organism.

3.11 Data Analysis

All statistical analyses were performed using the software SPSS package (IBM SPSS statistics 21, USA). The results were analyzed by one way analysis of variance (ANOVA) for the comparison of means. The means and Standard error (SE) were used for reporting and a P-value of <0.05 was considered statistically significant. The data were presented as tables, charts, and plates as appropriate.

CHAPTER FOUR

RESULTS

4.1 Physicochemical and Heavy Metal Analyses

The mean values of the physicochemical parameters of samples from Saon-gari local government area (LGA) were compared in table 4.1. The well water samples had significantly higher nitrate content (13.05mg/l) than other sample sources ($P<0.05$), while the content of the boreholes, packaged and stream water samples were statistically similar. The BOD and DO of the tap water samples were lower tha those of the other sample types (1.0 and 2.5mg/l respectively). The mean pH of the samples ranged from 6.9 – 7.11, and they were not statistically different from each other ($P>0.05$). The Iron content of the stream water samples was lower than the other samples, while the borehole samples had the highest Iron content(0.57mg/l).The total dissolved solids and conductivity values of the stream samples were also significantly higher than other sources ($P<0.05$). However, the lead content of the borehole samples (0.37mg/l) was higher than all others while it was not detected in stream samples.

Analysis of the mean values of the physicochemical parameters of the water samples from Ikara LGA showed that the values for the parameters varied from one sample type to the other but there was no statistical significance ($P> 0.05$) between the values of each category. However, the wells had the highest lead and nitrate contents (17.6 and 14.20 mg/l respectively), while lead was not detected in the packaged water samples (Table 4.2). The borehole samples from this location had the highest total dissolved solids content of 401.4ppm. The tap water samples had the lowest chromium content of 0.005mg/l while packaged water had the highest content of 0.09mg/l.

Table 4.1: Physicochemical quality of samples from Sabon-gari LGA

Parameter (mean ± SEM)	Boreholes	Packaged water Mean ± SEM	Stream water	Tap water	Wells	P-value
BOD(mg/l)	0.55 ± 0.25[a]	0.50 ± 0.00[a]	0.50 ± 0.00[a]	1.00 ± 0.00[a]	0.80 ± 0.10[a]	0.105
DO(mg/l)	1.15 ± 0.05[a]	1.07 ± 0.025[a]	1.15 ± 0.05[a]	2.5 ± 0.00[a]	1.70 ± 0.80[a]	0.169
Nitrate(mg/l)	8.60 ± 2.30[ab]	10.05 ± 0.05[ab]	11.20 ± 0.00[ab]	6.00 ± 0.00[a]	13.05 ± 1.05[b]	0.044*
Conduc.(µmHos/cm)	397.1 ± 158.9[ab]	120.50 ± 0.50[ab]	501.50 ± 0.50[b]	70.0 ± 0.00[a]	227.35 ± 30.7[ab]	0.034*
pH	7.0 ± 0.1[a]	7.1 ± 0.00[a]	7.11 ± 0.01[a]	7.0 ± 0.00[a]	6.9 ± 0.4[a]	0.917
Iron(mg/l)	0.57 ± 0.23[a]	0.32 ± 0.00[a]	0.012 ± 0.002[a]	0.11 ± 0.002[a]	0.094 ± 0.57[a]	0.157
Chromium(mg/l)	0.01 ± 0.009[a]	0.09 ± 0.002[a]	0.047 ± 0.002[a]	0.113 ± 0.003[a]	0.094 ± 0.06[a]	0.148
Lead(mg/l)	0.37 ± 0.36[c]	0.20 ± 0.01[b]	0.00[a]	0.22 ± 0.001[b]	0.22 ± 0.04[b]	0.001*
TDS(ppm)	198.5 ± 79.4[ab]	60.5 ± 0.5[ab]	250.5 ± 0.50[b]	35.0 ± 0.00[a]	113.6 ± 15.32[ab]	0.034*

ANOVA, Tukey HSD test, P≤ 0.05.

For each parameter, values with different superscript are significantly different

KEY

Conduc.- conductivity

SEM = Standard Error of means; *Significant at P ≤ 0.05

Table 4.2: Physicochemical quality of samples from Ikara LGA

Parameter	Boreholes	Packaged water	Stream water	Tap water	Wells	P-value
		mean ± SEM				
BOD(mg/l)	0.40 ± 0.1[a]	0.70 ± 0.00[a]	0.46 ± 0.60[a]	0.80 ± 0.30[a]	1.0 ± 0.0[a]	0.086
DO(mg/l)	1.5 ± 0.15[a]	1.46 ± 0.03[a]	1.63 ± 0.39[a]	2.25 ± 0.65[a]	2.90 ± 0.0[a]	0.148
Nitrate(mg/l)	9.25 ± 1.25[a]	11.45 ± 0.05[a]	11.26 ± 1.53[a]	9.40 ± 2.80[a]	14.20 ± 0.05[a]	0.315
Conduc.(µmHos/cm)	802.8 ± 517.2[a]	86.4 ± 0.05[a]	192.4 ± 31.2[a]	101.6 ± 41.6[a]	259.5 ± 0.5[a]	0.221
pH	6.85 ± 0.05[a]	7.05 ± 0.05[a]	6.93 ± 0.33[a]	7.35 ± 0.35[a]	7.0 ± 0.0[a]	0.752
Iron(mg/l)	0.84 ± 0.32[a]	0.69 ± 0.001[a]	2.9 ± 1.32[a]	0.72 ± 0.39[a]	0.25 ± 0.004[a]	0.278
Chromium(mg/l)	0.05± 0.05[a]	0.09 ± 0.001[a]	0.023± 0.01[a]	0.005 ± 0.005[a]	0.06 ± 0.002[a]	0.325
Lead(mg/l)	0.14 ± 1.48[a]	0.00[a]	0.113 ± 0.11[a]	0.126 ± 0.12[a]	17.6 ± 16.83[a]	0.380
TDS(ppm)	401.4 ± 258.6[a]	43.2 ± 0.0[a]	96.2 ± 15.60[a]	50.8 ± 20.8[a]	130.5 ± 0.5[a]	0.221

ANOVA, Tukey HSD test, $P \leq 0.05$.

For each parameter, values with different superscript are significantly different

KEY

Conduc.-conductivity; SEM=Standard Error of means

Table 4.3 shows the physicochemical quality of samples from Kaduna North LGA. The BOD of the stream samples were significantly higher than that of other sources ($P<0.05$). Lead was not detected in boreholes and packaged water samples, and the lead contents of the taps and wells were also similar. Chromium was also not detected in the stream water samples. The conductivity and TDS of the well water samples were higher than all others (803.0 and 401.5mg/l respectively), however the differences were not statistically significant ($P>0.05$). The mean value of the nitrate content of the various samples showed that taps and wells had similar values, but the tap water nitrate content was the highest (16.8mg/l).

Lead was not detected in any of the samples from Kajuru LGA (table 4.4). Tap and stream water samples were not available during the sampling period. The packaged water had higher nitrate content (20.05mg/l) than other sample sources. Borehole samples had the highest total dissolved solids content and also conductivity (174.5ppm and 348.9 µmHos/cm). There was however no significant difference between the mean values of the various water surces collected in the location ($P>0.05$).

The tap water samples from Jema'a LGA had a significantly higher conductivity and TDS values ($P<0.05$), as compared to other sample sources. The highest lead content was observed in packaged water samples (0.27mg/l), while none was detected in the well water samples (Table 4.5). Chromium was detected in little amounts in all sample sources, with the highest content being in tap water samples (0.56mg/l). The hghest dissolved oxygen content and biological oxygen demand was observed for borehole samples (2.1 and .25mg/l respectively).

Table 4.3: Physicochemical quality of samples from Kaduna North LGA

Parameter	Boreholes	Packaged water	Stream water	Tap water	Wells	P-value
		mean ± SEM				
BOD(mg/l)	0.20 ± 0.0[a]	0.30 ± 0.00 [ab]	0.50 ± 0.0 [c]	0.40 ± 0.0 [bc]	0.35 ± 0.05 [b]	0.02*
DO(mg/l)	1.1 ± 0.0[a]	0.59 ± 0.005 [a]	0.79 ± 0.005 [a]	0.8 ± 0.1 [a]	1.0 ± 0.3 [a]	0.242
Nitrate(mg/l)	12.4 ± 0.05[a]	11.5 ± 0.0 [a]	13.15 ± 0.05 [a]	16.80 ± 2.30 [a]	16.0 ± 2.5 [a]	0.193
Conduc.(µmHos/cm)	400.5 ± 0.5[a]	99.6 ± 0.05 [a]	269.2 ± 0.0 [a]	100.5 ± 18.1[a]	803.0 ± 352.0[a]	0.106
pH	7.23 ± 0.03 [a]	7.61 ± 0.05 [a]	7.7 ± 0.0 [a]	7.26 ± 0.26 [a]	7.3 ± 0.27 [a]	0.309
Iron(mg/l)	0.12 ± 0.0 [a]	0.11 ± 0.001 [a]	0.26 ± 0.001 [a]	0.26 ± 0.02 [a]	0.26 ± 0.137 [a]	0.104
Chromium(mg/l)	0.07± 0.001 [a]	0.048 ± 0.001[a]	0.0 [a]	0.05 ± 0.049[a]	0.03 ± 0.01 [a]	0.387
Lead(mg/l)	0.00 [a]	0.00 [a]	0.32 ± 0.005 [a]	0.19 ± 0.19 [a]	0.16 ± 0.16[a]	0.323
TDS(ppm)	200.2 ± 0.25 [a]	49.8 ± 0.025 [a]	134.5 ± 0.05 [a]	50.27 ± 9.07 [a]	401.5 ± 176.0 [a]	0.106

ANOVA, Tukey HSD test, P≤ 0.05.

For each parameter, values with different superscript are significantly different

KEY

Conduc.- conductivity

SEM = Standard Error of means; *Significant at P ≤ 0.05

Table 4.4: Physicochemical quality of samples from Kajuru LGA

Parameter	Boreholes	Packaged water	Wells	P-value
		mean ± SEM		
BOD(mg/l)	0.90 ± 0.1[a]	0.50 ± 0.40 [a]	0.55 ± 0.15 [a]	0.485
DO(mg/l)	1.75 ± 0.05[a]	1.29 ± 0.005 [a]	1.56 ± 0.05 [a]	0.095
Nitrate(mg/l)	14.0 ± 3.50[a]	20.05 ± 7.55 [a]	17.10 ± 6.10 [a]	0.656
Conduc.(µmHos/cm)	348.9 ± 260.1[a]	97.9 ± 42.3 [a]	330.5 ± 19.5[a]	0.501
pH	7.23 ± 0.03 [a]	7.61 ± 0.05 [a]	7.3 ± 0.27 [a]	0.626
Iron(mg/l)	0.12 ± 0.0 [a]	0.11 ± 0.001 [a]	0.26 ± 0.137 [a]	0.631
Chromium(mg/l)	0.07± 0.001 [a]	0.048 ± 0.001[a]	0.03 ± 0.01 [a]	0.310
Lead(mg/l)	0.00	0.00	0.00	-
TDS(ppm)	174.5 ± 130.1 [a]	48.9 ± 21.1 [a]	165.3 ± 9.75 [a]	0.500

ANOVA, Tukey HSD test, P≤ 0.05.

For each parameter, values with different superscript are significantly different

KEY

Conduc.- conductivity

SEM = Standard Error of means; -= no P-value generated

Table 4.5: Physicochemical quality of samples from Jema'a LGA

Parameter	Boreholes	Packaged water	Stream water	Wells	Taps	P-value
		mean ± SEM				
BOD(mg/l)	1.25 ± 0.85[a]	0.70 ± 0.11 [a]	0.95 ± 0.15 [a]	0.90 ± 0.0[a]	0.25 ± 0.05[a]	0.454
DO(mg/l)	2.1 ± 0.7[a]	1.96 ± 0.17 [a]	1.90 ± 0.4 [a]	1.50 ± 0.0[a]	1.65 ± 0.65 [a]	0.857
Nitrate(mg/l)	11.5 ± 0.65[a]	12.30 ± 1.13 [a]	20.30 ± 8.8 [a]	10.5 ± 0.0 [a]	11.95 ± 0.55 [a]	0.416
Conduc.(µmHos/cm)	572.0 ± 16.0[bc]	99.13 ± 14.49 [a]	239.3 ± 156.7 [ab]	159.7 ± 0.05[a]	656.5 ± 19.5[c]	0.002*
pH	6.64 ± 0.35 [a]	7.73 ± 0.048 [a]	7.43 ± 0.87 [a]	6.6 ± 0.0 [a]	7.25 ± 0.95 [a]	0.625
Iron(mg/l)	1.05 ± 0.54 [a]	0.18 ± 0.079 [a]	0.57 ± 0.52 [a]	0.36 ± 0.005[a]	0.121 ± 0.003 [a]	0.309
Chromium(mg/l)	0.01± 0.001 [a]	0.003 ± 0.001[a]	0.52 ± 0.027 [a]	0.04 ± 0.002 [a]	0.56 ± 0.01 [a]	0.066
Lead(mg/l)	0.173 ± 0.17 [a]	0.27 ± 0.14 [a]	0.19 ± 0.19 [a]	0.00[a]	0.204 ± 0.20[a]	0.793
TDS(ppm)	286.0 ± 8.0[bc]	49.5 ± 7.24 [a]	119.6 ± 78.35 [ab]	79.85 ± 0.05 [a]	328.3 ± 9.75 [c]	0.002*

ANOVA, Tukey HSD test, $P \leq 0.05$.

For each parameter, values with different superscript are significantly different

KEY

Conduc.-conductivity; SEM=Standard Error of means; *Significant at $P \leq 0.05$

For the samples collected in Kagarko LGA, the physicochemical quality results indicated that lead was not detected in stream and well water samples, but was detected in packaged and borehole water samples (0.06 and 0.08mg/l). Similarly, the nitrate values of the packaged and borehole samples were similar, though lower than the values of other sample types. Tap water was not available in this location at the time of sampling (Table 4.6). The stream water samples had the highest nitrate cntent (19.8mg/l), pH (7.30), chromium content (0.38mg/l), conductivity (298.2µmHos/cm) and TDS (149.0ppm). There was however no significant statistical difference between the mean values of all the parameters for all sample sources at $P>0.05$.

All borehole water samples from all locations were compared in table 4.7. The nitrate values of Kajuru and Kagarko were similar and significantly higher than other locations ($P<0.05$). Lead was not detected in the borehole samples from Kaduna North and Kajuru LGAs. The samples from Kaduna North had the highest mean pH (7.23) and chromium content (0.07mg/l). The borehole samples from Ikara LGA had the highest conductivity, TDS and iron content (802.8µmHos/cm, 401.4ppm and 0.85mg/l respectively).

In the same vein, comparism of mean values of the physicochemical parameters of the stream samples from all locations indicated that the BOD of the Jema'a LGA samples were significantly higher that those of the other locations ($P=0.009$). Chromium was not detected in the Kaduna North samples and lead was not detected in the Sabon-gari and Kagarko stream samples (Table 4.8). The nitrate content of the samples from Jema'a LGA were higher than other locations (20.3mg/l). However, the difference is not statistically significant ($P>0.05$).

Table 4.6: Physicochemical quality of samples from Kagarko LGA

Parameter	Boreholes	Packaged water	Stream water	Wells	P-value
		mean ± SEM			
BOD(mg/l)	0.35 ± 0.15[a]	0.53 ± 0.18 [a]	0.20 ± 0.10 [a]	0.35 ± 0.25 [a]	0.635
DO(mg/l)	0.90 ± 0.60[a]	1.13 ± 0.40 [a]	0.450± 0.10 [a]	0.85 ± 0.55 [a]	0.719
Nitrate(mg/l)	12.4 ± 1.90[a]	12.36 ± 0.66 [a]	19.80 ± 8.0 [a]	13.85 ± 1.55 [a]	0.492
Conduc.(µmHos/cm)	107.4 ± 3.15[a]	136.0 ± 11.97 [a]	298.2 ± 57.8 [a]	256.5 ± 142.4[a]	0.249
pH	6.95 ± 0.15 [a]	6.93 ± 0.20 [a]	7.30 ± 0.20 [a]	6.95 ± 0.35 [a]	0.677
Iron(mg/l)	0.07 ± 0.03 [a]	0.19 ± 0.068 [a]	1.25 ± 0.06 [a]	2.20 ± 2.11 [a]	0.400
Chromium(mg/l)	0.02± 0.01 [a]	0.058 ± 0.02[a]	0.38 ± 0.003 [a]	0.013 ± 0.003 [a]	0.389
Lead(mg/l)	0.08 ± 0.007 [a]	0.06 ± 0.06 [a]	0.00 [a]	0.00[a]	0.688
TDS(ppm)	53.67 ± 1.57[a]	68.0 ± 5.98 [a]	149.0 ± 28.9 [a]	128.3 ± 71.2 [a]	0.249

ANOVA, Tukey HSD test, $P \leq 0.05$.

For each parameter, values with different superscript are significantly different

KEY

Conduc.- conductivity

SEM = Standard Error of means

Table 4.7: Physicochemical quality of borehole samples by location

Parameter	Sabon-gari	Ikara	Kaduna North	Kajuru	Jema'a	Kagarko	P-value
		mean ± SEM					
BOD(mg/l)	0.55 ± 0.25[a]	0.40 ± 0.10 [a]	0.20 ± 0.005 [a]	1.00 ± 0.0 [a]	1.10 ± 0.51 [a]	0.35 ± 0.15[a]	0.380
DO(mg/l)	1.15 ± 0.005[a]	1.55 ± 0.15 [a]	1.11 ± 0.01 [a]	1.90 ± 0.0 [a]	1.93 ± 0.44 [a]	0.9 ± 0.6[a]	0.285
Nitrate(mg/l)	8.60 ± 2.30[a]	9.25 ± 1.25 [a]	12.25± 0.25 [ab]	17.45 ± 0.05 [b]	11.2 ± 0.51 [ab]	12.4 ±1.90[b]	0.017*
Conduc.(µmHos/cm)	397.1 ±158.9[a]	802.8±517.2 [a]	400.5 ± 0.5 [a]	88.80 ± 0.0[a]	584.3 ± 15.4[a]	107.4±3.15[a]	0.443
pH	7.0 ± 0.1 [a]	6.85 ± 0.05 [a]	7.23 ± 0.03 [a]	6.75 ± 0.05 [a]	6.77 ± 0.24 [a]	6.95 ± 0.15[a]	0.216
Iron(mg/l)	0.57 ± 0.24 [a]	0.85 ± 0.32 [a]	0.12 ± 0.01 [a]	0.02 ± 0.001 [a]	0.73 ± 0.45 [a]	0.06 ± 0.03[a]	0.352
Chromium(mg/l)	0.01± 0.01 [a]	0.05 ± 0.054[a]	0.07 ± 0.001 [a]	0.008 ±0.001[a]	0.02 ± 0.01 [a]	0.03 ± 0.02[a]	0.373
Lead(mg/l)	0.37 ± 0.04 [a]	0.15 ± 0.15 [a]	0.00 [a]	0.00 [a]	0.12 ± 0.11[a]	0.08 ± 0.07[a]	0.150
TDS(ppm)	198.6 ±79.5 [a]	401.4±258.6 [a]	200.7 ± 0.25 [a]	44.4 ± 0.02 [a]	292.2 ± 7.7 [a]	53.67±1.57[a]	0.216

ANOVA, Tukey HSD test, P≤ 0.05.

For each parameter, values with different superscript are significantly different

KEY

Conduc.- conductivity

SEM = Standard Error of means

*Significant at P ≤ 0.05

Table 4.8: Physicochemical quality of stream samples by location

Parameter	Sabon-gari	Ikara	Kaduna North	Jema'a	Kagarko	P-value
		mean ± SEM				
BOD(mg/l)	0.50 ± 0.0[a]	0.46 ± 0.06 [a]	0.50 ± 0.00 [a]	0.76 ± 0.20 [b]	0.20 ± 0.10[a]	0.009*
DO(mg/l)	1.60 ± 0.0[a]	1.63 ± 0.39 [a]	0.80 ± 0.0 [a]	1.70 ± 0.30 [a]	0.40 ± 0.1[a]	0.068
Nitrate(mg/l)	11.2 ± 0.0[a]	11.26 ± 1.50 [a]	13.20± 0.0 [a]	20.3 ± 5.17 [a]	19.8 ±8.0[a]	0.516
Conduc.(µmHos/cm)	501.5 ±0.5[a]	192.4±31.3 [a]	269.1± 0.1 [a]	207.3 ± 95.9[a]	298.2±57.8[a]	0.716
pH	7.1± 0.01 [a]	6.9 ± 0.33 [a]	7.65 ± 0.05 [a]	7.35 ± 0.50 [a]	7.30 ± 0.20[a]	0.110
Iron(mg/l)	0.01 ± 0.002 [a]	2.95 ± 1.33 [a]	0.27 ± 0.001 [a]	0.80 ± 0.37 [a]	1.25 ± 0.06[a]	0.220
Chromium(mg/l)	0.04± 0.002 [a]	0.02 ± 0.01[a]	0.0[a]	0.03 ±0.023 [a]	0.038±0.03[a]	0.135
Lead(mg/l)	0.0 [a]	0.11 ± 0.1 [a]	0.33±0 .005 [a]	0.13 ± 0.12[a]	0.0 [a]	0.303
TDS(ppm)	250.5 ±0.5 [a]	96.2±15.6 [a]	134.5 ± 0.05 [a]	103.6 ± 47.9 [a]	149.1±28.9[a]	0.110

ANOVA, Tukey HSD test, P≤ 0.05.

For each parameter, values with different superscript are significantly different

KEY

Conduc.- conductivity

SEM = Standard Error of means

*Significant at P ≤ 0.05

The tap water samples from Ikara LGA had the highest iron content (0.73mg/l), while the tap water samples from Kaduna North had the highest nitrate content (16.8mg/l). Though lead was not detected in the samples from Jema'a LGA, they had the highest conductivity (159.7µmHos/cm) and TDS (79.8ppm) values. Sabon-gari LGA tap samples had the highest led content, closely followed by that of Kaduna North LGA (Table 4.9). However, there was no statistically significant difference between all the values of the physicochemical parameters of the tap water samples.

When the packaged water samples from various locations were compared (table 4.10), the samples from Jema'a LGA had a significantly higher nitrate content, the same also for the high chromium content observed in Sabon-gari samples ($P<0.05$). The TDS and conductivity values for the samples from Kagarko LGA were higher than other packaged water samples, and the pH of the Kagarko samples were also lower than those from other locations (6.93). Samples from Jema'a LGA had a significantly higher dissolved oxygen content (1.96 mg/l) as compared to the samples from all other locations ($P<0.05$).

In table 4.11, the well water samples from various locations were compared and it was observed that the BOD of the Ikara LGA samples were significantly higher than that of other locations ($P<0.05$). The highest nitrate content was observed in Kajuru LGA samples (17.10mg/l), while lead was not detected in samples from Kajuru and Kagarko LGAs. Just like the packaged water samples, the wells from Sabon-gari LGA also had the highest chromium content (0.09 mg/l). The samples from Kaduna North LGA had the highest conductivity and TDS contents, while the samples from Kajuru LGA had the highest iron content (2.71 mg/l).

Table 4.9: Physicochemical quality of tap water samples by location

Parameter	Sabon-gari	Ikara	Kaduna North	Jema'a	P-value
		mean ± SEM			
BOD(mg/l)	1.00 ± 0.0[a]	0.8 ± 0.3 [a]	0.40 ± 0.00 [a]	0.9 ± 0.0 [a]	0.153
DO(mg/l)	2.50 ± 0.0[a]	2.3 ± 0.65 [a]	0.80 ± 0.1 [a]	1.50 ± 0.0 [a]	0.067
Nitrate(mg/l)	6.0 ± 0.0[a]	9.4 ± 2.8 [a]	16.80± 2.3 [a]	10.5 ± 0.0 [a]	0.055
Conduc.(µmHos/cm)	70.0 ±0.0[a]	101.6±41.6 [a]	100.5± 18.1 [a]	159.7 ± 0.05[a]	0.211
pH	7.0± 0.0 [a]	7.35 ± 0.35 [a]	7.26 ± 0.26 [a]	6.6 ± 0.0 [a]	0.178
Iron(mg/l)	0.37 ± 0.004 [a]	0.73 ± 0.39 [a]	0.26 ± 0.02 [a]	0.37 ± 0.005 [a]	0.459
Chromium(mg/l)	0.011±0.002 [a]	0.005± 0.005[a]	0.54±0.049[a]	0.04 ±0.002 [a]	0.145
Lead(mg/l)	0.21 ±0.001 [a]	0.126 ± 0.10 [a]	0.19±0 .19 [a]	0.0[a]	0.590
TDS(ppm)	35.0 ±0.0 [a]	50.8±20.8 [a]	50.3 ± 9.07 [a]	79.8 ± 0.005 [a]	0.178

ANOVA, Tukey HSD test, P≤ 0.05.

For each parameter, values with different superscript are significantly different

KEY

Conduc.- conductivity

SEM = Standard Error of means

*Tap water was not available in Kajuru and Kagarko LGAs at the time of sampling

Table 4.10: Physicochemical quality of packaged water samples by location

Parameter	Sabon-gari	Ikara	Kaduna North	Kajuru	Jema'a	Kagarko	P-value
		mean ± SEM					
BOD(mg/l)	0.50 ± 0.005[a]	0.7 ± 0.0 [a]	0.30 ± 0.00 [a]	0.50 ± 0.4 [a]	0.70 ± 1.20 [a]	0.53 ± 0.18[a]	0.653
DO(mg/l)	1.15 ± 0.05[ab]	1.50 ±0.005 [ab]	0.60 ± 0.0 [a]	1.75 ± 0.05 [ab]	1.96 ± 0.18 [b]	1.13 ± 0.4[ab]	0.045*
Nitrate(mg/l)	10.05 ± 0.05[a]	11.5 ± 0.0 [a]	11.50± 0.0 [a]	20.10 ± 7.55 [a]	12.3 ± 1.14 [a]	12.37±0.67[a]	0.258
Conduc.(µmHos/cm)	119.5 ±0.50[a]	86.35±0.05 [a]	99.7± 0.05 [a]	97.9± 42.3[a]	99.13 ± 14.5[a]	136.01±11.9[a]	0.403
pH	7.05± 0.05 [a]	7.15 ± 0.05 [a]	7.61 ± 0.01 [a]	7.5 ± 0.6 [a]	7.73 ± 0.48 [a]	6.93 ± 0.20[a]	0.506
Iron(mg/l)	0.32 ± 0.01 [a]	0.69 ± 0.01 [a]	0.01 ± 0.001 [a]	0.84 ± 0.83 [a]	0.19 ± 0.08 [a]	0.19 ± 0.07[a]	0.390
Chromium(mg/l)	0.09± 0.002 [b]	0.009±0.001[a]	0.049± 0.00[ab]	0.02 ±0.01[a]	0.003±0.003 [a]	0.06±0.02[ab]	0.009*
Lead(mg/l)	0.21± 0.01 [a]	0.00 [a]	0.00[a]	0.00 [a]	0.27 ± 0.13[a]	0.07±0.06	0.180
TDS(ppm)	60.0±0.001 [a]	60.45±17.3 [a]	49.8 ± 0.03 [a]	48.9 ± 21.3[a]	49.5 ± 7.24 [a]	68.03±5.98[a]	0.680

ANOVA, Tukey HSD test, P≤ 0.05.

For each parameter, values with different superscript are significantly different

KEY

Conduc.- conductivity

SEM = Standard Error of means

*Significant at P ≤ 0.05

Table 4.11: Physicochemical quality of well water samples by location

Parameter	Sabon-gari	Ikara	Kaduna North	Kajuru	Jema'a	Kagarko	P-value
		mean ± SEM					
BOD(mg/l)	0.80 ± 0.1[ab]	1.0 ± 0.0 [b]	0.35 ± 0.05 [ab]	0.55 ± 0.15[ab]	0.25 ± 0.05 [a]	0.35 ± 0.25[a]	0.034*
DO(mg/l)	1.70 ± 0.80 [a]	2.90 ± 0.005 [a]	1.00 ± 0.30 [a]	1.45 ± 0.21 [a]	1.65 ± 0.65 [a]	0.85 ± 0.55[a]	0.188
Nitrate(mg/l)	13.05 ± 1.05[a]	14.25 ± 0.05 [a]	16.0± 2.50 [a]	17.10 ± 6.10 [a]	11.95± 0.55 [a]	13.9 ±1.55[a]	0.796
Conduc.(µmHos/cm)	227.3±30.65 [a]	259.5±0.50 [a]	803.0±352.0 [a]	330.5 ± 19.5 [a]	656.5 ± 19.5 [a]	256.5±142.4[a]	0.151
pH	6.9 ± 0.4[a]	7.0± 0.0 [a]	7.37 ± 0.27 [a]	7.0 ± 0.2 [a]	7.25 ± 0.95 [a]	6.95 ± 0.35[a]	0.968
Iron(mg/l)	0.28 ± 0.14 [a]	0.25 ± 0.004 [a]	0.26 ± 0.13 [a]	2.71 ± 2.66 [a]	0.12 ± 0.003 [a]	2.2± 2.11[a]	0.151
Chromium(mg/l)	0.09± 0.06[a]	0.07 ± 0.002[a]	0.03 ± 0.02 [a]	0.043 ±0.02[a]	0.06 ± 0.01 [a]	0.14 ± 0.03[a]	0.647
Lead(mg/l)	0.29 ± 0.04 [a]	0.33 ± 0.02[a]	0.16 ± 0.16 [a]	0.00 [a]	0.20 ± 0.20[a]	0.00 [a]	0.419
TDS(ppm)	113.7 ±15.33[a]	121.5±0.5 [a]	401.5±176.0 [a]	165.3 ±9.8 [a]	328.3 ± 9.8 [a]	128.3±71.2[a]	0.267

ANOVA, Tukey HSD test, P≤ 0.05.

For each parameter, values with different superscript are significantly different

KEY

Conduc.- conductivity

SEM = Standard Error of means

*Significant at P ≤ 0.05

The percentage of samples form various sources that had their physicochemical quality within acceptable limits as specified by the WHO and the NIS were indicated in table 4.12. All samples from various sources had their BOD and TDS values within acceptable limits. However, it was observed that 62.5% of the tap water samples were not within acceptable limits for chromium (0.05 mg/l). There was a general low compliance in all samples as regards to lead content with a percentage of 50-69.2% of the samples having their lead contents within acceptable limits.

The physicochemical quality of the water samples collected in the wet and dry seasons were compared in table 4.13. There was a significantly higher content of the pH (7.27), nitrate (15.38 mg/l) and lead (0.22 mg/l) during the wet season ($P<0.05$). The chromium, DO and BOD contents of the samples were higher during the dry season. On the other hand, the conductivity, iron and TDS contents of the samples were higher in the wet season, though the difference s were not statistically significant ($P<0.05$).

4.2 Bacteriological analysis and isolation of target organisms

From all the 500 samples analysed, 178 bacterial isolates were characterized and identified (Table 4.14). *E. coli* was isolated from 73 samples while *Salmonella enterica* was isolated from 12 samples. Other isolates include *Proteus mirabilis, Klebsiella pneumoniae, Citrobacter freundii. Enterobacter aerogenes* and a host of others.

Table 4.12: Percentage samples with physicochemical quality within acceptable limits (WHO and NIS)

Parameter	WHO standard[a]	NIS standard[b]	Borehole (%)	Taps (%)	Stream (%)	Wells (%)	Packaged water(%)
BOD (mg/l)	6	NG	100	100	100	100	100
pH	NG	6.5-8.5	99	100	100	99	100
Nitrate(mg/l)	10	50	9.1[a], 100[b]	37.5[a],100[b]	7.7[a],100[b]	100 [a,b]	7.1[a] ,100 [b]
Conductivity (µmHos/cm)	250	1000	36.4[a],90.1 [b]	100 [a, b]	61.5[a],100[b]	16.7[a],91.7[b]	100 [a ,b]
Iron(mg/l)	NG	0.3	45.5	37.5	46.2	66.7	50
Chromium(mg/l)	0.05	0.05	81.8	62.5	84.6	66.7	71.4
Lead(mg/l)	0.01	0.01	63.6	50	69.2	50	64.2
TDS(ppm)	500	500	98	100	100	100	100

KEY: TDS- Total dissolved solids; NG- Guideline not specified; WHO-World Health Organization; NIS-Nigerian Industrial Standard; n-number of samples that met the standard;%-percentage=no of samples that met standard/total number of samples tested x 100.

The superscript letters a and b show the percentage of samples that had their values within acceptable limits as specified by the WHO and NIS respectively.

Table 4.13: Physicohemcial quality of all water samples from all locations by season

Season	Dry	Wet Mean±SEM	P-value
Nitrate(mg/l)	11.49±0.34	15.38±1.29	0.0035*
pH	7.00±0.08	7.27±0.11	0.029*
DO(mg/l)	1.52±0.11	1.40±0.16	0.269
BOD(mg/l)	0.63±0.08	0.53±0.06	0.163
Conduct.(µmhos/cm)	276.92±36.91	297.09±65.82	0.396
Iron (mg/l)	0.47±0.18	1.04±0.29	0.054
Chromium (mg/l)	0.04±0.01	0.03±0.01	0.265
Lead (mg/l)	0.03±0.02	0.22±0.03	0.00*
TDS (ppm)	138.46±18.45	148.54±32.91	0.396

T-test, P≤ 0.05.

In each category, values with different superscript are significantly different

KEY

Conduct.- conductivity

TDS- Total dissolved solids

SEM = Standard Error of means

*Significant at P ≤ 0.05

Table 4.14: Overall isolation rate of enteric bacteria in all water samples

Bacterial isolate	Number (%)
Acinetobacter baumanii	2(1.12)
Acinetobacter haemolyticus	1(0.56)
Acinetobacter iwofii	1(0.56)
Citrobacter diversus	3(1.68)
Citrobacter freundii	12(6.74)
Enterobacter aerogenes	1(0.56)
Enterobacter gergoviae	9(5.06)
Enterobacter cloacae	4(2.24)
Enterobacter sakazakii	1(0.56)
Escherichia coli O157	6(3.37)
Non- O157 *E. coli*	67(37.6)
Hafnia alvei	2(1.12)
Klebsiella ozaenae	4(2.24)
Klebsiella oxytoca	10(5.61)
Klebsiella pneumoniae	4(2.24)
Morganella morganii	1(0.56)
Pantoea agglomerans	2(1.12)
Providencia rettgeri	1(0.56)
Providencia stuartii	2(1.12)
Proteus mirabilis	19(10.67)
Proteus vulgaris	2(1.12)
Pseudomonas spp	5(2.80)
Salmonella enterica	6(3.37)
Salmonella arizonae	6(3.37)
Serratia liquefaciens	6(3.37)
Yersinia enterolitica	1(0.56)
Total	178(100)

N= number of isolates; %= percentage; number of the organism isolated/total number of organisms isolated ×100.

The organisms of interest were isolated from samples of various locations. *E. coli* was isolated more (19.28%) in samples collected in Kaduna North LGA while Jema'a LGA had the least (4.6%). Boreholes and wells contained more *E. coli* isolates than other sample sources (Table 4.15). *Salmonella enterica* was isolated from three samples each in Sabon-gari, Ikara and Jema'a while none was isolated from Kajuru LGA (Table 4.16). Also, the organism was not obtained in any packaged water samples.

From the two hundred and fifty-four (254) samples were collected during the dry season, *E. coli* was isolated from 52 (20.47%) of the samples, while 21 of the samples collected in the wet season were contaminated with the organism (table 4.17). The highest occurrence in the dry season was from samples from Kaduna North LGA (28.8%) while the least was from Jema'a LGA (5.8%). *Salmonella enterica* was isolated from 2(0.79%) of the samples collected in the dry season, while 10 of the samples collected in the wet season were contaminated with the organism (table 4.18). The highest occurrence of the organism was in samples collected from Jema'a LGA in the wet season (83.3%). No *Salmonella enterica* isolate was obtained from Kaduna North, Kajuru, Jema'a and Kagarko LGAs.

A higher count of total coliforms (TCC) was obtained during the dry season than the wet season in samples from Sabon-gari LGA. The highest count in the dry season was for packaged water samples (3.2×10^6 cfu/100ml), while the lowest were for tap water samples (3.3×10^3 cfu/100ml). For the faecal coliform counts, none was obtained for packaged and well water during the dry season, while a count of 1.8×10^5 cfu/100ml was obtained for well water samples during the wet season. The T-test showed no significant difference between the counts of the wet and dry seasons (Table 4.19).

Table 4.15: Isolation frequency of *Escherichia coli* from water samples from various locations

Location (N)	***Escherichia coli* n (%)**	**Source(x)**
Sabon-gari(88)	14(15.91)	Borehole(3), Packaged(4), tap(1),wells(5), stream(1)
Ikara(80)	13(16.25)	Borehole(2), packaged(2), tap(1),wells(6), stream(2)
Kaduna North(83)	16(19.28)	Borehole(5), packaged(2),taps(6), wells(2), stream(1)
Kajuru(82)	13(15.85)	Borehole(7), packaged(1), wells(5)
Jema'a(87)	4(4.60)	Borehole(1),packaged(0) tap(1),wells(1), stream(1)
Kagarko(80)	13(16.25)	Borehole(2),packaged(1), Well(10),streams(0)
TOTAL -500	**73(14.60)**	

N= number of isolates; %= percentage Isolation frequency n= Number of samples containing pathogens/number of samples analysed × 100; x= number of samples that contained *Escherichia coli*

Table 4.16: Isolation frequency of *Salmonella enterica* from water samples from various locations

Location (N)	***Salmonella enterica* n(%)**	**Source(x)**
Sabon-gari(88)	3(3.41)	Borehole(1),Packaged(0), tap(0),wells(2), stream(0)
Ikara(80)	3(3.75)	Borehole(1),packaged(0), tap(0),wells(1), stream(1)
Kaduna North(83)	1(1.20)	Borehole(0),packaged(0),taps(0), wells(1), stream(0)
Kajuru(82)	0(0)	Borehole(0), packaged(0), wells(0)
Jema'a(87)	3(3.45)	Borehole(1),packaged(0) tap(1),wells(1), stream(0)
Kagarko(80)	2(2.50)	Borehole(0),packaged(0), Well(1),streams(1)
TOTAL -500	**12(2.40)**	

N= number of isolates; %= percentage Isolation frequency n= Number of samples containing pathogens/number of samples analysed × 100; x=number of samples that contained *Salmonella enterica*

Table 4.17: Isolation frequency of *Escherichia coli* from all locations by season

Location(N)	Dry season n(%)	Source	Wet season	Source
Sabon-gari(14)	11(21.2)	Borehole(3), packaged(3), Tap(1), well(4),stream(0)	3(14.3)	Borehole(0),packaged(1), Tap(0), wells(1), streams(1)
Ikara(13)	8(15.3)	Borehole(1), packaged(0), Tap(1), well(5),stream(1)	5(23.8)	Borehole(1), packaged(2), Tap(1), well(1),stream(1)
Kaduna North(16)	15(28.8)	Borehole(5), packaged(2), Tap(5), well(2),stream(1)	1(4.8)	Borehole(0), packaged(0), Tap(0), well(1),stream(0)
Kajuru(13)	7(13.5)	Borehole(5), packaged(1), well(1)	6(28.6)	Borehole(2), packaged(0), well(4)
Jema'a(4)	3(5.8)	Borehole(1), packaged(0), Tap(1), well(1),stream(0)	1(4.8)	Borehole(0), packaged(0), Tap(0), well(0),stream(1)
Kagarko(13)	8(15.4)	Borehole(2), packaged(1), well(5),stream(0)	5(23.8)	Borehole(0), packaged(0), well(5),stream(0)
Total(73)	52(71.2)		21(28.8)	

N=number of samples in which isolate was detected; n= number of isolates obtained in the season; %=percentage isolation frequency=number of isolate detected in the season/total number of isolates obtained in the season × 100

Table 4.18: Isolation frequency of *Salmonella enterica* from all locations by season

Location(N)	Dry season n(%)	Source	Wet season	Source
Sabon-gari(3)	1(50)	Borehole(0), packaged(0), Tap(0), well(1),stream(0)	2(20)	Borehole(1),packaged(0), Tap(0), wells(1), streams(0)
Ikara(3)	1(50)	Borehole(0), packaged(0), Tap(0), well(1),stream(0)	2(20)	Borehole(1), packaged(0), Tap(0), well(0),stream(1)
Kaduna North(1)	0(0.0)	Borehole(0), packaged(0), Tap(0), well(2),stream(1)	1(10)	Borehole(0), packaged(0), Tap(0), well(1),stream(0)
Kajuru(0)	0(0.0)	Borehole(0), packaged(0), well(0)	0(0.0)	Borehole(0), packaged(0), well(0)
Jema'a(3)	0(0.0)	Borehole(0), packaged(0), Tap(0), well(1),stream(0)	3(30)	Borehole(1), packaged(0), Tap(1), well(1),stream(0)
Kagarko(2)	0(0.0)	Borehole(0), packaged(0), well(0),stream(0)	2(20)	Borehole(0), packaged(0), well(1),stream(1)
Total(12)	2(16.7)		10(83.3)	

N=number of samples in which isolate was detected; n= number of isolates obtained in the season; %=percentage isolation frequency=number of isolate detected in the season/total number of isolates obtained in the season × 100.

Table 4.19: Mean total and faecal coliform counts of Sabon-gari LGA samples by season

	Sample source	Dry	Wet	P-value
	Borehole	$6.3\times10^{3}\pm4.6\times10^{3}$	$4.9\times10^{4}\pm3.1\times10^{4}$	0.280
	Packaged water	$3.2\times10^{6}\pm2.9\times10^{6}$	$1.1\times10^{5}\pm2.6\times10^{4}$	0.077
TCC± SEM	**Streams**	$6.6\times10^{3}\pm5.6\times10^{3}$	$4.3\times10^{5}\pm3.6\times10^{3}$	0.156
(cfu/100ml)	**Taps**	$3.3\times10^{3}\pm1.6\times10^{2}$	$2.4\times10^{4}\pm5.6\times10^{3}$	0.279
	Wells	$2.0\times10^{4}\pm4.1\times10^{3}$	$9.4\times10^{5}\pm3.9\times10^{5}$	0.344
	Borehole	$3.8\times10^{3}\pm2.6\times10^{3}$	$2.1\times10^{4}\pm1.9\times10^{4}$	0.421
FCC± SEM	**Packaged water**	0.00	$9.1\times10^{3}\pm4.4\times10^{3}$	0.242
(cfu/100ml)	**Streams**	$5.6\times10^{1}\pm2.4\times10^{1}$	$1.2\times10^{2}\pm3.2\times10^{1}$	0.344
	Taps	$1.3\times10^{1}\pm1.4\times10^{1}$	$1.2\times10^{2}\pm5.5\times10^{1}$	0.354
	Wells	0.00	$1.8\times10^{5}\pm1.2\times10^{5}$	0.530

t-test, $P\leq 0.05$

KEY

TCC= Total coliform count; FCC= Faecal coliform count ; SEM= Standard error of means * Significant at $P\leq0.05$.

The mean counts of total coliform counts (TCC) and faecal coliform counts (FCC) of well water samples from Ikara LGA were higher than other sources (table 4.20). No faecal coliform was detected in the boreholes, packaged water, streams and taps of this location during the dry season, but were detected in all except the tap water samples during the wet season. There was a statistically significant higher value of the FCC of borehole and stream water samples collected in the wet season ($P<0.05$). Similarly, the counts of total coliforms obtained in the wet season for boreholes, streams, taps and wells were significantly higher than those of the dry season ($P<0.05$).

Table 4.21 shows the comparison of the mean TCC and FCC of various samples collected from Kaduna North LGA in both seasons. The counts for all water sources were higher during the wet season, with these differences being statistically significant for the FCC of taps and wells, and the FCC of packaged water, streams and wells ($P<0.05$). Faecal coliforms were not detected in packaged water and stream water samples during the dry season.

Similarly, for the samples collected from Kajuru LGA, the mean counts of the total and faecal coliforms were generally higher in the wet season, except for the packaged water samples where the reverse was the case. The FCC of the packaged water samples were significantly higher in the dry season ($P<0.05$) and the counts were higher than those of other sample sources (table 4.22).

Table 4.20: Mean total and faecal coliform counts of Ikara LGA samples by season

	Sample source	Dry	Wet	P-value
	Borehole	$7.7\times10^{2}\pm7.7\times10^{2}$	$1.1\times10^{6}\pm5.1\times10^{5}$	0.018*
	Packaged	$2.5\times10^{3}\pm2.9\times10^{2}$	$1.0\times10^{5}\pm7.5\times10^{4}$	0.374
TCC± SEM	**water**			
(cfu/100ml)	**Streams**	$9.3\times10^{3}\pm8.8\times10^{3}$	$8.9\times10^{5}\pm5.0\times10^{3}$	0.000*
	Taps	$1.2\times10^{4}\pm6.5\times10^{3}$	$1.8\times10^{5}\pm5.1\times10^{4}$	0.004*
	Wells	$2.1\times10^{4}\pm2.2\times10^{4}$	$1.4\times10^{6}\pm3.6\times10^{5}$	0.000*
	Borehole	0.0	$1.3\times10^{4}\pm5.4\times10^{3}$	0.008*
FCC± SEM	**Packaged**	0.0	$1.6\times10^{4}\pm6.0\times10^{3}$	0.148
(cfu/100ml)	**water**			
	Streams	0.0	$2.1\times10^{5}\pm5.0\times10^{3}$	0.000*
	Taps	0.0	0.0	---
	Wells	$8.5\times10^{3}\pm3.6\times10^{3}$	$2.3\times10^{5}\pm1.1\times10^{5}$	0.017*

t-test, $P\leq 0.05$

KEY

TCC= Total coliform count; FCC= Faecal coliform count ; SEM= Standard error of means

* Significant at $P\leq0.05$.

Table 4.21: Mean total and faecal coliform counts of Kaduna North LGA samples by season

	Sample source	Dry	Wet	P-value
	Borehole	$3.6\times10^{3}\pm2.6\times10^{3}$	$4.3\times10^{4}\pm2.7\times10^{4}$	0.183
	Packaged	$3.3\times10^{2}\pm3.3\times10^{2}$	$1.1\times10^{6}\pm5.6\times10^{5}$	0.249
TCC± SEM	**water**			
(cfu/100ml)	**Streams**	$3.2\times10^{5}\pm2.8\times10^{5}$	$8.5\times10^{7}\pm7.2\times10^{7}$	0.474
	Taps	$1.2\times10^{4}\pm6.5\times10^{3}$	$1.8\times10^{5}\pm5.1\times10^{4}$	0.004*
	Wells	$3.6\times10^{4}\pm2.5\times10^{4}$	$1.7\times10^{6}\pm4.2\times10^{5}$	0.000*
	Borehole	$2.2\times10^{3}\pm2.1\times10^{3}$	$1.4\times10^{5}\pm9.0\times10^{3}$	0.165
FCC± SEM	**Packaged**	0.0	$2.6\times10^{5}\pm1.2\times10^{5}$	0.014*
(cfu/100ml)	**water**			
	Streams	0.0	$2.1\times10^{5}\pm5.0\times10^{3}$	0.000*
	Taps	$7.0\times10^{3}\pm4.9\times10^{3}$	$3.8\times10^{4}\pm2.2\times10^{4}$	0.183
	Wells	$9.2\times10^{2}\pm9.1\times10^{2}$	$6.8\times10^{5}\pm3.1\times10^{5}$	0.025*

t-test, $P\leq 0.05$

KEY

TCC= Total coliform count; FCC= Faecal coliform count ; SEM= Standard error of means

* Significant at $P\leq0.05$.

Table 4.22: Mean total and faecal coliform counts of Kajuru LGA samples by season

	Sample source	Dry	Wet	P-value
	Borehole	$3.6\times10^{3}\pm2.6\times10^{3}$	$4.3\times10^{4}\pm2.7\times10^{4}$	0.183
TCC±SEM	**Packaged water**	$2.0\times10^{4}\pm3.1\times10^{2}$	$2.1\times10^{1}\pm1.6\times10^{1}$	0.423
	Wells	$1.1\times10^{3}\pm3.1\times10^{3}$	$2.1\times10^{5}\pm1.3\times10^{5}$	0.186
	Borehole	$9.1\times10^{3}\pm6.4\times10^{3}$	$1.1\times10^{4}\pm1.0\times10^{4}$	0.892
FCC±SEM	**Packaged water**	$1.4\times10^{4}\pm1.3\times10^{4}$	$1.5\times10^{3}\pm1.5\times10^{2}$	0.017*
	Wells	$4.3\times10^{2}\pm2.1\times10^{2}$	$4.8\times10^{2}\pm3.3\times10^{2}$	0.901

t-test, $P\leq 0.05$

KEY

TCC= Total coliform count; FCC= Faecal coliform count ; SEM= Standard error of means
* Significant at $P\leq0.05$.

The trend of samples collected in the wet season having higher counts was also observed for samples collected from Jema'a LGA (table 4.23). The packaged water samples collected in the dry season had no detectable faecal coliforms. There was a significantly higher TCC of tap and well water samples and FCC of streams and wells during the wet season. The highest count observed was for tap water samples collected in the wet season (3.1×10^5 cfu/100ml).

Faecal coliforms were not detected in any of the packaged water samples collected from Kagarko LGA (table 4.24). There were higher counts of total and faecal coliforms observed in borehole samples from this location during the dry season (1.9×10^4 and 4.2×10^3cfu/100ml respectively). For the other water sources, the counts were higher in the wet season, though the difference in the counts by season were not statistically significant ($P>0.05$).

Table 4.25 shows the mean total and faecal coliform counts of the well water samples form the six locations. The TCC of samples from Sabon-gari and Kaduna North LGAs were significantly higher than those of other locations (7.9×10^5 and 7.7×10^5 cfu/100ml respectively). The highest FCC observed was also in samples from Kaduna North LGA (3.09×10^5 cfu/100ml). The lowest counts observed were for samples from Kajuru and Kagarko LGAs. This shows that the well water sampled in Kaduna North LGA were very contaminated with faecal matter.

Table 4.23: Mean total and faecal coliform counts of Jema'a LGA samples by season

	Sample source	Dry	Wet	P-value
	Borehole	$7.1\times10^{4}\pm4.2\times10^{4}$	$1.7\times10^{5}\pm4.4\times10^{4}$	0.153
	Packaged	$5.0\times10^{3}\pm5.0\times10^{3}$	$1.5\times10^{5}\pm7.4\times10^{4}$	0.203
TCC± SEM	**water**			
(cfu/100ml)	**Streams**	$4.8\times10^{4}\pm5.0\times10^{3}$	$3.1\times10^{5}\pm1.0\times10^{1}$	0.471
	Taps	$4.4\times10^{4}\pm1.4\times10^{4}$	$3.2\times10^{5}\pm2.1\times10^{4}$	0.001*
	Wells	$4.9\times10^{4}\pm1.5\times10^{4}$	$3.1\times10^{5}\pm1.9\times10^{4}$	0.002*
	Borehole	$9.4\times10^{3}\pm6.9\times10^{3}$	$6.4\times10^{4}\pm3.0\times10^{4}$	0.165
FCC± SEM	**Packaged**	0.0	$4.3\times10^{4}\pm4.1\times10^{4}$	0.472
(cfu/100ml)	**water**			
	Streams	$3.2\times10^{4}\pm5.0\times10^{2}$	$3.1\times10^{5}\pm5.0\times10^{3}$	0.000*
	Taps	$6.4\times10^{2}\pm1.3\times10^{1}$	$5.2\times10^{2}\pm1.1\times10^{1}$	0.432
	Wells	$1.9\times10^{3}\pm5.3\times10^{2}$	$1.6\times10^{5}\pm2.5\times10^{4}$	0.000*

t-test, $P\leq 0.05$

KEY

TCC= Total coliform count; FCC= Faecal coliform count ; SEM= Standard error of means

* Significant at $P\leq0.05$.

Table 4.24: Mean total and faecal coliform counts of Kagarko LGA samples by season

	Sample source	Dry	Wet	P-value
	Borehole	$1.9\times10^4\pm1.2\times10^4$	$1.4\times10^4\pm8.7\times10^3$	0.727
	Packaged	0.0±0.0	$1.0\times10^3\pm1.0\times10^3$	0.203
TCC± SEM	**water**			
(cfu/100ml)	**Streams**	$3.5\times10^4\pm5.0\times10^3$	$1.3\times10^6\pm1.2\times10^6$	0.474
	Wells	$6.6\times10^4\pm1.8\times10^4$	$8.6\times10^4\pm1.8\times10^4$	0.472
	Borehole	$4.2\times10^3\pm1.5\times10^3$	$6.9\times10^2\pm3.9\times10^2$	0.029
FCC± SEM	**Packaged**	0.0±0.0	0.00±0.0	-----
(cfu/100ml)	**water**			
	Streams	$1.1\times10^4\pm5.0\times10^2$	$3.3\times10^4\pm3.3\times10^4$	0.632
	Wells	$1.5\times10^4\pm7.6\times10^3$	$7.0\times10^3\pm2.3\times10^3$	0.427

t-test, $P\leq 0.05$

KEY

TCC= Total coliform count; FCC= Faecal coliform count ; SEM= Standard error of means

* Significant at $P\leq0.05$.

Table 4.25: Mean total and faecal coliform counts (cfu/100ml) of well samples from various locations

Location(n)	Mean TCC/ml ± SE	Mean FCC/ml ± SE
Sabon-gari (26)	$7.9\times10^{5} \pm 3.40\times10^{5b}$	$1.5 \times 10^{5} \pm 1.04 \times 10^{5ab}$
Ikara (36)	$5.5\times10^{5} \pm 1.70\times10^{5ab}$	$9.65\times10^{4} \pm 4.7 \times 10^{4ab}$
Kaduna North (22)	$7.7\times10^{5} \pm 2.50\times10^{5\,b}$	$3.09 \times 10^{5} \pm 1.50 \times 10^{5b}$
Kajuru (48)	$1.2\times10^{5} \pm 7.30\times10^{4a}$	$4.58 \times 10^{2} \pm 2.0\times 10^{2}$ a
Jema'a (39)	$1.80\times10^{5} \pm 2.40\times10^{4ab}$	$8.50 \times 10^{4} \pm 1.80 \times 10^{4ab}$
Kagarko (41)	$7.40\times10^{4} \pm 1.30\times10^{4a}$	$1.10 \times 10^{4} \pm 4.90 \times 10^{3a}$
P-value	**0.001***	**0.005***

ANOVA, Tukey HSD test, $P \leq 0.05$.

In each category, values with different superscript letters are significantly different

KEY
N= number of samples
TCC= Total coliform count
FCC= Faecal coliform count
SE = Standard Error
ANOVA- Analysis of variance

* Significant at $P \leq 0.05$.

Table 4.26 shows that the mean TCC and FCC of tap water samples from the locations (except Kajuru and Kagarko where it was unavailable during sampling). It was observed that the total and faecal coliform counts from Jema'a LGA were the highest (1.84×10^5 and 7.20×10^4 cfu/100ml). There was no significant difference between the TCC of tap samples from various locations, but the FCC of Sabon-gari LGA was significantly lower than those of other locations ($P<0.05$).

Lastly, the TCC and FCC of all the stream water samples were compared in table 4.27, and it was observed that the streams from Ikara LGA had the highest total and faecal coliform count (5.60×10^7 and 4.30×10^6 cfu/100ml respectively). However, the mean TCC and FCC of the stream samples from various locations were not significantly different ($P>0.05$). The World Health Organization (WHO) standard for faecal and total coliforms in drinking water states that they should not be detected (0/100ml).

Table 4.28 shows the comparisons of the mean total and feacal coliform counts of tap water samples collected from all six locations. The total coliform count (TCC) of the samples from Sabon-Gari were significantly lower than that of all other locations ($P<0.05$). The feacal coliform counts (FCC) however were statistically similar. There was no statistical difference between the TCC and FCC of all the stream samples from all the various locations. However, the counts from Ikara were higher than the counts from other locations (Table 4.29).

Figure 4.1 indicates the number of all samples that had their TCC and FCC within the limits as specified by the WHO. None of the stream water samples met the criteria and 187 of the wells also.

Table 4.26: Mean total and faecal coliform counts (cfu/100ml) of borehole samples from various locations

Location(n)	Mean TCC/ml ± SE	Mean FCC/ml ± SE
Sabon-gari (20)	$3.18\times10^{4} \pm 1.88\times10^{4ab}$	$1.25 \times 10^{4} \pm 1.19 \times 10^{4ab}$
Ikara (22)	$4.4\times10^{5} \pm 2.30\times10^{5b}$	$5.30\times10^{3} \pm 2.50 \times 10^{3a}$
Kaduna North (19)	$2.44\times10^{4} \pm 1.45\times10^{4ab}$	$7.50 \times 10^{3} \pm 4.90 \times 10^{3ab}$
Kajuru (31)	$8.71\times10^{4} \pm 4.22\times10^{4ab}$	$9.90 \times 10^{3} \pm 6.0\times 10^{3ab}$
Jema'a (17)	$1.26\times10^{5} \pm 3.24\times10^{4ab}$	$4.12 \times 10^{4} \pm 1.88 \times 10^{4b}$
Kagarko (21)	$1.60\times10^{4} \pm 7.20\times10^{3a}$	$2.30 \times 10^{3} \pm 8.20 \times 10^{2a}$
P-value	**0.027***	**0.052**

ANOVA, Tukey HSD test, $P \leq 0.05$.

In each category, values with different superscript letters are significantly different

KEY
N= number of samples
TCC= Total coliform count
FCC= Faecal coliform count
SE = Standard Error
ANOVA- Analysis of variance

* Significant at $P \leq 0.05$.

Table 4.27: Mean total and faecal coliform counts (cfu/100ml) of packaged water samples from various locations

Location(n)	Mean TCC/ml ± SE	Mean FCC/ml ± SE
Sabon-gari (20)	$8.60\times10^{5} \pm 7.40\times10^{5a}$	$6.80 \times 10^{3} \pm 3.30 \times 10^{3a}$
Ikara (22)	$8.50\times10^{3} \pm 4.30\times10^{5a}$	$1.17\times10^{4} \pm 4.80\times10^{3a}$
Kaduna North (19)	$1.36\times10^{5} \pm 6.60\times10^{4a}$	$1.0\times10^{5} \pm 5.40\times10^{4a}$
Kajuru (31)	$1.30\times10^{4} \pm 1.30\times10^{4a}$	$9.30\times10^{3} \pm 8.80\times10^{3a}$
Jema'a (17)	$9.30\times10^{4} \pm 5.50\times10^{4a}$	$2.60\times10^{4} \pm 2.49\times10^{4a}$
Kagarko (21)	$6.60\times10^{3} \pm 6.60\times10^{3a}$	0.00^{a}
P-value	**0.934**	**0.161**

ANOVA, Tukey HSD test, $P \leq 0.05$.

In each category, values with different superscript letters are significantly different

KEY
N= number of samples
TCC= Total coliform count
FCC= Faecal coliform count
SE = Standard Error
ANOVA- Analysis of variance

Table 4.28: Mean total and faecal coliform counts (cfu/100ml) of tap water samples from various locations

Location(n)	Mean TCC/ml ± SE	Mean FCC/ml ± SE
Sabon-gari (11)	$3.30\times10^{4} \pm 2.30\times10^{4a}$	$7.10 \times 10^{3} \pm 6.50 \times 10^{3a}$
Ikara (05)	$6.30\times10^{4} \pm 4.80\times10^{4b}$	$1.00 \times 10^{1\ a}$
Kaduna North (20)	$9.58\times10^{4} \pm 3.15\times10^{4b}$	$2.20\times10^{4} \pm 1.14\times10^{4a}$
Jema'a (24)	$1.84\times10^{5} \pm 3.50\times10^{4b}$	$7.22 \times 10^{4} \pm 2.39 \times 10^{4a}$
P-value	**0.028***	**0.065**

ANOVA, Tukey HSD test, $P \leq 0.05$.

In each category, values with different superscript letters are significantly different

KEY
N= number of samples
TCC= Total coliform count
FCC= Faecal coliform count
SE = Standard Error
ANOVA- Analysis of variance

* Significant at $P \leq 0.05$.

Table 4.29: Mean total and faecal coliform counts (cfu/100ml) of stream water samples from various locations

Location(n)	Mean TCC/ml ± SE	Mean FCC/ml ± SE
Sabon-gari (03)	$2.95\times10^{6} \pm 1.50\times10^{5a}$	$3.15 \times 10^{4} \pm 5.0 \times 10^{3a}$
Ikara (03)	$5.60\times10^{7} \pm 4.88\times10^{7a}$	$4.30 \times 10^{6} \pm 2.80 \times 10^{6a}$
Kaduna North (03)	$2.30\times10^{5} \pm 2.20\times10^{5a}$	$5.00\times10^{4} \pm 5.0 \times 10^{4a}$
Jema'a (03)	$1.79\times10^{5} \pm 1.31\times10^{5\ a}$	$1.70 \times 10^{5} \pm 1.30 \times 10^{5a}$
Kagarko(03)	$9.83 \times 10^{5} \pm 9.0 \times 10^{5a}$	$2.70\times 10^{4} \pm 2.42 \times 10^{4a}$
P-value	**0.688**	**0.445**

ANOVA, Tukey HSD test, P≤ 0.05.

In each category, values with different superscript letters are significantly different

KEY
N= number of samples
TCC= Total coliform count
FCC= Faecal coliform count
SE = Standard Error
ANOVA- Analysis of variance

* Significant at P≤0.05.

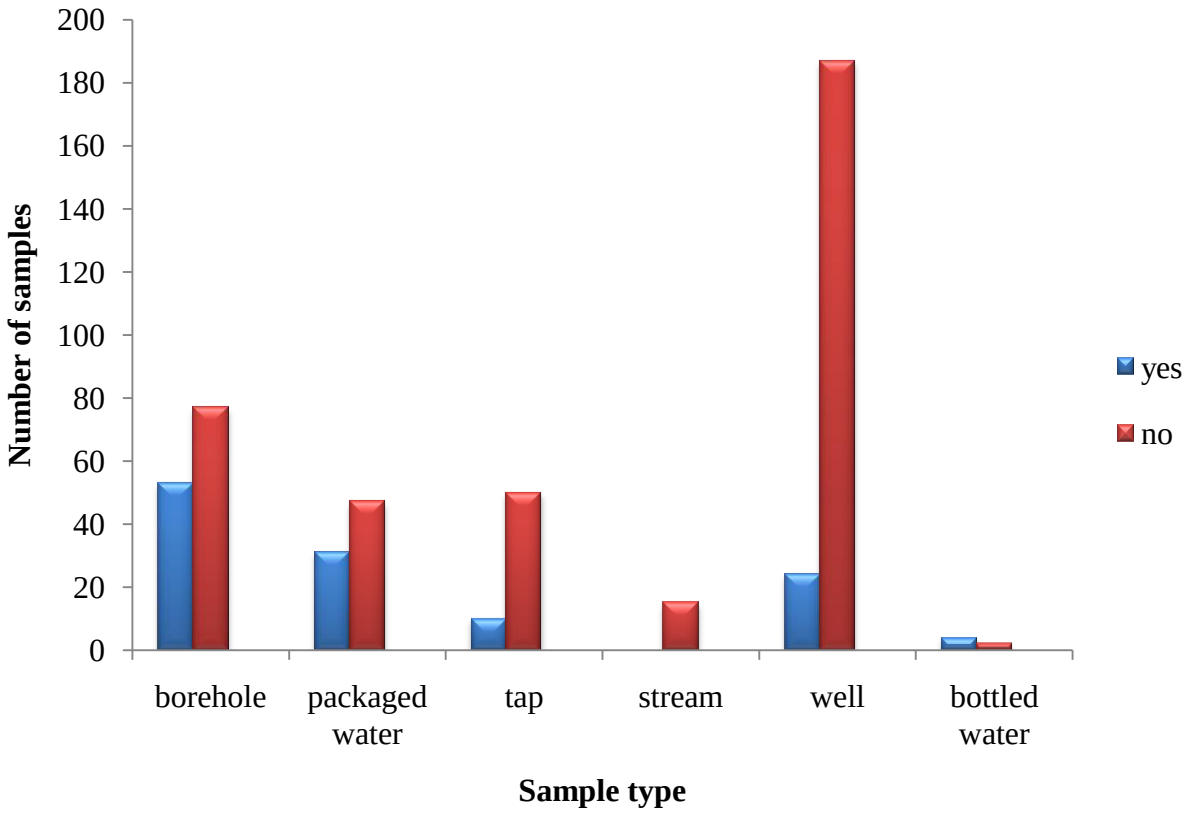


Figure 4.1: Number of each water type that met the WHO standard of zero/100ml total coliform count

Similarly, Figure 4.2 shows how many of the samples met the standard for feacal coliform count in drinking water as specified by the WHO. All the bottled water samples had their counts within the limits while 30 of the packaged water samples had FCC above the specified limits.

4.3 Antibiotic susceptibility tests

The antibiotic that had the most resistance from *E.coli* and *Salmonella* sp was Tetracycline (56.1 and 77.8% respectively). Forty (97.6%) of the *E.coli isolates* were susceptible to Chloramphenicol and Gentamicin, while all the 9 *Salmonella enterica* isolates (100%) were susceptible to Amoxycillin, Amoycillin-clavulanic acid, Ampicillin, and Gentamicin (Table 4.30).

The overall susceptibility of all the isolates to the antibiotics used is shown in Figure 4.3. The highest susceptibility of the isolates was to gentamicin (98%), followed by chloramphenicol, while the highest resistance was observed for tetracycline, ampicillin and amoxicillin. The resistance pattern of the isolates to the antibiotics showed that ten isolates were resistant to one antibiotic, 6 were resistant to two antibiotics, 10 were resistant to three antibiotics, 5 were resistant to 4 antibiotics, four were resistant to 5 antibiotics, one was resistant to six antibiotics while 3 were resistant to 7 antibiotics (Table 4.31).

Multidrug resistance was taken as resistance to 3 or more antibiotics. The percentage multidrug resistance of *E.coli* isolates showed that a total of 25 isolates were resistant to 2 or more antibiotics. Approximately 28% of the isolates were resistant to 3 antibiotics and 4% were resistant to 6 antibiotics. Also, 12% were resistant to seven of the ten antibiotics (Figure 4.4). For the *Salmonella* isolates, two (66.67%) of the isolates were resistant to 3 antibiotics while 1(33.33%) was resistant to 2 antibiotics (Figure 4.5).

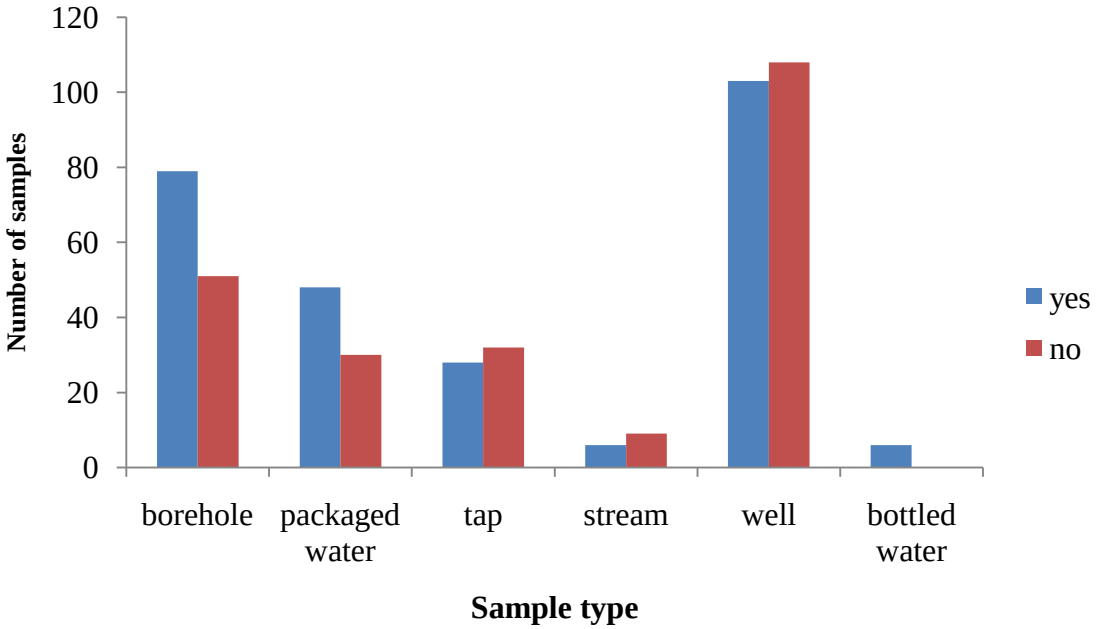


Figure 4.2: Number of each sample type that met the WHO standard of zero/100ml for faecal coliform counts in drinking water

Table 4.30: Antibacterial susceptibility patterns Of *Escherichia coli* and *Salmonella enterica* isolates from water samples

Antibacterial agent	Symbol	Disc content (µg)	*E.coli*(N=41) R n(%)	I n(%)	S n(%)	*Salmonella enterica* (N=9) R n(%)	I n(%)	S n(%)
Amoxicillin	AML	10	21(51.2)	5(12.2)	15(36.6)	0(0)	0(0)	9(100)
Amoxicillin-clavulanic acid	AMC	30	4(9.8)	3(7.3)	34(82.9)	0(0)	0(0)	9(100)
Ampicillin	AMP	10	18(43.9)	6(14.6)	17(41.5)	0(0)	0(0)	9(100)
Chloramphenicol	C	30	1(2.4)	0(0)	40(97.6)	0(0)	1(11.1)	8(88.9)
Ciprofloxacin	CIP	10	5(12.2)	27(65.9)	9(21.9)	1(11.1)	4(44.4)	4(44.4)
Cefotaxime	CTX	30	3(7.3)	0(0)	38(92.7)	1(11.1)	0(0)	8(88.9)
Gentamicin	GN	30	1(2.4)	0(0)	40(97.6)	0(0)	0(0)	9(100)
Nalidixic acid	NA	30	14(34.1)	9(22)	18(43.9)	3(33.3)	2(22.2)	4(44.4)
Sulphamethoxazole-trimethoprim	SXT	25	14(34.1)	0(0)	27(65.9)	1(11.1)	0(0)	8(88.9)
Tetracycline	TE	30	23(56.1)	3(7.3)	15(36.6)	7(77.8)	1(11.1)	1(11.1)

KEY

N-Number of isolates tested R- Resistant; I-Intermediate; S-Susceptible; n-Number of isolates susceptible/intermediate/resistant

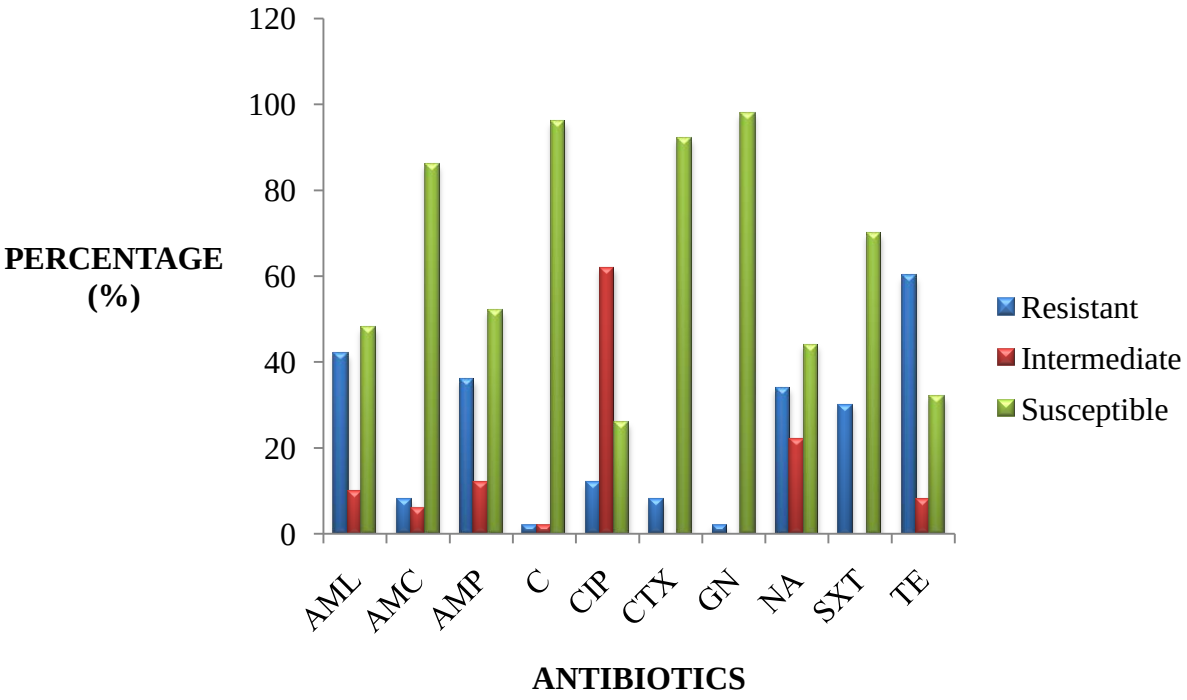


Figure 4.3: Overall percentage susceptibility pattern of the *E. coli* and *Salmonella enterica* isolates to the antibiotics

KEY

AML-Amoxycillin
AMC- Amoxycillin-clavulanic acid
AMP- Ampicillin
C-Chloramphenicol
CIP-Ciprofloxacin
CTX-Cefotaxime
GN-Gentamicin
NA-Nalidixic acid
SXT-Sulphamethoxazole-trimrthoprim
TE-Tetracycline

Table 4.31: Resistance patterns of the *E. coli* and *Salmonella enterica* isolates to antibiotics

Pattern of resistance	***E. coli* (n)**	***Salmonella enterica(*n)**
TE	3	3
NA	1	0
SXT	1	0
AMC	1	0
AML	1	0
TE,AML	1	0
TE,SXT	1	0
NA,CIP	1	0
NA,TE	2	1
NA,AML,AMC	0	1
NA,CIP,CTX	0	1
GN,AMP,AML	1	0
TE,AMP,AML	4	0
TE,NA,SXT	1	1
AMP,CTX,AML	1	0
TE,NA,SXT,AMP	1	1
TE,SXT,AMP,AML	3	0
TE,NA,SXT,AMP,AML	4	0
TE,NA,CIP,AMP,CTX,AML	1	0
TE,NA,C,CIP,AMP,CTX,AML	1	0
TE,NA,AMC,SXT,CIP,AMP,AML	2	0

KEY:n-Number of isolates resistant AML-Amoxycillin,AMC- Amoxycillin-clavulanic acid,AMP- Ampicillin,C-Chloramphenicol,CIP-Ciprofloxacin,CTX-Cefotaxime,GN-Gentamicin,NA-Nalidixic acid,SXT-Sulphamethoxazole-trimrthoprim,TE-Tetracycline

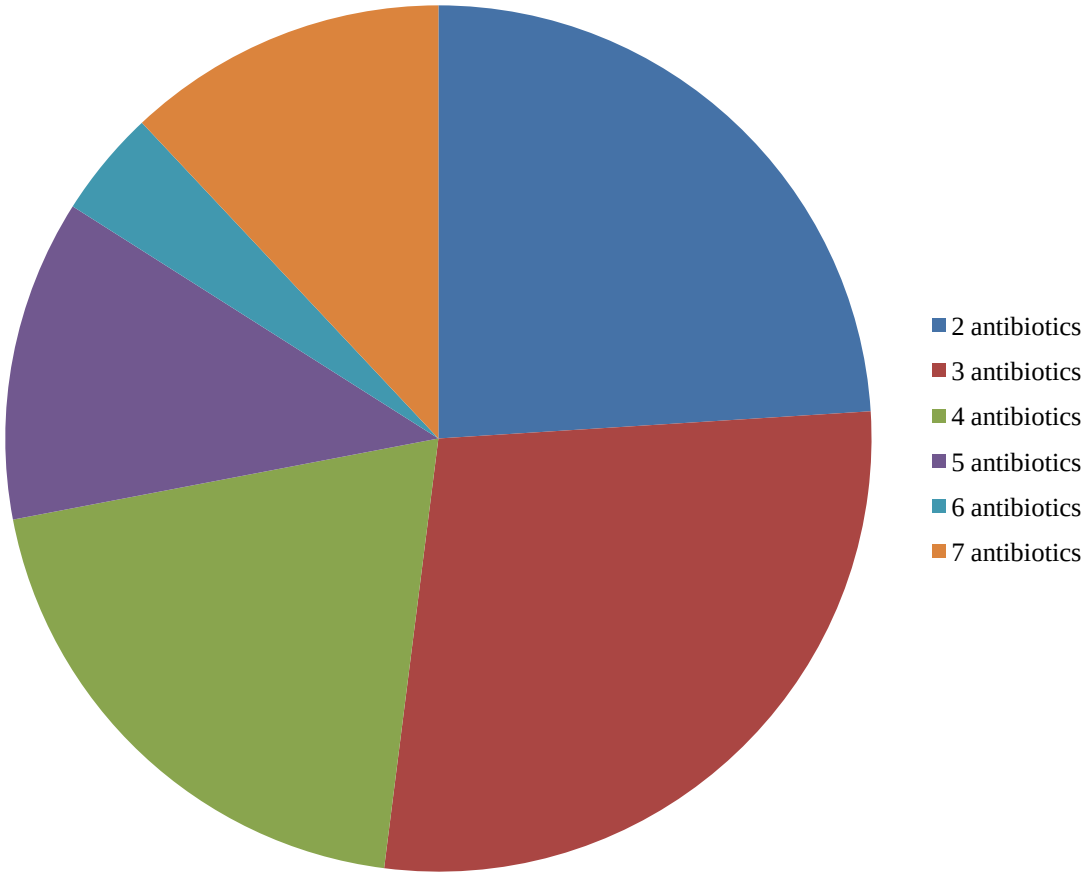


Figure 4.4: Percentage multidrug resistance of *E. coli* isolates (N=25)

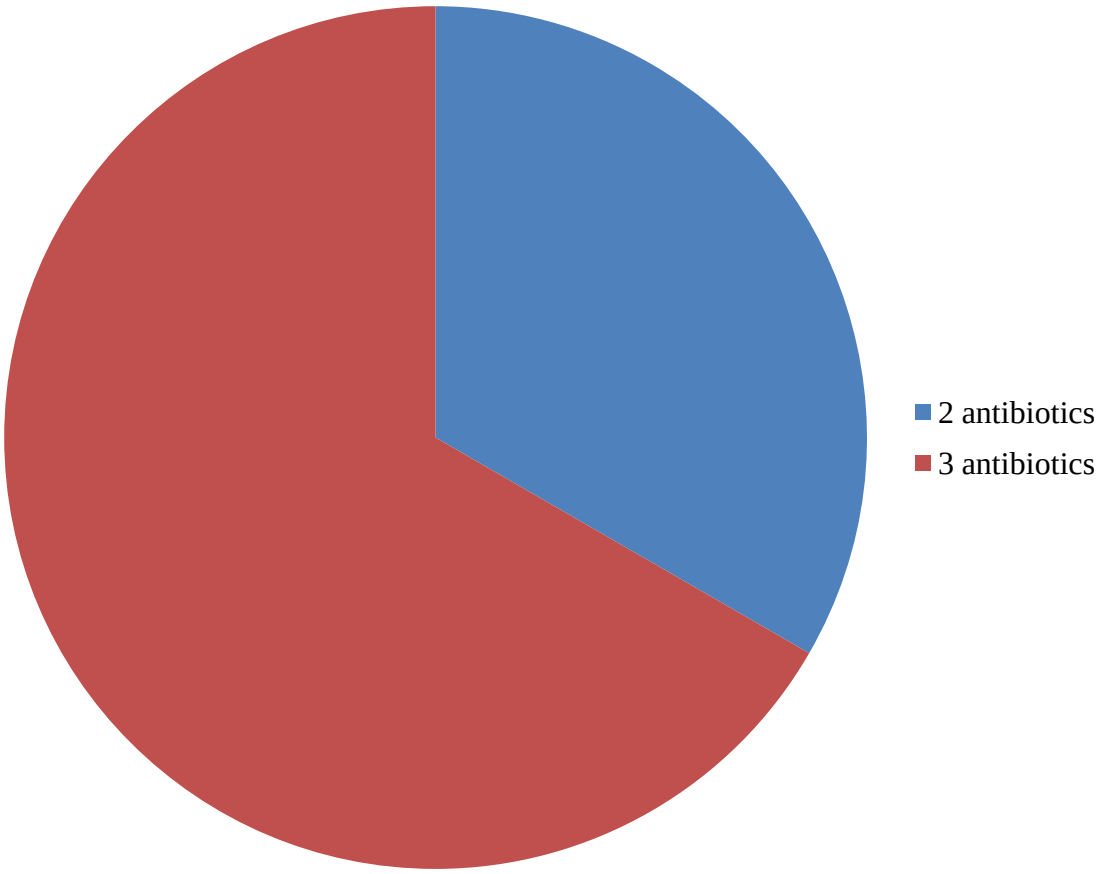


Figure 4.5: Percentage multidrug resistance of *Salmonella enterica* isolates

The multiple antibiotic resistance (MAR) indices ranged from 0.3 – 0.7. These multidrug resistant isolates were gotten from various water sources. Eight of them were isolated from borehole water, six from packaged water and one from stream water (Table 4.32).

Nine (30%) of the *E. coli* isolates had a MAR index of 0, seven (17.1%) had a MAR index of 0.1 and 3(7.31%) had a MAR index of 0.7. For the *Salmonella enterica* isolates, 4(44.4%) had a MAR inde of 0.1 while 2(22.2%) had a MAR index of 0 and 0.3 respectively (Table 4.33).

4.4 Polymerase chain reaction targeting some virulence genes in *E. coli* and *Salmonella enterica* isolates

The gel picture of the PCR targeting the stx1 gene in *E. coli* isolates is shown in Plate I. A 1kb plus ladder (Invitrogen) was used as a molecular marker and a negative control comprising of the master mix, and primers was used. Sixteen isolates of *Escherichia coli* were used in this PCR and the gene had an expected amplicon size of 614kb.

Plate II shows the gel picture of the PCR targeting the Stx2 gene in some *Escherichia coli* isolates. The expected amplicon size was 1181bp. Seven of the isolates produced amplicons for this gene a 1kb plus ladder (Invitrogen) was used as a molecular marker. A negative control was also used (Lane 2) which produced no band. Some other bands showed above the specified base pair size for this gene and they can be said to be non-specific bands.The eaeA gene was also assayed for in the *Escherichia coli* isolates (Plate III). The picture shows that the target gene with a base pair size of 229bp was amplified in only one of the isolates (Lane 1). Some other non-specific bands were amplified at base pair sizes far above the target. A 1kb plus ladder was used as a molecular marker.

Table 4.32: Antibiotics resistance patterns and multiple antibiotic resistance (MAR) index of *E. coli* and *Salmonella enterica* isolated from water

Isolate no.	Sample location	Sample source	Resistance pattern	MAR Index
198E.C	Ikara	borehole	TeAml	0.2
242E.C	kajuru	borehole	TeSxt	0.2
90E.C	Kaduna North	Tap	NaAmlAmc	0.3
276E.C	Kaduna North	packaged water	NaCip	0.2
156E.C	Sabon-gari	packaged water	NaTe	0.2
117S	Jama'a	well	NaTe	0.2
277E.C	Kaduna North	tap	NaTe	0.2
111S	Sabon-gari	borehole	NaCipCtx	0.3
207E.C	Ikara	Stream	GnAmpAml	0.3
153E.C	Kajuru	well	TeAmpAml	0.3
31E.C	Sabon-gari	packaged water	TeAmpAml	0.3
131E.C	Kagarko	borehole	TeAmpAml	0.3
227E.C	Kagarko	well	TeAmpAml	0.3
269E.C	Kaduna North	packaged water	TeNaSxt	0.3
118S	Jama'a	tap	TeNaSxt	0.3
251E.C	Kajuru	well	AmpCtxAml	0.3
236E.C	Kagarko	packaged water	TeSxtAmpAml	0.4
239E.C	Kagarko	borehole	TeSxtAmpAml	0.4
234E.C	Kagarko	borehole	TeSxtAmpAml	0.4
158E.C	Kajuru	well	TeNaSxtAmpAml	0.5
221E.C	Kaduna North	borehole	TeNaSxtAmpAml	0.5
275E.C	Kaduna North	borehole	TeNaSxtAmpAml	0.5
279E.C	Kaduna North	well	TeNaSxtAmpAml	0.5
197E.C	Ikara	well	TeNaCipCtxAmpAml	0.6
185E.C	Sabon-gari	packaged water	TeNaCCipCtxAmpAml	0.7
261E.C	Kaduna North	tap	TeNaAmcSxtCipAmpAml	0.7
262E.C	Kaduna North	tap	TeNaAmcSxtCipAmpAml	0.7

KEY: E.C- *E.coli,* S-*Salmonella enterica,* MAR Index=no of antibiotics isolate resisted/total number of antibiotics tested. AML-Amoxycillin,AMC- Amoxycillin-clavulanic acid,AMP- Ampicillin,C-Chloramphenicol,CIP-Ciprofloxacin,CTX-Cefotaxime,GN-Gentamicin,NA-Nalidixic acid,SXT-Sulphamethoxazole-trimrthoprim,TE-Tetracycline

Table 4.33: Frequency of MAR index among *E.coli* and *Salmonella enterica* isolates

MAR Index	Frequency of mar index(%)	
	***E.coli* (n=41)**	***Salmonella enterica*(n=9)**
0	9(30)	2(22.2)
0.1	7(17.1)	4(44.4)
0.2	5 (12.19)	1(11.1)
0.3	8 (19.51)	2(22.2)
0.4	5 (12.19)	0(0)
0.5	3 (7.31)	0(0)
0.6	1 (2.43)	0(0)
0.7	3 (7.31)	0(0)
0.8	0(0)	0(0)
0.9	0(0)	0(0)
1.0	0(0)	0(0)

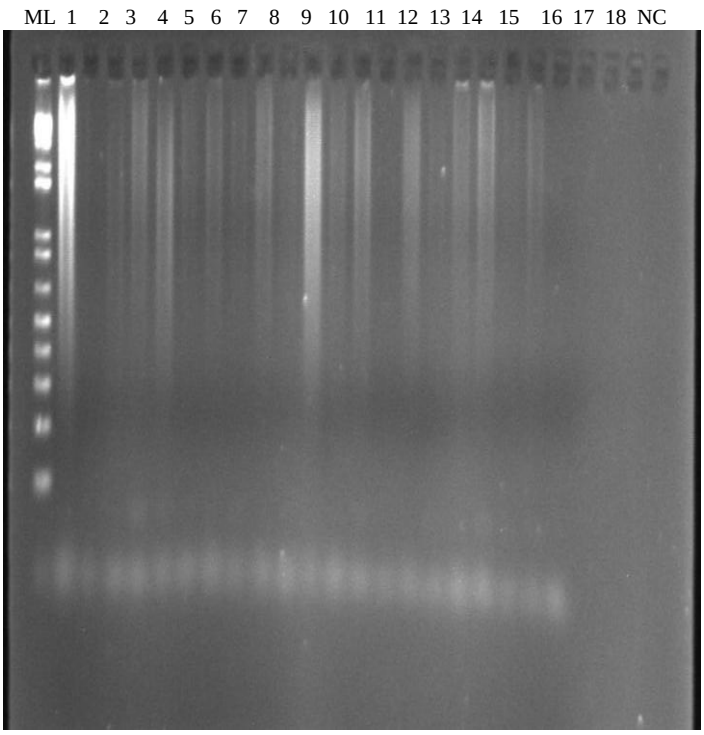


Plate I: Gel image of PCR for stx1 gene in *E. coli* isolates

Lanes 1-16: *E. coli;* all negative for the stx1 gene

ML: 1Kb plus molecular ladder

NC: Negative control

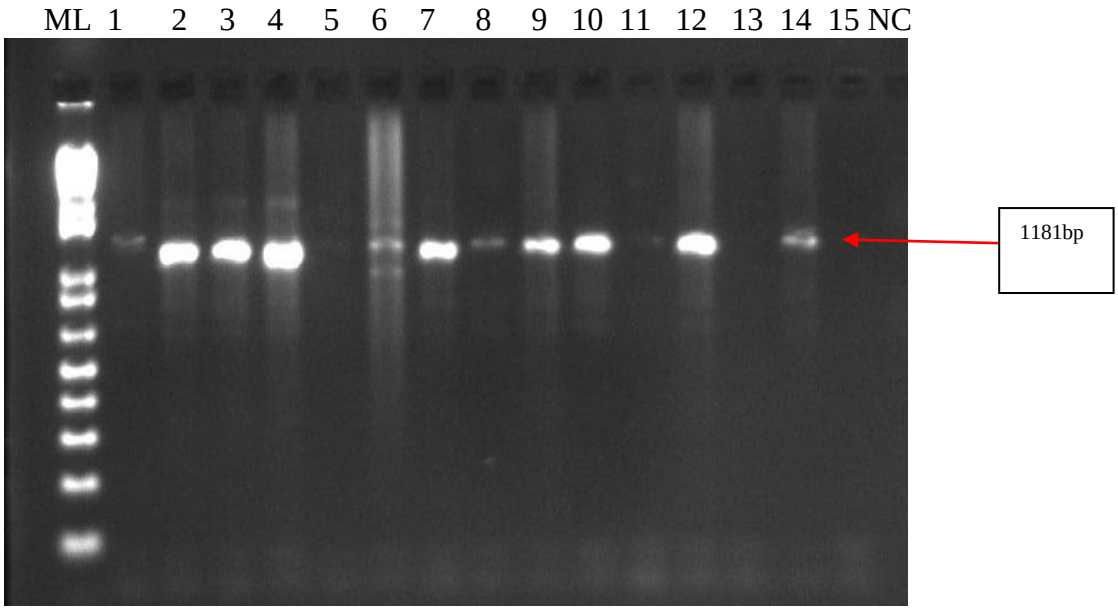


Plate II: Gel image of stx2 amplicons in *E. coli* isolates

ML- 1Kb plus molecular ladder

NC- Negative control

Lanes 1- 15- *E. coli* isolates

Lanes 2-4, 6-10, 12, 14: positive for the Stx2 gene

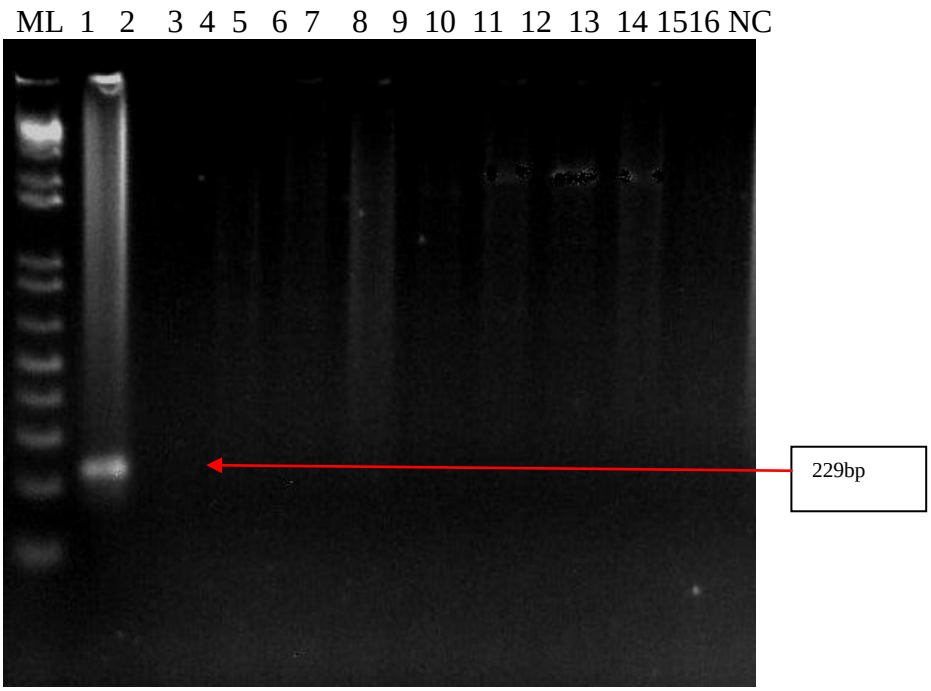


Plate III: Gel image of eaeA amplicons in *E. coli* isolates

NC- Negative control

Lanes 1-16: *E. coli* isolates

Lane 1: positive for eaeA gene

The gel picture of the PCR targeting the hlyA gene in the *E. coli* isolates is shown in Plate IV. The expected amplicon size of this gene was 534bp. Fifteen of the isolates produced amplicons for this gene. A negative control and a 1kb plus molecular ladder were used.

The invA gene was targeted in the PCR carried out on the *Salmonella enterica* isolates (Plate V). All six isolates (Lanes 2 -7) showed amplicons at the expected size of 244bp. A 1kb plus ladder was used as molecular marker. This confirmed that the isolates belong to the *Salmonella* genus.

Plate VI shows the gel picture of the PCR products for the target gene SpvA in the *Salmonella enterica* isolates. The gene had an expected amplicon size of 604bp. None of the isolates produced an amplicon for the gene after the PCR this indicates that none of the *Salmonella* isolates in this study possessed the spvA gene.

4.5 Polymerase chain reactions targeting some antibiotic resistance genes in *E. coli* and *Salmonella enterica* isolates

The antibiotic resistant gene sul1 was assayed for using the PCR. Plate VII shows the gel picture of the PCR products of the sul1 gene in selected *E. coli* and *Salmonella enterica* isolates that showed phenotypic resistance to Sulphamethoxazole- trimethoprim. The expected amplicon size was 416bp. Ten of the isolates (Lanes 1 and 2, 4 and 5, and 7- 11) produced amplicons for this gene.

Some of the *E. coli* and *Salmonella enterica* isolates isolates which were resistant to the beta-lactams were subjected to PCR assaying for the blaTEM gene. The gel picture is shown in Plate VIII. The expected amplicon size was 964bp. Two *Salmonella* isolates produced amplicons for this gene while none of the *E. coli* isolates produced amplicon.

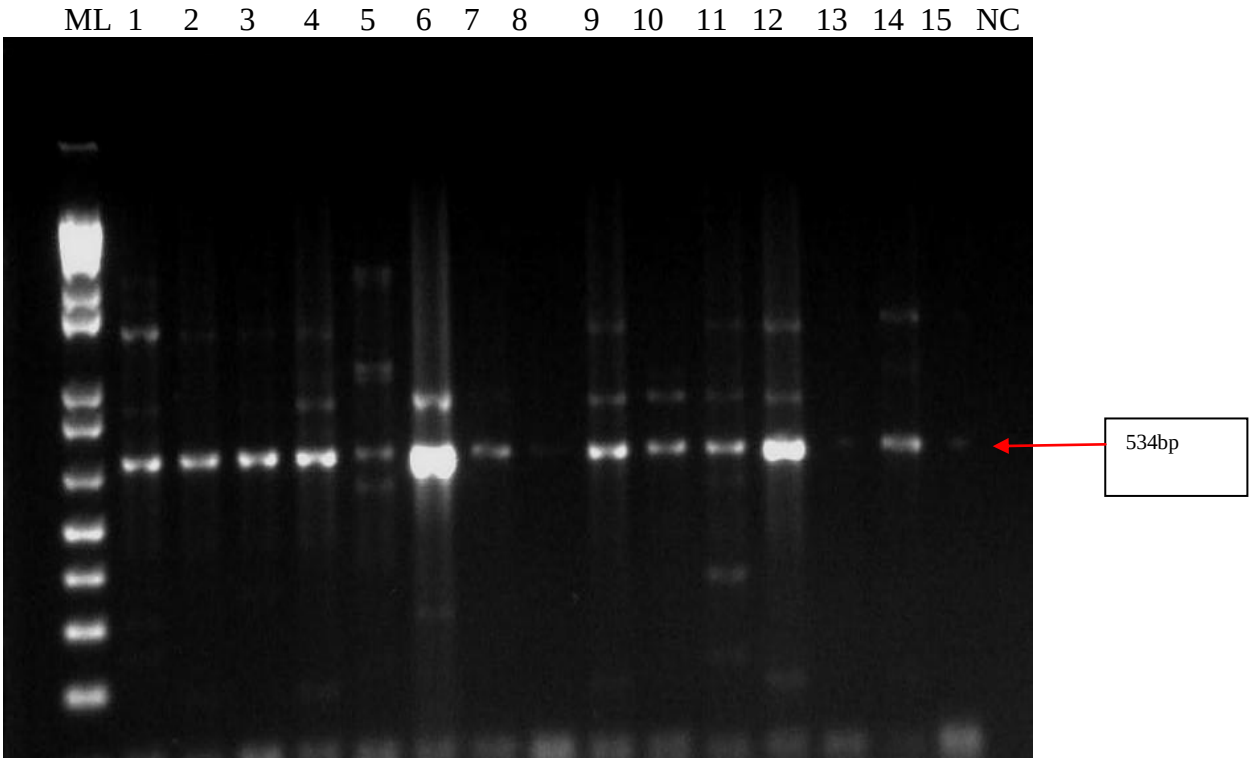


Plate IV: Gel image of hlyA amplicons in *E. coli* isolates

NC- Negative control

Lanes 1-15: *E. coli* isolates, all positive for the HlyA gene

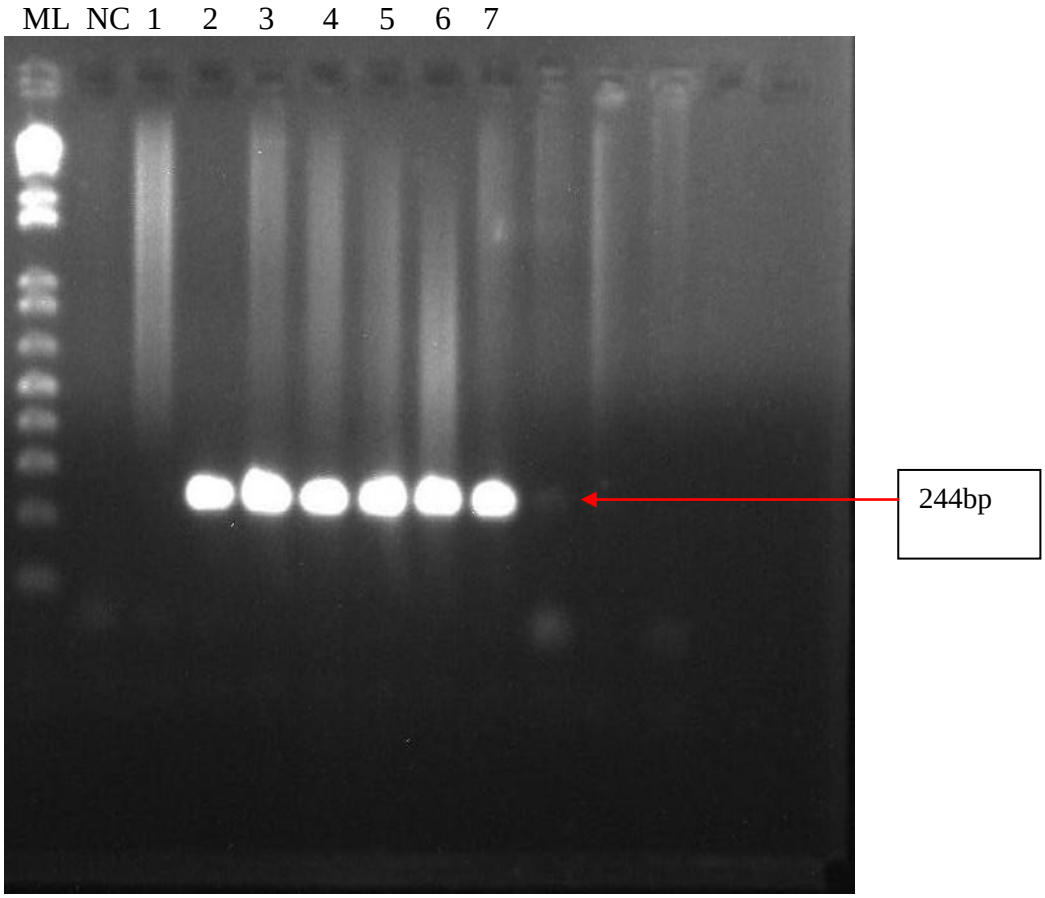


Plate V: Gel image of invA amplicons in *Salmonella enterica* isolates

ML- 1 Kb plus molecular ladder

NC-negative control

Lane 1- *Escherichia coli*

Lanes 2-7: *Salmonella* enterica isolates

Lanes 2-7: positive for the invA gene

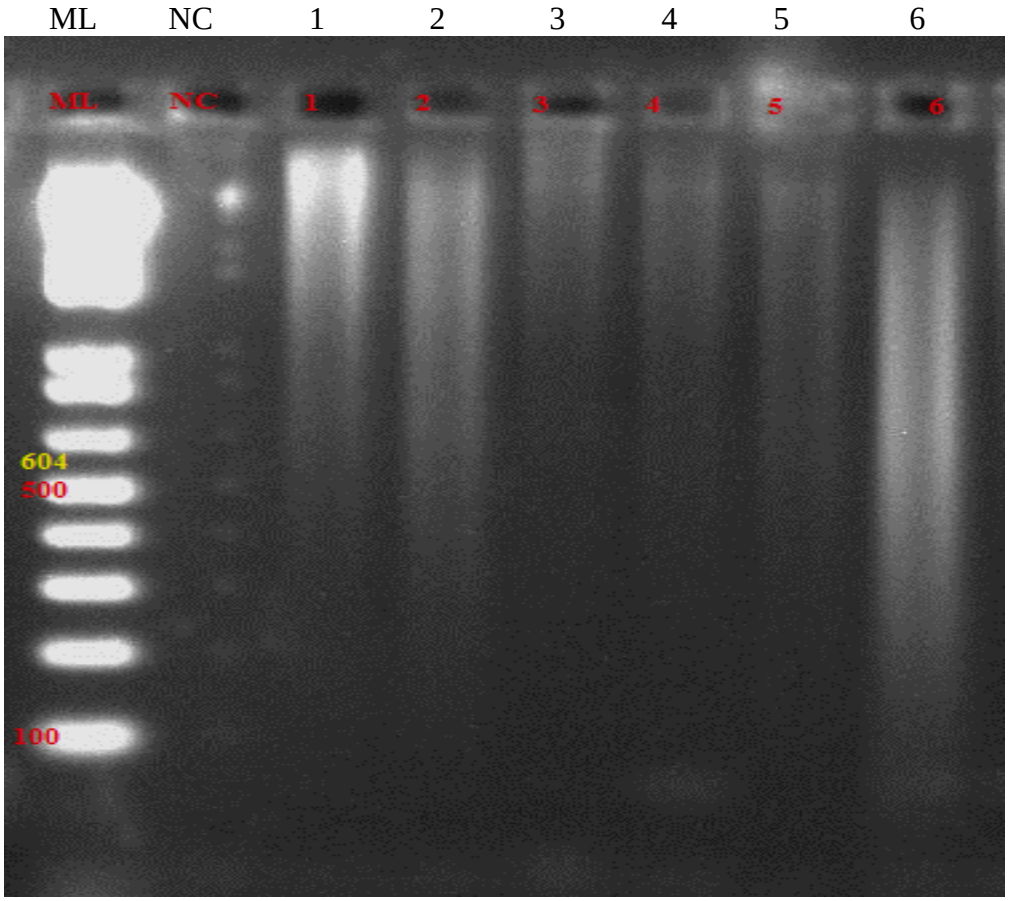


Plate VI- Gel image of PCR for spvA gene in *Salmonella enterica* isolates

ML- Molecular ladder

NC- Negative control

Lanes 1 -6: negative for spvA gene

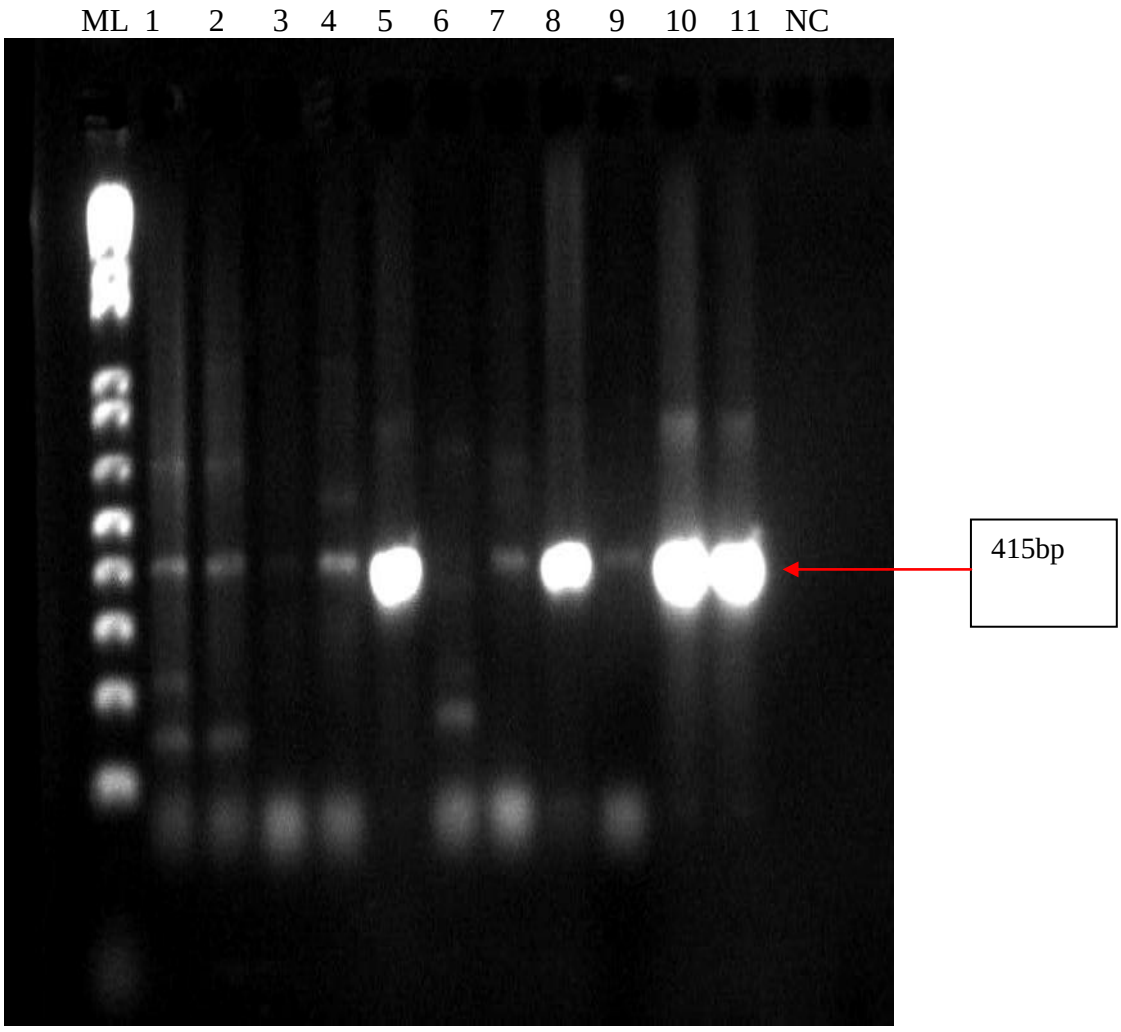


Plate VII: Gel image of sul1 amplicons in *E. coli* and *Salmonella enterica* isolates

ML- Molecular ladder

Lanes 1 and 2- *Salmonella* enterica

Lanes 3-11- *E. coli*

Lane 12- negative control

Lanes 1-5, 7-11: positive for the sul1 gene

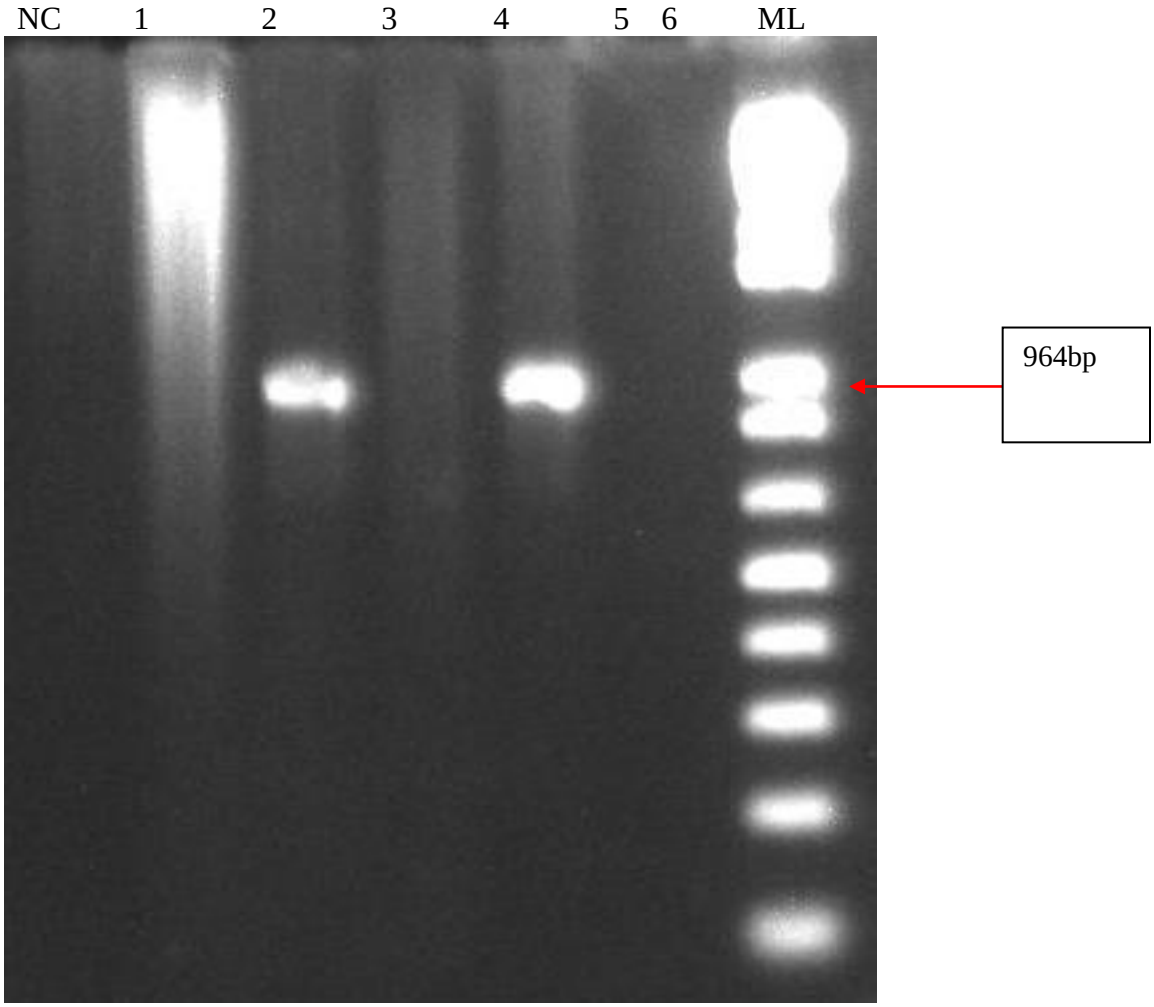


Plate VIII: Gel image of blaTEM amplicons in *E. coli* and *Salmonella enterica* isolates

ML= Molecular ladder

NC= Negative control

Lanes 1,2,4 and 6- *Escherichia coli*

Lanes 3 and 5- *Salmonella enterica*

Lanes 2 and 4 are positive for the blaTEM gene

The tetracycline resistance gene tetA was assayed for and plate IX shows the agarose gel electrophoresis of amplified gene products in *E. coli* and *Salmonella enterica* isolates. The expected amplicon size was 577bp. Five (5) isolates produced amplicons for the tetA gene. They included 2 *E. coli* isolates (lanes 3 and 4) and 3 *Salmonella enterica* isolates.

Some of the *E. coli* and *Salmonella enterica* isolates that showed phenotypic resistance to tetracycline were subjected to PCR testing for the tetracycline resistance gene tetB. The result is shown in the gel electrophoresis picture in Plate X. The expected amplicon size was 415bp. Two *E. coli* isolates (lanes 7 and 14) produced bands for the tetB gene.

4.6 Polymerase chain reaction targeting the 16SrRNA gene in *E. coli* and *Salmonella* isolates, BLAST and phylogenetic analysis

Plate XI shows the gel electrophoresis of amplified 16S rRNA gene from selected in *E. coli* and *Salmonella enterica* isolates. The expected amplicon size was 1500bp. All the isolates used in this PCR showed bands at the expected amplicon size.

The sources of the *E. coli* that possess virulence genes are shown in Table 4.34. Packaged water from Kagarko, Sabon-gari and Kaduna North LGAs contained isolates that possessed one or more virulence genes. Similarly, borehole water, tap water, well water and stream water all contained isolates that possessed one or more virulence gene.

Table 4.35 shows the sources of *Salmonella enterica* isolates that possess virulence genes. Most of the *Salmonella* enterica isolates that contained the virulence gene invA were isolated from well water while the remaining two were isolated from tap and borehole water samples, both from Jema'a LGA.

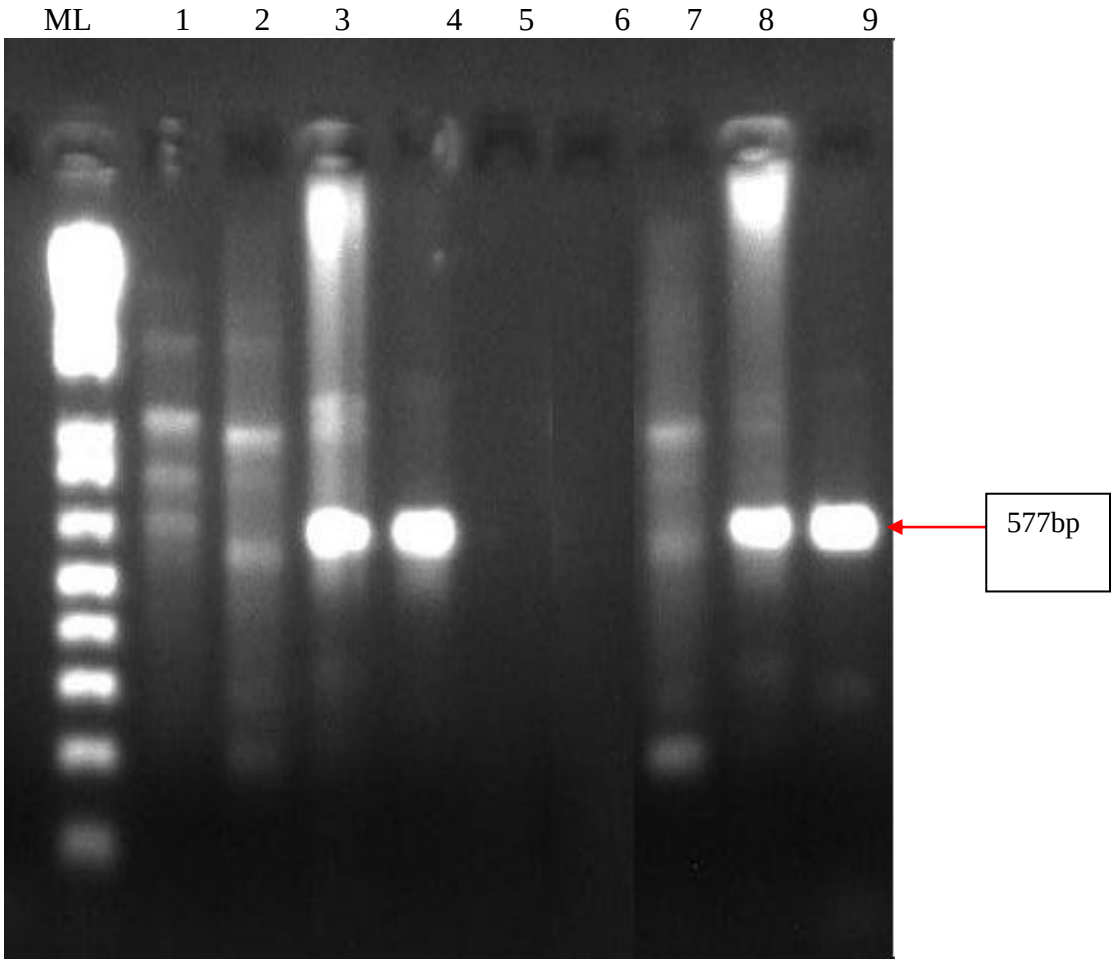


Plate IX- Gel iimage of tetA amplicons in *E. coli* and *Salmonella enterica* isolates

ML- Molecular ladder

Lanes 1-4- *Salmonella enterica*

Lanes 5- negative control

Lanes 6-9- *E. coli*

Lanes 3,4,7,8 and 9- positive for tetA gen

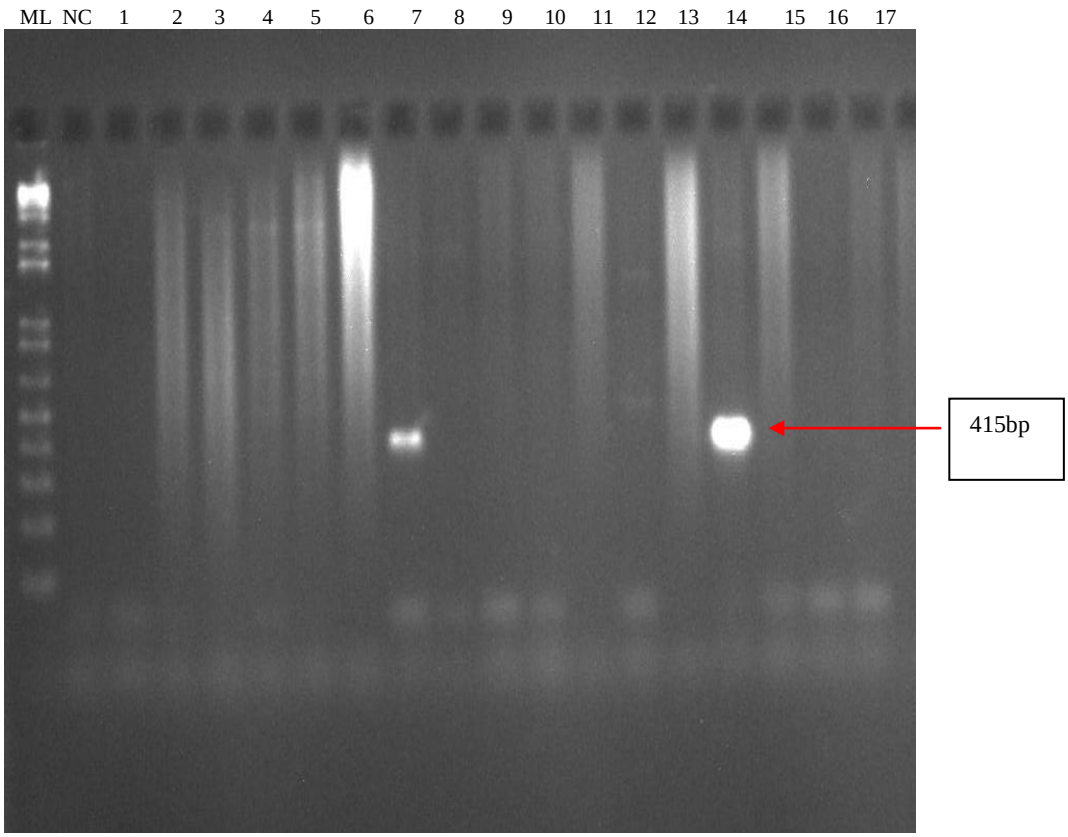


Plate X: Gel image tetB amplicons in *E. coli* and *Salmonella enterica* isolates

ML= Molecular ladder (1kb plus)

NC= Negative control

Lanes 1-5= *Salmonella* enterica

Lanes 6-17= *Escherichia coli*

Lanes 7 and 14- positive for tetB gene

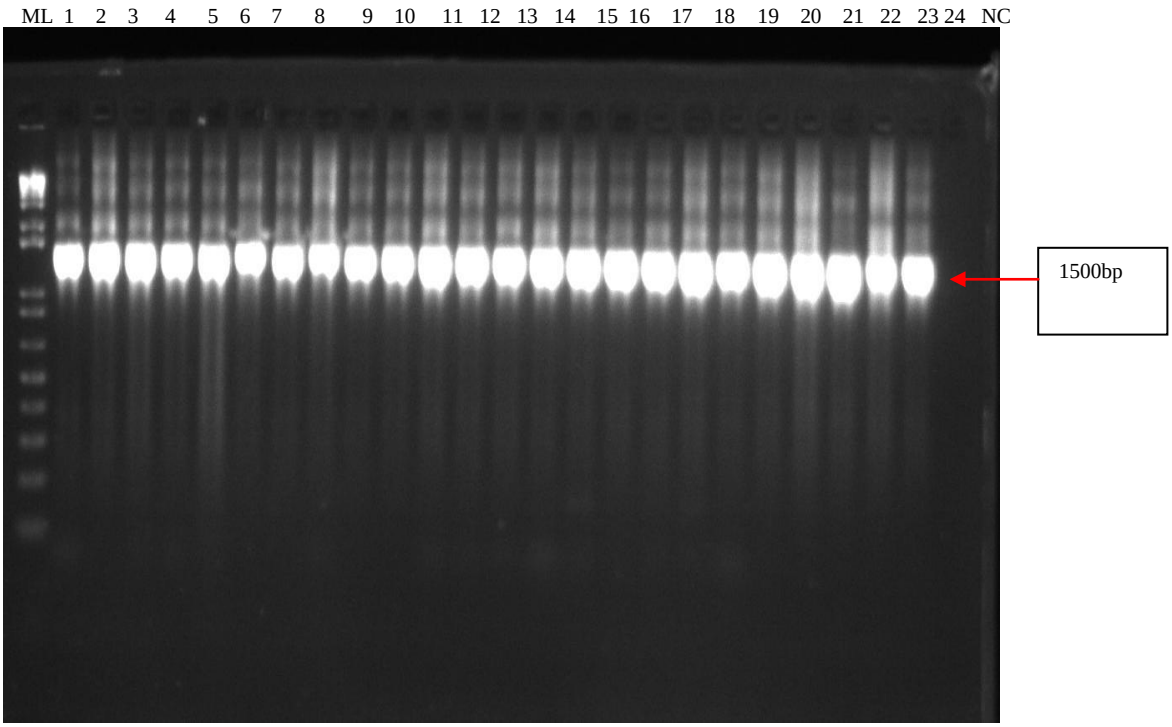


Plate XI: Gel image of 16S rRNA amplicons in *E. coli* and *Salmonella enterica* isolates

ML- Molecular ladder

Lanes 1 – 24- *E. coli and Salmonella* isolates

NC- Negative control

Table 4.34: Sources of *E. coli* isolates that possess virulence and resistance genes.

s/no	Identity	Location	Source	AR pattern		Virulence genes detected	AR genes detected
31	*E. coli* O104:H4	Sabon-gari		packaged water	TE,AMP,AML	Stx2, hlyA	TetA,
158	*E. coli*	Kajuru		well water	TE,NA,SXT,AMP,AML	hlyA	Sul1, tetA
185	*E. coli* IAI39	Sabon-gari		packaged water	TE,NA,C,CIP,CTX,AMP,AML	hlyA	TetA,
188	*E. coli* O157:H7	Sabon-gari		borehole water	SXT	Stx2, hlyA	-
196	*E. coli* O157:H7	Ikara		Well water	TE,NA,CIP,AMP,CTX,AML	Stx2, hlyA	-
198	*E. coli* O157:H7	Ikara		well water	TE,AML	Stx2, hlyA, eaeA	TetA,
207	*E. coli* O104:H4	Ikara		stream water	TE,AMP,AML	Stx2, hlyA	-
235	*E. coli* IAI39	Kagarko		borehole water	TE,NA,SXT,AMP,AML	hlyA	TetA,
236	*E. coli* O157:H7	Kagarko		packaged water	TE,SXT,AMP,AML	hlyA	tetA
239	*E. coli* O157:H7	Kagarko		borehole water	TE,SXT,AMP,AML	hlyA	-
261	*E. coli*	Jema'a		tap water	TE,NA,AMC,SXT,CIP,AMP,AML	hlyA	TetA, TetB,
262	*E. coli* O157:H7	Jema'a		tap water	TE,NA,AMC,SXT,CIP,AMP,AML	Stx2, hlyA	Sul1, TetA, TetB, bla_{TEM}
267	*E. coli* IAI39	Kaduna North		stream water	TE,NA,SXT,AMP,AML	Stx2, hlyA	Sul1, TetA,
269	*E. coli* IAI39	Kaduna North		packaged water	TE,NA,SXT	Stx2, hlyA	-
279	*E. coli* IAI39	Kaduna North		well water	TE,NA,SXT,AMP.AML	Stx2, hlyA	Sul1, bla_{TEM}

Key: A.R= Antibiotic Resistance; - = no gene detected

Table 4.35: Source of *Salmonella enterica* isolates that possessed virulence and resistance genes.

Isolates/no	Identity	Location	Source	Antibiotic resistance pattern	Virulence genes detected	Antibiotic resistance genes detected
108	*Salmonella enterica* ser Typhimurium	Sabon-gari	Well water	TE	invA	TetA,
115	*Salmonella enterica* ser Typhimurium	Jema'a	Borehole	TE	invA	-
117	*Salmonella enterica* ser Typhi	Jema'a	Well water	TE	invA	Sul1, TetA,
118	*Salmonella enterica* ser Typhimurium	Jema'a	Tap water	TE,NA,SXT	invA	Sul1, TetA,
190	*Salmonella enterica* ser Typhi	Ikara	Well water	Susceptible to all antibiotics tested	invA	-
200	*Salmonella enterica* ser Typhi	Ikara	Well water	Susceptible to all antibiotics tested	invA	-

Key: - : Antibiotic resistance gene not detected

Table 4.36 shows the identity of the sequenced isolates after BLAST on the NCBI website. The various *Salmonella enterica* and *E. coli* isolates 16S rRNA sequences were subjected to BLAST on the National Center for Biotechnology Information (NCBI) website and the closest relatives are shown in the table, together with their accession numbers. Their percentage identity scores range from 89 – 98%. The expected values (E-values) for all the sequences that were subjected to BLAST was 0.00. The sequences of the isolates obtained in this study have been deposited in the NCBI GenBank, and they have been assigned accession numbers which are shown in table 4.37.

Figure 4.6 is an Unweighted Pair Group Method with Arithmetic mean (UPGMA) tree showing evolutionary relationship between 16S rRNA sequences of *E. coli* strains isolated from water samples in Kaduna state, while figure 4.7 is a maximum parsimony tree showing evolutionary relationship between 16S rRNA sequences of the *Salmonella enterica* strains.The trees were rooted with *Geitlerinema* spp, a genus from the *Cyanobacteria* group. The sequences of reference strains obtained from the Genbank database are indicated in boldtype with their respective accession numbers in parantheses. The scale bars represent the nucleotide differences between the species; bootstrap tests were carried out for 500 replicates and the values at the nodes indicate the bootstrap value in percent.

Table 4.36: Identification of sequenced organisms using BLAST

Isolates/no	Identification	Identity score (%)	E-value	NCBI accension no.
31	*Escherichia coli* O104:H4	98	0.00	NC_018658.1
185	*Escherichia coli* IAI39	97	0.00	NC_011750.1
188	*Escherichia coli* O157:H7	96	0.00	NC_002695.1
196	*Escherichia coli* O157:H7	97	0.00	NC_002695.1
198	*Escherichia coli* O157:H7	98	0.00	NC_002695.1
207	*Escherichia coli* O104:H4	97	0.00	NC_018658.1
235	*Escherichia coli* IAI39	97	0.00	NC_011750.1
236	*Escherichia coli* O157:H7	98	0.00	NC_011750.1
239	*Escherichia coli* O157:H7	98	0.00	NC_002695.1
262	*Escherichia coli* O157:H7	96	0.00	NC_002695.1
267	*Escherichia coli* IAI39	94	0.00	NC_011750.1
269	*Escherichia coli* IAI39	96	0.00	NC_011750.1
279	*Escherichia coli* IAI39	96	0.00	NC_011750.1
108	*Salmonella enterica* subsp *enterica* ser Typhimurium	97	0.00	NC_003197.1
115	*Salmonella enterica* subsp *enterica* ser Typhimurium	97	0.00	NC_003197.1
117	*Salmonella enterica* subsp *enterica* ser Typhi	89	0.00	NC_003198.1
118	*Salmonella enterica* subsp *enterica* ser Typhimurium	95	0.00	NC_003197.1
190	*Salmonella enterica* subsp *enterica* ser Typhi	98	0.00	NC_003198.1
200	*Salmonella enterica* subsp *enterica* ser Typhi	97	0.00	NC_003198.1

Table 4.37: Genbank accession numbers assigned to *E. coli* and *Salmonella enterica* 16S rRNA sequences from this study

Isolate s/no	Identification	Genbank accession numbers
31	*Escherichia coli* O104:H4	KX_602657
185	*Escherichia coli* IAI39	KX_602656
188	*Escherichia coli* O157:H7	KX_602655
196	*Escherichia coli* O157:H7	KX_602654
198	*Escherichia coli* O157:H7	KX_602653
207	*Escherichia coli* O104:H4	KX_602652
235	*Escherichia coli* IAI39	KX_602651
236	*Escherichia coli* O157:H7	KX_602650
239	*Escherichia coli* O157:H7	KX_602649
262	*Escherichia coli* O157:H7	KX_602648
267	*Escherichia coli* IAI39	KX_602647
269	*Escherichia coli* IAI39	KX_602646
279	*Escherichia coli* IAI39	KX_602645
115	*Salmonella enterica* subsp *enterica* ser Typhimurium	KT_737738
108	*Salmonella enterica* subsp *enterica* ser Typhimurium	KU_255189
117	*Salmonella enterica* subsp *enterica* ser Typhi	KT_737737
118	*Salmonella enterica* subsp *enterica* ser Typhimurium	KT_737736
190	*Salmonella enterica* subsp *enterica* ser Typhi	KU_255190
200	*Salmonella enterica subsp enterica ser Typhi*	KU_255191

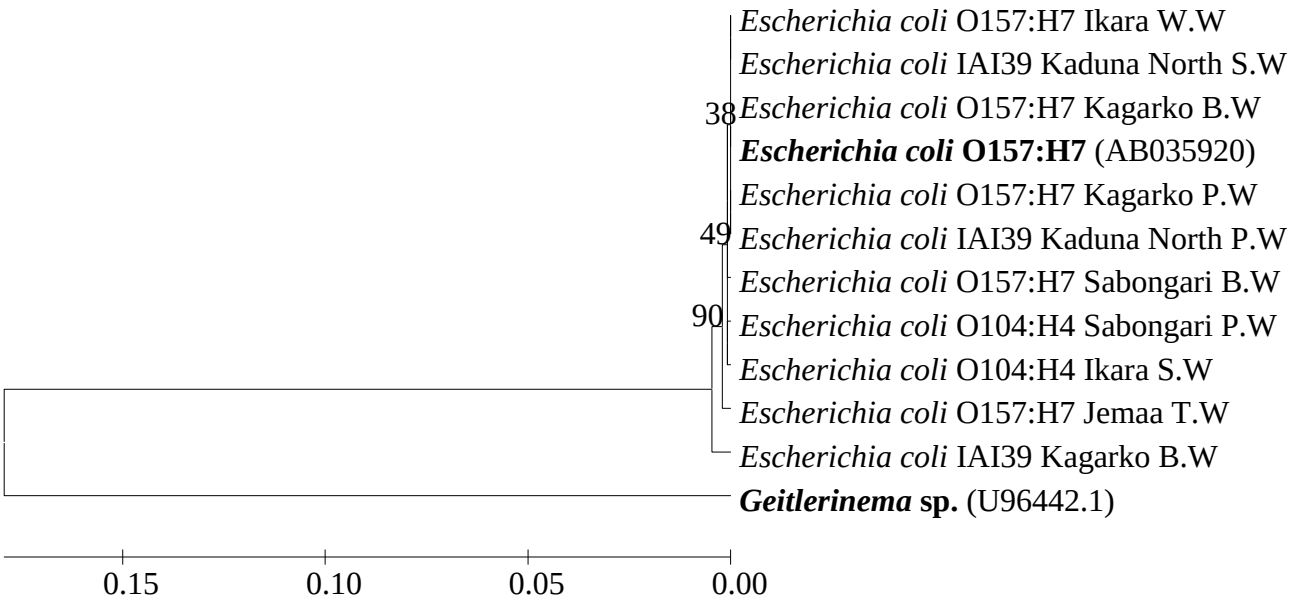


Fig. 4.6: Phylogenetic tree showing relationship between 16S rRNA sequences of *E. coli* isolates

KEY:

W.W: well water; S.W: stream water; B.W: borehole water; P.W: packaged water; T.W: tap water

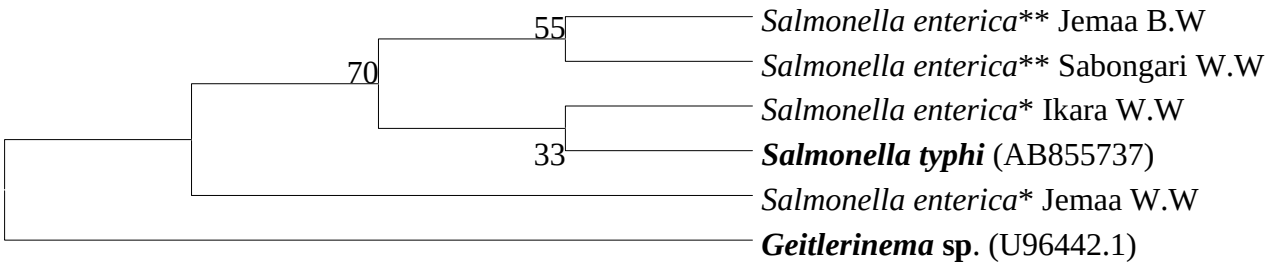


Fig. 4.7: Phylogenetic tree showing relationship between 16S rRNA sequences of *Salmonella enterica* isolates

KEY:W.W: well water; B.W: borehole water

* *Salmonella enterica* subsp *enterica* ser Typhi; ** *Salmonella enterica* subsp *enterica* ser Typhimurium

CHAPTER FIVE

DISCUSSION

5.1 Physicochemical Quality of Water Samples

5.1.1 Nitrate

The nitrate content of the tap samples from Sabon-gari LGA was significantly lower than other sample types while the nitrate content of well water samples was the highest in the area. This trend followed, except in Kaduna North LGA where tap water samples had the highest nitrate content. Furthermore, the tap water samples from Kaduna North LGA had the highest nitrate content as compared to tap water samples from other locations. This suggests that the water treatment in the plant that supplies water to this LGA is probably inadequate. The higher nitrate content in the samples collected in the wet season could be attributed to the use of chemical fertilizers in farming (which is more frequent in the wet season), indiscriminate animal grazing, and improper disposal of animal and human wastes.

This agrees with a study carried out in Benue State which showed that the nitrate level was higher in the wells sampled in the wet season than in the dry season (Ocheri *et al*., 2010). The water source with the highest nitrate content overall was stream water while the lowest was borehole. This conforms to findings in literature that nitrate concentrations in groundwater decrease with increasing depth. The higher nitrate content in packaged water, wells and borehole samples from Kajuru LGA also could be as a result of agricultural practices in that area and pollution of the water sources. Also it indicates that the packaged water samples were not properly treated before they were packaged for sales. The values

observed in this study were far higher than those observed in drinking water in Ilorin, Kwara State and lower than that observed in Makurdi, Benue state (Ocheri *et* al., 2010; Akinwumi *et al*., 2012)

Nitrate is the most highly oxidized form of nitrogen and the major sources of nitrates in drinking water are runoff from fertilizer use; leakage from septic tanks, sewage; and erosion of natural deposits (EPA, 2014). Babies who are less than six months of age that consume water with high nitrate levels could become very ill, and if they are not treated, they may die. Symptoms include shortness of breath and blue baby syndrome (EPA, 2014). Drinking water contaminated with nitrates is also a risk factor for developing many cancers (Njeze *et al.,* 2014). The following treatment methods have proven to be effective for removing nitrate to below 10 mg/l: ion exchange, reverse osmosis and electrodialysis (EPA, 2014).

The WHO set the maximum contaminant level of nitrate in drinking water as 10mg/l. Going by this standard, an alarmingly low percentage of all sample types (except wells) had nitrate contents beyond permissible limits. In a similar study carried out in Ile-ife, Osun State, it was observed that ground water had very high content of nitrate (above 45mg/l) in most of the ground water samples, but low in the surface waters and pipe-borne water (Oluyemi et al., 2010). Regulation of drinking-water quality is so important that the United States Congress passed the safe drinking water act in 1974. This law requires the environmental protection agency (EPA) to set the safe levels of contaminants in water, at which no adverse health effects are likely to occur (Alhassan and Ujoh, 2012; Njeze *et al*.,

2014). As a result of that law, the regulation for nitrate became effective in 1992 when the EPA and WHO set the maximum contaminant level (MCL) for nitrate as 10 mg/l.

The possible health risks and exposure over a lifetime with an adequate margin of safety were considered in deciding this maximum acceptable contaminant level. Surprisingly, the current national standard for drinking water in Nigeria, allows a maximum contaminant level of 50 mg/l for nitrate (NSDWQ, 2007). This should be considered too high and therefore water that has nitrate values above 10mg/l should be considered unsafe for ingestion because current water standard for nitrate is based on levels considered low enough to protect infants from methaemoglobinemia.

In this light, the water sampled from various sources had a low percentage of compliance (7.7-37.5%) to the WHO standard for nitrate in drinking water. Of particular worry, is the nitrate content of tap and packaged water samples which are supposed to have undergone adequate treatment.

5.1.2 pH

The higher pH values observed in the wet season agrees with some results of the study carried out by Makwe and Chup (2013). Furthermore, almost all the samples (with the exception of one well in Ikara LGA which had a higher pH) had their pH within the acceptable range specified by the WHO and NIS (6.5 - 8.5). This was the trend for the samples from all locations and across all sample types. The pH values, which give the indication of acidity and alkalinity of the waters show that the water samples are safe for agricultural, recreational and domestic uses. This result agrees with other studies done in

other parts of Nigeria, where the pH of water samples were also within acceptable limits (Esemikose and Akoji, 2014; Oluyemi *et al*., 2010 and Oluyege *et al*., 2014).

In general, water with a low pH (< 6.5) could be acidic, soft, and corrosive. Therefore, the water could leach metal ions such as iron, manganese, copper, lead, and zinc from the aquifer, plumbing fixtures, and piping (EPA, 2014). Drinking water with a pH level above 8.5 indicates that high levels of alkaline minerals are present. High alkalinity does not pose a health risk, but can cause aesthetic problems, such as an alkali taste to the water, scale build-up in plumbing, and lowered efficiency of electric water heaters (EPA, 2014).

5.1.3 Dissolved oxygen (DO)

The values of DO from all locations ranged 0.3- 2.9 mg/l. The DO of the samples collected in the dry season was higher than those of the wet season. This may be because DO varies with water temperature and altitude. Cold water holds more oxygen than warm water (EPA, 2014) and the temperature of water would naturally be cooler in the dry season than in the wet season.

Tap water had the highest mean DO content in Sabon-gari and Ikara. This agreed with the study carried out by Oluyemi *et al.* (2010) where tap water had a higher DO content as compared to the other water sources sampled. This may be due to aeration process during water treatment. The DO of the borehole samples and packaged water from Jema'a LGA were higher than that of other sources. It is a possibility that the packaged water companies in the area make use of borehole water samples, hence the similarity in the values. Dissolved oxygen is important parameter in water quality assessment and biological

processes prevailing in the water. The DO values indicate the degree of pollution in the water bodies (Nirmala *et al*., 2012).

5.1.4 Biological oxygen demand (BOD)

The BOD of stream samples from Jema'a LGA were significantly higher than those of other locations which indicate that the strams sampled in Jema'a were more polluted than those of other locations ($P<0.05$). The mean value of samples collected in the dry season was higher than those collected in the wet season, though the difference was not statistically significant. All the samples had their BOD within the specified limit set by the WHO (6mg/l). This agrees with the findings of Oluyemi *et al.* (2010) and suggests that the water from the areas sampled is less polluted by organic matter and they could support aquatic life. The BOD could be affected by various parameters such as temperature, pH, the presence of certain kinds of microorganisms, and the type of organic and inorganic material in the water.

5.1.5 Electrical conductivity (EC)

The higher mean value in borehole samples is most likely due to the presence of soluble minerals from the bedrock of the soils which is more pronounced in the wet season. (Oluyemi *et al.,*2010). The tap water samples in Jema'a LGA had significantly higher EC values than those of other sources in the area ($P<0.05$). This indicates that the treatment was not adequate enough or there was some contamination along the distribution pipes.The electrical conductivity of water is directly related to the concentration of dissolved solids in the water. Ions from the dissolved solids in water influence its ability to conduct an electrical current, (EPA, 2014).

The values observed in this study agree with that of other researchers (Oluyemi *et al.*, 2010; Ibrahim and Ajibade, 2012; Abakpa *et* al., 2013; and Oluyege *et al.,* 2014). Thirty (60%) of the samples tested met the WHO standard, while 48(96%) met the NIS standard. This indicates that the water should be treated before being consumed.

5.1.6 Total dissolved solids (TDS)

The TDS values of all samples tested ranged from 27.85 - 660mg/l. The mean values by sample sources showed that packaged and tap water samples from most of the locations had the lowest mean value (52.84mg/l) probably because of the sedimentation and filteration processes that it had undergone. The TDS of tap water samples from Jema'a LGA was significantly higher than those of other sources in the area, just like the value of electrical conductivity. This is not surprising because the alue of TDS is directly related to the values of conductivity. The mean TDS value of the wet season samples was higher than that of the dry season. This is probably due to the run-offs associated with the rains.

This result is in agreement with that of Oluyemi *et al*. (2010) and Ibrahim and Ajibade (2012). Only one of the boreholes sampled in Ikara had a TDS value that exceeded the maximum limit, some treatments such as addition of coagulants may be required to make this water suitable for domestic purposes. High concentrations of total dissolved solids (TDS) may cause adverse taste effects. Highly mineralized water may also deteriorate domestic plumbing and appliances. It is recommended that waters containing more than 500 mg/l of dissolved solids not be used if other less mineralized supplies are available

(WHO, 2012). Water containing more than 500mg per litre of TDS causes gastrointestinal irritation. High value of TDS influences the taste, hardness and corrosive property of the water (Nirmala *et al*., 2012).

5.1.7 Iron

The mean value of samples collected in the wet season was higher than the mean of those collected in the dry season but it was not statistically significant (P> 0.05). This probably may due to influence of rainfall infiltrating and dissolving iron mineral in rocks and soil which are leached into groundwater sources (Ocheri, 2010).

The percentage of all the sample sources that had their values within the specified NIS limit of 0.3mg/l ranged from 37.5-66.5% of the samples tested. This agrees with the study carried out by Ibrahim and Ajibade (2012). This means a greater percentage of samples had iron contents higher than the permissible limits which may pose some health complications if the concentration rises above these levels in the long run. This is because heavy elements are known to be non-biodegradable and bioaccumulate progressively in aquatic organisms and human cells when there is prolonged exposure. Also, the water samples may have taste and aesthetic problem.

The presence of heavy elements in the water sources can be explained by pluvial processes which washed man-made debris that contained these metals into the water sources during wet season as well as release of some elements from geologic processes (Butu and Bichi, 2013).

5.1.8 Chromium

The high content of chromium in a few of the samples could be due to wide range use of chromium in metal plating, pipes or from the wastes that get to the surface and ground water through run-offs. The content in packaged water from Sabon-gari LGA was significantly higher than the packaged water from all other locations ($P<0.05$), the metal was however not detected in stream water samples from Kaduna North LGA. Chromium is a trace element that occurs as an abundant element in the earth crust, its compounds are found in the environment due to erosion of chromium- containing rocks and from other man-made sources. People who use water containing total chromium in excess of the maximum contaminant level over many years could experience allergic dermatitis. Furthermore, some forms of chromium are believed to be carcinogenic when ingested (EPA, 2014).

This finding was in contrast to the study carried out by Oluyemi *et al.* (2010) where only 5% of the drinking water samples tested had their chromium content within acceptable limits. It however agrees with the findings of Butu and Bichi (2013).

5.1.9 Lead

The mean lead content of the samples collected during the wet season was significantly higher than that of the dry season ($P\leq 0.05$), probably because of the pluvial processes that occur more in the wet season. The element was not detected in stream samples of Sabon-gari LGA and in packaged water in Kaduna North, Ikara and Kajuru LGAs. Between 50-69.2% of the samples tested had their lead content within permissible limits as stipulated by

the WHO and NIS (0.01mg/l). This finding is in contrast to that of Oluyemi *et al.* (2010) but similar to findings obtained by Agbaire and Obi (2012). The higher amounts of lead in well water samples puts consumers of the water at great risk of lead poisoning which has been reported in recent times in neighbouring Niger and Zamfara States (Abdu and Yusuf, 2013). Lead contamination of water has also been reported in dam water in Zaria (Butu and Bichi, 2013), river water in Kaduna State (Egbe and Ahunaya, 2016) and other parts of Nigeria such as Enugu (Ogbu and Onyekwere, 2005), Lagos (Ehi-Eronmonsele and Okiei, 2012), and Abakaliki (Ignatius *et al*., 2012), among others.

Lead in drinking water can result in a variety of adverse health effects. In babies and children, exposure to lead in drinking water above the permissible level can result in delays in physical and mental development, along with slight deficits in attention span and learning abilities. Adults who drink this water over many years could develop kidney problems and high blood pressure. Lead enters tap water through corrosion of plumbing materials (EPA, 2014). Generally, borehole and packaged water samples in this study had better physicochemical quality as compared to other water sources.

5.2 Bacteriological Quality of Water Samples

5.2.1 Isolation of coliforms

A host of coliform bacteria were isolated from the water samples during this study. They include *Acinetobacter baumanii* (1.12%), *Citrobacter freundii* (6.74%), *Enterobacter gergoviae* (5.06%), *Escherichia coli* O157 (3.37%), *Klebsiella pneumoniae* (2.24%), *Proteus mirabilis* (10.67%), *Salmonella enterica* (3.37%) and many others. A total of 178

isolates were obtained and characterized biochemically. Ideally, bacteria should not be isolated from potable water, but it is not surprising that these bacteria were isolated because the water samples were suitable for support microbial growth based on their physicochemical quality. It is however of great concern that water that has been treated (tap and packaged water) contains bacteria. This result is similar to that gotten by Oyedeji *et al.* (2011), where it was observed that several sachet drinking water manufacturers in Nigeria utilised well water as their source of water with little or no prior treatment.

A previous survey of the wells in some parts of the study area showed that many of them had defective or no casings, no well covers, and were located very close to latrines and septic tanks. Also, animals such as sheep, dogs, goats and so on roam freely around water sources (Olukosi *et al.*, 2008). Besner *et al.* (2002) observed that wells could have contaminant seepage through defective well cap-seals, holes in the well casing and other defects. The indiscriminate use of the buckets used for drawing water out of the well for other purposes could also be a potential source of contamination as these may have had contact with human faecal matter. Rain water can also pick harmful bacteria and other pollutants on the land surface. If this water pools near the wells and seeps through, it could pose potential health problems (Besner *et al*., 2002).

5.2.2 Isolation of target organisms

Escherichia coli was isolated from water samples of all the six locations with samples from Kaduna North having the highest isolation rate (30.8%). The isolates were obtained in all water sources. On the other hand, *Salmonella enterica* was isolated from all locations

except Kajuru LGA. However, the organism was not detected in any packaged water samples, probably because it is very sensitive to the treatments that the packaged water is believed to have undergone. The isolation frequency observed shows that hygiene is generally poor in all the parts of Kaduna state that were sampled.

From all the 500 samples collected, *E. coli* was isolated from 73(14.6%) while *Salmonella enterica* was isolated from 12(2.4%) of them. Six isolates of *E. coli* O157:H7 were obtained. The presence of these organisms is an indicator of possible faecal contamination of the water sources and the likely presence of other harmful organisms like *Vibrio cholerae,* Rotavirus, *Cryptosporidium spp* and so on (Halablab *et al.*, 2010). The presence of faecal coliform is an index of the bacteriological quality of water (Chigor *et al*., 2012). These organisms have also been isolated by other researchers who worked with drinking water in other parts of the country (Edema *et al.,* 2011; Ibiene *et al.,* 2012; Lateef *et al.,* 2012; Egbe *et al*., 2013; Tula and Osaretin, 2014; Oluyege *et al.,* 2014; Aboh *et al.,* 2015).

The isolation of these organisms from water sources is of public health concern as it shows that there is a potential risk to consumers especially those who drink the water without any further treatment. The isolation of *E. coli* O157:H7 from the environment is significant given its low infectious dose. A survey of Kaduna state showed that there is improper sewage disposal in most of the parts, indiscriminate defeacation and improper siting of wells and boreholes close to latrines and soak-aways (Olukosi *et al.*, 2008).

In a similar study done in Ile-Ife, Osun state, all the studied wells were observed to be prone to encroachment by roaming domestic animals which litter the well environment with

their droppings thereby reducing the hygienic conditions of the wells. Well water should therefore be subjected to adequate disinfection treatments and buckets used for drawing water must be kept clean and retained specifically for the purpose. Wells should not be sited close to pit latrines while drainages around wells should be sanitized frequently (Oyedeji *et al.*, 2011).

It was observed that the mean total coliform and faecal coliform counts were higher in the wet season than the dry season. This could be due to the high amount of these coliform during the wet season as the water availability and run-offs during rainfall favours the movement and reproduction of the organisms. This finding agrees with that from a study carried out by Makwe and Chup (2013). Also Chigor *et al.* (2012) reported that there could be a continuous faecal pollution from point sources; domestic drain, waste from abattoirs, sewage and mechanic workshop both in the dry and wet seasons, hence the high isolation rate of *Salmonella enterica* in the dry season. This supports the findings of Kistemann *et al*. (2002) who observed that substantial shares of the total microbial loads in water courses and drinking water reservoirs in Germany resulted from rainfall and runoff events.

The total and faecal coliform counts from all locations were high but the counts of samples collected from Ikara LGA were higher than the rest though it was not statistically significant. This may be due to the fact that it's a rural area and hygiene level is expected to be lower. Furthermore, pipe-borne water not readily available there at the time of sampling, so the populace rely on wells and boreholes for their water supply.

The mean counts for total and faecal coliforms for stream water samples were statistically significantly higher than the mean counts of other water sources. This is not surprising as streams are more exposed to sources of contamination such as runoffs during rainfall and livestock and other animals could also drink from the streams, contaminating them in the process. The high counts observed for packaged water agrees with the findings of Oyedeji *et al*. (2010) and Tagoe *et al*. (2011). It could be attributed to the fact that many manufacturers seal raw water from wells or boreholes with little or no treatment and also, many companies do not carry out routine cleaning of their equipment which could end up being a source of contamination of their products.

The presence of faecal coliforms and *E. coli* in sachet water indicates faecal contamination. Sachet water is considered by most people as being cleaner compared to ordinary tap water. Unfortunately, it was observed in this study that most of the sachet water samples were not fit for consumption. This is in conformity to works by Obiri-Danso *et al*.(2003); Dodoo *et al*. (2006); Oyedeji *et al*. (2010) and Akinyemi *et al*.(2011). The faecal contamination of the sachet water may be due to the fact that the water may have been sourced from shallow and contaminated boreholes or wells and may not be properly treated before sealing.

Although documented evidence is scarce, improper storage, poor handling of sachet water by the vendors and back-seepage into bags that are not well sealed are some of the identified possible sources of contamination. This finding agrees with a report that the

quality of sachet water, despite having the mandatory NAFDAC certification number, can be compromised significantly due to handling as it moves from the manufacturer to the consumer, and must not be assumed to be generally safe for drinking (Dada, 2009). In addition to this, *Salmonella enterica* are capable of adhering to surfaces, hereby forming biofilms, a major factor responsible for the survival of *Salmonella* in drinking water (Holt and Chaubal, 1997).

High demand for packaged water has led to springing up of small scale entrepreneurs who engage in production of packaged waters without due regard to hygienic practices in the production processes. The implication of this is a lack of guarantee that the products will meet set standards for drinking water quality (Tagoe *et al*., 2011). The contamination of tap water could be attributable to leakage in pipes and inadequate treatment. Six bottled water samples were analysed and majority of them had their faecal and total coliform counts conforming to the WHO standard. This shows that bottled water could be a better though more expensive alternative to sachet water. The absence of faecal indicator bacteria in all brands of bottled drinking water that were sampled could be attributed to better hygienic practices observed in the industry compared to the sachet water producing industry. These include use of protective sealed caps on bottles, improved and hygienic filling system and use of non-returnable plastic containers (Oyedeji *et al*., 2011).

It was observed that a higher percentage of all sample types did not meet the WHO requirement for total coliforms in drinking water which is zero/100ml. These results conform to the study carried out by various researchers (Dodoo *et al.*, 2006; Tagoe *et al*.,

2011; Oyedeji *et al*., 2011). Inadequate sanitation and unhygienic practices account for the major source of microbial contamination of any potable water (Sahota, 2005). This results in microbial contamination of the emerging product.

5.3 Antibiotic Susceptibility Pattern of the Isolates

For the *E. coli* isolates, 83% were susceptible to Amoxycillin-clavulanic acid, 97.6% were susceptile to Chloramphenicol and Gentamicin, 92.7% to Cefotaxime and 65.9% to Sulphamethoxazole-trimethoprim. The highest resistance observed was to Tetracycline (56.1%), while the least resistance was to Gentamicin and chloramphenicol (2.4%). For the *Salmonella enterica* isolates, all 9 of them were susceptible to Amoxycillin, Amoxycillin-clavulanic acid, Ampicillin and Gentamicin. Furthermore, 88.9% of them were susceptible to Chloramphenicol, Cefotaxime and Sulphamethoxazole-trimethoprim. The highest resistance observed was to Tetracycline (77.8%). The high resistance rate to tetracycline could be explained due to that antimicrobials are used in food animals to treat or prevent disease and also to promote growth (Lapierre *et al.*, 2006), while the low resistance to gentamicin could be due to the fact that it is administered parenterally and therefore cannot be easily taken without the aidd of a skilled medical personnel.

The extremely low toxicity of the antimicrobial agents in the tetracycline, sulfonamide and β-lactam classes has resulted in their overuse in the medical world, hence the observed increased resistance. The relatively high level of resistance to antimicrobial agents in the environment depicts a true reflection of their indiscriminate and excessive usage (Titilawo, 2015). Overall, when the two organisms were combined, it was observed that the highest

susceptibility was to Gentamicin (98%), while the least susceptibility was to Ciprofloxacin (26%). Ten(10) of the isolates were resistant to 1(one) antibiotic, six were resistant to 2 antibiotics, 10 were resistant to 3 antibiotics, 5 were resistant to 4 antibiotics, 4 were resistant to 5 antibiotics, 1 was resistant to 6 antiiotics and 3 were resistant to 7 antibiotics. Twenty-eight percent of the multidrug resistant *E. coli* were resistant to 3 antibiotics while 4% were resistant to 6 antibiotics. 12% were resistant to 7 antibiotics. For *Salmonella enterica,* 66.7% of the multidrug resistant isolates were resistant to 3 antibiotics while 33.3% of them were resistant to 2 antibiotics.

The highest number of MDR isolates was isolated from borehole samples (8). Seven were isolated from well water samples, 6 from packaged water samples, 5 from tap water and one from stream water. Most of the resistant isolates showed the TeNaAmpAml resistance pattern. These antibiotics belong to the tetracycline, quinolone and Penicillin families. Six were resistant to ciprofloxacin which is a fluoroquinolone. This result slightly differed from that obtained by Oyedeji *et al*. (2011) where none of the isolates from water was resistant to ciprofloxacin. Also, the resistances that were observed in that study were more from stream water. However, they also reported high level of resistance to ampicillin, tetracycline. Similar results were also reported by some researchers (Patoli *et al.,* 2010; Nimri and Azaizeh, 2012; Tula and Osaretin, 2014).

The results show that the isolates showed 12 resistance patterns, and are resistant to 3-7 antibiotics. The most common resistance patterns are: TeAmpAml and TeNaSxtAmpAml with four different isolates each. The high level of resistance is an evidence of abuse and overuse of antibiotics in the environment and in the hospitals (Okeke *et al*., 2007). This

could be worsened by the over the counter sale of antibiotics, inappropriate prescription by clinicians and poor regulations on the retail and purchase of antibiotics (Tula and Osaretin, 2014). This could also be attributed to the fact that it has been discovered that in sub-saharan Africa, the health practitioners prescribe ampicillin, chloramphenicol, erythromycin, gentamicin, penicillin, tetracycline and trimethoprim-sulphathoxazole as the first-line drugs in treating infections. The available second line antibiotics vary with locality but will often include amikacin, amoxicillin-clavulanic acid, cefuroxime, ciprofloxacin and nalidixic acid (Hart and Kariuki, 1998; Fasehun,1999).

When people use sub-therapeutic doses of antibiotics, as obtains when the dosage for the drugs are not strictly adhered to, highly resistant strains of the organisms are selected sequentially. Poor quality drugs can provide sub-inhibitory selective pressure, of which neither the patient nor the prescriber are aware. There have been numerous reports of sub-standard drugs in Nigeria. These reports describe drug preparations containing between 0-80% of the stated label claim. Some of them contain such low concentrations that they can only be considered counterfeit, meaning that they were deliberately manufactured with low or no active drug content. Others may have complied with pharmacopoeia standards at some time but have, in the course of distribution and display, been degraded by heat and humidity (Okeke and Sosa, 2003).

The isolation of antibiotic resistant bacteria in drinking water samples is of public health concern because of the possibility of transferring multiple antibiotic resistances to normal

flora in the intestinal tract leading to more complicated antibiotic resistance. They may also act as reservoirs which will contribute to the maintenance and spread of antibiotic resistance genes (Guardabassi *et al*., 2004; Constanzo *et al*., 2005). Many isolates in this study showed resistance patterns indicative of acquired resistance, such as multiple resistances to antibiotics in different groups and resistance patterns indicative of extended spectrum beta lactamase production. The high rate of resistance to ampicillin found in the isolates is consistent with the work of Oyedeji *et al.* (2011). According to Sayah *et al*. (2005), the source of water contamination has a significant role to play in determining the extent of antimicrobial resistance as contaminating bacteria could come from domestic, wild animals or human sewage. Antibiotic resistance poses a threat to everyone most especially the children, elderly and the immunocompromised who are more vulnerable to bacterial illnesses. For the general public as a whole antibiotic resistance reduces the number of effective drugs available for treatment of the sick. Therefore, treatment may only be achieved through the use of new but costly antibiotics (Tula and Osaretin, 2014).

Data relating to *Salmonella enterica* prevalence in surface and drinking water in developing countries are quite rare. Nevertheless, data on water- borne outbreaks as well as case control studies investigating the risk factors for endemic typhoid fever confirmed the relevance of water as source for the transmission of this disease (Abakpa *et al.*, 2013). Most commonly used antibiotics for the treatment of typhoid fever are fluoroquinolone such as ciprofloxacin, ofloxacin and pefloxacin and third generation cephalosporins such as ceftriaxone. Some variants of *Salmonella* have developed multidrug resistance as an integral part of their genome, hence are likely to retain their drug-resistant genes even when

antimicrobial drugs are no longer used, making the pathogen more virulent and potent (Marathe *et al.*,2012).

There is therefore the need to control faecal pollution of water supplies to avert the occurrence of waterborne diseases outbreak. Through effective public health education by relevant Government agencies, the people should be educated on the implications associated with the consumption of contaminated water for drinking and other domestic purposes. Public health education aimed at improving personal, household and community hygiene is imperative. Furthermore, there is a need to educate people regarding the irrational use of antibiotics and safe disposal of antibiotic containing waste (Patoli *et al.*, 2010).

5.4 PCR Targeting Virulence Genes in *E. coli* and *Salmonella enterica* Isolates

The Shiga toxin type 1 (Stx1) gene was not detected in any of the *E. coli* isolates while the Shiga toxin type 2 (Stx2) gene was detected in 8 of the *E. coli* isolates. This corroborates the results of a study carried out in the USA, where the Stx1 gene was not detected in any strains tested while 93.1% of the isolates were found to carry the Stx2 gene (Shekarforoush *et al*., 2008). The Stx1 and Stx2 genes code for the production of the shiga toxin which is a virulence factor. Shiga toxin is considered to play a prominent role in the pathogenesis of STEC (Shiga toxigenic *Escherichia coli*) and is presently classified into two broad types, *stx1* and *stx2*, which have similar structure in their molecular structure and enzymatic activity but are antigenically distinct.

Stx1 is highly homologous to Stx of *Shigella dysenteriae* type 1 and highly conserved, although, two sequence variants have been reported (Burk *et al*., 2003). Stx2 shares approximately 56% amino acid homology with Stx1 but is relatively more heterogeneous. Several related variants of *stx2* have been described. They include Stx2c, Stx2d, Stx2e, Stx2f and Stx2g, moreover, Stx2c can be classified into two groups: Stx2vha, and Stx2vhb (Beutin *et al*., 2008; Prager *et al*., 2009). These other subtypes of Stx1 and Stx2 were not assayed for.

The pathogenesis of STEC O157:H7 infection in humans depends on many bacterial virulence factors including Stx1, Stx2, enterohemorrhagic *E. coli* (EHEC) - haemolysin, and intimin among others (Wang *et al*., 2008). But the most important factor is Stx2 (Kawano *et al*., 2008). In human, Shiga toxins are the major virulence factors of STEC responsible for haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS). The association of STEC with HC and HUS implies that Stx2 is more closely to these diseases than stx1 (Siegler *et al*., 2003). Within the human disease-associated strains, those producing Stx2 appear to be more commonly responsible for serious complications such as HUS than those producing only Stx1. Previous studies have shown that stx1 genotype is one of the important factors of clinical outcome of STEC O157 infection and that pathogenicity for humans was higher in the stx2 genotype strains (Kargar and Homayoon, 2015).

Some types of assays may have problem due to instability of phages carrying Stx genes. Karch *et al.,* (1999) tested Stx genes stability in forty-five (45) Stx positive strains immediately after isolation. After passage on trypticase soy agar or other routine culture

media, 15 of 45 strains lost one of the Stx1 or Stx2 genes. Loss of Stx genes in serial cultures is seen after long term shedding of the organism (Mazaheri *et al*., 2005). Therefore, it is also possible that the Stx1 gene was lost during culturing of the isolates. Some studies have revealed that strains possessing only stx_2 are potentially more virulent than strains harbouring stx_1 even strains carrying both stx_1 and stx_2 .

Stx2 has been found to be approximately 400 times more toxic (as quantified by LD50 in mice) than Stx1 (Tahamtan *et al.*, 2010). *E. coli* O157:H7 strains without virulence factors have been also isolated from faeces and beef carcasses, but isolates not possessing these major virulence factors are not considered to be pathogenic (Barkocy-Gallagher *et al*., 2004).

The intimin gene (eaeA) was observed in just one *E. coli* O157:H7 isolate. This gene is responsible for the intimate attachment of STEC to epithelial cells of the intestine, thereby causing attaching and effacing lesions in the intestinal mucosa. It is also responsible for initiation of host signal transduction pathways. It is a 94-kDa outer membrane protein, which is encoded by the eae gene on a ca. 34 kb chromosomal pathogenicity island termed the locus of enterocyte effacement (LEE). However, it has been observed from other studies that many human STEC isolates including those from patients with haemolytic uraemic syndrome and haemorrhagic colitis did not contain eaeA. This is an indication that this gene might not be essential for human infection (Blanco *et al.*, 2006).

The enterohemolysin gene (hlyA) was detected in all 15 of the *E. coli* isolates that were tested. The enterohemolysin, also called enterohemorrhagic E. coli haemolysin (EHEC-

HlyA), encoded by the hly gene is another virulence factor of *E. coli*. Enterohemolysin production (hlyA gene product) might have a direct association with the capacity of a given STEC strain to result ins serious disease (Paton & Paton 1998b). The hlyA gene is not confined to serogroup O157 (Paton and Paton, 1998b), but it has been suspected to have a role in pathogenicity, because it occurs in the majority of the pathogenic STEC strains tested and was reactive to sera of patients with HUS (Pradel *et al*., 2001; Grauke *et al*., 2002).

The invA gene was detected in all six of the *Salmonella enterica* isolates that were tested. On the contrary, none of them showed the presence of the spvA gene. The 100% detection rate of *inv* gene by PCR suggests that the gene is conserved among *S. enterica* isolates (Murugkar *et al*., 2003). Furthermore, it is a predominant virulence gene necessary for the serovars of *S. enterica* to express virulence in the host (Das *et al*., 2012). This gene is essential for full virulence in *Salmonella enterica* (Mezal *et al.,* 2013). This gene has also been amplified in other studies involving *Salmonella* from food and water (Abakpa *et al.,* 2015; Smith *et al.,* 2015). This further confirms that the isolates obtained in this study are potentially pathogenic as amplification of invA gene is now recognized as an international standard procedure for the detection of *Salmonella* genus (Amini *et al*., 2010).

Another important virulence factor located on a plasmid previously shown to be common among predominant non-typhoidal serovars of *Salmonella enterica* is the spv operon which contains five genes (spvRABCD*)*. One main function of the *spv* operon is to potentiate the systemic spread of the pathogen (Gebreyes *et al*., 2009). None of the *Salmonella enterica* isolates in this study produced amplicons for the spvA gene. SpvA is one of the most

important virulence genes in the spv operon, but it is however not present in all *Salmonella* isolates.

5.5 PCR Targeting Antibiotic Resistance Genes in *E. coli* and *Salmonella* Isolates

The tetracycline resistance genes that were assayed for were tetA and tetB. The amplicons for the tetA gene were observed in two isolates each of the *Salmonella enterica* and *E. coli* isolates. Only two (2) isolates of *E. coli* produced amplicons for tetB gene. The tetracycline resistance is a common and developing aspect of resistance among bacteria and the tetracycline resistance genes (including tetA and tetB) have been detected from environmental samples (Titilawo *et al*., 2015).

Tetracycline and other antibiotics have been frequently used to treat diseases, but unfortunately their repeated use has resulted in the development of resistant strains. Tetracycline resistance in bacteria is mediated by four mechanisms: efflux, ribosomal protection, enzymatic inactivation, and target modification (Patterson *et al*., 2007). The tetB gene has been identified as the most prevalent tetracycline resistance determinant with a wide host range due to the fact that it resides on highly mobile genetic elements that are readily transferred between different genera of bacteria. The gene tetA on the other hand, is located on conjugative plasmids. In this study, tetA was observed from more isolates than tetB. This agrees with the findings of other workers who have reported the ability of tetA gene to freely spread in farm animals than tetB, and that this determinant

could indicate that tetA gene can be spread more easily in the environment than tetB gene (Koo and Woo, 2011; Skocková *et al*., 2012).

Tetracyclines are antibiotics which inhibit bacterial growth by interfering with protein synthesis. Three different mechanisms of tetracycline resistance have been identified so far: tetracycline efflux, ribosome protection, and tetracycline modification. The most common resistance mechanism in Gram-negative bacteria is the energy-dependent efflux pump system which is encoded by the genes tet*A*, tetB, tetC, tetD, and tet*G*, with tetA and tetB genes being the most frequently described (Olowe *et al*., 2013).

The sul1 gene was amplified in almost all the isolates (*Salmonella enterica* and *Escherichia coli*) that showed resistance to Sulfamethoxazole-trimethoprim. This gene has also been observed in other molecular studies on *Enterobacteriaceae* (Hendriksen *et al*., 2008; Dessie *et al*., 2013). Bacteria may acquire several genes for a metabolic pathway which ultimately produces a metabolite that no longer contains the binding site of an antimicrobial agent. Commonly, sulfonamide resistance in gram-negative bacteria generally arises from the acquisition of 2 genes, sul1 or sul2, encoding forms of dihydropteroate synthase that are not inhibited by the drug (Enne *et al*., 2001).

The blaTEM gene which codes for resistance to beta-lactams was observed in two *Salmonella enterica* isolates. Production of extended-spectrum β-lactamases (ESBLs) is one of the main mechanisms of resistance to broad-spectrum β-lactam antibiotics among

the *Enterobacteriaceae* (Bradford, 2001; Bush, 2008). Four main classes of β-lactamase enzymes have been reported in this group of microbes. Class A enzymes are derived from the older, broad broad-spectrum β-lactamases (e.g.,TEM-1, TEM-2, SHV-1) and have an extended substrate profile that permits hydrolysis of all cephalosporins, penicillins and aztreonam.

These enzymes are most commonly produced by *Klebsiella* spp and *Escherichia coli* but may be produced by other Gram-negative bacteria, including Enterobacter, *Salmonella, Proteus and Citrobacter* spp (Rayamajhi *et al.,* 2008). ESBLs are derivatives of simple lactamase (TEM or SHV) enzymes that are harboured mostly by Gram-negative bacilli (Satheesh *et al*., 2006). This gene has also been observed in *E. coli* isolated from river water in Osun state, Nigeria (Titilawo *et al.*, 2015).

Bacteria harboring resistance genes have a selective advantage over those that do not in the presence of the antibiotic in question. If a population of bacteria that comprises predominantly of susceptible strains, with a few resistant ones is exposed to an antibiotic, the sensitive strains are killed off. The resistant ones are left to flourish, thereby replacing the old population. This simple fact has been responsible for the success of resistant bacteria in a world where antibiotics are used for a broad range of purposes (Okeke and Sosa, 2003). The consequences of antimicrobial use are also caused by prophylactic treatment of animals to prevent infection or antimicrobials employed as growth promoters. Multi drug resistant (MDR) bacteria are troublesome because it is possible for a pathogen to be resistant to all of the antimicrobial compounds that are used to treat the infections it

causes (Alanis, 2005). The dramatic increase in antimicrobial resistance in different species of bacteria, particularly multidrug-resistance in *Salmonella enterica* and *Escherichia coli*, continue to emerge throughout the world because antimicrobials are extensively used for therapeutic and prophylactic purposes in animals and humans (Hsu *et al*., 2006).

The sources of *E. coli* isolates that possess virulence genes ranged from stream water, borehole water, tap water, well water and packaged water. This indicates that most of the drinking water sources used in the areas sampled contained organisms that are a threat to public health. Of particular interest is isolate 262 which is resistant to 7 antibiotics and possesses 2 virulence genes and 4 resistance genes. It was isolated from tap water which is assumed to have been treated and of better quality than the stream and well water. The *Salmonella enterica* were also isolated from well, tap and borehole water, none was isolated from packaged water. This also connotes a public health threat and shows the contamination of the water sources by human and/or animal faecal matter. *Salmonella* Typhi is strictly a human pathogen that causes invasive fever (typhoid fever), whereas most other *Salmonella* serotypes cause mainly gastrointestinal symptoms without systemic invasion (Braden, 2006).

5.6 Sequencing of Isolates, BLAST Results and Phylogenetic Analysis

The *E. coli* isolates that were sequenced were confirmed to be *E. coli* O157:H7, *E. coli* O104:H4 and *E. coli* IAI39 serogroups. Strain IAI39 is an extraintestinal pathogenic E.coli strain (ExPEC) isolated from the urine of a patient with urinary tract infection in France in the 1980s. It belongs to phylogenetic group D and is serotype O7:K1. The *Salmonella*

enterica were confirmed to belong to the *Salmonella enterica* subgroup and the typhi and typhimurium serovars.

The E- values shown on the table is an acronym for “expect value” which is indicative of the number of hits or results that can be observed by chance when searching a database of a particular size. It essentially describes the background noise. The smaller the value the more reliable the results obtained. All the sequences had E-values of 0.00. This indicates that the results were very real and reliable, and not as a result of random occurrences or chance (Agostino, 2013).

The 16S rRNAsequences of the isolates from this study have been successfully deposited in the GenBank of the NCBI and they have been assigned accession numbers. Bacterial species have at least one copy of the 16S rRNA gene which contains highly conserved regions together with hyper variable regions, which is used for identification of new strains. However a considerable variation can occur between species in both the length and the sequence of 16S-23S rDNA region therefore this region is useful in characterization of bacterial species (Magray *et al.*, 2011). Relationships among organisms are often presented as evolutionary trees. The resolution obtained in a given phylogenetic tree is generally thought to be a schematic picture of our understanding of the evolutionary history of these organisms.

A group of species appears as a clade when they all descend from a node from which no other species that is not a member of this group also descend. The difference in nucleotides of the *E. coli* sequences can be seen to be very little as indicated by the relatively short horizontal lines between the species. The strain isolated from a borehole water sample in Kagarko L.G.A (*Escherichia coli* IAI39) appears to have evolved earlier than all the strains as indicative by its position at the bottom of the tree. It also forms a different cluster from all the other strains. Several *E. coli* O157:H7 sequences appear in different clusters. This is because serogroup does not correspond to genotype. This agrees with the result gotten by Fukushima *et al*. (2002) where *E. coli* isolates with O serogroups associated with EPEC and EHEC appear in different clusters or different pathotypes are grouped in one cluster. These results indicate lateral transfer of genes for O antigens (Wang and Reeves, 2000), as well as genes for H antigens among *E. coli* strains.

The *Salmonella enterica* strain isolated from a well water in Jema'a L.G.A, (*Salmonella enterica* subsp *enterica* ser Typhi) is observed to be at the bottom the clade indicating that it evolved earlier than all the other strains, while the strain isolated from a borehole water sample in the same local government area (*Salmonella enterica* subsp *enterica* ser Typhimurium) appears to be the most recently evolved strain as indicated by at its position at the top of the clade. The two different serotypes, typhi and typhimurium formed two different clusters however the ref strain of *S. typhi* formed a cluster with the strain isolated from well water in Ikara.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

In conclusion, water used for drinking in the parts of Kaduna State that were sampled were generally of low quality as they had contents of some nitrate and heavy metals that were above the maximum contamination limit.

Many of the water sampled exceeded the recommended limits for total and faecal coliforms as stipulated by the World Health Organization, and are therefore not fit for human consumption, except with additional treatment. Pathogenic *E. coli and Salmonella enterica* were isolated from quite a number of the samples indicating that the water had been contaminated by faecal matter.

For the *E. coli* isolated in this study, the drugs of choice are: Gentamicin, Cefotaxime, Chloramphenicol and Amoxycillin- clavulanic acid. For the *Salmonella enterica* isolated, they are Gentamicin, Cefotaxime, Chloramphenicol and Amoxycillin- clavulanic acid. Most of the isolates showed resistance to Tetracycline.

The presence of *tet*A and *tet*B genes among the *E. coli* isolates suggests that these genes are the major determinant of resistance to tetracycline in this environment. Also the presence of blaTEM and sul1 genes among some of the isolates shows that they are part of the genes responsible for resistance to ampicillin, ceftriaxone and sulphamethoxazole-trimethoprim respectively.

The *E. coli* isolates possessed virulence genes such as stx2, eaeA and hlyA, while the *Salmonella enterica* contained the invA gene. This indicates that the isolates might be pathogenic. These organisms often carry resistance genes that can be spread to pathogens and therefore constitute a hidden reservoir of antibiotic resistance

The *E. coli* isolated in this study belonged to the O157:H7, O104:H4, and IAI39 serogroups while the *Salmonella enterica* isolates belonged to the Typhi and Typhimurium serovars.

The 16S rRNA sequences of isolates from this study have been successfully deposited in the GenBank of the National Centre for Biotechnology Information (NCBI) website and they have been assigned accession numbers.

6.2 Recommendations

1. The contamination of drinking water by heavy metals and other organic and inorganic substances needs to be checked and corrective measures should be put in place to avoid the short and long-term effects of pollution by heavy metals. There is also the need to control faecal pollution of water supplies to avert the occurrence of waterborne diseases outbreak.
2. Kaduna State inhabitants that live in the area that were studied should be advised to consume water especially well, tap and stream water only after proper treatment.
3. The government should be intimated so that potable water safe for consumption can be made much more readily available to the general populace. For example, the pipes that deliver tap water to households need to be regularly checked to discover any leaks which could serve as a source of contamination of the water supplies.

4. Sources of drinking water such as boreholes and wells should be constructed properly and should also be situated far away from livestock and domestic animals, gutters, latrines, soak-aways and any other sources of contamination.
5. The government through the National Agency for Food and Drug Control (NAFDAC) needs to put more checks in place to ensure that water that is commercially sold always meet up to standard.
6. These results confirms the importance of emphasizing on the proper use of antimicrobials in the study environment to reduce the selection and spread of resistant strains of *Salmonella enterica* and *E. coli* and to sustain the usefulness of the antibiotics in controlling the diseases on a long-term basis. This underlines the need for integrated surveillance systems of antibiotic resistance in poultry, human and other animal hosts in Nigeria.
7. The general populace should be educated on the dangers of abuse and misuse of drugs. Mandatory laws should be put in place by the authorities aiming at making a more prudent use of antibiotics in both human and veterinary medicine.

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APPENDICES

Appendix I: Microgen GN A substrate reference table

Well	Reaction	Description	Positive	Negative
1	Lysine	Lysine decarboxylase: Bromothymol blue changes to green/blue indicating the production of the amine cadaverine	Green/blue	Yellow
2	Ornithine	Ornithine decarboxylase: Bromothymol lue chanes to blue indicating the production of the amine putrescin	blue	Yellow/green
3	H_2S	H_2S production: thiosulphate is reduced to H_2S that reacts with ferric salts to give a black precipitate	Brown/black	straw
4-6	Glucose/mannitol/xylose	Fermentation: bromothymol blue changes from blue to yellow as a result of the acid produced from carbohydrate fermentation.	yellow	Blue/green
7	ONPG	Hydrolysis: ONPG hydrolysis by β-galactosidase results in the production of ortho-nitrophenol	Yellow	colourless
8	Indole	Indole is produced from tryptophan and gives a pink/red complex when Kovac's reagent is added	Pink/red	colourless
9	Urease	Hydrolysis of urea results in the formation of ammonia leading to an increase in Ph which changes phenol red from yellow to pink	Deep pink	Straw to pale salmon pink colour
10	VP	Acetoin production from glucose is detected by the formation of a pink/red complex after the addition of alpha naphthol and creatine in the presence of KOH.	Deep pink/red	Colourless to pale pink
11	Citrate	Utilization of citrate as the only carbon source leads to a colour change in bromothymol blue form green to blue	Blue	Yellow/pale green
12	TDA	Indolepuryvic acid is produced from tryptophan by tryptophan deaminase giving a cherry red colour when ferric irons are added.	Cherry red	Straw colour

Key: H_2S- Hydrogen Sulphide; ONPG- Ortho-nitrophenol-galactosidase; TDA- Tryptophan deaminase acid

Appendix II: Biochemical tests and Microgen GN A Identification results

Mot	TSI	Oxd	Lys	Orn	H_2s	Glu	Man	Xyl	ONPG	Ind	Ure	VP	Cit	TDA	Octal code	Identity score (%)	Identity
+	A/A_g	-	+	+	-	+	+	+	+	+	-	-	-	-	6760	97.11	*E. coli*
+	A/A_g	-	-	-	-	+	+	+	-	+	-	-	-	-	0760	90.61	*E. coli inactive*
+	A/A_g	-	+	+	-	+	+	+	+	+	-	-	+	-	6762	84.29	*E. coli*
+	A/A_g	-	-	+	-	+	+	+	+	-	+	+	+	-	2756	86.76	*E. cloacae*
+	A/A H_2S	-	+	--	+	+	+	+	-	-	+	-	+	-	1712	99.94	*C. freundii*
+	A/A_g	-	+	+	+	+	+	+	+	-	+	+	+	+	7757	93.1	*E. gergoviae*
+	A/A H_2S	-	+	+	-	+	+	+	+	+	+	-	+	-	6772	84.93	*C. diversus*
+	A/A_g	-	+	+	-	+	+	+	+	-	-	+	+	-	6746	50.5	*E. aerogenes*
-	K/K	-	+	-	-	-	-	-	+	-	-	+	-	-	4044	50.45	*A. iwoffii*
+	K/K	-	+	+	-	+	+	+	+	-	-	-	-	-	6740	85.89	*H. alvei*
-	K/K	-	+	-	-	+	-	-	-	-	+	-	+	-	4412	99.85	*A. baumanii*
-	K/K	-	+	-	-	-	-	-	-	-	-	-	+	-	4002	98.92	*A.haemolyticus*
-	A/A_g	-	+	+	+	+	+	+	+	+	+	+	+	+	6777	83.02	*Klebsiella oxytoca*
-	A/A_g	-	-	-	-	+	+	+	+	-	-	-	-	-	0740	71.83	*Klebsiella ozaenae*

Appendix II contd: Biochemical tests and Microgen GN A Identification results

Mot	TSI	Oxd	Lys	Orn	H_2S	Glu	Man	Xyl	ONPG	Ind	Ure	VP	Cit	TDA	Octal code	Identity score (%)	Identity
-	K/Ag H2S	+	+	-	+	+	+	+	-	-	-	-	+	-	5702	91.32	*Salmonella spp*
-	K/Ag H2S	+	+	+	+	+	+	+	-	+	-	+	+	-	7726	78.67	*Salmonella spp*
-	K/Ag H2S	+	+	+	+	+	+	+	+	+	-	-	+	-	7762	98.69	*Salmonella arizonae*
-	A/Ag	-	+	-	+	+	+	+	+	-	+	+	+	+	5757	89.46	*Klebsiellapneumoniae*
+	K/Ag H2S	-	-	+	+	+	+	+	-	-	+	+	+	+	3717	100	*Proteus mirabilis*
+	K/Ag H2S	-	-	-	+	+	-	-	-	-	+	-	-	-	1410	74.63	*Proteus vulgaris*
+	K/A	-	-	-	-	+	-	-	-	+	+	-	+	+	0433	98.97	*Providencia stuartii*
+	K/A	-	+	+	-	+	+	+	+	+	-	+	+	-	6766	80.26	*Serratia liquefaciens*
-	K/A	-	-	-	-	+	+	+	-	-	-	+	+	-	0706	95.53	*Pantoea agglomerans*

KEY: Mot- motility; TSI- Triple sugar iron test; Oxd- oxidase;Lys- lysine; Orn- ornithine; H_2S- Hydrogen sulphide; Glu- glucose; Man- mannitol; Xyl- xylose; ONPG-Ortho-nitrophenol-galactosidase; Ind- indole; Ure- urease; VP- Voges- Proskaeur; Cit- citrate; TDA- Tryptophan deaminase acid

Appendix III: Zone Diameter Interpretative standard breakpoints for *Enterobacteriaceae* for antibiotics used in this study

Antimicrobial agent	Disk potency(µg)	Zone diameter			Testing conditions
		R ≤	I	S≥	
Gentamicin	30	12	13-14	15	**Medium:** Mueller-Hinton agar
tetracycline	30	11	12-14	15	
Nalidixic acid	30	13	14-18	19	**Innoculum:** direct colony suspension equivalent to 0.5 McFarland standard
Amoxicillin-clavulanic acid	30	13	14-17	18	
Sulfamethoxazole-trimethoprim	25	10	11-15	16	**Incubation**: at 37°C , ambient air; 16-18h
Chloramphenicol	30	12	13-17	18	
Ciprofloxacin	5	20	21-30	31	
Ampicillin	10	13	14-16	17	
Cefotaxime	30	22	23-25	26	
Amoxycillin	10	13	14-16	17	

Key: R- resistant; I- intermediate; S- susceptible.

Source: CLSI, 2014

Appendix IV: Preparation of stock solution of Primers

Primer	Volume of water/buffer to make 100µm (µl)
Stx1-F	288.88
Stx1-R	410.7
Stx2-F	412.22
Stx2-R	366.28
eaeA-F	413.17
EaeA-R	473.75
HlyA-F	384.86
HlyA-R	422.01
InvA-F	443.03
InvA-R	422.18
SpvA-F	389.4
SpvA-R	348.3
TetA-F	445.99
TetA-R	311.8
TetB-F	332.99
TetB-R	425.27
Sul1-F	295.52
Sul1-R	394.91
BlaTEM-F	417.84
BlaTEM-R	343.22

Appendix V: Media Formulation

1. **Brilliant Green Lactose Bile Broth(Oxoid, UK)**

Formulation	**g/l**
Peptone	10.0
Lactose	10.0
Ox bile(purified)	20.0
Brilliant green	0.0133
Final pH (at 25°C) 7.4 ± 0.2	

2. **Eiosin Methylene Blue (EMB) agar (Lifesave Biotech, USA)**

Formulation	**g/l**
Peptic digest of animal tissue	10.0
Dipotassium phosphate	2.0
Lactose	5.0
Sucrose	5.0
Eiosin-Y	0.4
Methylene blue	0.065
Agar	13.5
Final pH (at 25°C) 7.2 ± 0.2	

3. **Methyl red Voges Proskauer broth (Scharlau, Spain)**

Formulation	**g/l**
Peptone	7.0
Dextrose	5.0
Potassium phosphate	5.0
Final pH (at 25°C) 7.0 ± 0.2	

4. **Mueller-Hinton agar (Lifesave Biotech, USA)**

Formulation	**g/l**
Infusion from meat	2.0
Casein hydrolysate	17.5
Starch	1.5
Agar-agar	13.0
Final pH (at 25°C) 7.4± 0.2	

5. **Nutrient agar (Lifesave Biotech, USA)**

Formulation	**g/l**
Peptic digest of animal tissue	5.00
Beef extract	1.50
Yeast extract	1.50
Sodium Chloride	5.00
Agar	15.00
Final pH (at 25°C) 7.4± 0.2	

6. **Peptone water (Scharlau, Spain)**

Formulation	**g/l**
Peptone	10.0
Sodium chloride	5.0
Disodium phosphate	3.5
Potassium phosphate	1.5
Final pH (at 25°C) 7.2 ± 0.2	

7. **Salmonella Shigella agar (TM Media, India)**

Formulation	**g/l**
Agar	15.0
Sodium chloride	10.0
Lactose	10.0
Bile salts	18.5
Sodium thiosulphate	8.5
Beef extract	5.0
Peptic digest animal tissue	5.0
Ferric citrate	1.0
Neutral red	0.025
Brilliant green	0.00033
Final pH (at 25°C) 7.0 ± 0.2	

8. **Selenite F broth (TM Media, India)**

Formulation (Part I)	**g/l**
Sodium phosphate	10.0
Lactose	4.0
Casein enzymic hydrolase	5.0
Final pH (at 25°C) 7.0 ± 0.2	

Formulation (Part II)	**g/l**
Sodium hydrogen selenite	4.0

9. Simmon's citrate medium (Oxoid, England)

Formulation	**g/l**
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	1.0
Sodium citrate	2.0
Sodium chloride	5.0
Bromothymol blue	0.08
Agar	15.0

Final pH (at 25°C) 6.8 ± 0.2

10. Sorbitol Macconkey agar (supplemented with Cefuxime-tellurite) (Oxoid, UK)

Formulation	**g/l**
Peptone	20.0
Sorbitol	10.0
Bile salts no. 3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0

Final pH (at 25°C) 7.1± 0.2

Cefixime-tellurite supplement

Formulation	**mg/l**
Potassium tellurite	2.5
Cefixime	0.05

11. Tryptic Soy broth (Merck, Germany)

Formulation	**g/l**
Peptone from casein	17.0
Peptone from Soymeal	3.0
D(+) Glucose monohydrate	2.5
Sodium chloride	5.0
Di-Potassium hydrogen phosphate	2.5

Final pH (at 25°C) 7.3 ± 0.2

12. Triple Sugar Iron agar (Oxoid, UK)

Formulation	**g/l**
Lab lemco powder	3.0
Yeast extract	3.0
Peptone	20.0
Glucose	1.0
Lactose	10.0
Sucrose	10.0
Ferrous sulfate	0.3
NaCl	5.0
Sodium thiosulfate	0.3
Phenol red	0.024
Agar	12.0

Final pH (at 25°C) 7.4 ± 0.2

13. Urease media

Formulation	**g/l**
Peptone	1.0
Glucose	1.0
Sodium chloride	5.0
Disodium phosphate	1.2
Potassium dihydrogen phosphate	0.8
Phenol red	0.012
Agar	15.0

Final pH (at 25°C) 6.8 ± 0.2

Appendix VI: Composition of reagents

1. **Kovac's Indole reagent**

Para-dimethylaminobenzaldehyde	2g
Isoamyl alcohol (3-methyl-1-butanol)	30ml
Hydrochloric acid (concentrated)	10ml

2. **Methyl red solution**

Methyl red	0.05g
Absolute ethanol	28ml
Distilled water	22ml

3. **5% alpha-napthol**

Alpha-napthol	5g
Absolute ethanol	100ml

4. **40% Potassium hydroxide**

Potassium hydroxide	40g
Distilled water	100ml

5. **McFarland's standard number 0.5**

1% Barium chloride	0.05ml
1% Sulphuric acid	9.95ml