WATER QUALITY ASSESSMENT AND CHARACTERIZATION OF ANTIBIOTIC RESISTANCE GENES IN SALMONELLA SPECIES FROM SELECTED DAMS IN ZARIA, KADUNA STATE, NIGERIA

BY

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SEPTEMBER, 2018

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Ph.D/SCIE/31216/2012-2013

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

DEPARTMENT OF MICROBIOLOGY,

FACULTY OF LIFE SCIENCES,

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ZARIA

SEPTEMBER, 2018

DECLARATION

I declare that the work in the thesis entitled "Water quality assessment and characterization of antibiotic resistance genes in *Salmonella*spp from selected dams in Kaduna State, Nigeria." was carried out by me in the Department of Microbiology under the supervision of Prof.O.S.Olonitola, Prof. H.I. Inabo and Dr. E.E. Ella.

The information derived from 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.

AregbeElizabeth Adenike	**Signature**	**Date**

CERTIFICATION

This thesis entitled "WATER QUALITY ASSESSMENT AND CHARACTERIZATION OF ANTIBIOTIC RESISTANCE GENES IN SALMONELLA SPECIES FROM SELECTED DAMS IN KADUNA STATE, NIGERIA" by Aregbe, Elizabeth Adenike meetsthe regulations governing the award of the Degree of Doctor of Philosophy (Microbiology) of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

Prof O.S. Olonitola
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Dean, School of Postgraduate Studies **Signature**_____ **Date**_____

DEDICATION

To the Glory of God. This work is dedicated to my sweetheart, Bunmi, and my lovely kids Oluwatobi, Omotola and Sophia for their support, prayers and encouragement, God bless you.

ACKNOWLEDGEMENT

I appreciate the Almighty God for seeing me through this work successfully. I sincerely appreciate the contributions of Prof.O.S. Olonitola under whose supervision this work was carried out. His fatherly advice, encouragement and constructive criticisms are highly appreciated.

My profound gratitude goes to Prof. H.I. Inabo for her great support and kind advice throughout my studies. I also appreciate Dr. E.E. Ellafor his constant support, guidance and motivation.

I sincerely appreciate the encouragement and academic advice received from Prof.J.K.P.Kwaga. My gratitude also goes to Prof.S.O. Mohammed (DG, National Space Research Development Agency) for his encouragement during this research work.Worthy of mention is the contribution of Prof.N.Esiobu of ABNL Molecular laboratory, Abuja who gave me free access to the Laboratory bench space.

The contributions of Dr. H.M.Raji, Dr. A.U. Asuke, Dr. S.L. Afegbuaand Mr H.G. Bishop are highly appreciated. My gratitude goes to all academic, technical and non-academic staff of the Department of Microbiology A.B.U, Zaria for their various contributions to the successful completion of this work. Not to be forgotten are the contributions of Mr. E.S.Alex, Mr. W.M. S Stephen, Mr. S.Jumare and Mr. A.M. Shittu.

My gratitude also goes to all staff of the Water Quality Laboratory, A.B.U, Zaria particularly Mr. P.C.Alika, Mal. C.S.Adamu and other technical staff of Department of Water Resources Engineering Ahmadu Bello University, Zariafor their companionship and for providing a pleasurableand friendly working atmosphere.

Finally, I thank my parent for their constant prayers for my success throughout this programme, May God Almighty grant them long life and abundance of health to enjoy the fruits of their labour. I thank my husband, Mr. Ezekiel BunmiAregbe, my children, Oluwatobi, Omotolaand SophiaAregbe, for their support, patience and endurance in the course of this programme.

To my brother, sisters and in-laws, Mr Ade Bello, Mrs. Temi Bello,Mr. Peter Jack, Mrs. Shade Peter Jack, Mr. YemiAkinpeluMrs. Tayo Akinpelu, Mr. Andrew Ebohor, Mrs. Tosin Ebohor with Juliana Bello, I say thank you so much for your support. I appreciate the efforts of ToluAregbe, BidemiAregbe, KoredeAregbe, Ayo Aregbe and Shola Aregbe; May God see you through your endeavors in life.

I must specially thank my friends particularly Dr. Gladys Imokhe, Mrs. Aisha Okunade, Mrs.Ebere Adedoyin, Mrs. Cecilia Odigie,Dr. Marie, Mrs. Vivien Ameso, Mrs. YuanaAwua and Mrs. Mercy Itoro for their unimaginable moral and financial support.

I thank every other person who has contributed to the success of this programme who I could not mention for limited space. May God be with you.

ABSTRACT

This study is aimed at assessing the water quality and characterizing the antibiotic resistance genes in *Salmonella*spp from selected dams in Kaduna State, Nigeria. A 10- month study was carried out on the physicochemical and bacteriological parameters of water samples and the detection of antibiotic resistance genes in *Salmonella* spp from selected Dams in Zaria, Kaduna State, Nigeria. Four hundred and fifty (450) water samples were collected from threeselecteddamsin Zaria. The locations were Kubanni Dam, Galma Dam and Markwa Dam. The physicochemical and bacteriological analysis of water samples were carried out by assessing twelve (12) parameters both in the dry and wet seasons. The bacterial load of water samples from the selected dams in Kaduna State, Nigeria were determined using the pour plate method.*Salmonella*spp were isolated from the water samples on selective culture media. Pure isolates were identified by conventional biochemical test and characterized using Microgen identification. TheAntibiotic susceptibility profiles of the *Salmonella* isolates were determined using the disc diffusion methods as described in official monographs. The antibiotic resistance genes in selected isolates using Polymerase Chain Reaction (PCR) were detected.Thephysicochemical parameters of water samples showed three parameters exceeded standard drinking water quality values of World Health Organization(2011) and Nigerian Drinking Water Quality Standards permissible limits (2007).Turbidity ranged from 20.18±26.17 (Markwa dam) to 37.39±35.69) (Galma), hardness ranged from 233.46±82.10 Galma to

238.55±104.31mg/l Markwa and Chemical Oxygen Demand ranged from 238.80±48.95mg/lGalma to 274.25±97.90mg/l Kubannidam.The results for the bacterial load of water was highest for the month of May for the three dams.Markwa dam had the highest bacterial counts during the wet season($1.53\times10^{8}\pm2.71\times10^{6}$cfu/ml).A total of 101 (22.4%) bacterial isolates was obtained from the water samples among which 43(9.5%) were identified target isolates. The isolation rates of *Salmonella* Serovars was 9.5% with *S.*Arizonae, *S.*Pullorum, and *S.*Choleraesuis as the most frequently isolated species. Of these 43 target isolates, 27(6.0%) were *S.* Arizonae,*S.*Pullorum 14(3.1%)and 2(0.4%) *S.*Choleraesuis isolates. Wet season had occurrence of the isolates 22(4.8%) than the dry season 21 (4.6%).*S.*Arizonae, *S.*Pullorum *and S.*Choleraesuiswere 100% susceptible to Imipenem (10µg) and Ceftriaxone (30µg) respectively.*S.*Choleraesuis isolates were 100% susceptible to Ciprofloxacin (5µg). Most isolates were resistant to Ampicillin (10µg), Nalidixic acid (30 µg) and Amoxicillin-clavulanic acid (30µg),Cefoxitin(30µg) and Cotrimoxazole (25µg). The presence of antibiotic resistance genes, *catP, sul2,tem*, and *gyrB* genes was demonstrated in 5(33.3%),1(6.7%), 2(13.3%) and 9(60.0%) respectively of *S.*Arizonae, *catP, sul2,tem,*and *gyrB*genes in 4(26.7%), 1(6.7%), and 1(6.7%) and 6(40.0%)respectively of *S.*Pullorumand none in*S.*Choleraesuisby PCR. The presence of multidrug resistant enteric bacterial pathogens suggests that the water samples were contaminated with faecal matters and that antibiotics were used in the environment

indiscriminately. Therefore policy should be put in place in water quality monitoring to warn against hazards to public health and vulnerable water resources.

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ABBREVIATIONS AND ACRONYMS

APHA	American Public Health Association
BOD	Biochemical Oxygen Demand
CDC	Centers for Disease Control and Prevention
COD	Chemical Oxygen Demand
^{o}C	Degree Celsius
DO	Dissolved Oxygen
DNA	Deoxyribonucleic Acid
FDA-CVM	Center for Veterinary Medicine
LPS	Lipopolysaccharide
ML	Molecular Ladder
MAR	Multiple Antibiotic Resistance
MARI	Multiple Antibiotic Resistance Index
ONPG	Ortho-Nitro phenyl-β-galactosidase
NARMS	National Antimicrobial Resistance Monitoring System
NDWQS	Nigeria Drinking Water Quality Standard
NTS	Non Typhoidal *Salmonella*
PCR	Polymerase Chain Reaction
QRDR	Quinolone Resistance Determinant Region
TDS	Total Dissolved Solids
USDA-FSIS	Food Safety and Inspection Service and Agricultural Research Service
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Water quality includes all the physical, chemical and biological factors that influence beneficial use of water for various purposes to which the reservoir was built (Amadi *et al.,* 2014). The quality of water is important in drinking water supply, irrigation, fish production, recreation and other purposes (Amadi *et al.,* 2014).

The contaminants in water are divided roughly into three categories: physical, chemical and biological, but environmental risk assessment reveals that the exposure to biological contaminants especially water-borne microbial pathogens needs to be given higher priority in treatment for domestic water supplies (Okonko *et al*., 2008).

The family *Enterobacteriaceae* are of utmost concern and presents significant concern; The members includes *Salmonella*, *Shigella*, *Klebsiella*, and *Escherichia coli* (Adeogun *et al.,* 2012). These have been documented to pose significant threats to human health as they serve as the causative agents of several gastrointestinal symptoms like vomiting, nausea and diarrhoea and even more chronic disease like kidney damage especially in susceptible or immunocompromised individuals (Adeogun *et al.,* 2012).

The genus *Salmonella* is classified into two species *S.enterica* and *S.bongori.*and identified into serotypes according to the White-Kauffmann-Le Minor scheme; there are more than 2,500 *Salmonella* serotypes that have been described and reported (Wattiau *et al.,* 2011). Most of the serotypes pathogenic to humans and animals belong to *S.enterica* (Pui *et al.,* 2011).

The *Salmonellae* are constantly found contaminating environmental samples and in almost all types of aquatic environment, as a result of faecal contamination from humans, pets, farm animals, and wild life.

Their presence in water, therefore, indicates faecal contamination. Municipal sewage effluents, pollution from agricultural products, storm water runoff from contaminated environments and direct deposit of faecal materials from wild animals and birds are the main sources of these pathogens in natural waters (Abulreesh, 2013, Bhatta *et al*., 2007 Haley *et al.,* 2009).

Salmonella causes four clinically distinguishable forms of infections in humans. These are gastroenteritis, enteric fever, bacteremia and other complications of non typhoidal Salmonellosis as well as chronic carrier state in people of all ages. They also cause severe invasive disease in infants, elderly persons and immuno-compromised persons. The transmission of *Salmonella* species takes place through the faecal-oral route, by means of contaminated food and water (Barry *et al.,* 2012).

Antimicrobial resistance is a global health concern responsible for rising incidences of both debilitating and lethal diseases (WHO, 2014). Several antibiotics are used in the treatment of *Salmonella* infections in both human and veterinary medicine and the overuse of these antibiotics has led to the emergence of antibiotic drug resistance which are often released into the environment leading to longer hospital stays, higher medical costs and increased mortality (Zhang *et al.,* 2009).

Some of the antibiotic resistance are mediated by the acquisition of resistance genes which are often located on mobile genetic elements such as plasmids, transposons and integrons. Horizontal gene transfer accounts for the main pathway by which antibiotic resistance genes are spread to same or different species (Pruden *et al*., 2006).

Molecular methods have provided major breakthroughs employing polymerase chain reaction (PCR) for rapid, sensitive and specific diagnosis of *Salmonella* infections. Therefore, the use of PCR for targeting specific genes such as *gyrA*, *gyrB*, *tem*, *sul2* and *catP* genes in *Salmonella* isolates would provide more information about its genetic capabilities in the study area and Nigeria at large (Asma *et al.,* 2005).

1.2 Statement of Research Problem

The pollution of rivers and reservoirs has increased water contaminants such as heavy metals, nutrients, organic matter, soluble ions, oil and grease, and organic chemicals such as pesticides due to urbanization, intensive agriculture, recreation, and the manufacturing industry (Ekiye and Zejiao, 2010).

After almost sixty years of water supply development in Nigeria, it is regrettable that only 60% of the population have access to safe drinking water, and in rural areas less than 50% of the households have access to portable water (Abdulmumuni *et al.*, 2014). At least 27% of Nigerians, most of whom live in the North Central and the far North of Nigeria, depended absolutely on streams, ponds, rivers, dams and rainwater as their drinking water source (Taiwo *et al*., 2012).

Salmonella species have been found in almost all types of aquatic environments that received faecal contamination, that includes drinking water (Bhatta *et al.,* 2007), rivers (Haley *et al.,* 2009), lakes (Sharma and Rajput, 1996), ponds, marine waters (Matinez *et al.,* 2005), run-off water (Claudon *et al.,* 1971), treated and untreated wastewater (Mafu *et al.,* 2009) worldwide. (Martinez *et al.,*2005).

Salmonellosis is common in most parts of the world (Kong *et al.*, 2002) and prevalent in Europe and North America (Randall *et al.*, 2004), Latin America, the Middle East and Africa (Kariuki *et al.*, 2006). Countries such as India, Japan and the United States also had cases of *Salmonella* outbreaks (Van *et al.*, 2008). Several studies have documented isolation of *Salmonella* from humans and poultry in different parts of Nigeria (Akinyemi *et al.*, 2011).

Outbreaks of Salmonellosis caused by *Salmonella* Serovars Gallinarum, Pullorum, Typhimurium and Enteritidis have also been reported in Nigeria (Agbaje *et al.*, 2010). High

prevalence of waterborne diseases such as cholera, diarrhoea, dysentery, salmonellosis and hepatitis have been seen with thousands of children and even adults dying every year (Oguntoke *et al.*, 2009; Raji and Ibrahim, 2011).

Waterborne salmonellosis (non-typhoidal species) are now the second leading cause of gastroenteritis around the world and according to the US Centre for Disease Control and Prevention, 1.4 million cases of salmonellosis occur annually in the USA with self-limiting infections and higher risk of invasive infection, higher frequency and duration of hospitalization, longer illness, and death (WHO, 2014).

The treatment of Salmonellosis with antibiotics like Sulphonamides, Tetracyclines, Aminoglycosides, or Quinolone had only been successful in reducing mortality and temporarily improving clinical recovery, as multi-drug resistant *salmonella* had emerged in recent years and very few antibiotics appear to completely eliminate the organism. (WHO, 2014). The use of these drugs in animal food can select for antibiotic resistant bacteria and high rates of resistance among *Salmonella* infections (Akinyemi *et al.*, 2006).

Antibacterial drug resistance in *Salmonella* species has been a serious problem for public health worldwide and are of concern (WHO, 2014). Multidrug-resistant *Salmonellae* are associated with increased morbidity and mortality, since they are much less susceptible to antibiotic therapy (WHO, 1998).

1.3 Justification for the Study

Every living thing on earth needs water to survive and without any doubt, inadequate quantity and quality of water have serious impact on sustainable development (Taiwo *et al.*, 2012). Water quality

assessment is a fundamental tool necessary for the management of freshwater resources, particularly rivers, streams and dams (Adah and Abok, 2013).

Two major sources of water whose quality are normally assessed are the surface (streams, reservoirs, rivers, ponds, lakes) and ground waters (wells, boreholes). It has been reported that surface waters are generally poor in quality due to Indiscriminate disposal of industrial effluents into surface water bodies, impoundment of surface water for irrigation purposes and use of fertilizers for agriculture has resulted in pollution of water by leachates (Akindele, 2013).

Water quality assessment in reservoirs is therefore paramount especially to safeguard the public health and also to protect the water resource since it induces physical, chemical and biological changes in the stored water (Obeta, 2016). The chemical composition of water within a reservoir can be significantly different from that of other reservoirs. The water discharged from reservoirs can be of different compositions and show a different seasonal pattern to that of other reservoirs (Akindele *et al.,* 2013).

Organic matter deposited in water bodies leads to nutrient enrichment which promotes growth of heterotrophic bacteria, that include various pathogens such as *Shigella* spp, *Salmonella enterica* and *Vibrio cholerae* (WHO, 2008). In many developing countries with inadequate sanitation, contaminations of environmental waters by enteric pathogens such as *Salmonella* are very common and poses an increased health risk to individuals that consume the water (Taiwo *et al*., 2012).

The increasing treatment failure with the empirical therapy among patients with Salmonellosis necessitates the need for frequent assessment and reporting of antibacterial drug resistance patterns of *Salmonella* species in our environment since the use of this antibiotics is accompanied by the increases in rates of morbidity and mortality (Agbaje *et al.,* 2010).

The emerging of Antibitic Resistance Genes (ARGs) in the water environment is becoming an increasing worldwide concern. Hundreds of various ARGs encoding resistance to a broad range of antibiotics have been found in microorganisms distributed not only in hospital wastewaters and animal production wastewaters, but also in sewage, wastewater treatment plants, surface water, groundwater, and even in drinking water (Zhang *et al.,* 2009).

In Nigeria, several studies have been carried out on the water quality assessments but only few studies had looked into the detection of resistance genes in *Salmonella* isolates in dams in the northern part of the country hence the need for this study. Outcome of this study may suggest new ways of improving the quality of water in the dams and develop adequate approaches in ensuring potability of water for its intended users resident in Kaduna State, Nigeria.

1.4 Aim of the Study

The aim of the study was to assess water quality and to characterize antibiotic resistance genes in *Salmonella* spp isolated from selected dams in Kaduna State, Nigeria.

1.5 Objectives of the Study

The specific objectives were to:

1. determine the physicochemical parameters of water samples from selected dams in parts of Kaduna State, Nigeria.
2. determine bacterial load of water samples from the selected dams in Kaduna State, Nigeria.
3. identify the *Salmonella* isolates using conventional, commercial and rapid diagnostic methods.
4. determine the antibiotic resistance patterns of the isolates by the disc diffusion technique
5. detect antibiotic resistance genes in selected isolates using Polymerase Chain Reaction (PCR).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Uses Of Water

Water is regarded as the universal solvent primarily due to its chemical and physical properties. It is one of the substances essential to life and the ecosystem. All living organisms on the earth need water for their survival and growth.The earth is the only planet in the universe that have about 70 % of water. But due to increased human population, industrialization, use of fertilizers in the agriculture and man-made activity becomes highly polluted with different harmful contaminants (Basavaraja *et al*., 2011; Sagar *et al*., 2015).

There are two main sources of water: surface water and groundwater. Surface water is found in lakes, rivers, and reservoirs. Groundwater lies under the surface of the land, where it travels through and fills openings in the rocks. The rocks that store and transmit groundwater are called aquifers. Groundwater must be pumped from an aquifer to the earth's surface for use(Sagar *et al*., 2015).Water is generally used for drinking, fishing, recreation, agriculture, industrial and domestic use.The availability of good quality water is an indispensable feature for preventing diseases and improving quality of life (Patil *et al*., 2012).

Natural water are often laden with several impurities and these impurities are introduced into the water through different means such as weathering of rocks and leaching of soils, dissolution of aerosol particles from the atmosphere and from several human activities, including mining, processing and the use of metal-based fertilizer in agricultural revolution could result in continued rise in concentration of heavy metals in fresh water reservoir due to run-off (Patil *et al*., 2012). Also, faecal pollution of drinking water sources hascaused water borne diseases which led to the death of millions of people (Adefemi and Awokunmi, 2010).

2.2 Water Quality and Physicochemical properties

Water quality refers to the chemical, physical and biological characteristics of water (Diersing, 2009). The quality of water is affected by a wide range of natural and human influences. The most important of the natural influences are geological, hydrological and climatic, since these affect the quantity and the quality of water available. (Johnson *et al*., 1997).

The following different physico-chemical parameters are to be tested regularly for monitoring quality of water.

2.2.1 pH and Temperature

pH is a measure of the amount of free hydrogen ions in water. Specifically, it is the negative logarithm of the molar concentration of hydrogen ions. It can be measured with a scale from 0 -14. pH of 7 is neutral, pH less than 7 is acidic and pH greater than 7 is basic. The pH of drinking water is in the range of 6.5-8.5 (WHO, 2011).

Temperature on the other hand is an important environmental factor which affect on metabolic rate and the reproductive activities of plants and animals. Water temperature expresses how cold or hot a water is. Metabolic activities increases with a rise in temperature, thus increasing fish's demand for oxygen; however, an increase in water temperature also causes a decrease in dissolved oxygen, limiting the amount of oxygen available to these aquatic organisms. A rise in temperature can also provide conditions for the growth of disease-causing organisms (Sagar *et al.,* 2015).

Water temperature varies with season, elevation, geographic location and climatic conditions and is influenced by stream flow, stream side vegetation, ground water inputs and water effluent from industrial activities (WHO, 2011). The temperature of water depends on the depth of the water column, climatic and topographic changes (Sagar *et al*., 2015).

2.2.2 Colour and Turbidity

Good water should be transparent and colourless. The colour of water is expressed in True Colour Units (TCU) which correspond to the colouration of a series of platinum/cobalt salt dilutions (Sagar *et al.,* 2015). Turbidity measures the loss of transparency of a solution. The presence of colloidal solids such as suspended silt and clay, organic matter, and plankton contributes to turbidity and gives water a cloudy appearance which reduces the transparency. Turbidity measures the amount of solid that is scattered or absorbed in water. Photoelectric turbidometers measure turbidity in nephelometric turbidity units (NTU) (Sagar *et al*., 2015).

2.2.3 Electrical Conductivity

Electrical conductivity (EC) is the ability of water to conduct an electric current due to dissolved salts present in it. The dissolved salts produce ions that migrate in solution and then generate electric currents. The conductivity of water is a more or-less linear function of the concentration of dissolved ions. Conductivity itself is not a human or aquatic health concern, but because it is easily measured, it can serve as an indicator of other water quality problems, if it suddenly increases; it indicates that there is a source of dissolved ions in the water. It is used to estimate the ionic or soluble salt concentration in soils and water, fertilizer solution and chemical solution. It is measured with the help of electrical conductivity meter which measures the resistance offered by the water between two platinized electrodes. (Sagar *et al*.,2015).

2.2.4 Alkalinity

Alkalinity is a chemical measurement of water's ability to neutralize acid. Alkalinity is also a measure of water buffering capacity or its ability to resist changes in pH upon the addition of acids or bases (Sagar *et al.,* 2015). Alkalinity of natural water is due to the presence of weak acid salts, although strong bases

may also contribute (i.e. OH-) in the extreme environment. Bicarbonate represents the major form of alkalinity in natural water, its source being the partitioning of CO_2 from the atmosphere and the weathering of carbonate minerals in rocks and soil. Other salts of weak acids, such as borate, silicates, ammonia, phosphate, and organic bases from natural organic matter may be present in small amounts. Waters with high alkalinity are unpalatable and can cause gastrointestinal discomfort (Sagar *et al*., 2015).

2.2.5 Dissolved Oxygen

Dissolved oxygen (DO) is the measure of the concentration of free (not chemically combined) molecular oxygen (a gas) dissolved in water usually expressed in milligram per liter, part per million, or percent saturation. Dissolved oxygen level is considered the most important and commonly employed measurement of water quality and indicator of a water body ability to support desirable aquatic life (Sagar *et al.,* 2015).The amount of dissolved oxygen in reservoirs, lake or river is dependent on the water temperature, the quantity of sediments in the stream, the amount of oxygen taken out of the system by respiration and decaying organisms, and the amount of oxygen put back into the system by photosynthetic plants, stream flow and aeration (Sagar *et al.,* 2015). It is measured in milligrams per litre.Water with low dissolved oxygen has an unpleasant taste while water with high dissolved oxygen is good for drinking purposes. Dissolved oxygen is the most important indicator of the health of water bodies and its capacity to support a balanced aquatic ecosystem of plants and animals (Sagar *et al.,* 2015). Warm water released from industrial outlets, flowages or storm sewers can also reduce dissolved oxygen levels. Dissolved oxygen may play a large role in the survival of aquatic life in lakes and reservoirs (Adefemi and Awokunmi, 2010).

2.2.6 Total Hardness

Total Hardness (TH) is defined as the sum of calcium and magnesium in water.Hardness of water is measured in mg/l. A high concentration of hardness may be due to the presence of dissolved polyvalent metallic ions from sedimentary rocks, seapage and runoff from soils. Calcium and magnesium, the two principal ions, are present in many sedimentary rocks, the most common being limestone and chalk (WHO,2011). The permissible limit for total hardness of water according to WHO is 150 mg/ l and water containing calcium carbonate at concentrations below 60 mg/l is generally considered as soft; 60–120 mg/l, moderately hard; 120–180 mg/l, hard; and more than 180 mg/l, very hard (Todd and Mays,2005). High concentration of hardness may cause the problem of heart disease,osteoporosis, nephrolithiasis (kidney stones), colorectal cancer, hypertension and stroke, coronary artery disease, insulin resistance and obesity,these disorders have treatments, but no cures (WHO,2011).

2.2.7 Total Dissolved Solids

Total Dissolved Solids (TDS) are the total amount of mobile charged ions, including minerals, salts or metal dissolved in a given volume of water in mg/l. TDS is directly related to the purity of water and the quality of water purification system and it affects every thing that consumes, lives in or uses water. Common inorganic salts can be found in water bodies. The cations include calcium, magnesium, potassium and sodium, while the anions are carbonates, nitrates, bicarbonates, chlorides and sulphates (Patil *et al*., 2012).

2.2.8 Chemical Oxygen Demand and Biochemical Oxygen Demand

The standard method for indirect measurement of the amount of pollution in a sample of water is the chemical oxygen demand.The test procedure is based on the chemical decomposition of organic and inorganic contaminants, dissolved or suspended in water. A low value of dissolved oxygen and higher

values of Biochemical oxygen demand and Chemical Oxygen Demand can be as a result of the discharge of effluents and non-point source of pollution (Mahesh and Prabhahar, 2012).

Biochemical Oxygen Demand (BOD) is a measure of organic matter contamination in water, specified in mg/l. It measures the amount of oxygen that microorganisms consume while decomposing organic matter, it also measures the chemical oxidation of inorganic matter (Sagar *et al.,* 2015). Microorganisms are responsible for decomposing organic waste.The waste water treatment plants, failing septic systems, and agriculture and urban run-off, act as a food source for water-borne bacteria, When biochemical oxygen demand levels are high, dissolved oxygen levels decrease which means aquatic organisms may not survive if the demand for oxygen by the bacteria is high because the oxygen that is available in the water is being consumed by bacteria. On the other hand, if there is no organic waste present in the water, there won't be as many bacteria present to decompose it and thus the biochemical oxygen demand will tend to be lower and the dissolved oxygen level will tend to be higher. Typically the test for biochemical oxygen demand is conducted over a five-days period while chemical oxygen demand takes a few hours (Sagar *et al*., 2015).

2.2.9 Chloride

Chloride, the ionized form of chlorine, is one of the most abundant inorganic ions in natural water and wastewater. Their presence in natural water result from the leaching of chloride-containing rocks and soils with which the water comes in contact. Chloride found in domestic sewage is derived from kitchen wastes, human faeces and urinary discharge. Human faeces, for example, contain about 6g of chloride per person per day (Sagar *et al.,* 2015). Water softeners also add large quantities of chlorides. Large amount of chlorides may also enter in wastewater from industries like ice cream plant, meat salting. The chloride of wastewater can be measured by titrating the sample of wastewater with standard silver nitrate solution, using potassium chromate as indicator which is known as the potentiometric method of

chloride analysis. The effect of chloride on stomach causes discomfort and Eye/nose irritation (Sagar *et al*., 2015).

2.3 Water Quality of Reservoirs

A dam is an obstruction constructed across a stream or river. Reservoirs created by man not only suppress floods but also provide water for activities such as irrigation, human consumption, industrial use, aquaculture, navigability and hydropower generation (Bazza, 2006). A dam can be used to collect water or store water that can evenly be distributed between locations. Dams generally serve the primary purpose of retaining water, while other structures such as floodgates or levees (also known as dikes) are used to manage or prevent water flow into specific land regions (Bazza, 2006).

The form and operation of reservoirs influences water quality characteristics. Reservoirs in densely populated or agricultural areas which receive little management control, have a tendency to be highly enriched (WHO, 1997). Reservoirs show many of the same basic hydrodynamic, chemical and biological characteristics as the natural lakes . However, even when reservoirs are formed by the damming of a single river, creating water bodies which may look very much like natural lakes, the purpose for which the reservoirs were created (e.g. hydro-power generation, irrigation or domestic water supply) may significantly alter their physico-chemical character and biological responses. These responses may also be made more difficult to interpret as a result of the changing demands placed on such reservoirs over time (such as additional recreational uses). In most instances, the peculiar form of a reservoir, its mode of operation, and an unnatural location and shape may cause considerable variation of the basic limnological behaviour (WHO,1997).

Reservoirs formed by river impoundment undergo great changes in water quality during the early stages of their formation whilst a new ecological balance is becoming established.The release of organically-bound elements from flooded vegetation, excreta and soils could result in an initial high level of

biological production (sometimes called a trophic surge), suggesting a very different water quality than would actually be seen in the future. Careful assessment of the water quality is essential for the effective management of the reservoir.

2.4 Bacteriological Quality of Water

Microorganisms especially bacteria are the most common pathogens that contaminate water bodies and also serve as agents of several diseases world wide. It is known that water serves as a vehicle for a diverse array of microbial life through which these microbes gain access through food to humans and animals. Water is necessary and must be given prominence in microbial studies and analysis. Of the many infectious microorganisms found in the environment, bacteria (such as *Shigella, Escherichia coli, Vibrio*, and *Salmonella*), viruses (such as Norwalk virus and Rotaviruses), and protozoans (such as *Entamoeba*, *Giardia*, and *Cryptosporidium*) may be found in water (Johnson *et al*., 2014).

Bacteriological water analysis is a method of analyzing water to estimate the number of bacteria present and, if needed, to find out what sort of bacteria they are. It represents one aspect of water quality. It is a microbiological analytical procedure which uses samples of water and from these samples determine the concentration of bacteria. It is then possible to draw inferences about the suitability of the water for various purposes. This process is used, for example, to routinely confirm that water is safe for human consumption or that bathing and recreational waters are safe for use (Hunter *et al*., 2010).

From microbial perspective, the main public health concern associated with drinking water is enteric disease because it is impractical to look for all known enteric pathogens that may contaminate drinking water, microbial safety is assessed through detection of indicators of faecal pollution (Hunter *et al.,*2010)

Microbial indicator of faecal pollution is when an organism occurs in high numbers in human or animal faeces. If detected, indicates potential faecal (human or animal) contamination of the water body or distribution system under investigation and the consequent potential presence of enteric pathogens. Four main indicators of microbial safety of drinking water have been used: Heterotrophic Plate Counts (HPC), Total Coliform (TC), Faecal Coliform (FC) and *Escherichia coli* (*E.coli*) (Hunter *et al.,*2010).

The heterotrophic group of bacteria encompasses a broad range of bacteria that uses organic carbon sources to grow. Colony counts of heterotrophic bacteria, referred to as HPC, provide an indication of the general load of aerobic and facultative anaerobic bacteria of a water sample (Hunter *et al.,*2010). This indicator is also known as Standard Plate Count (SPC), Aerobic Plate Count (APC) and Total Plate Count (TPC).The total coliform group is a large collection of bacteria that are mostly found in the environment (Hunter *et al.,*2010). The feacal coliform group is a subset of the total coliform group that principally exists in faeces, while *E. coli* belongs to the faecal coliform group and is the only member that is specific to the intestinal tract of warm-blooded animals (Clasen *et al*., 2007).

2.4.1 Heterotrophic Plate Count (HPC)

HPC reflects the load of general aerobic bacteria in the water system. Although HPC was used in the late 1800s to assess the “purity” of source water, today, significant changes in HPC serve as an alert for possible deterioration of water quality, triggering further investigation (Clasen *et al*., 2007). An increase in HPC in finished water can indicate a problem with water treatment or a change in quality of the water source, prior to treatment (Clasen *et al*., 2007).

2.4.2 Total Coliforms (TC)

Total Coliform are no longer used as an indicator of faecal contamination because of the advances in science which show that they are not specific to the intestine of humans or warm-blooded mammals

and can also be found in the environment (Clasen *et al.,* 2007). The presence of TC in water samples can indicate the presence of a biofilm or can serve as an indicator of treatment efficiency because of their sensitivity to chlorine. However, HPC is a better indicator for that purpose because it covers a wider range of bacteria. The faecal coliform group or thermotolerant coliform is a subset of the total coliform group and comprises bacteria such as *E. coli* (Clasen *et al.,* 2007).

Analysis of water are usually performed using culture, biochemical and sometimes optical methods. When indicator organisms levels exceed pre-set triggers, specific analysis for pathogens may then be undertaken and these can be quickly detected (where suspected) using specific culture methods or molecular biology (Clasen *et al*., 2007; Hunter *et al*., 2010).

2.5 *Salmonella* as a Pathogen Associated with Water

Salmonella was first visualized in 1880 by Karl Eberth (Abatcha *et al*., 2014). However, it was Georg Theodor Gaffky that successfully grew the pathogen in pure culture four years later in 1884 (Hardy, 1999). A year after that, medical research scientist Theobald Smith discovered what would be later known as *Salmonella enterica* (var. Choleraesuis) (Abatcha *et al*., 2014). At that time, Smith was working as a research laboratory assistant in the Veterinary Division of the United States Department of Agriculture. The department was under the administration of Daniel Elmer Salmon, a Veterinary Pathologist. Initially, *Salmonella* Choleraesuis was thought to be the causative agent of hog cholera, so Salmon and Smith named it "Hog-cholerabacillus". The name *Salmonella* was not used until 1900, when Joseph Leon Lignières proposed that the pathogen discovered by Daniel Salmon's group be called *Salmonella* in his honor (Heymann *et al*., 2006; Abatcha *et al*., 2014).

2.5.1 Classification and Nomenclature of *Salmonella*

The nomenclature of *Salmonella* is controversial and still evolving. The Centers for Disease Control and Prevention (CDC) uses the nomenclatural system of *Salmonella* recommended by the World Health Organization (WHO) collaborating centre (Popoff *et al*., 2003). According to this system, the genus *Salmonella* is classified into two species, *Salmonella enterica* (type species) and *Salmonella bongori*, based on differences in their 16S rRNA sequence analysis. The type species, *S. enterica*, can be further classified into six subspecies based on their genomic relatedness and biochemical properties (Reeves *et al*., 1989). The subspecies are denoted with roman numerals: I, *S. enterica* subsp. *enterica*; II, *S. enterica* subsp. *salamae*; IIIa, *S. enterica* subsp. *arizonae*; IIIb, *S. enterica* subsp. *diarizonae*; IV, *S. enterica* subsp. *houtenae*; and VI, *S. enterica* subsp. *indica*. Among all the subspecies of *Salmonella*, *S. enterica* subsp. *enterica* (I) is found predominantly in mammals and contributes approximately 99% of *Salmonella* infections in humans and warm-blooded animals. In contrast, the other five *Salmonella* subspecies and *S. bongori* are found mainly in the environment and also in cold-blooded animals, and hence are rare in humans (Brenner *et al*., 2000). In addition to the classification of subspecies based on phylogeny, Kauffman and White evolved a scheme to further classify *Salmonella* by serotype based on three major antigenic determinants: somatic (O), capsular (K) and flagellar (H) (Brenner *et al*., 2000). The heat-stable somatic O antigen is the oligosaccharide component of lipopolysaccharide located at the outer bacterial membrane.

A specific serotype of *Salmonella* can express more than one O antigen on its surface (Hu and Kopecko, 2003). The heat-labile H antigens are found in the bacterial flagella and are involved in the activation of host immune responses. Most *Salmonella* species contain two distinct genes that encode for the flagellar proteins; these bacteria have the special ability of expressing only one protein at a time and are, therefore, called diphasic (phase I and II). Each serotype expresses specific phase I, H antigens which

are responsible for its immunological identity, whereas phase II antigens are non-specific antigens that can be shared by many serotypes (McQuiston *et al*., 2008). The surface K antigens are heat-sensitive polysaccharides located at the bacterial capsular surface and are the least common antigens found in the serotypes of *Salmonella*. Virulence (V_i) antigens, a special subtype of K antigen, are found only in three pathogenic serotypes: Paratyphi C, Dublin and Typhi. A formal identification of a specific serotype can be carried out by comprehensive serotyping of all the antigenic determinants of the bacterium. However, most clinical laboratories prefer to conduct simple agglutination reactions to antibodies or antisera specific to the somatic O antigens with the intention of grouping Salmonellae into six serogroups, designated A, B, C1, C2, D and E. This grouping system provides valuable information for epidemiological studies and allows genus identification of *Salmonella* infections (Wattiau *et al*., 2011).*Salmonella* can be further subdivided by phage typing, this method in conjunction with serotyping, pulse field electrophoresis (PFGE), determination of antibiotic resistance patterns and plasmid profiling are methodologies that provide significant information for the assessment of *Salmonella* prevalence and epidemiology (Molbak, 2004). To date, over 2500 serotypes have been identified; more than 50% of these serotypes belong to *S. enterica* subsp. *enterica*, which accounts for most of the *Salmonella* infections in humans (Guibourdenche *et al*., 2010). The term 'serovar', which is synonymous to serotype, is commonly used in the literature. The differential characteristics of *Salmonella* species and subspecies are given in Tables 2.1. although the species name '*Salmonella enterica*' has been adopted by the CDC and World Health Organization for years, it has not been accepted officially by the Judicial Commission. Therefore, the naming of a particular *Salmonella* serotype usually omits the subspecies; *Salmonella enterica* subspecies *enterica* serotype Typhi, for example, is shortened to *Salmonella* ser. Typhi or *S*. Typhi in the literature (Brenner *et al*., 2000).

Table 2.1: Species and Subspecies in the *Salmonella* Genus

***Salmonella* species**	**Subspecies**	**Number of Serovars**
Salmonnella enterica	Enterica	1,504
	Salamae	502
	Arizonae	95
	Diarizonae	333
	Houteane	72
	Indica	13
Salmonella bongori		22
Total		2,541

Source: Abulreesh, (2012).

2.5.2. Identification and Characterization of *Salmonella*

Salmonella are facultative anaerobic, gram negative motile, small rods. (Bell and Kyriakides, 2002; Molbak, 2004). Temperature for growth ranges from 8°C to 45°C, strains can stand pH between 4- 9 and is able to grow at water activities above 0.94. *Salmonella* is heat labile so the organism can be inactivated at ordinary cooking temperatures (> 70 °C) although the cooling time and values for temperature and time could change depending on the serotype and the food matrix. In addition

Salmonella has shown to tolerate up to 20% salt concentration (Molbak *et al.,* 2006; Bell and Kyriakides, 2002). Under freezing conditions (from -23°C to -18°C) this microorganism is able to survive as long as seven years (Bell and Kyriakides, 2002). The difficulty in controlling *Salmonella* is due to its ability to survive extreme environmental conditions (Molbak *et al.,* 2006).

The biochemical characteristics of *Salmonella* indicate that they are able to reduce nitrates to nitrites, produce gas from glucose (not always), produce hydrogen sulfide on triple-sugar iron agar, and are usually able to use citrate as the sole carbon source (Bell and Kyriakides, 2002; Molbak *et al*., 2006).

2.5.3 Pathogenenesis of *Salmonella*

The severity of *Salmonella* infections in humans varies depending on the serotype involved and the health status of the human host (Shu-Kee *et al*., 2015). Children below the age of 5 years, elderly people and patients with immunosuppression are more susceptible to *Salmonella* infection than healthy individuals (Bakowski *et al*., 2008; Shu-Kee *et al*., 2015). Almost all strains of *Salmonella* are pathogenic as they have the ability to invade, replicate and survive in human host cells, resulting in potentially fatal disease. *Salmonella* displays a remarkable characteristic during its invasion of non-phagocytic human host cells (Hansen-Wester *etal*., 2002) whereby it actually induces its own phagocytosis in order to gain access to the host cell.

The remarkable genetics underlying this ingenious strategy is found in *Salmonella* pathogenicity islands (SPIs), gene clusters located at the large chromosomal DNA region and encoding for the structures involved in the invasion process (Grass and Finlay, 2008). When the bacteria enter the digestive tract *via* contaminated water or food, they tend to penetrate the epithelial cells lining the small intestinal wall. SPIs encode for type III secretion systems, multi-channel proteins that allow *Salmonella* to inject its effectors across the intestinal epithelial cell membrane into the cytoplasm. The bacterial effectors then

activate the signal transduction pathway and trigger reconstruction of the actin cytoskeleton of the host cell, resulting in the outward extension or ruffle of the epithelial cell membrane to engulf the bacteria (Grass and Finlay, 2008; Shu-Kee *et al*., 2015).

The morphology of the membrane ruffle resembles the process of phagocytosis (Takaya *et al*., 2002). The ability of *Salmonella* strains to persist in the host cell is crucial for pathogenesis, as strains lacking this ability are non-virulent (Bakowski *et al*., 2008). Following the engulfment of *Salmonella* into the host cell, the bacterium is encased in a membrane compartment called a vacuole, which is composed of the host cell membrane. Under normal circumstances, the presence of the bacterial foreign body would activate the host cell immune response, resulting in the fusion of the lysosomes and the secretion of digesting enzymes to degrade the intracellular bacteria. However, *Salmonella* uses the type III secretion system to inject other effector proteins into the vacuole, causing the alteration of the compartment structure. The remodeled vacuole blocks the fusion of the lysosomes and this permits the intracellular survival and replication of the bacteria within the host cells. The capability of the bacteria to survive within macrophages allows them to be carried in the reticuloendothelial system (RES) (Monack *et al*., 2004).

2.5.4 Clinical Manifestations of *Salmonella*

Based on the clinical patterns in human salmonellosis, *Salmonella* strains can be grouped into typhoid *Salmonella* and non-typhoid *Salmonella* (NTS). In human infections, the four different clinical manifestations are enteric fever, gastroenteritis, bacteraemia and other extraintestinal complications (Sheorey and Darby, 2008).

2.5.4.1 Enteric fever

Salmonella Typhi is the aetiological agent of typhoid fever, while paratyphoid fever is caused by *S.* Paratyphi A, B and C. Since the clinical symptoms of paratyphoid fever are indistinguishable from typhoid fever, the term 'enteric fever' is used collectively for both fevers, and both *S.* Typhi and *S.* Paratyphi are referred as typhoid *Salmonella* (Connor and Schwartz, 2005). Humans are the sole reservoir for the two strains of typhoid *Salmonella*.

The organisms are transmitted *via* the ingestion of food or water contaminated with the waste of infected individuals. Enteric fever is characterized by an incubation period of one week or more, with prodromal symptoms such as headache, abdominal pain and diarrhoea (or constipation), followed by the onset of fever (Bhan *etal*., 2005). Diarrhoea is more commonly observed in children, whereas patients with immunosuppression are more likely to develop constipation (Thielman and Guerrant, 2004). During the illness, enteric fever displays a specific fever pattern with an initial low-grade fever (>37.5°C to 38.2°C) which slowly develops to high-grade fever (>38.2°C to 41.5°C) in the second week. If the patient is left untreated, fever can persist for a month or more (Patel *et al*., 2010). Besides fever, infected patients may also develop myalgia, bradycardia, hepatomegaly (enlarged liver), splenomegaly (enlarged spleen), and rose spots on their chest and abdomen (Kuvandik *et al*., 2009). In endemic regions, approximately 15% of the infected patients develop gastrointestinal complications which include pancreatitis, hepatitis and cholecystitis (Kuvandik *et al*., 2009; Patel *et al*., 2010).

Haemorrhage is one of the most severe gastrointestinal complications that occur as a result of perforation of Peyer's patches, lymphatic nodules located at the terminal ileum, resulting in bloody diarrhoea. also, the ability of typhoid *Salmonella* to survive and persist in the reticuloendothelial system results in relapse in approximately 10% of the infected patients (Parry *etal*., 2002).

2.5.4.2 Gastroenteritis

Salmonella strains other than *S.* Typhi and *S.* Paratyphi are referred to as Non Typhoidal *Salmonella* (NTS), and are predominantly found in animal reservoirs. NTS infections are characterized by gastroenteritis or 'stomach flu', an inflammatory condition of the gastrointestinal tract which is accompanied by symptoms such as non-bloody diarrhoea, vomiting, nausea, headache, abdominal cramps and myalgias. Symptoms such as hepatomegaly and splenomegaly are less commonly observed in patients infected with NTS (Hohmann, 2001). Compared to typhoid infections, NTS infections have a shorter incubation period (6–12 h) and the symptoms are usually self-limiting and last only for 10 days or less (Crump *et al*., 2008). Gastrointestinal complications of NTS infections include cholecystitis, pancreatitis and appendicitis, while the perforation of the terminal ileum has no association with NTS infections (Hohmann, 2001). Infants, young children, elderly people and immunocompromised patients are highly susceptible to NTS infections and develop more severe symptoms than normal individuals (Scallan *et al*., 2011).

2.5.4.3 Bacteraemia and other extraintestinal complications

Salmonella bacteraemia is a condition whereby the bacteria enter the bloodstream after invading the intestinal barrier. Almost all the serotypes of *Salmonella* can cause bacteraemia, while *S.* Dublin and *S.* Cholearaesuis are two invasive strains that are highly associated with the manifestations of bacteraemia (Woods *et al*., 2008).

Similar to enteric fever, high fever is the characteristic symptom of bacteraemia, but without the formation of rose spots as observed in patients with enteric fever. In severe conditions, the immune response triggered by bacteraemia can lead to septic shock, with a high mortality rate (Hohmann,2001). The clinical manifestation of bacteraemia is more commonly seen in NTS infections than in typhoid *Salmonella* infections. The difference in the clinical manifestation is believed to be associated with the presence of the *spv* (*Salmonella* plasmid virulence) gene in NTS which causes non-typhoidal

bacteraemia, based on genetic analysis (Guiney and Fierer, 2011). Although the mechanism of the gene to enhance the virulence traits of NTS remains unclear, expression of the gene is required to prolong apoptotic cell death and this may allow the bacteria to persist in the host cells for a longer period (Gulig *et al*., 1993).

Approximately 5% of patients infected with NTS develop bacteraemia and, in some cases, extraintestinal complications occur, with the lung being the most commonly compromised organ. Other extraintestinal complications include cellulitis, urinary tract infections, pneumonia, endocarditis and meningitis (Shimoni *et al*., 1999; Arii *et al*., 2002).

2.5.4.4 Chronic Carrier State

The status of chronic carrier is defined as the shedding of bacteria in stools for more than a year after the acute stage of *Salmonella* infection (Shu-Kee *et al*., 2015). Since humans are the only reservoir of typhoid *Salmonella*, carriers of *S.* Typhi and *S.* Paratyphi are responsible for the spreading of enteric fever in endemic regions, as the common transmission route is the ingestion of water or food contaminated with the faeces of chronic carriers (Bhan *et al*., 2005). About 4% of patients with enteric fever, predominantly infants, elderly people and women, may become chronic carriers (Gonzalez-Escobedo *et al*., 2011). In contrast, the carrier state of NTS is less frequent, with an occurrence rate of 0.1% in patients with non-typhoidal salmonellosis. This is because the primary reservoir of NTS is animals, instead of humans (Hohmann, 2001; Shu-Kee *et al*., 2015).

2.6 Diagnosis and Detection of *Salmonella* in Environmental Samples

There are four steps for the recovery of injured *Salmonella* cells from the environment.

The first is the pre-enrichment, where buffered peptone water or lactose broth can be used,followed by growth on a non-selective broth. This is followed by enrichment in selective broth, such as Rasspaport-

Vasilliadis (RV) Broth, Selenite Cysteine Broth (SC), or tetrationate Broth (TT). Finally the subsequent isolation is done on selective medium such as Brillant green agar, Bismuth sulfite agar, Hektoen agar (HA) or Xylose Lysine Desoxycholate (XLD) agar (Molbak *et al*., 2006).

Some strains of *Salmonella* could present different reactions to the combinations of inhibitory substances in the isolation media incubation temperatures, selective enrichment broths and media (Cardinale *et al*., 2003). Some *Salmonella* serotypes (*S*. Anatum, *S*. Tennessee, *S*. Newington and *S*.Senftenberg) are lactose positive cultures (Chen *et al.,* 2004), for that reason it is important not to relyonly on lactose to distinguish *Salmonella* from other microorganisms present but to utilize alternative selective media such as Mannitol Lysine Crystal VioletBrillant Green (MLCB) or Bismuth Sulphite Agar (Bell and Kyriakides, 2002).

There are a wide variety of methods commercially available for *Salmonella* detection and identification. These include the use of antibodies to *Salmonella* antigens (Enzyme-Linked Immuno-sorbent assay (ELISA), immuno-chromatography, chemiluminescent immunoassay, antibody coated dipsticks, latex agglutination), electrical conductance methods, and polymerase chain reaction (PCR) (Bell and Kyriakides, 2002; Molbak *et al*., 2006). The principle limitation is that all of these techniques need a pre-enrichment step to reach detectable numbers of cells inthe sample (10^4 -10^5 cells/ml). This factor makes it very difficult to develop a truly rapid method for detection and identification of *Salmonella* that would allow processing thesample in a normal 8-hours work day (Bell and Kyriakides, 2002, Molbak *et al*., 2006). The capability to serotype or fingerprint is of importance for surveillance, inspection, and investigation of outbreaks (Molbak *et al.,* 2006).

*Salmonella*s subtyping can be accomplished by biotyping, phage typing, antibiotic resistant patterns, pulse field gel electrophoresis and ribotyping (Chen *et al.,* 2004). The development of DNA-based methods for detection of *Salmonella,* have allowed for novel approaches in this field. The foundation of

these methods is the hybridization oftwo complementary single –stranded molecules (one in the form of a probe, primer, DNA fragment or oligonucleotides developed in the laboratory and the other strand corresponds to the target microorganism) to obtain double- stranded nucleic acid molecules under defined physical and chemical conditions. Other diagnostic tools for *Salmonella* are the DNA microarrays. These are biochips, which enables hybridization by the presence of immobilized oligonucleotides to a solid base. Results can be analyzed automatically with use of an appropriate device (Molbak *et al*., 2006). Numerous probes can be placed on a DNA chip and that number is expanding because of the continued growth of fully sequenced organisms (Molbak *et al*.,2006).

Molecular methods have been investigated to provide increased specificity and sensitivity for the detection of low cell numbers compared to culture based methods (Harwood *et al.*, 2004). Molecular methods are mostly independent of culturability of bacteria, and require no additional conformational steps (Kimura *et al.,* 1999). Among the different molecular methods, PCR has proved very useful for detecting low amounts of a specific DNA against a large background of prokaryotic and eukaryotic cells, and organic materials present in environmental samples (Temelli *et al*., 2010). DNA has to be isolated and substatially purified before it is amplified with PCR. Bacterial DNA purification consequently becomes an indispensable preliminary step for any PCR reaction (Nam *et al*., 2005). The availability of effective DNA extraction methods is essential for environmental microbiology methods (Espy *et al.,* 2006).

2.6.1 Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction is a rapid, oligonucleotide primer-directed *in vitro* method for replicating defined DNA sequences from target organisms (Lee *et al.,* 2009). The Polymerase Chain Reaction has been used to detect a wide variety of microorganisms directly in clinical specimen including bacteria in blood, cerebrospinal fluid (CSF), and tissue. It is also been used to characterize antimicrobial resistance

genes in bacterial isolates. Most PCR methods typically amplify DNA fragments of up to 10 kilo base pairs (kb). Some PCR methods can copy DNA fragments of up to 40kb in size which is still much less than the total nuclear DNA content of eukaryotic cell – for comparison, the haploid genome of a human cell consists of about three billion DNA base pairs (3Gb). With the development of the polymerase chain reaction technique, it is now possible to amplify any piece of DNA.

Polymerase Chain Reaction, as currently practiced, requires several basic components. As a consequence of the speed, specificity and sensitivity of the PCR, the procedure has become one of the most widely used assays for direct detection of low levels of pathogenic microbes in environmental samples as opposed to the cultural methods (Daum *et al.*, 2002).

The efficiency of PCR detection however depends on several factors. The selection of an appropriate DNA extraction method and the pre-PCR procedures are very crucial factors (Yan *et al*., 2014). Since PCR analyses are based on detection of intact nucleic acids rather than intact viable cells, the possibility exists that positive pathogen PCR amplifications may arise from either dead cells or non-infectious cells (Yan *et al.,* 2007).

2.7 The Use of Antibiotics

Traditionally, the term 'antibiotic' refers to substance produced by microorganisms that at low concentration kill or inhibit the growth of other microorganisms but cause little or no host damage. The term antimicrobial agent refers to any substance of natural, synthetic, or semisynthetic origin that at low concentration kills or inhibits the growth of microorganisms but causes little or no host damage. Neither antibiotics nor antimicrobial agents have activity against viruses (Reeves, 2012;European Centre for Disease Prevention and Control, 2014; Rowland, 2015).

A limited number of antibiotics also possess antiprotozoal activity (Reeves, 2012). Antibiotics are not effective against viruses such as the common cold or influenza, and their inappropriate use allows the emergence of resistant organisms (European Centre for Disease Prevention and Control ECDC, 2014).

Sometimes the term antibiotic (which means "opposing life") is used to refer to any substance used against microbes, synonymous with antimicrobial (Reeves, 2012). Some sources distinguish between antibacterial and antibiotic; antibacterial are used in soaps and disinfectants, while antibiotics are used as medicine (Reeves, 2012).

Antibiotics revolutionized medicine in the 20th century, and have together with vaccination led to the near eradication of diseases such as tuberculosis in the developed world. However, their effectiveness and easy access led to overuse (Reeves, 2012), especially in livestock production, prompting bacteria to develop resistance. This has led to widespread problems with antimicrobial and antibiotic resistance, so much as to prompt the World Health Organization to classify antimicrobial resistance as a "serious threat that is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect any one, of any age, in any country" (WHO, 2014).

2.7.1 Classes of Antibiotics

Antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity. Most target bacterial functions or growth processes (Calderon and Sabundayo, 2007). Those that target the bacterial cell wall (penicillins and cephalosporins) or the cell membrane (polymyxins), or interfere with essential bacterial enzymes (rifamycins, lincomycins, quinolones, and sulfonamides) have bactericidal activities.

Those that target protein synthesis (macrolides, lincosamides and tetracyclines) are usually bacteriostatic (with the exception of bactericidal aminoglycosides) (Finberg *et al.*, 2004). Further

categorization is based on their target specificity. "Narrow-spectrum" antibiotics target specific types of bacteria, such as gram-negative or gram-positive, whereas broad-spectrum antibiotics affect a wide range of bacteria. Following a 40-year break in discovering new classes of antibacterial compounds, four new classes of antibiotics have been brought into clinical use in the late 2000s and early 2010s: cyclic lipopeptides (such as daptomycin), glycylcyclines (such as tigecycline), oxazolidinones (such as linezolid), and lipiarmycins (such as fidaxomicin) Cunha, 2009: Srivastava*et al*., 2011)

2.7.2 Antibiotic Resistance

Antibiotic resistance is defined as the ability of bacteria to stop the inhibitory (bacteriostatic) or killing bactericidal) effects of antibiotics to which it is previously sensitive and susceptible (Lee *et al.,* 2009). An organism can be resistant intrinsically to an antibiotic agent due to changes in the genetic constituent and it can also be susceptible naturally (Forbes *et al.,* 2002). The antimicrobials are characterized by their specificity of targeting entities or organisms; an example is antiviral agent, antibacterial drugs and antifungal (CDC, 2008).

In the early 20th century, penicillin was isolated from the fungus *Penicillium notatum*. A decade later, the mass pharmaceutical production of antibiotics started that saved many lives and transformed the beginning for modern medicine (Abatcha *et al*., 2014). Penicillin was widely available commercially in 1943, and the demand grew drastically. It was perceived as a miracle drug and people developed generalized expectations for a quick and rapid cure. Unfortunately, the overuse of the antibiotics led to the emergence of resistant strains (Levy, 2002). Over the years, there was increasing concern about the prevalence of resistance that was reported in many pathogens, spreading rapidly in different geographical regions of the world (Byarugaba, 2005). This has been as a result of selective pressures of antimicrobial use, changes in microbial characteristics, and technological changes that enhanced the modern development and transmission of drug-resistant microbes. At the same time, antimicrobial

resistance is a natural occurring phenomenon, and it is often enhanced as a result of infectious etiological agents' adaptation and exposure to antimicrobials used in humans and veterinary medicine or widespread abuse in agricultural farm and disinfectants at household levels (Walsh, 2000).

2.8 Resistance as an Emerging Problem

Resistance to antimicrobials and particularly multidrug resistance is an emerging problem in Enterobacteriaceae in both developing and developed countries (White, 2005; Odeyemi, 2012). Resistant microorganisms have emerged as a result of improper use of antibiotics in human health as well as in agricultural practices (Akinyemi *et al.,*2006). For example, in United States it has been reported that most of the antibiotics produced are fed to farm animals as growth promoters and to obtain a better meat to feed ratio (WHO, 2014). Over the time these low doses of antimicrobials confer the ability of microorganisms to evolve mechanisms of defence, therefore making them less susceptible to the effect of the drug and contributing to treatment failure.

2.8.1 Antibiotic Resistant *Salmonella*

The misuse of antimicrobial agents as chemotherapy in human and veterinary medicine or as growth promoter in food animals can potentially lead to widespread dissemination of antimicrobial resistant *Salmonella* and other pathogens via mobile genetic elements (Bouchrift *et al.,* 2009). Recent findings showed the consequences of antibiotic resistance on human health (Abatcha *et al*., 2014). These consequences can be divided into two categories: first, the infections that may not have occurred and secondly, the high incidence of treatment failures and increase in the severity of disease (WHO, 2009). Moreover, resistance can spread from non-human sources to human by various routes such as animal, water and contaminated foods. Contact with *Salmonella* carrier animal is the most important pathway in transmission of resistance to humans. Resistance to combinations of many classes of antimicrobial

agents in *Salmonella* has led to the re-emergence of Multidrug Resistance *Salmonella* (MDR) strains that may pose risk to humans (White *et al*., 2001; O'Brien, 2002). Many researches in the last two decades indicated the occurrence of MDR strains in different *Salmonella* serovars (Abatcha *et al*., 2014; WHO, 2014). MDR *Salmonella* strains have been found to be of many serotypes such as Agona, Anatum, Pullorum, Schwarzengrund, Choleraesuis, Derby, Dublin, Heidelberg, Kentucky, Newport, Senftenberg, Typhimurium, and Uganda (Chen *et al.,* 2004; Gebreyes and Thakur, 2005; Zhao *et al.,* 2008). Nowadays, interest in antimicrobial resistance *Salmonella* has increased. The emergence and persistence of antibiotic resistance in *Salmonella* species continue to pose serious risks to human health (Joseph *et al*., 2008). The most common *Salmonella* serotype associated with multidrug resistance is *S.* Typhimurium definitive phage type DT104 found to display a phenotype of resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT), these are the most common drug classes used in veterinary medicine (Mulvey *et al.,* 2006).The World Health Organization's Categorization of Antimicrobials of Critical Importance to Human Medicine is shown in Table 2.2**.**

This strain is followed by other serotypes such as *S*. Typhi, *S*. Paratyphi, *S*. Infantis, *S*. Uganda, *S*. Agona, and *S*. Newport (Holt *et al.*, 2007; Nógrády *et al*., 2007; Zhao *et al*., 2007).In Nigeria, morbidity associated with illnesses due to *Salmonella* continues to be on the increase and, in some cases, resulting in death. New concerns have been identified. Since the beginning of the 1990s, strains of *Salmonella* which are resistant to a range of antimicrobials, including first-choice agents for the treatment of humans, such as chloramphenicol and cotrimoxazole, and the third-generation cephalosporins, have emerged and are threatening to become a serious public health problem (Akinyemi *et al.,* 2006). Although Fluoroquinolones have been demonstrated to be efficatious both *in vitro* and *in vivo* in the treatment of severe *Salmonella*-associated illnesses, strains with reduced susceptibility to ciprofloxacin among travelers have been reported in some parts of the globe (Hakanen *et al.,* 2001). Multi-drug resistance to critically important antimicrobials is compounding the problems. *Salmonella,* have been

widely documented to possess resistance to several antibiotics used in medical treatment. For instance, antibiotic-resistant *Salmonella* accounted for an annual mortality estimate of 4,760 deaths in the U.S alone (WHO, 1998).

Antibiotic resistance has an important social and economic impact, and there is a need for stronger scientific and public health efforts to better regulate, control and monitor the use and abuse of antimicrobials (WHO, 2014). Due to the concern over increasing resistance, the CDC, FDA-CVM, (Center for Veterinary Medicine) and USDA-FSIS (Food Safety and Inspection Service and Agricultural Research Services) established the National Antimicrobial Resistance Monitoring System (NARMS) for Enteric Bacteria to monitor antimicrobial resistance among food borne enteric bacteria isolated from humans and foods (CDC, 2009). In 2006, the NARMS published a list containing the categories of antimicrobials of importance for human health antimicrobials in this list are classified based on whether the evaluated antimicrobial is unique or one of the few alternatives for treatment of human diseases (NARMS, 2006). This report also details two multidrug resistance patterns. 5.5 % of non-Typhi *Salmonella* are thought to be resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole/ sulfisoxazole, and tetracycline (ACSSuT) (NARMS, 2006). This percentage is lower than the 8.8% observed in 1996 due to the overall reduction of resistance in some serovars. However other *Salmonella* serovars appear to be acquiring resistance to these antibiotics, such as *S*. Newport. Evaluated in 2006, the resistance of this serotype increased 6% from 1996. According to The CDC (2009), a second multidrug resistance pattern (Ampicillin, Streptomycin, Chloramphenicol, Sulfamethoxazole, Tetracycline, Amoxicillin clavulanic acid, and ceftiofur) was not detected in any serotype in 1996. In 2006, a 2% increase was observed among non- typhi *Salmonella* serovars (NARMS, 2006).

Table 2.2: World Health Organization Categorization of Antimicrobials of Critical Importance to Human Medicine

	Categorization of Antimicrobial	Subclass
Critically Important	Aminoglycosides	Amikacin
		Gentamicin
		Streptomycin
	Aminopenicillins	Ampicillin
	B-Lactamase inhibitor combinations	Amoxicillin-clavulanic acid
	Cephalosporins(3rd generation)	Ceftriaxone
	Ketolides	Telithromycin
	Macrolides	Azithromycin
		Erythromycin
	Quinolones	Ciprofloxacin
		Nalidixic acid
Highly Important	Aminoglycosides	Kanamycin
	Cephalosporin (1st generation)	Cephalothin
	Cephamycins	Cefoxitin
	Folate pathway inhibitor	Trimethoprim-sulfamethoxazole
	Phenicols	Chloramphenicol
	Sulfonamides	Sulfamethoxazole
		Sulfisoxazole
Important	Tetracyclines	Tetracycline

Lincosamides	Clindamycin

Source: (NARMS, 2006).

2.8.2 Mechanism of Antibiotic Resistance by *Salmonella*

The mechanisms for antibiotic resistance can be categorised as (i) modification or destruction of the antimicrobial agent, (ii) pumping the antimicrobial agent out from the cell by efflux pumps, (iii) modification or replacement of the antibiotic target, and (iv) decrease in cell membrane permeability (Abatcha *et al*., 2014).

Thus, microorganisms are developing resistance mechanisms by developing mutations in the gene locations of target proteins or acquiring mobile genetic elements carrying resistance genes such as plasmid, integrons and transposons (Walsh, 2003).

There are many classes of antimicrobial drugs, but the most common antimicrobials that *Salmonella* has developed resistance are namely; aminoglycosides, β- lactams, chloramphenicol, quinolones, sulfonamides and trimethoprim (Abatcha *et al*., 2014).

2.8.2.1 Aminoglycoside Drugs

The aminoglycosides are active and effective against gram negative bacteria. It can also be used in combination with other antibiotics drugs to have broad-spectrum of activity (Gonzalez and Spencer, 1998). Majority of aminoglycosides are bactericidal, while others like spectinomycin inhibit the bacterial growth and are bacteriostatic by mode of action (Mascaretti, 2003).

There are three mechanisms by which bacteria become resistant to aminoglycosides:Theses include reduction in antibiotic uptake or decreased permeability, alteration of ribosomal binding sites and antibiotic modifications.

The *Salmonella* uses mechanisms such as expression of plasmid-mediated aminoglycoside modifying enzymes against aminoglycoside (Gebreyes and Altier, 2002; Guerra *et al*., 2002). According to Shaw *et al*. (1993), in their short review of aminoglycosides, these enzymes are categorised into three groups and are named based on reactions they perform; this includes acetyltransferases, phosphotransferases, and nucleotidyltransferases.

Aminoglycoside acetyltransferases (AAC), catalyze acetyl CoA-dependent aceytylation of an amino group (Shaw *et al*., 1993; Mascaretti, 2003). There are four groups of this enzyme based on the areas that they alter: AAC (1), AAC (2'), AAC (3), and AAC (6') (Mascaretti, 2003). Also, genes encoding these enzymes are also typically designated aac (Vanhoof *et al.,* 1998). Many of this genes have been found in varieties of *Salmonella* subtypes, including Agona, Typhimurium, Newport, Typhimurium var. Copenhagen Kentucky and 4,5,12 (Chen *et al.,* 2004; Doublet *et al*., 2004; Levings *et al*., 2005; Mulvey *et al*., 2006). The aac genes have been found as part of *Salmonella* pathogenic islands (Doublet *et al*., 2004), integrons (Pai *et al*., 2003; Levings *et al*., 2005), and plasmids (Guerra *et al*., 2001). Aminoglycoside acetyltransferases provide resistance to tobramycin, gentamicin, and kanamycin (Mascaretti, 2003).

Enzymes such as Aminoglycoside phosphotransferases (APH), catalyzes ATP-dependent phosphorylation of a hydroxyl group (Shaw *et al*., 1993; Mascaretti, 2003). This is classified into groups depending on the specific sites of phosphorylation. Groups APH (3") and APH (6), provide resistance to streptomycin (Mascaretti, 2003) and have been found encoded on plasmids harbored by *Salmonella* (Gebreyes and Altier, 2002). Most genes having these encoding enzymes are designated as aph (Vanhoof *et al.,* 1998) and these genes, aph (3")-Ib and aph (6-Id), Ib and Id refers to the type of modification .Theys are

commonly known as *strA* and *strB*, (Streptomycin resistance) respectively (Shaw *et al.*, 1993; Madsen *et al*., 2000). *Salmonella* serotypes Blockely, Bredeney, Agona, Anatum, Derby, Give, London, Saintpaul, Hadar, Heidelberg, and Typhimurium have been found to possess genes from both families (Madsen *et al*., 2000; Pezzalla *et al*., 2004). Genes encoding enzymes of the APH (3') subgroup provide resistance to kanamycin and neomycin (Mascaretti, 2003), and have been found in several *Salmonella* subtypes such as Enteritidis, Haardt, Derby (Chen *et al*., 2004), Typhimurium (Gebreyes and Altier, 2002), and Typhimurium var. Copenhagen (Frech *et al.,* 2003).

The nucleotidyl transferase is the final group of enzymes providing aminoglycoside resistance (Shaw *et al*., 1993; Mascaretti, 2003). These enzymes are divided into several groups based on the site of modification and also target the hydroxyl groups. Genes encoding these enzymes are usually designated aad (Vanhoof *et al.,* 1998), some are also designated as ant. The gene (amino glycoside O adenyl transferase) aad A, is referred to as ant (3") (Shaw *et al*., 1993), found in *Salmonella* providing resistance for streptomycin (Mascaretti, 2003). Also many of these variants genes have been found in serotypes Bredeney, Derby, Agona, Anatum, Enteritidis, Give, Heidelberg, Saint Paul, and Typhimurium (Madsen *et al.,* 2000; Chen *et al*., 2004; Pezzella *et al.,* 2004). The aad B gene, also known as ant(2')-Ia (Shaw *et al.*, 1993), confers resistance to tobramycin and gentamicin (Mascaretti, 2003). It has been found in serotypes Typhimurium and Typhimurium var Copenhagen (Frech *et al*., 2003; Antunes *et al*., 2005). Both aad A and aad B have been found in integron-borne gene cassettes (Winokur *et al*., 2001; Pezzalla *et al*., 2004).

2.8.2.2 Beta-lactam Antibiotics

These family or groups comprise penicillins derivatives, cephalosporins, carbapenems and monobactams (Petri, 2006; Queenan and Bush, 2007). Their mechanism of action is by interfering Penicillin-Binding Proteins (PBPs), a group of seven proteins. These proteins facilitate the synthesis of peptidoglycan, an

important component of the bacterial cell wall. Beta lactams are generally considered bactericidal; also the activity varies among betalactam organisms, and target PBP. Furthermore for enteric bacteria such as *Salmonella* and *E. coli*, it appears that inhibition of the essential PBPs, 1 through 3, leads to bactericidal activity. Many organisms are now becoming resistant to ampicillin and methicillin due to their wide clinical use (Angulo *et al*., 2000). Also, as a consequence of this, cephalosporins were developed as second class of beta lactams. There is close structural similarity between Cephalosporins and penicillins, but they have a 6, rather than a 5 member beta- lactam ring (Hornish and Kotarski, 2002). These structural differences provide cephalosporins with a wide range of efficacy and stability. Cephalosporins are grouped into four generations according to their spectrum of activity and the time of the agent's introduction (Hornish and Kotarski, 2002). The increased use of the drugs for treatment has made microorganisms more resistant. The carbapenems are more effective against gram positive and gram negative bacteria and other beta-lactam family, and are very stable against beta-lactamases. They diffuse easily in bacteria they are considered as broad spectrum β-lactam antibiotic. For this reason, their clinical use are reserved for a multidrug resistance bacteria.

There is an indication of resistance to carbapenems such as imipenem by *Salmonella* species (Arman-Lefevre *et al*., 2003; Miriagou *et al*., 2003). Beta-lactams transverse the bacterial cell wall to reach their targets PBP by using two porins OmpC and OmpF which facilitate the passage (Jaffe *et al.*, 1982). Studies have shown that decreases in either OmpF or OmpC porin levels have generated an increase in resistance (Medeiros *et al*., 1987; Bellido *et al.,* 2002). In other report, decrease in porin content found that the reduction in OmpF and OmpD porin expression actually leads to decreased resistance to most beta-lactams other than mecillinam and imipenem in *Salmonella* envB mutants, this is due to the other effect of envB mutation on the organisms (Oppezzo *et al*., 1991).

Beta-lactam resistance is mediated by Extended-Spectrum β-Lactamase (ESBL) genes that are mostly encoded by plasmids. ESBLs, a heterogeneous group of enzymes, are encoded by genes which have been described that confer resistance to third and fourth generation cephalosporins and monobactams but are inhibited by β-lactamase inhibitors (Abatcha *et al*., 2014). Cefotaximase (CTX-M), temoneira (TEM) and sulfhydryl variable (SHV) are class A ESBLs. To date, there is growing concern about more than 340 beta lactamases resistance genes, such as *blaTEM, blaOXA, blaPER, blaPSE, blaSHV, blaCTX-M,* and *blaCMY* with *blaTEM* being the most common among *Salmonella*., while some are more prevalent in *Salmonella* globally (Armand-Lefevre *et al.*, 2003; Abatcha *et al*., 2014). The genes *bla*TEM-1 and *blaTEM*-52 have been found in many *Salmonella* serotypes including Enteritidis, Dublin, Haadrt, Muenchen, Panama and Typhimurium (Chen *et al.,* 2004, Gebreyes and Thakur, 2005). In *Salmonella,* the secretion of a beta-lactamase is the common mechanism of resistance to beta-lactams. This enzyme acts by hydrolyzing the structural rings of the Beta lactam, by producing beta amino acids with no antimicrobial activity. In *Salmonella* encoding genes are found or carried on the plasmid (Mascaretti, 2003). Ambler classification of beta lactamases is the most widely used. It divides β-lactamases into four classes (A, B, C and D) based upon their sequences of amino acid (Mascaretti, 2003). Moreover, in *Salmonella* the class A beta- lactamases are the most commonly found class of beta-lactamases. They provide a range of resistance against penicillins, cephalosproins, and carbapenems and are plasmid encoded. The *blaKPC-2* is a class A beta- lactamase gene, which confers resistance to imipenem, was discovered in a *Salmonella* serotype Cubana isolate (Miriagou *et al.,* 2003). The emergence of another class A beta-lactamases, known as cefotaximases (CTX-M), which confer resistance to ampicillin and cephalosporins, is of clinical importance (Batchelor *et al.,* 2005). Also in *Salmonella* serotypes Anatum, Enteritidis, Stanley, Typhimurium, and Virchow, *BlaCTX-M* variants have been identified (Weill *et al*., 2004; Batchelor *et al.,* 2005).

The class C beta-lactamases is the second most common class of beta-lactamases that provides resistance against cephalosporins such as cefoxitin and ceftiofur that are encoded by chromosomal *ampC* genes. These genes are harboured in plasmids carried by *Salmonella* (Morosini *et al*., 2000). However, more research is primarily focused on *bla*CMY-2 which is present and has been associated with resistance to ceftiofur (Alcaine *et al.,* 2005). Ceftiofur is a group of third generation cephalosporin and are closely related to ceftriaxone. The resistance to this drugs and the spread of the gene is a public health problem worldwide as ceftriaxone is a drug of choice for treating *Salmonella* infection in infant. Many *Salmonella* serotype such as Typhimurium, Agona, and Newport (Doublet *et al*., 2004; Alcaine *et al.,* 2005), have been found to carry these resistance genes (Winokur *et al.,* 2001; Alcaine *et al*., 2005).

The Class B beta-lactamases like Metallo-beta-lactamases, provide resistance to all beta-lactam antibiotics, including carbapenems (imipenem) and are usually encoded chromosomally, though plasmid mediated class B beta-lactamases, such as IMP-1 and VIM-1, have been reported (Mascaretti, 2003). These Class B beta-lactamases are not commonly found in *Salmonella*.

At last Class D beta-lactamases are uncommon among *Salmonella.* This class of enzymes provides resistance to lactams closely related to oxacillin, such as cloxacillin and methicillin. The gene *bla*OXA-1 was found in a *Salmonella* serotype Paratyphi (Cabrera *et al.,* 2004) and *bla*OXA-30 has been found in serotypes Muenchen and Typhimurium (Hanson *et al*., 2002; Antunes *et al.,* 2006).

2.8.2.3 Chloramphenicol Antibiotics

Chloramphenicol is a broad-spectrum antibiotics against both the gram negative and gram positive bacteria and its effectiveness and ability to cross the blood-brain barrier makes it the drug of choice for systemic infections therapy.

Chloramphenicol is specific and potent inhibitor of protein synthesis by binding to the peptidyltransferase center of the 50s ribosomal unit, thus preventing formation of peptide bonds (Mascaretti, 2003). As a result of the binding to the relevant enzymes, the drugs will prevent elongation of the peptides.

Chloramphenicol has been used in human and veterinary medicine for the treatment of Salmonellosis for a long time and this has given rise to resistance strains. There are two mechanisms by which *Salmonella* resistance to chloramphenicol are conferred: (i) by the plasmid-borne enzyme called chloramphenicol acetyltransferases (CAT) or nonenzymatic chloramphenicol resistance gene cm1A and (ii) Efflux pump in which the antibiotic is removed. *Salmonella* Typhi isolates have been found to encode genes for CAT and are plasmid-borne (Guerra *et al.,* 2000). However, CAT genes, such as *cat1* and *cat2*, have also been found in *Salmonella* serotypes such as Derby, Haardy, Enteritidis and Typhimurium (Chen *et al.,* 2004). The *cmlA* (Cabrera *et al.,* 2004) and *floR* (White *et al.,* 2001) are closely related genes encoded in Chloramphenicol eflux pumps in *Salmonella* that have been reported. Also *floR genes* appear to be very wide spread in *Salmonella*, whereas *cmlA* is less widely distributed. There are various *Salmonella* serotypes such as Agona, Kiambo, Albany, Newport, Typhimurium, Typhimurium var Copenhagen that have been found to carry *floR* (Meunier *et al.,* 2003; Cabrera *et al*., 2004; Doublet *et al.,* 2004; Alcaine *et al.,* 2005). This genes has been found in *Salmonella* pathogenicity islands (Weill, *et al*., 2004), as well as in many different plasmids due to high mobility (Meunier *et al*., 2003), and closely associated with multi-drug resistance (Doublet *et al.,* 2004; Alcaine *et al.,* 2005) most likely due to its presence on plasmids carrying multiple resistance genes.

2.8.1.4 Quinolones Drugs

There are many generations of quinolones, which are more effective against bacterial infection. However, their mode of action varies, the early and late generation of quinolones target DNA gyrase and DNA topoisomerase IV (Mascaretti, 2003).

Quinolones and fluoroquinolones bind to and prevent DNA processing enzymes such as topoisomerases from assisting in DNA replication and maintenance (Hopkins *et al.,*2005). The mutations occur in the quinolone resistance determining region (QRDR) which is a conserved site in these enzymes targeted by these antibiotics. Resistance to nalidixic acid and to fluoroquinolones builds in a stepwise process of mutations in the QRDR region producing an enzyme with a target region that quinolones cannot bind to Chen *et al.,*2007; Zaki and Karande, 2011).Quinolones do not actually bind to the topoisomerase, but to the double stranded DNA in the topoisomerase complex (Shen and Pernet, 1985). There are reports of *Salmonella* with low-level resistance to quinolones and resistance to nalidixic acid (Molbak *et al.,* 1999; Breuil *et al*., 2000; Olsen *et al*., 2001). *Salmonella* resistance to quinolone has been classified into two mechanisms. mediated by the product of *gyrA* and *gyrB*, genes which encode for the subunits of DNA gyrase, targeting mutations in the quinolone resistance-determining region (QRDR), and in the *parC* subunit of topisomerase IV (Casin *et al*., 2003; Baucheron *et al*., 2004; Levy *et al*., 2004). Also the second mode of action involves changes in the AcrAB-TolC efflux system expression, as a result of mutations in the regulator genes of this system. Over expression of this efflux system decreases sensitivity to quinolone (Levy *et al.,* 2004; Olliver *et al*., 2005). However, it is an accumulation of all these mutations that provides resistance, because no single mutation confers high-level resistance to quinolones (Heisig, 1993). However, acquiring multiple of unlinked mutations by *Salmonella* is necessary because some of those mutations reduce fitness, particularly those involved in the regulation of the efflux pump (Giraud *et al*., 2003). In some bacteria such as *E. coli* and *Klebsiella pneumoniae* (Wang *et al*., 2004), the expression of a plasmid mediated gene called *qnr* has also been linked to quinolone resistance (Li, 2005). The gene expresses a protein that appears to bind to DNA-gyrase and prevent quinolone inhibition (Li,

2005). Martinez *et al.* (2005), reported that plasmids harboring *qnr* could be transferred via conjugation from other bacterial species to *Salmonella*.

2.8.2.5 Sulfonamide and trimethoprime Antibiotics

These classes of antibiotics are bacteriostatic and their mode of action is by competitively inhibiting enzymes involved in the synthesis of tetrahydrofolic acid. Sulfonamides inhibit dihyrdropteroate synthetase (DHPS), while trimethoprim inhibits dihydrofolate reductase (DHFR) (Mascaretti, 2003; Abatcha *et al*., 2014). The resistance of *Salmonella* to sulfonamide has been attributed to the presence of an extra *sul* gene which expresses an insensitive form of DHPS (Mascaretti, 2003; Antunes *et al*., 2005). The *sul1*, *sul2*, and *sul3* are the three main genes that have been indentified; The gene *sul1* has been harboured by a wide range of *Salmonella* serotypes such as Enteritidis, Hadar, Heidelberg, Orion, Rissen, Agona, Albany, Derby, Djugu, and Typhimurium (Chen *et al*., 2004; Doublet *et al*., 2004; Antunes *et al.,* 2005). The class I integrons that contain other resistance gene, have often been associated with this gene (Sandvang *et al.,* 1998; Guerra *et al*., 2002). These integron gene cassettes have also been found to be located on transferable plasmids (Guerra *et al.,* 2002) and as part of *Salmonella* genomic island variants (Boyd *et al*., 2001; Doublet *et al.,* 2004).

The *sul2* gene appears to relate with plasmids, but not class I integrons (Antunes *et al*., 2005). Many *Salmonella* serotypes; Enteritidis, Agona, and Typhimurium isolates have been found to habour *sul2* (Chen *et al.,* 2004).

Also *sul3* has only been recently known to be associated with plasmids and class I integrons in *Salmonella* (Guerra *et al.,* 2002; Antunes *et al*., 2005), and found in many serotypes; Anatum, Bradenburg, Heidelberg, Rissen, Agonaand Typhimurium (Guerra *et al.,* 2002; Antunes *et al*., 2005).

Trimethoprim resistance is attributed to the activity of DHFR (Mascaretti, 2003). Serotypes known to have trimethoprim resistance genes are Agona, Djugu, Hadar, Newport, Rissen Albany, Derby, and Typhimirium (Doublet *et al.*, 2004; Martinez *et al*., 2005; Antunes *et al*., 2006). Likewise, these genes have been found as part of integron-borne gene cassettes also associated with *sul*1 and *sul*3 (Antunes *et al.,* 2005), on transferable plasmids carrying other resistance genes (Villa and Carattoli, 2005), and *Salmonella* genomic islands (Doublet *et al.,* 2004).

2.9 Prevention and Control Strategies for *Salmonella*

Historical surveillance data suggest that enteric fever was endemic in Western Europe and North America and that rate decreased in parallel with the introduction of treatment of municipal water, pasteurization of dairy products, and the exclusion of human faeces from food production (Crump and Mintz, 2010). At present, enteric fever prevention focuses on improving sanitation, ensuring the safety of food and water supplies, identification and treatment of chronic carriers of *S.* Typhi, and use of typhoid vaccines to reduce the susceptibility of hosts to infection (WHO, 2014).

2.9.1 Vaccines Measures

There are two vaccines available for the prevention of Typhoid fever. The Ty21a vaccine is a live, attenuated, oral vaccine containing the *S.* Typhi strain Ty21a, and the parenteral Vi vaccine is based on the *S.* TyphiVi antigen. Ty21a is available as enteric capsules and is licensed in the United States for use

in children 6 years of age and else where including Nigeria, for children as young as 2 years of age. The Vi-based vaccine is licensed in the United States for children aged 2 years and the effectiveness of parenteral Vi vaccine has recently been confirmed in young children (Sur *et al*., 2009).

A new conjugate vaccine is under development, VirEPA, includes Vi antigen which bound to a nontoxic recombinant protein that is antigenically identical to *Pseudomonas aeruginosa* exotoxin. In addition, efforts are underway to develop and evaluate improved live, attenuated, oral vaccines with the goals of maintaining safety while improving efficacy and reducing the number of doses required (Marathe *et al*., 2012).

2.9.2 Non-vaccine Measures

The improvement of sanitation and the availability of safe water and food that was achieved in industrialized countries centuries ago to low- and middle income countries has proved to be a challenge in developing countries although, United Nations Millennium Development Goal sets a target the proportion of the population without sustainable access to safe drinking water and basic sanitation to halve, by 2015. Evidence suggests that interventions to improve the quality of drinking water may be relatively more important for the prevention of enteric infection relative to sanitation measures than was previously thought (Clasen *et al*., 2007; Ugboko and De, 2014). Improving water quality at the house hold level, as well as at the source, can significantly reduce diarrhoea (Clasen *et al*., 2007). It is possible that interventions that reduce the rate of diarrhoeal diseases transmitted through contaminated water, food, and poor hygiene would have similar effects on rates of enteric fever (Ugboko and De, 2014).

The identification and treatment of *S.* Typhi carriers, particularly those involved with food production, has proven to be an important strategy for the control of typhoid fever in low-incidence settings. Although carriers can be identified by serial culture of stool specimens, this approach is labour intensive.

Anti-Vi antibody assays have proven to be a useful alternative to stool culture for identifying carriers in outbreak settings (Zige *et al*., 2013). However, when used at the community level in an area where typhoid is endemic, the high background levels of anti-Vi antibody appear to render the method impractical (Gupta *et al*., 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Kaduna State, Nigeria on three selected dams: Galma dam, Kubanni dam and Markwa dam (Figure 1). The main tributaries of Galma River are Shika River in the middle course and the Rivers Kinkiba and Likarbu in its lower course. Galma dam is located on latitudes 11˚07'45"E to 11˚08'20"E and longitudes 07˚46'N to 07˚48'N. The Galma reservoir which is popularly called Zaria dam was constructed across the Galma River in 1975 (Patrick *et al.,* 2015).

The Kubanni Dam, popularly called A.B.U Dam was constructed on River Kubanni, Zaria, Nigeria in 1973 with coordinates Latitude: 11°83.18" Longitude: 7°39'15.67". The Kubanni dam is a small earth dam built by excavating the valley of River Kubanni and using the over burden as an embankment across the valley at about 300 meters downstream of Kubanni confluence (Iguisi, 1997).

The Markwa Reservoir also known as University College Dam is located in Zaria, Kaduna State with coordinates (10˚39' 36.87"N, 7˚36'02.22"E. It is an impounding reservoir constructed in early 1970s' for irrigation. It lies within some 2.5 km to the north from the Ahamadu Bello University Teaching Hospital (ABUTH), on the Ahmadu Bello University land, with original total storage of 636,300 m^3 (Baba, 2007).

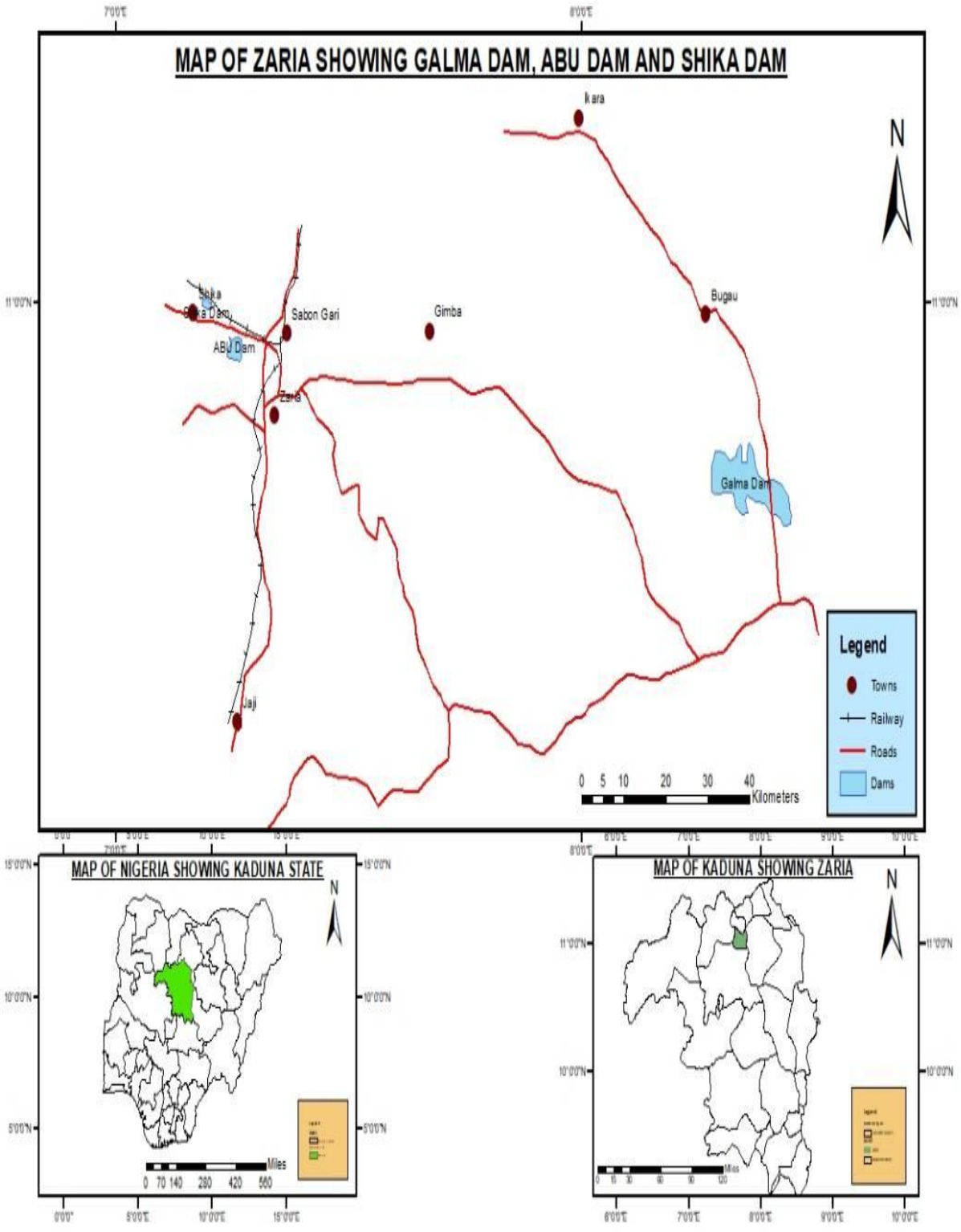


Figure 1. Map of Zaria Metropolis and its Environment Showing dams (Extracted and Modified from Google Earth, 2017)

3.2 Sample Size

The sample size was calculated using the equation below which is derived from UNICEF (2006):This was made up to 450 to capture the three dams for a 10 months period consisting of Dry season (October-March) and Wet season (April-July).The calculated size was 319.62 but a total of 450 samples was collected to accomodate sample error and ensure precision.

$$n = z^2 p q/d^2$$

n = required number of samples (sample size)

P= previous prevalence =40% (Jasini *et al.,*2016)

q = (1 – p) =1-0.40

Z= 1.96

d = precision (allowable error), taken as 5% = 0.05

$$= \frac{1.96^2(0.40)\ 0.52}{0.05^2}$$

$$= 319.62 = 450$$

3.3 Collection of Samples

The water samples were collected in three different dams in Kaduna State, Nigeria which included the Galma Dam, the Kubanni Dam and the Shika Dam. A total of 450 water samples were collected during a 10 month period using 5 litre raw water samples which were collected into sterile containers from the reservoirs. The water samples were collected in batches and each batch of the samples was transported

in ice containers to the Water Quality Laboratory and the Water Resources Engineering Ahmadu Bello University, Zaria for Microbiological and Physicochemical analyses within 4hours of collection.

3.4 Physicochemical Analysis of Water Samples

3.4.1 Determination of pH

A bench top pH meter (HI -2210-02) was used with a combined electrode. Known buffer solutions of pH 4 and pH 9 were prepared and used to standardize the equipment. Thereafter, the meter was used to determine the pH of water using standard protocols (APHA, 2012).

3.4.2 Determination of Colour

This was done using Spectrophotometer (HACHDR 2000). According to the standard procedures, 10ml of distilled water was measured into the cuvette and standardized. The instrument was blanked. Thereafter 10 ml of the water sample was added into another cuvette and the absorbance determined at 455 nm (APHA, 2012).

3.4.3 Determination of Temperature

The water temperatures were measured at the site prior to collection and confirmed in the laboratory using Hana instrument (HI98129 Model) which was calibrated in degree centigrade (APHA, 2012).

3.4.4 Determination of Turbidity

Turbidity were determined using Turbidometer (HACHDR 2000).The meter was calibrated with standard cuvettes and 10 ml of water sample were added into the cuvette thereafter the cuvette were placed inside the meter and the readings were taken.Turbidity Unit is in nephelometric unit (APHA, 2012).

3.4.5 Determination of Electrical Conductivity/Total Dissolved Solid

Hana instrument (HI98129) was used to determine the conductivity and Total Dissolved Solids of the water samples. The power key and the conductivity key/TDS (Total Dissolved Solids) meter was switched on. The probe was dipped into the water and the stable reading on the meter were recorded for both Electrical Conductivity and the Total Dissolved Solids (APHA, 2012).

3.5 Chemical Analysis of Water Samples

3.5.1 Determination of Total Hardness

Total Hardness was determined using Standard Titration Method (APHA, 2012).

Twenty five (25 ml) of distilled water was added to 25 ml of water sample in a conical flask. Two ml phosphate buffer solution with pH 10.4 was added; followed by addition of 0.1g Eriochrome black T dye and titrated with EDTA titrant (0.01 Molar). The volume of EDTA used was noted and the hardness of water sample in ppm (as $CaCO_3$) calculated using the equation as follows. The colour change was from wine red to sky blue

$$\text{Hardness} = \frac{(\text{ml titrant used}) \times (\text{molarity of titrant}) \times 100}{(\text{ml of Sample})}$$

Where: molarity of titrant =0.01

$CaCO_3$=100

3.5.2 Determination of Total Alkalinity

Alkalinity was determined using Standard Titrimetric Method (APHA, 2012).

One hund ml of water sample was transferred into a conical flask with two drops of phenolphthalein indicator and titrated with 0.02N H_2SO_4 and a pink color was observed. Thereafter, two drops of bromocresol green and two drops of methyl red was added. The mixture was swirled and titrated with same solution of 0.02N H_2SO_4 until colour change was observed from pink to yellow. Total alkalinity in $CaCO_3$ mg/l was determined by: Titre value ×10 through a titration method.

$$\text{Total Alkalinity} = \frac{A \times N \times 50000}{\text{ml of sample}}$$

where A = ml of H_2SO_4

N= Molarity of acid used (0.02m)

3.5.3 Determination of Chloride

Chloride was measured using Standard Method Titrations (APHA,2012).

50 millitre of water sample was measured in a conical flask and 1ml of potassium dichromate indicator was added to the water sample. The mixture was titrated against 0.01$AgNO_3$ until there was a pinkish - yellow colour change.

$$\text{Chloride mg/l} = \frac{A \times N \times 35.5 \times 100}{\text{ml of sample}}$$

where A = ml of $AgNo_3$= (50)

N = Molarity of titrant used (0.0141m)

3.5.4 Determination of Biochemical Oxygen Demand (BOD) and Dissolved Oxygen (DO)

Three hundred millitre 300-ml glass Biological Oxygen Demand (BOD) stoppered bottle (brim-full) was filled with sample to avoid air bubbles.The stoppers were removed and 2ml of manganese sulphate solution was added immediately to the collection bottle by inserting the calibrated pipette just below the surface of the liquid followed by the addition of 2 ml of alkali-iodide-azide reagent which was added in the same manner. This was stoppered to avoid spills and inverted several times to homogenize. It was left to stand for about two minutes and 2 ml of H_2S0_4 was added and allowed to stand for 10 mins and inverted again.

Thereafter, 2ml of mixture was dispensed into 250 ml conical flask and titrated against 0.025N Sodium sulphate using 1ml of starch solution as indicator to give the first DO .The samples were then incubated in BOD bottles for 5 days and the above procedure was repeated to give the second DO. The subtraction between the two DOs gave the BOD of the samples.

The BOD was calculated using the formular: $(BOD)_5$ in mg/l = DO_1-$DO_{5.}$) (Radojeric and Baskin, (1999).

3.5.5 Determination of Chemical Oxygen Demand (COD)

Ten ml of water sample was dispersed in three 100 ml of conical flask labeled as test 1,2,and 3 respectively. Similarly,distilled water was dispersed into another set of three 100ml conical flask and labeled as blank 1,2,and 3 resp. Five ml of potassium dichromate was added to each of the six conical flasks .The flasks were kept in water bath at 100^{o}C for one hour and allowed to cool for 10 mins. Five millitre (5ml) of potassium iodide was added to each flask and 10ml of sulphuric acid was added to each flask to form a blue color. Each of the solution in the flask was titrated with 0.1N sodium sulphate until color disappeared completely (Radojeri and Baskin, (1999).

3.6 Bacteriological Analysis of Water

3.6.1 Enumeration of Total Bacterial Count

Total Bacterial Count for the water samples were obtained using pour plate method. In this method, serial dillutions of the sample were made in sterile bijou bottles containing sterile distilled water .dilutions of 10^5 and 10^7 from the samples were taken and one ml of each dilution were inoculated into the cooled plate count agar which were then mixed thoroughly in the petri dish in duplicates and incubated at 37^0C for 24 hours. The petri dishes from dilutions containing between 30-300 discrete colonies were counted and the result expressed as the numbers of bacteria per milliliter (Cheesbrough, 2006).

3.6.1.1 Enrichment and isolation of *Salmonella* spp from water samples

Approximately 10 ml of each water sample were enriched in 10 ml Selenite cystine broth (Difco) and incubated for 24 hour at 37^0C. It was sub cultured onto *Salmonella-Shigella* agar (Oxoid) (SSA) and plates were incubated at 37^0C for 48 hour (Cheesbrough, 2006).

3.6.1.1 Purification of isolates

Representative colonies from *Salmonella-Shigellae* agar (Oxoid) (SSA). Plates showing growth for *Salmonella* were examined based on cultural and morphological characteristics that are transparent colonies with black centre. For the Gram staining procedure, a drop of sterile distilled water was placed on a clean dry slide and a smear of the test organism was prepared by emulsifying a loopful of the bacterial culture on the slide. It was then allowed to air dried and heat fixed by passing through a Bunsen flame.This was stained with crystal violet (primary stain) and allowed to stand for 60 seconds, followed by addition of Lugol's iodine and allowed for another 60 seconds. It was washed off under slow running tap water. Then decolorized with 95% alcohol for 3 seconds and was immediately rinsed with the slow running tap water. It was further counter-stained with dilute safrarnin for 30 seconds then

drained and blot dried. The slide was then examined using x100 oil immersion objective. A violet or purple colour indicated a Gram-positive while a pink/red colour indicated a Gram-negative organism.The presumptive isolates were purified by repeated sub-culturing on nutrient agar medium (Cheesbrough, 2006).

3.7 Biochemical Characterization of Isolates

The following biochemical tests were done to identify *Salmonella* Species

3.7.1 Primary Identification Tests

3.7.1.1 Sugar Fermentation in Triple Sugar Iron (TSI) Agar

The media were prepared according to the manufacturer's instructions and dispensed into test tubes and sterilized by autoclaving at 1210C for 15 mins after sterilization. the tubes were placed on a rack, slanted and allowed to solidify until they became solid. The slants were inoculated with isolates by streaking over the entire surface of the slant and then stabbing deep into the butt and incubation at 37^0C for 24 hours .*Salmonella* isolates indicated an alkaline over acid reaction (Cheesbrough, 2006).

3.7.1.2 Hydrogen Sulphide Formation (H_2S) Test

Formation of H_2S was determined by the blackening of the whole butt or a streak or ring of blackening at the slant butt junction in TSI (Cheesbrough, 2006).

3.7.1.3 Citrate Utilization Test

Simmons citrate agar slants were prepared according to the manufacturers instruction and was inoculated with the test organism. Isolates of *Salmonella* developed a deep blue colour in the medium which indicated a positive reaction (Cheesbrough, 2006).

3.7.1.4 Oxidase Test

The oxidase test were performed using oxidase reagent (phenylenediamine) (PL.390) from Mast diagnostics (Nesto, Wirral, UK) in accordance with the manufacturer published protocol. A pure colony was placed on a filter paper using a sterile wire loop. A drop of test oxidase reagent was added on to it and mixed. After 30 seconds, the filter paper was observed for no colour change which was negative for presumptive isolates of *Salmonella* (Cheesbrough, 2006).

3.7.1.5 Indole Test

Five (5) millilitres of tryptone water were inoculated with the organism and the culture was incubated at 37^0C for 24hours. A few drops of Kovacs reagent (0.2 ml) was added to the culture and shaken gently. A red color development at the surface showed a positive result. Presumptive *Salmonella* isolates were indole negative with a yellow color development (Cheesbrough, 2006).

3.7.1.6 Motility Test

A premixed commercially available motility medium were prepared according to manufacturers' instruction. The medium was brought to boil, dispensed into 10 ml test tube and sterilized by autoclaving at 121^oC for 15 minutes. Sterilized medium were cooled in an upright position on racks.Using a sterile inoculating needle, the motility medium was inoculated with well isolated colonies of the tests organism by making a fine stab to a depth of about 1-2 cm short of the bottom of the tube. The tubes were then incubated at 37^oC for 24 hours .The line of inoculation was not sharply defined and the rest of the medium was some what cloudy if the organism was motile. The growth of non-motile organisms were restricted to the line of inoculation which became sharply defined and the rest of the medium remained clear (Cheesbrough, 2006).

3.7.1.7 Urease Test

Urea agar were prepared according to the manufacturer's instructions and were inoculated with the test organisms**.** The slant were incubated at 37°C for 24 hours. The development of a deep red colour with ammonia fumes indicated a positive reaction. Presumptive *Salmonella* isolates showed a negative reaction with no color change (Cheesebrough, 2006).

3.7.1.8 Methyl Red Test

MR/VP broth were inoculated with the pure culture of the test isolates and incubated at 37^0C for at least 24 hours .A few drops of 0.04% methyl red solution was added. Thereafter Presumptive *Salmonella* isolates showed a positive reaction with a magenta -red colour while a yellow colour showed a negative result. (Cheesebrough, 2006).

3.7.1.9 Voges-Proskauer Test

MR/VP broth were inoculated with a pure culture of the test organism and incubated at 37^0C for 24 hours. At the end of the incubation period, 1ml of broth was added to a clean test tube containing 0.6ml of 5% alpha naphthol and 0.2 mL of 40% potassium hydroxide.The tube was allowed to remain for 10 to 15 minutes after which it was incubated at 37^0C for 24 hours. A positive test showed a cherry -red color. Presumptive *Salmonella* isolates showed a negative reaction with no color change (Cheesebrough, 2006).

3.7.1.1 Lysine Decarboxylase Test

The lysine decarboxylase medium were prepared according to the manufacturer's instruction. The broth was incubated at 37°C for 24 hours after inoculation with the test organism. A negative test showed a yellow color. *Salmonella* isolates showed a positive reaction with a blue color (Cheesbrough, 2006).

3.7.2 **Identification of the Isolates using the Microgen Identification System Software**

The presumtive isolates were further subjected to a confirmation test using a standardized microgen test kit:The Microgen kit for enteric bacteria was used for *Salmonellae* confirmation. A single colony was emulsified from a 24 hour culture in 3ml sterile 0.85% saline for the micro well test strip. Using a sterile Pasteur pipette, 3-4 drops (approximately 100µl) of the bacterial suspension was added to each well of the micro well test strip(s). Before inoculation, wells 1,2 3 to 9 was overlaid with 3-4 drops of mineral oil. The wells were highlighted with a black circle around the well to assist in adding oil to the correct wells. The micro well test strip(s) were then sealed with the adhesive tape which was punctured and the adhesive tape wasover the wells 7, 11 and 12 in the GN A micro well test strip. . For well 10, One drop of Voges Proskauer (VP) l reagent and 1 drop of VP ll reagent were added and read after 30 minutes. Positive result was shown by the formation of a deep pink/red colour. In well 12, one drop of TDA reagent was added and read after 60 seconds. Formation of a cherry red colour indicated a positive result as shown from the images from Microgen Identification Test Kits of the Presumptive *Salmonella* isolates in Appendix I.The plates were then incubated at 37°C for 24 hours.

The isolates were then identified using the Microgen Identification System Software (MID-60) made of substrates organized into set of 3 reactions with each substrate assigned a numerical value (4, 2, and 1) and the sum of the positive reactions for each triplet forming a single digit of the Octal Code that was used to determine the identity of the isolate. The Octal Code was then entered into the Microgen Identification System Software which generated report of the most likely organisms in the selected

database. The software generated an identification based on probability, Percentage probability and likelihood with analysis of the quality of differentiation recorded.

3.7.3 Determination of Antibiotic Susceptibility profile of the Isolates

The antibiotic susceptibility pattern of the isolates were determined using the Kirby Bauer disk diffusion method on Mueller-Hilton agar according to Clinical and Laboratory Standard Institute guidelines for Antimicrobial Susceptibility Testing (CLSI, 2017).

3.7.4 Antibiotics Tested

The organisms were tested in vitro for susceptibility to the following commonly used antibiotics: Ampicillin, (AMP-10µg); Ciprofloxacin, (CIP-5µg); Sulphamethoxazole-trimethprim (Cotrimoxazole), (SXT-5µg); Nalidixic acid, (NAL-30µg); Imipenem (IMP-10µg), Chloramphenicol (C-30µg); Ceftriaxone (CTX-30µg), Cefoxitin (FOX-30µg); Gentamycin (CN-10µg) and Augmentin^{R}, (AUG-30µg) (CLSI, 2017).

3.7.5 Standardization of Bacterial Inoculum

Using a sterile wire loop, three discrete colonies that were freshly grown for 24 hours on Nutrient agar at 37^{o}C were picked and emulsified in sterile 10ml normal saline. The prepared turbidity were matched with 0.5 McFarland standards to have an equivalent suspension (1.5 x10^{8} cells/ml).

3.7.6 Disc Diffusion Susceptibility Test

Sterile swab were used to inoculate the suspension by streaking on the prepared and dried Mueller-Hinton agar plate evenly. It was then allowed to stand for 3 minutes. Sterile forceps were used to place the disc; the plate were then incubated at 37^{o}C for 24 hours. By using a ruler on the underside of the plate, the diameter of each zone of inhibition was measured in millimeters as shown from the images for Antibiotic resistance and susceptibility in Appendix II. The Zone diameter for standards were

compared with Clinical and Laboratory Standards Institute, CLSI, 2017 published limits; each isolate were classified as Sensitive (S), Intermediate (I) or Resistant (R) (WHO, 2011). Organisms that were observed to be resistant to at least four different antibiotics were classified as being multidrug resistant (Ezekiel *et al*., 2011).

The Multiple Antibiotic Resistance (MAR) index was calculated and interpreted according to Krumperman, (1983) using the formular a/b, where "a" represents the number of antibiotics to which a particular isolate was resistant and b" the total number of antibiotics tested.

3.8 Molecular Detection of Antibiotic Resistance genes in *Salmonella* isolates using Polymerase Chain Reaction

In this study, Polymerase Chain Reaction (PCR) was employed for the detection of resistance genes in the bacterial isolates as a confirmation to the phenotypic characters of resistance noted in the organisms.This study employed the conventional PCR (Zhang *et al.,* 2009).

3.8.1 Bacterial cell suspension

The preparation of the bacterial cell suspension was carried out using the method described by Zhang (2009). Luria and Bertani broth media was prepared with the following constituents: peptone (10g), NaCl (5g), 1N NaOH (10ml), yeast extract (5g), distilled water (1litre), pH 7.0 (adjusted with NaOH solution) and sterilized at 121^0C for 15 mins. The isolates from stored slants were subcultured onto the same medium and under the same incubation condition. Discrete colonies were picked from the fresh culture plates, placed into 3ml Luria and Baertani (LB) broth medium and incubated at 37^oC for 24h. Bacterial cells were then harvested by centrifugation at 8000rpm (6800xg) in a refrigerated micro centrifuge at 4^oC for 30 seconds in an Eppendorff's tube. The supernatant were decanted and cells harvested (Zhang *et al.,* 2009).

3.8.2 Genomic DNA Extraction Using ZR Genomic DNA TM- Tissue MiniPrep kit

DNA extraction was carried out using the method described by DNeasy Blood and Tissue Handbook (2006): The harvested cell pellets were dislodged and 20µl of proteinase K was added and mixed thoroughly by vortexing. The mixture were further incubated at 55^{o}C for 3h and vortexed occasionally using a thermo-mixer until the cells were completely lysed and properly mixed to prevent clogging of the DNeasy Mini spin column. Two hundred microlitres (200µl) of buffer AL were added to the sample and mixed thoroughly by vortexing. This were further accompanied by the addition of 200µl of absolute ethanol (96-100) and mixed again by vortexing to yield a homogenous solution. This mixture was then dispensed`into DNeasy Mini spin column placed in a 2ml collection tube and centrifuged at 8000rpm for 1 min. The flow-through and collection tube were then discarded. The DNeasy Mini spin column was transferred into a new 2ml collection tube and 500µl buffer AW1 were added and centrifuged again at 8000rpm for 1 min before the flow-through were discarded for the third time. DNeasy Mini spin column were placed in 2ml collection tube and 500µl buffer AW2 were added and centrifuged at 14000 rpm for 3minutes to dry the DNeasy membrane in order to remove the residual ethanol that might interfere with subsequent reactions. The flow-through and collection tube were removed and discarded carefully so that the column does not come into contact with the flow-through which could result in carry over of ethanol. The used DNeasy Mini spin column were now placed in a clean 2ml micro-centrifuge tube and 200µl buffer AE were introduced onto the DNeasy membrane to elute, and increase the final DNA concentration and yield in the elute. The column were then incubated at room temperature (30^{o}C+ 2^{o}C) for 1min before being centrifuged at 8000rpm for 1minute. The elution of the membrane were then repeated twice to ensure increased overall DNA yield in the same micro-centrifuge tube previously used, but care were

taken to prevent dilution of the first eluate inserted at the red bands to ensure easy view of the To ascertain the DNA were actually extracted, the eluent were subjected to agarose gel electrophoresis, and viewed using a U.V illuminator.

3.8.3 Agarose Gel Electrophoresis

To ascertain that the DNA were actually extracted, the eluent was subjected to agarose gel electrophoresis and it was prepared as follows:

The agarose gel was prepared by combining 1.0g of agarose in ten times concentration (10x) of Trisacetate ethylene diamine tetra-acetate (2ml 10x TAE) buffer and 98ml distilled water in a 250ml Beaker and heating in a microwave for 2 minutes until the agarose was dissolved. 20µl ethidium bromide (5.0mg/ml) was added to the dissolved agarose solution as dye and mixed. The gel was then poured onto a mini horizontal gel electrophoresis tank and the casting combs well while filling the plasmid DNA. It was then allowed to set for 30 minutes. The casting combs were carefully removed after the gel had completely solidified, one time concentration (1x) TAE electrophoresis buffer were then added to the reservoir until the buffer covered the agarose gel. Five microliters (5µl) of gel loading dye (Bromophenol blue) were added to 15µl of each sample with gentle mixing. Twenty microlitres (20µl) of the sample were loaded onto the wells of the gel, the mini horizontal electrophoresis gel set up were covered and the electrodes connected running from cathode (-) to anode (+). Electrophoresis were carried out at 100 mV for 45 minutes to allow easy separation of sample based on molecular weight. At the completion of the electrophoresis, the gel were removed from the buffer (Tris) and was viewed under a trans-illuminator UV light of wavelength 302nm. The band pattern of the DNA fragments were then photographed with a Polaroid camera**.** It was then documented using an electrophoresis gel documentation system. Electrophoresis was employed to identify the number of plasmid copies

present in different isolates. However, standard DNA molecular weight markers were used to estimate the size (1kb ladder and 100bp plus DNA ladder).

3.8.4 PCR Amplification and Identification of the Resistance Genes in Multidrug Resistant *Salmonell*a Isolates

Using various primer sets, PCR was carried out to detect the antibacterial drug resistance genes against the antibacterial drugs. PCRs was performed using appropriate primer combinations and cycling conditions. The PCR amplification for *Salmonella* spp was performed using a DNA thermo-cycler (Eppendorf Master cycler 5330) as described by Kong *et al*., (2002).

3.8.4.1 PCR Detection of catP Gene

Polymerase chain reaction was done for the detection of chloramphenicol drug resistance gene (*catP*) using 1X PCR buffer (Fermentas), 1.5mM MgCl2, 0.2mMdNTPs, 0.5mM both primers (catP F& catP R, Table (3.1), 0.5U Taq polymerase and 2µl of the template DNA. The total reaction volume was 25µl. The cycling condition for PCR were initial denaturing at 95^0C for 2 minutes followed by 35 cycler of 95^0C for 30 sec, 60^0C for 30 sec and 72^0C for 45 seconds and cooling at 4°C (Akinyemi *et al.,* 2011).

3.8.4.2 PCR Detection of tem (β-lactamase) Gene

Ampicillin drug resistant gene(*tem*) was detected by PCR using 1x PCR buffer (Fermentas) , 2.0mM MgCl2, 0.2mM dNTPs, 0.5mM each of forward and reverse primers (tem F & tem R,), 0.5U Taq polymerase and 2µl of the template DNA. The total reaction volume was 25µl. The thermocycler conditions for PCR reaction were initial denaturing at 95^0C for 2 minutes followed by 35cycler of 95^0C for 30sec, 55^0C for 30 sec and 72^0C for 1 min and cooling at 4°C (Asma *et al.,* 2005).

3.8.4.3 PCR Detection of sul-2 Gene

For the detection sulfonamide resistant gene (*sul-2*), a PCR was done using 1x PCR buffer (Fermentas), 1.5m MMgCl2, 0.2mMdNTPs, 0.5m primer (sul-2, F and sul-2 R Table -2), 0.5U Taq polymerase and 2µl of the template DNA. The total reaction volume was 25ul. Thermo cycler conditions for PCR reactions were initial denaturing at 95^0C for 2 minutes followed by 35 cycles of 95^0C for 30 seconds 60^0C for 30 seconds and 72^0C for 1 minute and cooling at 4°C (Cattoir *et al.,* 2007).

3.8.4.4 PCR Detection of gyrA Gene

For the detection of *gyrA* gene mutations responsible for fluoroquinlones resistance, a PCR was done using 1x PCR buffer (Fermentas), 1.5mM MgCl2 0.2mM dNTPs, 0.5mM of both primers (gyrA F & gyrA R,), 0.5U Taq polymerase and 2µl of the template DNA . The total reaction volume was 25µl. Thermo cycler conditions for PCR reaction were initial denaturing at 95 ^{0}Cfor 1minute followed by 35 cycles of 95^0C for 30 seconds, 56^0C for 1min and 72^0C for 1minuteand cooling at 4°C. PCR products were observed by gel electrophoresis and a photograph was taken (Cavaco *et al.,* 2008).

3.8.4.5 PCR Detection of gyrB Gene

For the detection of *gyrB* gene mutations responsible for fluoroquinlones resistance, a PCR was done using 1x PCR buffer (Fermentas), 1.5mM MgCl2 0.2mM dNTPs, 0.5mM of both primers (gyrB F & gyrB R,), 0.5U Taq polymerase and 2µl of the template DNA . The total reaction volume was 25µl. Thermo cycler conditions for PCR reaction were initial denaturing at 95 ^{0}C for 2minutes followed by 35 cycles of 95^0C for 30 seconds, 50^0C for 30 seconds and 72^0Cfor 1 minute and cooling at 4°C. PCR products were observed by gel electrophoresis and a photograph was taken (Chen *et al.,*2004).

3.8.5 Gel Electrophoresis and Gel Visualization

The 5µl of the PCR products was mixed with 2µl of loading dye and the mixture loaded into the wells on the electrophoresis gel. The loaded products were run at 100V on agarose gel (1.5% w/v gel in x1 TAE buffer) for 15 minutes. 5µl ethedium bromide in about 300ml water was used to stain the bands for 30 minutes on rocking shaker. The bands were viewed under UV lamp to establish amplification of target genes and photographed.

Table 3.1:List of Primer Sequences used for PCR identification.

Primer	Primer sequence	Target gene	Size (bp)	References
CAT F	3'CCT GCC ACT CAT CGC AGT 5'	*catP*	623	Akinyemi *et al.,* 2011
CAT R	5'CCA CCG TTG ATA TAT CCC 3'			
Sul F	3' TCA ACA TAA CCT CGG ACA GT 5'	*sul*	707	Cattoir *et al.,* 2007
Sul R	5'GAT GAA GTC AGC TCC ACC T 3'			
A F	3'GCA CGA GTG GGT TAC ATC GA 5'	*tem*	311	Asma *et al.,* 2005
A R	5' GGT CCT CCG ATC GTT GTC AG- 3'			
CIP F	3' TAC CGT CAT AGT TAT CCA CGA 5'	*gyrA*	312	Cavaco *et al.,* 2008
CIP R	5' GTA CTT TAC GCC ATG AAC GT 3'			

GYR F	3'GCG CTG TCC GAA CTG TAC CT 5'	*gyr B*	181	Chen *et al.,*2004
GYR R	5'TGA TCA GCG TCG CCA CTT CC 3'			

3.9 Statistical Analysis

The results were presented inform of tables, charts, graphs, and analyzed using One way ANOVA (Analysis of variance) followed by Duncan Multiple Range (DMRT) test for mean separation, $p< 0.05$ to determine the differences between the mean sampling locations and months. The association between the resistant rates of *Salmonella* isolates and also the antibiotic activities were also analysed using SPSS IBM (Version 20 for window) statistical package at $p< 0.05$ level of significance.

CHAPTER FOUR

4.0 RESULTS

4.1 Physicochemical Quality Of Water From Dams In Parts Of Kaduna State

The physicochemical parameters of water samples from the three dams (Markwa, Kubanni and Galma dam) are shown in Table 4.1. The results indicate significant differences amongst most of parameters tested among the dams except for Hardness, Biochemical Oxygen Demand and colour.The conductivity of water samples from the three dams ranged 133.97±30.33(Kubanni dam) to 148.80±22.64µS/cm (Markwa dam) while pH ranged from 7.15±0.20 (Markwa dam) to7.39±0.22 (Galma dam), Alkalinity ranged from 35.91±11.51 mg/l (Galma dam) to 41.83 mg/l (A.B.U dam). Turbidity ranged from 20.18±26.17 (Markwa dam) to 37.39±35.69) NTU (Galma), Total dissolved solid ranged from 63.40±9.01) mg/l (Galma dam), to 70.64±6.99 (Markwa dam), Chemical oxygen demand ranged from 238.80±48.95mg/l (Galma) to 274.25±97.90mg/l (Kubanni dam), dissolved oxygen ranges from 1.54± 0.56mg/l, (Markwa dam) to 1.81± 0.42 (Galma dam), Chloride ranged from 12.62±2.35 (Galma dam) mg/l to 18.03±3.57 (Markwa dam), Hardness ranged from 233.46±82.10 (Galma) to 238.55±104.31mg/l (Markwa) and Temperature ranged from 26.05±1.41^{0}C (Galma) to 26.33±1.18 (Markwa dam) . The physical, industrial and other factors within the area surrounding the dams are presented in Table 4.2

Table 4.1.The Mean Physicochemical Parameters of Water Samples from three Selected Dams in Kaduna State

Physicochemical parameters	Markwa dam ±SD	A.B.U dam ±SD	Galma dam ±SD	NSDWQ Standard	WHO Standard	p values
Colour (Pt/Co)	8.00±4.78	7.77±4.73	8.17±6.00	15	15	0.770
Temperature (0^0C)	26.33^{a}±1.18	26.14^{ab}±1.31	26.05^{b}±1.41	30-32.5	20-25	0.001
Electrical Conductivity (µs/cm)	148.80^{a}±22.64	133.97^{b}±30.33	139.26^{b}±29.20	1000	1000	0.001
pH	7.15^{c}±0.20	7.27^{b}±0.31	7.39^{a}±0.22	6.5-8.5	6.5-8.5	0.001
Alkalinity(mg/l)	40.83^{a}±14.20	41.83^{a}±9.98	35.91^{b}±11.51	100	100	0.001
Hardness (mg/l)	238.55±104.31	235.61±106.26	233.46±82.10	150	150	0.896
Turbidity (NTU)	20.18^{b}±26.17	37.16^{a}±29.47	37.39^{a}±35.69	5	5	0.001
Total dissolved solids (mg/l)	70.64^{a}±6.99	65.33^{b}±7.49	63.40^{c}±9.01	500	600	0.001
Dissolved oxygen (mg/l)	1.54^{c}±0.56	1.66^{b}±0.35	1.81^{a}±0.42	5	5	0.001
Biochemical Oxygen Demand (mg/l)	0.68±0.35	0.74±0.27	0.74±0.32	10	6	0.169
Chemical Oxygen Demand (mg/l)	254.08^{b}±64.99	274.25^{a}±97.90	238.80^{b}±48.95	10	10	0.001
Chloride (mg/l)	18.03^{a}±3.57	14.36^{b}±3.10	12.62^{c}±2.35	250	200	0.001

superscripts with the same letters in each row are not significantly different $p \geq 0.05$, KEY: Nigerian standard for drinking water quality (NSDWQ),World Health Organization (WHO) Standard deviation (SD)

The water parameters in the wet season had high values compared to the dry seasons except for colour. The values of the parameters vary significantly ($p < 0.05$) in relation to the location except turbidity and COD which did not differ significantly (Table 4.2).

The mean monthly physicochemical properties of water samples for Kubanni Dam in Table 4.3 showed there were high significant differences in all the parameters tested for the sampling months.Turbidity was highest in 98.80mg/l (June) and lowest in 11.88mg/l (May), Colour was highest in 18.33TCU (October), COD was highest in 365.79mg/l (October) and lowest in 206.00mg/l (November), Hardness was highest in 358.92mg/l (May) and lowest in 122.56(June) dissolved oxygen was highest in 1.93mg/l(July) and lowest in 1.31mg/l (November) BOD was highest in 0.99mg/l (October) and lowest in 0.59mg/l (November), pH was highest in 7.57 (March) and lowest in 7.13 (October), temperature was highest in 26.87^0C (October) and lowest in 24.21^0C(January), total dissolved solid was highest in 75.73mg/l (July) and lowest in November (55.73mg/l) alkalinity was highest in 54.33mg/l (June) and lowest in 37.53mg/l (November), chloride was highest in 18.36mg/l (April) and lowest in 11.00mg/l (November) and electrical conductivity was highest in 174.20µs/cm (May) and lowest in 100.47µs/cm (June).

Table 4.2 Seasonal Variation in Physicochemical Parameters of Water Samples from three Selected Dams in Kaduna State

Parameters	Markwa Dam		Kubanni Dam		Galma Dam		
	Dry± SD	Wet± SD	Dry± SD	Wet± SD	Dry± SD	Wet± SD	p value
Turbidity (NTU)	15.08±9.97	25.28±35.03	35.23±15.47	39.10±38.75	45.05±44.54	29.72±21.48	0.885ns
DO (Mg/l)	1.30±0.63	1.74±0.37	1.51±0.47	1.81±0.18	1.61±0.48	2.02±0.27	<0.001
BOD (Mg/l)	0.65±0.36	0.71±0.34	0.68±0.34	0.79±0.17	0.63±0.34	0.85±0.27	<0.001
COD (Mg/l)	253.30±86.70	254.80±31.42	268.49±131.70	280.0±43.43	242.53±64.98	235.07±23.96	0.790ns
pH	7.140 ±23	7.16±0.15	7.27±0.37	7.26±0.37	7.46±0.22	7.32±0.19	<0.090
Colour (Pt/Co)	9.67±5.66	6.33±2.89	9.67±5.66	5.87±2.38	10.67±7.55	5.67±1.71	<0.001
Temperature (^{0}C)	25.73±1.30	26.93±0.61	25.36±1.38	26.92±0.58	25.33±1.63	26.76±0.55	<0.001
TDS (Mg/l)	70.56±6.45	70.72±7.54	59.92±5.97	70.73±4.26	57.65±7.29	69.15±6.60	<0.001
Hardness (Mg/l)	216.37±107.26	260.74±96.98	202.41±92.63	268.82±109.97	204.84±95.23	262.08±53.32	<0.001
Alkalinity (Mg/l)	39.12±13.53	42.53±14.72	40.12±11.42	43.53±8.02	32.01±11.06	39.81±10.66	<0.001

Chloride (Mg/l)	17.15±4.70	18.92±1.43	12.29±2.79	16.42±1.69	11.78±2.95	13.46±1.00	<0.001
EC (µs/cm)	144.22±20.96	153.39±23.45	124.48±26.18	143.45±31.38	126.35±33.51	152.17±15.99	<0.001

Table 4.3 Mean Monthly Physicochemical Properties of Water Samples for Kubanni Dam in Kaduna State

Parameter	October 2016	November 2016	December 2016	January 2017	February 2017	March 2017	April 2017	May 2017	June 2017	July 2017	p-value
Turbidity (NTU)	58.89^{c}	39.85^{c}	32.51^{cd}	25.76^{de}	19.12^{ef}	12.30^{f}	12.73^{f}	11.88^{f}	98.80^{a}	59.73^{b}	<0.001
DO (Mg/l)	1.75^{ab}	1.31^{c}	1.39^{c}	1.42^{c}	1.71^{b}	1.75^{ab}	1.77^{ab}	1.68^{b}	1.89^{ab}	1.93^{a}	<0.001
BOD (Mg/l)	0.99^{a}	0.59^{de}	0.62^{cde}	0.56^{e}	0.65^{cde}	0.77^{bcd}	0.73^{cde}	0.71^{cde}	0.91^{ab}	0.82^{abc}	<0.001
COD (Mg/l)	365.79^{a}	206.00^{c}	257.30^{c}	268.67^{bc}	244.67^{c}	241.33^{c}	267.33bc	279.33bc	337.33ab	274.67^{bc}	<0.002
pH	7.13^{e}	7.18^{de}	7.27^{cde}	7.34b^{cd}	7.44^{abc}	7.57^{a}	7.51^{ab}	7.43^{abc}	6.90^{f}	6.93^{f}	<0.001
Colour (Pt/Co)	18.33^{a}	13.33^{b}	6.67^{d}	5.00^{e}	5.00^{e}	5.00^{e}	5.00^{e}	5.00^{e}	9.33^{c}	5.00^{e}	<0.001
Temperature (^{0}C)	26.87^{bc}	26.45^{c}	24.41^{de}	24.21^{e}	24.86^{d}	26.85^{bc}	27.47^{a}	26.52^{c}	26.65^{bc}	27.09^{ab}	<0.001
TDS (Mg/l)	58.73^{e}	55.73^{f}	56.40^{ef}	62.07^{d}	66.67^{c}	67.67^{c}	65.73^{c}	70.80^{b}	73.73^{a}	75.73^{a}	<0.001
Hardness (Mg/l)	123.23^{d}	167.00^{c}	147.47^{cd}	243.09^{b}	331.24^{a}	256.90^{a}	353.53^{a}	358.92^{a}	122.56^{d}	152.18^{cd}	<0.001
Alkalinity (Mg/l)	41.87^{b}	37.53^{b}	41.40^{b}	36.67^{b}	43.13^{b}	42.13^{b}	38.33^{b}	38.87^{b}	54.33^{a}	44.00^{b}	<0.001

Chloride (Mg/l)	11.40^{g}	11.00^{g}	13.23^{ef}	12.06^{fg}	13.76^{e}	16.23^{bc}	18.36^{a}	17.30^{ab}	14.53^{de}	15.69^{cd}	<0.001
EC (µs/cm)	100.67^{e}	110.27^{d}	114.07^{d}	136.07^{c}	161.33^{b}	167.27^{ab}	161.53^{b}	174.20^{a}	100.47^{e}	113.80^{d}	<0.001

For each parameter means with the different letters (superscripts) are significantly different ($p<0.05$), using Duncan multiple range test for mean separation

Key: DO=Dissolved Oxygen, BOD=Biochemical Oxygen Demand, COD=Chemical Oxygen Demand and EC=Electrical Conductivity.SD=Standard Deviation, TDS=Total Dissolved Solids

Table 4.4 showed the analysis of the mean monthly physicochemical properties of water samples for Galma Dam showed there were significant differences in all the parameters tested for the sampling months. The Turbidity was highest in 128.85NTU (October) and lowest in 16.95NTU (March), Colour TCU (October) and lowest in 8.33 (June) Hardness was highest in 327.94mg/l (February) and lowest in 129.96mg/l (October). COD was highest in 264.67mg/l (October) and lowest in 220.0mg/l (June), dissolved oxygen was highest in 2.21mg/l (July) and lowest in 1.34mg/l (December) BOD was highest in 1.11mg/l (July) and lowest in 0.53mg/l (January), ph was highest in 7.58 (January) and lowest in 7.25(June), temperature was highest in 26.63^0C(May) and lowest in 24.39^0C (January), total dissolved solid was highest in 77.21mg/l (June)and lowest in October (50.33mg/l) alkalinity was highest in 53.13mg/l (July) and lowest in 26.47mg/l (January), chloride was highest in 13.89(July) and lowest in 9.43(January) and electrical conductivity was highest in 164.80µs/cm (March) and lowest in 114.53µs/cm(December)

Table 4.4 Mean Monthly Physicochemical Properties of Water Samples for Galma Dam in Kaduna State

Parameter	October 2016	November 2016	December 2016	January 2017	February 2017	March 2017	April 2017	May 2017	June 2017	July 2017	p-value
Turbidity (NTU)	128.85^{a}	31.07^{b}	22.13^{c}	22.16^{d}	21.03^{d}	16.95^{d}	18.25^{d}	19.63^{d}	71.25^{b}	22.53^{d}	<0.001
DO (Mg/l)	2.01^{abc}	1.41^{d}	1.34^{d}	1.51^{d}	1.78^{c}	2.06^{ab}	1.84^{bc}	1.93^{bc}	2.05^{ab}	2.21^{a}	<0.001
BOD (Mg/l)	0.86^{b}	0.55^{d}	0.57^{cd}	0.53^{d}	0.65^{cd}	0.77^{bc}	0.70^{bcd}	0.59^{cd}	1.06^{a}	1.11^{a}	<0.001
COD (Mg/l)	264.67^{a}	228.00^{ab}	232.00^{ab}	258.00^{ab}	230.00^{ab}	228.00^{ab}	240.00^{ab}	260.67^{a}	220.00^{b}	226.67^{ab}	<0.090
pH	7.35^{bc}	7.36^{bc}	7.50^{a}	7.58^{a}	7.53^{a}	7.45^{ab}	7.50^{a}	7.28^{c}	7.25^{cd}	7.17^{d}	<0.001
Colour (Pt/Co)	23.33^{a}	13.33^{b}	5.00^{d}	5.00^{d}	5.00^{d}	5.00^{d}	5.00^{d}	5.00^{d}	8.33^{c}	5.00^{d}	<0.001
Temperature (^{0}C)	26.51^{abc}	26.38^{bc}	24.59^{d}	24.39^{d}	24.81^{d}	26.54^{abc}	27.31^{a}	26.63^{abc}	26.17^{abc}	27.17^{ab}	<0.001
TDS (Mg/l)	50.33^{f}	59.73^{de}	56.67^{e}	61.13^{bcd}	60.40^{cde}	63.73^{bc}	64.60^{b}	65.00^{b}	77.21^{a}	75.13^{a}	<0.001
Hardness (Mg/l)	129.96^{f}	175.75^{d}	136.02^{ef}	254.54^{c}	327.94^{a}	274.07^{bc}	275.42^{bc}	305.05^{ab}	162.96^{de}	292.93^{b}	<0.001
Alkalinity (Mg/l)	34.53^{bc}	35.80^{b}	34.47^{bc}	26.47^{d}	28.80^{cd}	31.13^{bcd}	31.13^{bcd}	31.60^{bcd}	52.07^{a}	53.13^{a}	<0.001

Chloride (Mg/l)	11.93^{b}	13.76^{a}	13.43^{a}	9.43^{c}	10.33^{c}	12.73^{ab}	13.63^{a}	13.53^{a}	13.50^{a}	13.89^{a}	<0.001
EC (µs/cm)	96.68^{g}	119.87^{ef}	114.53^{f}	144.87^{bcd}	155.80^{abc}	164.80^{a}	159.67^{b}	164.60^{a}	130.87^{de}	140.93^{cd}	<0.001

For each parameter means with the different letters (superscripts) are significantly different ($p<0.05$), using Duncan multiple range test for mean separation

Key: DO=Dissolved Oxygen, BOD=Biochemical Oxygen Demand, COD=Chemical Oxygen Demand and EC=Electrical Conductivity.SD=Standard Deviation, TDS=Total Dissolved Solids

Table 4.5 showed the analysis of results for the mean monthly physicochemical properties of water samples for Markwa dam. There were significant differences for most of the parameters tested for the sampling months except for COD. The turbidity was highest in 93.55NTU (June) and lowest in 6.19NTU (May), hardnesswas highest in 348.82mg/l (February) and lowest in 131.31mg/l (June).dissolved oxygen was highest in 2.26mg/l (June) and lowest in 0.91mg/l (January) BOD was highest in 1.22mg/l (June) and lowest in 0.43mg/l (February), pH was highest in 7.29 (January) and lowest in 6.94 (October), colour was highest in 18.33TCU (October), temperature was highest in 27.59^0C (April) and lowest in 24.76^0C (December), total dissolved solid was highest in 75.00mg/l (April) and lowest in 59.20mg/l (June) alkalinity was highest in 64.73mg/l (June) and lowest in 32.33mg/l (April), chloride was highest in 18.83mg/l (May) and lowest in 14.34mg/l (November) and electrical conductivity was highest in 176.67µs/cm (May) and lowest in 176.67µs/cm (June).

Table 4.5 Mean Monthly Physicochemical Properties of Water Samples for Markwa Dam in Kaduna State

Parameter	October 2016	November 2016	December 2016	January 2017	February 2017	March 2017	April 2017	May 2017	June 2017	July 2017	P-value
Turbidity (NTU)	15.75^{bc}	19.73^{b}	19.48^{c}	10.16^{cd}	10.27^{cd}	6.82^{d}	6.68^{d}	6.19^{d}	93.55^{a}	13.14^{c}	<0.001
DO (Mg/l)	2.22^{ab}	1.38^{c}	1.11^{d}	0.91^{d}	1.03^{d}	1.50^{c}	1.47^{c}	1.48^{c}	2.26^{a}	2.00^{b}	<0.001
BOD (Mg/l)	1.05^{b}	0.80^{c}	0.50^{d}	0.47^{d}	0.43^{d}	0.50^{d}	0.45^{d}	0.47^{d}	1.22^{a}	0.90^{bc}	<0.001
COD (Mg/l)	230.00	274.00	247.33	262.00	253.33	263.33	264.00	267.33	242.67	236.67	<0.682
pH	6.94^{c}	7.11^{b}	7.07^{b}	7.29^{a}	7.25^{a}	7.19^{ab}	7.11^{b}	7.17^{ab}	7.09^{ab}	7.15^{ab}	<0.001
Colour (Pt/Co)	18.33^{a}	13.33^{b}	6.67^{d}	5.00^{e}	5.00^{e}	5.00^{e}	5.00^{e}	5.00^{e}	11.67^{c}	5.00^{e}	<0.001
Temperature (o^0C)	26.50^{cd}	26.77^{bc}	24.76^{e}	25.29^{e}	25.35^{e}	27.20^{ab}	27.59^{a}	26.23^{d}	26.57^{bcd}	27.07^{abc}	<0.001
TDS (Mg/l)	66.33^{c}	66.47^{c}	73.40^{b}	73.67^{b}	72.93^{b}	74.6^{b}	75.00^{b}	78.47^{a}	59.20^{d}	66.33^{c}	<0.001
Hardness (Mg/l)	137.37^{c}	167.40^{c}	139.39^{c}	288.88^{b}	348.82^{a}	329.96^{a}	331.98^{a}	350.16^{a}	131.31^{c}	160.26^{c}	<0.001
Alkalinity (Mg/l)	53.67^{b}	37.07^{c}	37.80^{c}	32.87^{c}	34.20^{c}	30.93^{c}	32.33^{c}	33.27^{c}	64.73^{a}	51.40^{b}	<0.001

Chloride (Mg/l)	16.03^{de}	14.34^{e}	22.33^{a}	16.06^{de}	16.93^{cd}	20.03^{b}	20.10^{b}	18.83^{bc}	17.56^{cd}	18.06^{bcd}	<0.001
EC (µs)	124.71^{d}	130.80^{d}	145.27^{c}	147.80^{c}	172.53^{ab}	167.47^{b}	165.60^{b}	176.67^{a}	116.60^{e}	140.60^{c}	<0.001

For each parameter means with the different letters (superscripts) are significantly different ($p<0.05$), using Duncan multiple range test for mean separation

Key: DO=Dissolved Oxygen, BOD=Biochemical Oxygen Demand, COD=Chemical Oxygen Demand and EC=Electrical Conductivity.SD=Standard Deviation, TDS=Total Dissolved Solids

4.2 Bacteriological Quality of Water from Dams in Parts of Kaduna State.

The mean monthly aerobic counts of bacteria of water samples from sampling sites from different dams are shown in Table 4.6. Sampling sites in Markwa had the highest in May 1.77×10^{8} CFU/ml and lowest in October 2.75×10^{7}CFU/ml. The mean monthly aerobic count of water from Kubanni dam had the highest in May 1.74×10^{8} CFU/ml and lowest in October 2.51×10^{7} CFU/ml, while bacterial count of water for Galma dam had the highest count in May and March 1.65×10^{8}CFU/ml and lowest in December1.15×10^{7} CFU/ml. Generally, the result showed significant difference in all the dams for aerobic bacteria counts.

The highest bacterial mean counts were recorded in the wet season and the lower counts were recorded in the dry season. There was a significant difference between the mean total bacterial counts of the wet and the dry seasons ($p<0.05$). The seasonal variations of the total bacterial counts differ significantly with Markwa dam having $1.53\times10^{8}\pm2.71\times10^{6}$CFU/ml, in the wet season while it had $1.09\times10^{8}\pm7.20\times10^{6}$CFU/ml in the dry season. Galma dam had ($1.52\times10^{8}\pm1.85\times10^{6}$) CFU/ml in the wet season and $9.98\times10^{7}\pm6.87\times10^{6}$CFU/ml in the dry season while Kubanni dam had $1.43\times10^{8}\pm3.62\times10^{6}$CFU/ml in the wet season and $9.60\times10^{7}\pm6.76\times10^{6}$CFU/ml in the dry season (Table 4.7).

Table 4.6 Mean Monthly Aerobic Bacterial Counts of Water Samples from three Selected Dams in Kaduna State, Nigeria

Month	Markwa Dam	Galma Dam	Kubanni Dam
	Aerobic counts (Cfu/ml)		
OCTOBER	2.75×10^{7e}	2.26×10^{7g}	2.51×10^{7d}
NOVEMBER	4.49×10^{7d}	6.16×10^{7f}	4.32×10^{7d}
DECEMBER	1.45×10^{8b}	1.15×10^{7e}	1.14×10^{8c}
JANUARY	1.48×10^{8b}	1.45×10^{8bcd}	1.36×10^{8b}
FEBRUARY	1.73×10^{8a}	1.56×10^{8abc}	1.61×10^{8a}
MARCH	1.68×10^{8a}	1.65×10^{8a}	1.67×10^{8a}
APRIL	1.66×10^{8a}	1.60×10^{8ab}	1.62×10^{8a}
MAY	1.77×10^{8a}	1.65×10^{8a}	1.74×10^{8a}
JUNE	1.17×10^{8c}	1.31×10^{7de}	1.01×10^{8c}
JULY	1.41×10^{8b}	1.41×10^{7cd}	1.14×10^{8c}

TOTAL	1.96×10^{10}	1.89×10^{10}	1.80×10^{10}

For each parameter means with the different letters (superscripts) are significantly different

($p < 0.05:0.001$)

Table 4.7 Seasonal Variations of Total Viable Bacterial Load of Water from the three Dam Sites in Zaria

	MeanTABC±SE(cfu/ml)Season		
LOCATION (N)	**DRY±SE**	**WET±SE**	**p value**
MARKWA DAM(150)	$1.09\times10^{8}\pm7.20\times10^{6(c)}$	$1.53\times10^{8}\pm2.71\times10^{6(a)}$	
KUBANNI DAM (150)	$9.60\times10^{7}\pm6.76\times10^{6(c)}$	$1.43\times10^{8}\pm3.62\times10^{6(b)}$	0.001
GALMA DAM(150)	$9.98\times10^{7}\pm6.87\times10^{6(d)}$	$1.52\times10^{8}\pm1.85\times10^{6(a)}$	
TOTAL MEAN	$1.01\times10^{8}\pm4.01\times10^{6(b)}$	$1.50\times10^{8}\pm1.64\times10^{6(a)}$	

Analysis of variance (ANOVA), Duncan multiple range test for mean separation, $p\leq0.05$

KEY:

N=number of samples

TABC =Total aerobic bacterial counts

SE=Standard error

A total of 101 bacterial isolates were isolated amongst which, 43 were *Salmonella* species. 58 other isolates were *Serratia rubidae*.**,** *Hafnia alvei*., *Enterobacter* georgoviae., *Klebsiella* spp., *Morganella morganii*., *Proteus mirabilis* , *E. coli, and Cronobacter sakazakii* (Table 4.8).

Out of the 43 *Salmonella* spp. isolated, *S.* Arizonae was the most predominant accounting for 27 (6.0%), followed by *S.* Pullorum 14(3.1%) and *S.* Choleraesuis 2*(*0.4%) respectively. There were significant differences among water samples which were positive for *Salmonella* spp ($p<0.05$);

From the four hundred and fifty (450) samples that were collected. *S.*Arizonae was isolated from 15(6.7%) and 12(5.3%) samples in the dry and wet season respectively*, S.* Pullorum from 6(2.7%) and 8(3.6%) samples in the dry and wet season respectively and only 2(0.9%) *S.*Choleraesuis were isolated in the wet season (Table 4.9).

No seasonal variations were observed in the frequency isolation of the target pathogens both in the wet 22 (4.8%) and dry seasons 21(4.6%) There was significant difference ($p<0.05$) in the occurrence of the target organisms between the two seasons (Table 4.10).

Table 4.8 Isolation rate of Bacteria in the Raw Water Samples.

Bacteria isolates	Number of isolates (%)
Citrobacter koseri	6(5.94)
Citrobacter freundii	4(3.96)

Citrobacter sakazakii	3(2.97)
Cronobacter sakazaki	2(1.98)
Enterobacter geogoviae	5(4.95)
Escherichia coli	8(7.82)
Hafnia alvei	6(9.90)
Klebsiella ozaenae	2(1.98)
Klebsiella oxytoca	3(2.97)
Salmonella Arizonae	27(26.7)
Salmonella Choleraesuis	2(1.98)
Salmonella Pullorum	14(13.96)
Morganella morganii	4(3.96)
Proteus mirabilis	8(7.82)
Serratia rubidae	7(6.93)
TOTAL	101(100)

KEY: %- percentage=number of the organism isolated / total number of organisms isolated x 100

Table 4.9 Distribution of *Salmonella* spp from Water Samples from three Selected Dams in Kaduna State

Location	*S.*Arizonae No. isolated (%)	*S.* Pullorum No .isolated (%)	*S.*Choleraesuis No. isolated (%)	p-value
Markwa Dam (150)	11(7.3)	9(6.0)	0(0.0)	0.015
Kubanni Dam (150)	4(2.7)	4(2.7)	1(0.7)	
Galma Dam (150)	12(8.0)	1(0.7)	1(0.7)	
Total Frequency	**27(6.0)**	**14(3.1)**	**2(0.4)**	

Table 4.10 Seasonal Variation in the Distribution of *Salmonella* spp in three Dams in Kaduna State

Sample Location	Number of samples	*S.*Arizonae No. isolated (%)		*S.*Pullorum No. isolated (%)		*S.*Cholearesuis No. isolated (%)		p-value
		Dry	Wet	Dry	Wet	Dry	Wet	
Markwa Dam	75	7(9.3)	4(5.3)	4(5.3)	5(6.67)	0(0.0)	0(0.0)	0.068
Kubanni Dam	75	0(0.0)	4(5.3)	2(2.7)	2(2.7)	0(0.0)	1(1.3)	
Galma Dam	75	8(10.7)	4(5.3)	0(0.0)	1(1.3)	0(0.0)	1(1.3)	
Total	225	15(6.7)	12(5.3)	6(2.7)	8(3.6)	0(0.0)	2(0.9)	

4.3 Antibiotic Susceptibility Pattern of the Isolates

Table 4.11 shows the antimicrobial susceptibility pattern of the 43 *Salmonella* spp isolated from water samples using ten antibiotics. The antibiotics effective against all the *Salmonella* isolates tested were ceftriaxone and imipenem (100%), In addition they were susceptible to Gentamicin (93%) and ciprofloxacin (74.4%). All the 43 isolates (100%) were resistant to ampicillin, nalidixic acid and augmentin^{R}. It was found that 41(95.3%) were resistant to cefoxitin, cotrimoxazole and chloramphenicol (60.5%).

The antibiotic resistance profiles of *S.*Arizonae*, S.*Choleraesuis and *S.* Pullorum from water samples to different antibiotics. High resistance to antibiotics were seen in most of the antibiotics used except for ceftriaxone and imipenem (100%), Gentamicin (93%) and ciprofloxacin (74.4%) (Table 4.12).

The resistance profile of selected *Salmonella* spp isolated from water samples in Table 4.13 showed the highest number of isolates resistant in about six of the antibiotics used while all the isolates were resistant to atleast four antibiotics.

The occurrence of Multiple Antibiotics Resistance Index (MARI) ranged from 0.4 – 0.7. Twenty four isolates with MARI 0.6 had the highest occurrence of 55.8%, followed by isolates with 0.5 (20.9%), 0.4(7.0%) and seven isolates with 0.7 MARI had the least occurrence of 16.3% (Table 4.14).

Table 4.15 showed the multiple antibiotic resistant patterns of *Salmonella* species isolated from water samples to different antibiotics.The highest levels of multidrug resistant were observed in 10 (2.2%) isolates of *S.*Arizonae and 8(1.8%) isolates of *S.*Pullorum which were both resistant to six antibiotics, while the least was seen in 1(0.2%) isolate for the *Salmonella* species.

Table 4.11 Antimicrobial Susceptibility Profile of *Salmonella* spp Isolated from three Selected Dams in Kaduna State

S/N	Antibiotic	Disc potency (µg)	Susceptibility (n=43)		
			Sensitive No (%)	Intermediate No (%)	Resistant No (%)
1	AMP	10	0(0.0)	0(0.0)	43(100)
2	NAL	30	0(0.0)	0(0.0)	43(100)
3	AMC	30	0(0.0)	0(0.0)	43(100)
4	FOX	30	1(2.3)	1(2.3)	41(95.3)
5	SXT	25	2(4.7)	0(0.0)	41(95.3)
6	C	30	8(18.6)	9(20.9)	26(60.5)
7	GEN	10	40(93.0)	0(0.0)	3(7.0)
8	IMP	10	43(100)	0(0.0)	0(0.0)
9	CIP	5	32(74.4)	1(2.3)	10(23.3)
10	CEF	30	43(100)	0(0.0)	0(100)

KEY:AMP= ampicillin, IMP=Imipenem., SXT= cotrimoxazole, NAL =nalidixic acid, AMC= amoxicillin &clavulanic acid , CN= gentamycin, FOX=cefoxitin, C=chloramphenicol, , CIP=ciprofloxacin and CEF=Ceftriaxone

Table 4.12 Antibiotic resistance profile of *S.*Arizonae, *S.*Pullorum and *S.*Choleraesuis Isolated from water samples in Kaduna State

	Percentage of resistant isolates		
	***S.*Arizonae**	***S.*Pullorum**	***S.*Cholearesuis**
	***(*n =27)**	**(n=14)**	**(n=2)**

Antibiotics	Disc potency		
AMP	100	100	100
AMC	100	100	100
SXT	92.6	100	100
NAL	100	100	100
FOX	92.6	100	100
GEN	7.4	7.1	100
IMP	0.0	0.0	0.0
CIP	29.6	14.3	0.0
C	55.5	71.4	50.0
CEF	0.0	0.0	0.0

n =Number of isolates that are resistant.

KEY:AMP= ampicillin, IMP=Imipenem., SXT= cotrimoxazole, NAL =nalidixic acid, AMC= amoxicillin and clavulanic acid , CN= gentamycin, FOX=cefoxitin, C=chloramphenicol, CEF=ceftriaxone, CIP=ciprofloxacin

Table 4.13 Antibacterial Resistance Profile of Selected *Salmonella* species Isolated from Water Samples

Resistance Phenotype	Number of isolates resistant
AMP SXT AMC FOX	1
AMP SXT NAL AMC	2
AMP NAL AMC FOX C	1
AMP SXT NAL AMC FOX	7
AMP NAL AMC FOX C	1
AMP SXT NAL AMC FOX C	19
AMP SXT NAL AMC CIP FOX	3
AMP SXT NAL AMC CN FOX	2
AMP SXT NAL AMC CN FOX C	1
AMP SXT NAL AMC CIP FOX C	6
	43

KEY:

AMP= ampicillin, SXT= cotrimoxazole, NAL =nalidixic acid, AMC= amoxicillin and clavulanic acid, CN= Gentamicin, FOX=cefoxitin, C=chloramphenicol, CIP=ciprofloxacin,

Table 4.14 Antibacterial Resistance Patterns of *Salmonella* species isolated from Water Samples to 10 different Antibiotics

Number of Antibiotics	Number of resistant isolates (%)	Resistant pattern	MARI
4	3(7.0)	AMP-NAL-AMC-FOX	0.4
		AMP-SXT-NAL-AMC	
5	9(20.9)	AMP-SXT-NAL-AMC-FOX	0.5
		AMP-NAL-AMC-FOX-C	
6	24(55.8)	AMP-SXT-NAL-AMC-FOX-C	0.6
		AMP-SXT-NAL-AMC-CIP-FOX	
		AMP-SXT-NAL-AMC-CN-FOX	
7	7(16.3)	AMP-SXT-NAL-AMC-CN-FOX-C	0.7
		AMP-SXT-NAL-AMC-CIP-FOX-C	

KEY:AMP= ampicillin, SXT= cotrimoxazole, NAL =nalidixic acid, AMC= amoxicillin and clavulanic acid, CN= Gentamicin, FOX=cefotaxime, C=chloramphenicol, CIP=ciprofloxacin

Table 4.15 Multiple Antibiotics Resistance Patterns of *Salmonella* spp from Water Samples

Number of antibiotics	Resistance phenotype	No. of bacterial isolates with patterns			
		***S.*Arizonae (%)**	***S.*Pullorum(%)**	***S.*Choleraesuis (%)**	**Total number of isolates resistant**
4	AMP SXT NAL AMC	2(0.4)	-	-	2
	AMP NAL AMC FOX	1(0.2)	-	-	1
5	AMP SXT NAL AMC FOX	4(0.8)	3(0.7)	1(0.2)	8
	AMP NAL AMC FOX C	1(0.2)	-	-	1
6	AMP SXT NAL AMC FOX C	10 (2.2)	8 (1.8)	1 (0.2)	19
	AMP SXT NAL AMC CIP FOX	2 (0.4)	1 (0.2)	-	3

	AMP SXT NAL AMC CN FOX				
		2(0.4)	-	-	2
7	AMP SXT NAL AMC CN FOX C	-	1 (0.2)	-	1
	AMP SXT NAL AMC CIP FOX C	5(1.1)	1 (0.2)	-	6
Total (%)		**27 (6.0)**	**14 (3.1)**	**2 (0.4)**	**43 (9.5)**

KEY: - None , AMP= ampicillin, SXT= cotrimoxazole, NAL =nalidixic acid, AMC= amoxicillin &clavulanic acid , CN= Gentamicin, FOX=cefotaxime, C=chloramphenicol, CIP=ciprofloxacin

4.4 Detection of Antibiotic Resistance Genes in the Isolates

The amplifications of these *gyrA, gyr B, tem, sul 2* and *catP* genes are related to fluoroquinolones and quinolones resistance, ampicillin resistance, sulphamethoxazole resistance and Chloramphenicol resistance. The presence of chloramphenicol resistance gene, *catP* was detected in 60% (9 / 15) of the *Salmonella* spp while it was not detected in the remaining six of the fifteen isolates. Similarly, *sul*2 gene coding resistance to sulphamethoxazole was detected in 13.3% (2/15) of the isolates other genes identified include the *tem* gene which was detected in 20% while no *gyrA* gene was detected in any of the isolate. On the other hand, *gyrB* gene was present in all the 15 isolates (Table 4.16). Isolate KUBANNI/5/S.P/Jan and MARKWA/70/S.A/Apr had combination of three genes (*cat* P, *sul*2 and *gyrB*) .

The presence of 9 *cat*P gene in *Salmonella* spp was confirmed by PCR amplification of 623-bp product in plate I.The PCR amplification of a 707 bp fragment of *sul-2*gene confirmed the presence of *sul-2* in 2 isolates of *Salmonella* spp.

Amplification of *gyrA* gene are related to fluoroquinlones resistance. None of the *gyrA* gene was confirmed by PCR amplification of 312bp product (Plate IV).Amplification of 311- bp *tem* gene revealed the presence of this gene in 3 isolates. The result is presented in Plate. III. Amplification of *gyrB* gene are related to Nalidixic acid resistance. Presence of *gyrB* gene was confirmed by PCR amplification of 181bpproduct for all the isolate tested (Plate V).

From the result presented in table 4.17, it was found that *S.*Arizonae and *S.*Pullorum harbored genes for *cat p*, *sul2*, *tem* and *gyrB* except *gyrA*. Non of the genes were detected in *S.*Choleraesuis.

Table 4.16: Distribution of Some Antibiotic Resistance Genes Among the *Salmonella* spp Isolates

S/N	Isolates	Genes expressed				
		*cat*P	*sul*2	*tem*	*gyr* A	*gyr*B
1	KUBANNI/20/S.P/Apr	-	-	-	-	+
2	KUBANNI/4/S.P/Feb	-	-	-	-	+
3	KUBANNI/64/S.A/May	-	-	-	-	+
4	KUBANNI/5/S.P/Jan	+	+	+	-	+
5	MAKWA/70/S.A/Apr	+	+	+	-	+
6	GALMA/50/S.A/Dec	+	-	-	-	+
7	MARKWA/11/S.A/Feb	+	-	-	-	+
8	GALMA/35/S.A/Feb	-	-	-	-	+
9	GALMA/114/S.A/May	+	-	-	-	+
10	GALMA/68/S.A/Oct	-	-	-	-	+
11	MARKWA/15/S.P/March	+	-	-	-	+
12	MARKWA/69/S.P/May	+	-	-	-	+
13	GALMA/25/S.A/Nov	+	-	+	-	+
14	KUBANNI/76/S.A/Apr	-	-	-	-	+
15	MARKWA/65/S.P/Oct	+	-	-	-	+

Percentage occurrence	60%	13.3%	20%	0.0%	100%

Key: *tem*= β-Lactamase gene, *gyr*A and B =fluoroquinolones genes, *catP*= Chloramphenicol gene and *sul* 2=sulphamethoxazole and trimethoprim gene. Key:location/isolate number/month

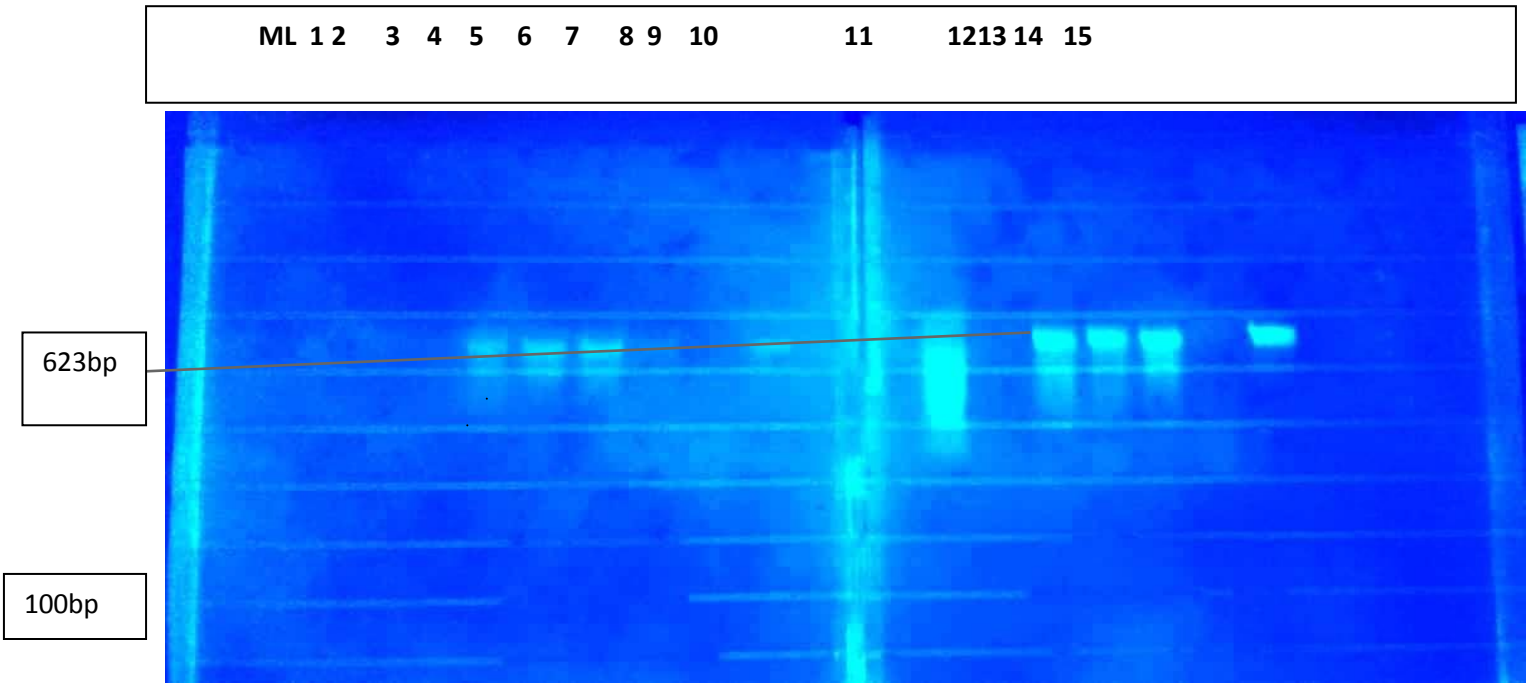


Plate I:Amplicon of *cat P* Gene of *Salmonella* spp Isolated from Water Samples

Size: 623bp.

KEY: Lane 1and 11 contains the (100bp)

2-17 *Salmonella* spp isolates

positive for lane 4-7,9,11,13&15

ML=Molecular Ladder

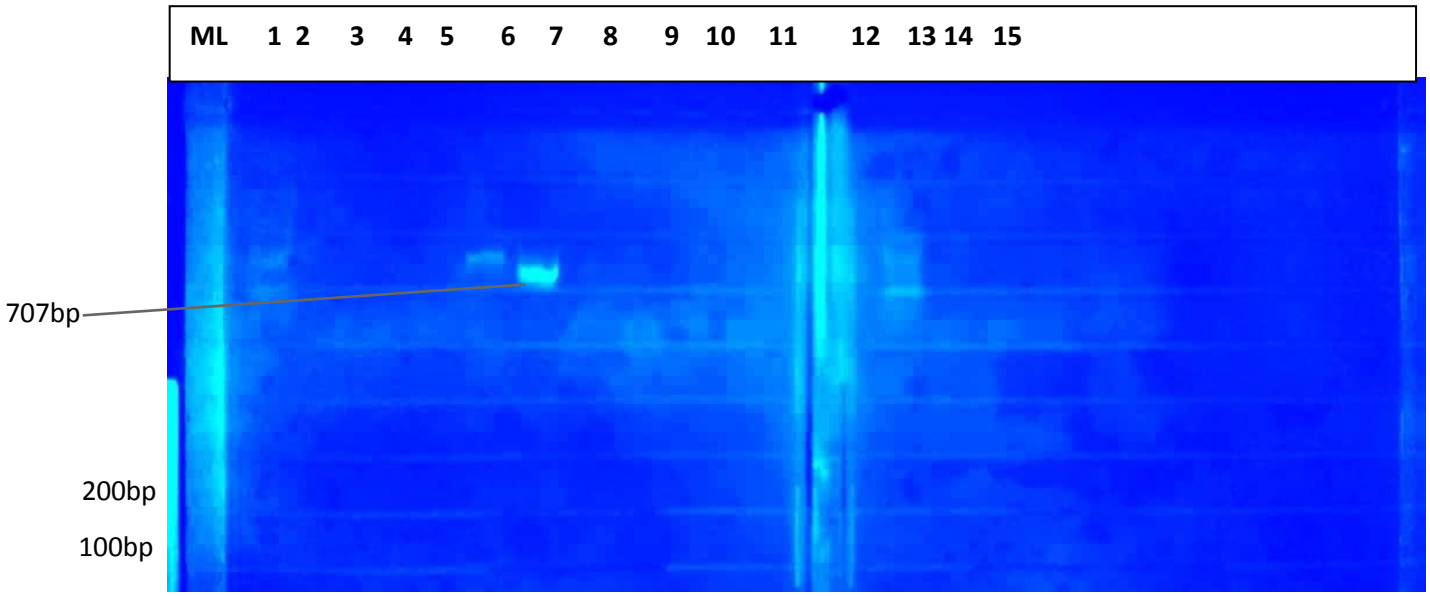


Plate II:Amplicon of *sul*2 Gene of *Salmonella* spp Isolated from Water Samples

Size:707bp.

KEY: Lane 1 and -11 (50bp).

2-17 *Salmonella* spp isolates.

positive for lane 4&5

ML=Molecular Ladder

Plate IV :Amplicon of *gyrA* Gene of *Salmonella* spp Isolated from Water sample

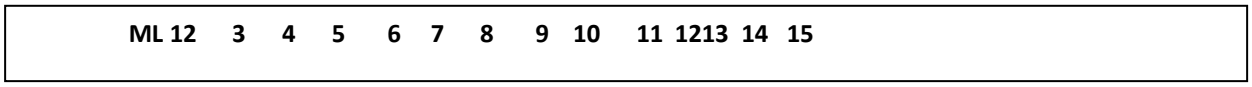
size: 312bp.

KEY: Lane 1,10 and 11(100bp)

S2-S17 *Salmonella* spp isolates.

no positive lane seen

ML=Molecular Ladder



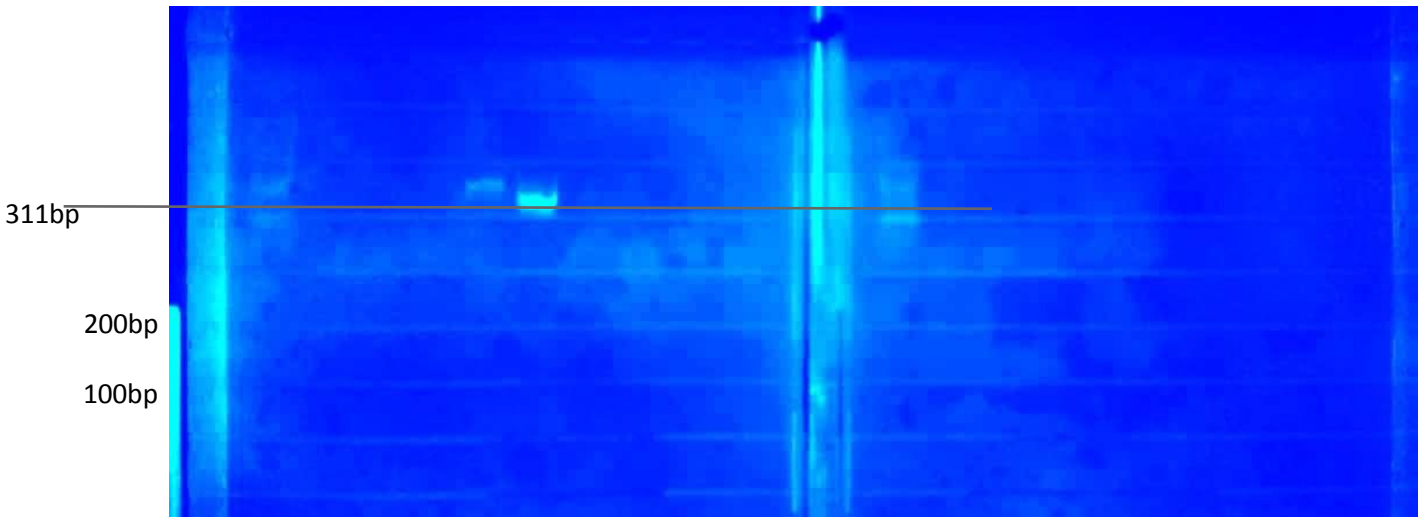


Plate III: Amplicon of *tem* gene of *Salmonella* spp Isolated from Water Samples

Size :311bp.

KEY: Lane 1,10 and 14(100bp).

Lane 2-17 *Salmonella* spp isolates.

positive for lane 4, 5 & 13

ML=Molecular Ladder

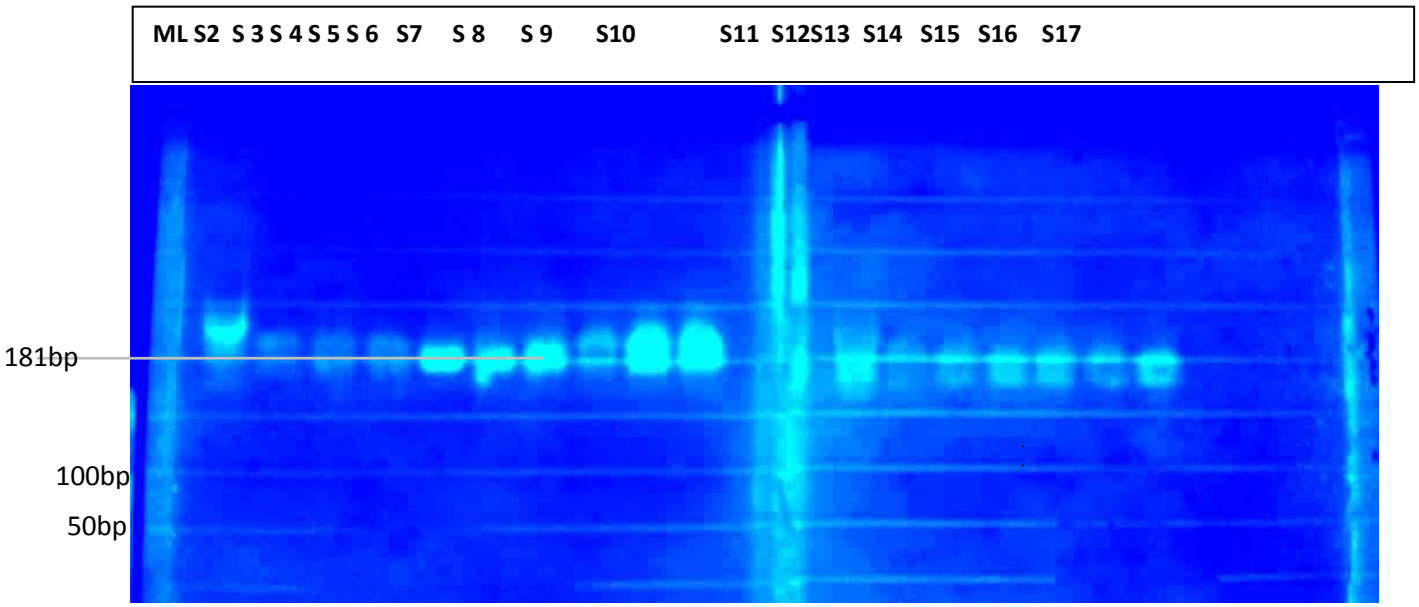


Plate V :Amplicon of *gyrB* Gene of *Salmonella* spp Isolated from Water sample

Size: 181bp

Key: Lane 1and 11 (100bp) .

S2-S17 *Salmonella* spp isolates.

positive for lane 2,3 4,5,6,7,8,9,10,12,13, 14 and 15

ML=Molecular Ladder

Table 4.17: Occurrence of Antibiotic Resistance Genes Among *Salmonella* spp Isolates

Resistance Genes	*S.*Arizonae(%) (n=15)	*S.*Pullorum(%) (n=15)	*S.*Choleraesuis(%) (n=15)
cat P	**5(33.3)**	**4(26.7)**	**0(0.0)**
sul 2	**1(6.7)**	**1(6.7)**	**0(0.0)**
tem	**2(13.3)**	**1(6.7)**	**0(0.0)**
gyr A	**0(0.0)**	**0(0.0)**	**0(0.0)**
gyrB	**9(60.0)**	**6(40.0)**	**0(0.0)**

Key: *Tem*= β-Lactamase gene, *gyrA* and *B* =fluoroquinolones genes, *CatP* = Chloramphenicol gene and *Sul2*= Sulphamethoxazole and trimethoprim gene

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physicochemical Properties of Water Samples from Dams

Water is one of the most important and abundant compounds of the ecosystem. All living organisms on the earth need water for their survival and growth (Patil *et al*., 2012).

The physicochemical properties recorded for the water samples obtained from each of the selected dams were all within the World Health Organization (2011) and Nigerian Standard for Drinking Water Quality (2007) limits except for turbidity, COD and hardness. Generally the water samples from Markwa dam were observed to be hard while Galma dam was found to be most turbid.

Each parameter, means were significantly different ($p<0.05$). There were significant differences amongst the parameters with reference to location, season and monthly variations for the three dams . However, there was no statistically significant difference observed for hardness and color for the three dams. Similar result was recorded by Chigor *et al.,(*2012), Fadaeifard *et al.,* (2012) and Patrick *et al.,* (2015).

The water temperature range for the three reservoir were within the range of (26.05±1.41-26.33±1.18) for the three dams. It compares well with the normal range for drinking water. The water temperature range mentioned in NDWQS (2007) and WHO (2011) standards. The result agrees with Okunlola *et al.*, (2014); Oyakhilome *et al*., (2012) and Patrick *et al.,* (2015). Which had mean temperature values within the optimal range but disagrees with Suleiman and Audu (2014) who reported high temperature range above 30^0C.

pH is considered as one of the most important water quality parameter. Measurement of pH relates to the acidity or alkalinity of the water (Sagar *et al.,* 2015). The normal drinking water pH range mentioned in WHO (2011) and NDWQS (2007) guidelines is between 6.5 and 8.5. The pH values of all the three dam water samples were found to be in the range of 7.15±0.20 and 7.39±0.22 which was neutral in all the location.This range is typical of most major drainage basins of the world (Genevieve and James 2006). Similar result agrees with the findings of (Adefemi *et al.,*2007) in water samples from Ureje, Egbe, Ero and Itapaji dams, South West of Nigeria.

The electrical conductivity is an indicator of how salt-free, ion-free or impurity free a water sample is (Aktar *et al.,* 2010). The mean values of electrical conductivity of the water were all within the permissible limit of 1000mg/l NDWQS (2007) and WHO (2011). This result agrees with Ajibade (2004), Adefemi *et al.,* (2007) , Ladipo *et al.,*(2011), Odeyemi *et al.,* (2011), Oyem *et al.,*2014.

Total Dissolved Solids (TDS) are the inorganic matters as well as small amounts of organic matter, which are present as solution in water. All the three dams had TDS levels for all samples below the maximum permissible limit of 600mg/l (NSDWQ, 2007) and (WHO, 2011) .The low TDS values measured reflect freshness of the water source .The result agrees with the work of Adefemi *et al.,(*2007), Ajibade (2004), Ladipo *et al.,*(2011), Oyakhilome *et al.,* (2012) and Oyem *et al.,*2014.

Chlorides are the ionized form of chlorine and their presence in natural water results from the leaching of chloride-containing rocks and soils with which the water comes in contact. All the three dams had chloride levels for all samples below the maximum permissible limit of 250mg/l (NSDWQ, 2007) and 200mg/l (WHO, 2011).The result agrees with Oyakhilome *et al.,* (2012) and Oyem *et al.,*2014.

Turbidity is the measurement of the cloudiness of water caused by a variety of particles and is another key parameter in water quality analysis. It may indicate the presence of disease-causing organismsin water, which may come from soil runoff (Basavaraja*et al.,* 2011).The high turbidity recorded in Galma dam (37.39±35.69) NTU which was the most turbid could be due to the dam location . Several anthropogenic activities (both domestic and agricultural) are going on around the area, although the other dams also had high turbidty values which could be attributed to the

presence of organic matter pollution. Suspended sediment such as silt or clay, inorganic materials, or organic matter such as algae, plankton and decaying material run-off with high suspended particles are discharged in the reservoirs especially during the torrential rains in the wet season or during the dry seasons due to the cool harmattan winds. Turbidity value agrees with the findings from Ajibade (2004), Adefemi *et al.*, (2007) Ladipo *et al.*,(2011) Odeyemi *et al.*, (2011) , Oyem *et al.*,2014, Saeed,2008) and Wakawa *et al.*,(2008). which were far greater than 5.0 NTU limit given by NSDWQ (2007) and WHO (2011). High turbidty could lead to unpleasant taste and odours, increase the amount of the disinfection by-products Trihalomethanes (THMs) in treated water and interfere with sunlight penetration, thus reducing photosynthesis and the production of oxygen for fish and aquatic life (Oyakhilome *et al.*, 2012) .

All the dam water sampled in all the months were hard for the three dams especially in the month of February.The values were above the permissible limit of 150mg/l (NSDWQ, 2007) and 200mg/l (WHO, 2011). According to Hardness Classification of Water by Sawyer and McCarty quoted in Todd and Mays (2005), the dam water is classified as hard which might be due to the presence of dissolved Ca^{2+} and Mg^{2+}ions. High concentration of hardness found in all the dams could be due to the presence of dissolved polyvalent metallic ions from sedimentary rocks, seepage and runoff from soils. High concentration of hardness may cause the problem of heart disease, osteoporosis, nephrolithiasis (kidney stones), colorectal cancer, hypertension and stroke, coronary artery disease, insulin resistance and obesity. Most of these disorders have treatments, but not cures (WHO,2011).This result agrees with the work of Oyakhilome *et al.,* (2012) and Sagar *et al*.,(2015)

Dissolved Oxygen (DO) content varies between 1.54 – 1.81mg/L within the sampling sites. DO plays a vital role in supporting the aquatic life and is susceptible to slight environmental changes. Oxygen depletion often results with time and DO is used as a parameter in water quality to evaluate the degree

of freshness of a river. It also indicates organic pollution in the water body (Ladipo *et al.,* 2011).Seasonal variation in DO content is related to temperature and biological activities (Chapman, 1996).The samples obtained were found to have DO values below the WHO limit of 10mg/l and low which could be due to urban runoff leading to aeration with continuous disturbance of the water from wind storms .The results agree with Oyakhilome *et al.,*(2012).

Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) are two common measures of water quality that reflect the degree of organic matter pollution of a water body (Genevieve and James, 2006). The BOD values of between 0.74-0.68mg/l for the three locations were recorded and are within WHO permissible level of 6mg/l. Therefore, a low BOD is an indicator of good quality water, while a high BOD indicates polluted water.

The chemical oxygen demand is an indication of the chemical decomposition of organic and inorganic contaminants, dissolved or suspended in water. All the dams had a high level of COD which could be as a result of leachate from dumpsites, agricultural and urban runoff, discharge of effluents and non-point source of pollution which comes from different sources as a result of rain fall carrying laboratory generated waste from various Faculties and Departments.The results agreed with Igbinosa and Okoh (2009) and Sagar *et al.*,(2015) who attributed the increased COD to addition of organic and inorganic substances from the environment as well as organic contaminants entering the system from municipal sewage treatment plants or non-point sources of pollution.

The mean monthly physicochemical properties of water samples for A.B.U dam showed variations in the parameters tested and were all significant. The variations could be due to the dam location and the activities around the dam. The high chemical oxygen demand, Turbidity

and Hardness in all the months could be due to the main pollution sources for example waste from dumpsites, the municipal waste water, storm water runoffs, the A.B.U sewage treatment plant, abattoir effluents and irrigation farms treated with chemical fertilizers (Chigor *et al.*,2012). All these makes A.B.U reservoir turbid and vulnerable to chemical pollution.

The mean monthly physicochemical properties of water samples for Galma dam showed variations in the parameters tested and were all significant ($p<0.05$).The variations could be as a result of run-offs from untreated discharge of pollutants from domestic sewers, storm water discharges, industrial wastewater, agricultural runoff and other various adjoining tributaries (most of which are seasonal); and contain myriads of toxic pollutant from few industrial activities in Chikaji and Dakace areas (Patrick *et al.*, 2015) pollution from different sources of anthropogenic activities along the river banks which flows into the dam (Butu and Bichi, 2012).

The mean monthly physicochemical properties of water samples for Markwa Dam showed variations in the parameters tested and were all statistically significant except for the COD.The high chemical oxygen demand, Turbidity and Hardness in all the months could be as a result of runoff from farmlands; these carry organic and inorganic pollutants that are used in agriculture, most especially towards the end of the rainy season when pesticides are sprayed on farms around the university college dam, illegal dumping of refuse, animal waste, storm water runoff and non point source pollution.

5.2 Mean Aerobic Bacterial Counts of Water Samples from Dams

The mean monthly aerobic counts of the three dam water samples varied significantly with the highest count in the month of May.This could be due to the presence of heterotrophs which are a

group of microorganisms that use organic carbon sources to grow in all types of water bodies. The accumulation of waste from irrigation farms, waste from farm animals especially cattle, human faeces on the slopes of the river valley might have been washed off in to the dams by rain during this period. Most of these organisms are opportunistic pathogens that could infect individuals with weakened immune systems causing nosocomial (hospital acquired) infections, including wound infections, urinary tract infections, post-operative infections, respiratory infections, and infections in burn patient. The findings compares to Chigor *et al.*,(2012) who reported higher counts ranging from 2.0×10^{1} to 1.6×10^{6}CFU/ml due to the abattoir effluents, storm water runoff and irrigation farms around the dams and of Oluyege *et al*., (2010) who reported higher bacterial counts of 4.7×10^{6} to 8.0×10^{8} CFU/ml due to the presence of bushes and shrubs around these dams and remarkably serving as hideout for mammals that frequently visit these water bodies to drink and subsequently pass their faeces around the river banks and are washed into the reservoirs. Adefemi and Awokunmi (2010), Oyakhilome *et al.*,(2012), also reported high bacteria counts which could be due to influx through runoff of microorganisms originating from vegetation decay, municipal sewage, garbage, abattoir activities domestic and faecal waste .

The seasonal variations of total viable bacteria load of water from the three dams showed that the bacterial load of water in the wet season were higher than the dry season which compares with the findings of Oluyege *et al.,(*2010), Odeyemi, (2011), Oyakhilome *et al.,*(2012); Oyem *et al., (*2014). The high bacteria counts could be as a result of the presence of heterotrophic bacteria in the raw water as a result of use of sewage effluent as fertilizer, aquacultural practices and effluent disposal around these dams for source of organic matter and plant nutrients especially during the raining season (Patil *et al.,* 2012).

5.3 Occurrence of *Salmonella* spp and other Enteric Bacteria in Water Samples from Dams

Salmonella is an enteric pathogen that is shed predominantly in faeces making faecal pollution the main source of water contamination (Roth, 2012).The study revealed the presence of *Salmonella* in dam samples analyzed with an overall isolation rate of 9.5% .This finding, in itself, is not surprising since higher isolation rates of *Salmonella* was reported from water samples in Nigeria (Jasini *et.al.,* 2016; Oguntoke *et al.,* 2009 and Oluyege *et al.,* 2009 Akinyemi *et al.,*2006) The low rates from this study could be due to the survival strategy of the organisms. *Salmonella* may survive and remain virulent for long periods in the environment, they may undergo a viable but nonculturable stage when they encounter environmental stress conditions (Martinez *et al.,* 2005). The 9.5% isolation rate of *Salmonella* obtained is also lower than the 16% isolation rate by Adzitey *et al.,* (2016) from dam water used for drinking in Tamale, Ghana.

The results also revealed that raw water samples from the dams were associated with enteric pathogenic bacteria such as *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp, *Proteus mirabilis, E.coli, S.rubidae, M.morganii, E.geogoviae* and *H. alvei* as seen in Appendix III.The occurrence of these pathogenic Gram-negative bacteria in the raw water suggest that there is a health risk for those drinking this water directly without treatment to develop gastrointestinal infections. However, diseases such as bacteremia, meningitis, urinary tract infections, cholera, respiratory infections and other opportunistic infections can also occur, especially for immunosuppressed individuals.

The distribution of *Salmonella* species showed that a total of forty three *Salmonella* isolates were obtained from the water samples collected from these selected dams and the most common species isolated in this study were *S.* Arizonae, *S.* Pullorum, and *S.*Choleraesuis. *S.* Arizonae and *S.* Pullorum, were isolated from all the three dam sites while *S.Choleraesuis* was isolated from Kubanni and Galma dam only. These results indicates contamination from faecal material of

either human or animal origin, sewage effluents, agricultural run-off and direct deposit of faecal materials from wild animals, reptiles and birds .The results agreed with the findings of (Ariie*t al.*, 2002; Chen *et al.*, 2004).

Although *S.* Arizonae, *S.* Pullorum, and *S.* Cholearesuis were the three most frequently isolated *Salmonella* spp in this study, other species vary with the sources of contamination (Parry *et al.,* 2002; Martinez *et al.,* 2005;). Seasonal variations in the frequency of occurrence of *Salmonella* species based on sample locations showed that no seasonal trends were observed. *Salmonella* serovars have unique seasonal trends. In the environment, generally they peak during wet seasons and are more associated with rainfall (Abulreesh, 2012).

5.4 Antibiogram of the *Salmonella* Isolates Obtained from Water Samples

From the results obtained in this study, the antimicrobial susceptibility pattern of the 43 *Salmonella* spp isolated from the water samples using ten antibiotics revealed that Ceftriaxone and Imipenem was the most effective with 43(100%) followed by Gentamycin and Ciprofloxacin with 93% and 74.4% respectively. 43 isolates were 100% resistant to Ampicillin, Nalidixic acid, and Amoxiclav followed by Cotrimoxazole and Cefoxitin with 95.3% while Chloramphenicol had 60.5% in order of decreasing resistance.The findings compares with Effa *et al.*,(2011),Tesfaw *et al.*,(2013) ; Poonia *et al.*, (2014); (Jasini*et al.*,(2016) who stated that third-generation cephalosporin such as ceftriaxone and fluoroquinlones are the drugs of choice for first line of treatment.The isolates susceptible to Ceftriaxone, Imipenem, and Gentamycin could be due to cost effective and parenteral route for administration of the drugs (Crump *et*

al.,2008).The origin of resistance in water samples can be traced to the use of drugs in the environment, deposits of hospital waste in dumpsites, use of drugs in animal husbandry for growth and treatment which could get washed in to these water bodies and subsequent transfer to the dams. The high rate of multidrug resistance by *Salmonella* isolates correlates with the works of Oluyege *et al.*,(2009) and Adley *et al*, (2011) which reported that *Salmonella* exhibit multi drug resistant patterns to more than four antibiotics.This result could be due to the overuse of antibiotics in animals in mass treatment and long-term administration of antimicrobial growth promoters to animals which may lead to the emergence of multidrug-resistant strains of enteric bacteria, including *Salmonella* spp. High resistance to Ampicillin, Nalidixic acid and Amoxicillin-clavulanic acid could also be due to the usage of these drugs both in human and animal medicine. This could have resulted in the development of resistance strains to these antibiotics through the horizontal gene transfer by mobile genetic elements. The high resistance could also be due to the use of these fake or expired drugs, abuse and misuse of the drugs among the general populace which could have been washed off in to these dams (Abdullahi *et al.*, 2013).

The isolates presented with several patterns of resistance to the antibiotics ranging from those that were resistance to 7 antibiotics to those that showed resistance to 4 antibiotics.In all,the highest levels of resistant isolates were observed in 24(55.8%) which showed resistance to six of the antibiotics while the least was 3(7.0%) which were resistant to four antibiotics. This could be as a result of the overuse of antibiotics in food-producing animals, mass treatment and long-term administration of antimicrobial growth promoters to animals may lead to the emergence of multidrug-resistant strains of enteric bacteria, including *Salmonella* spp (Collingnon, 2003).

The variation in the susceptibility and resistance level of the Salmonella isolates to different classes of antibiotics in this study could be due to differences in species from location to location. Antibiotic susceptibility patterns vary regionally and geographically and have been reported to change rapidly over time (Martinez*et al*., 2005).

Multiple Antibiotics Resistance (MAR) index is calculated as the ratio of the number of antibiotics to which organism is resistant to total number of antibiotics to which the organism is exposed (Poonia *et al.,*2014). The multiple antibiotics resistant patterns of *Salmonella* spp from water samples in this study is greater than 0.2 which implies that the strains of such *Salmonella* spp originated from an environment where several antibiotics are in use (Ehinmidu, 2003).

5.5 Antibiotic Resistance Genes

The mechanism of resistance in most organisms are coded by specific genes .These genes could be genomic and inherent or plasmid mediated and acquired from other organisms. The presence of these gene segments have been used to identify potential resistance to antibiotics .

The study shows genotypic identification of *gyrA, gyr B tem, sul 2* and *catP genes* among 15 *Salmonell*a isolates that were mostly resistant to Ciprofloxacin, Nalidixic acid, Ampicillin,sulphamethoxazole and chloramphenicol. However, genetic analysis has indicated that the source of resistance is frequently a transferable plasmid (Abdullahi *et al.*,2013).The antibiotic resistance could have occured either through transferable resistance genes, excessive

use of antibiotics in treatment leading to selective pressure or response to environmental drastic conditions. The resistance in *Salmonella* spp could arise from acquisitions of foreign genes that encode enzymes to destroy, chemically inactivate, pump out the drug out of the cell or provide alternative pathways to the targeted by the antibiotic.

Chloramphenicol resistance is conferred by an enzyme encoded by *cat*p gene .A total of nine isolate possess the *catP* gene which codes for the enzyme chloramphenicolacetyltransferase leading to resistance to chloramphenicol.This is a plasmid borne gene and its presence is worrisome as the use of chloramphenicol as the drug of choice for Salmonella infection is greatly hampered (Aaresthrup *et al.,*2003; prescott,2011).

Two of the isolates harboured the *sul*2 genes which is as a result of the acquisition of genes encoding enzymes that do not bind the drugs leading to Sulphamethoxazole resistance. The *sul2* genes, encode an insensitive DHPS enzyme and are found in *Salmonella* spp globally (Prescott,2000). Similar results was seen with Guerra *et al.,*2002.

Resistance gene analysis of the resistant isolates shows the presence of ampicillin resistance gene in three of the isolates. β-lactamase gene conferring resistance to β-lactam antibiotics all of which were resistant to Ampicillin, Amoxicillin-clavulanic acid and Cefoxitin. This result could be due to diverse point mutations in the *tem* gene. *Salmonella* spp may acquire genes encoding enzymes, such as β-lactamases, that destroy the antibacterial agent, similar resistance in these isolates as reported by(Chen *et al.,(*2004), Mooljuntee *et al*. (2010) and Momtaz *et al*. (2012). However, several other isolates with similar resistance pattern gave negative amplifications with these genes in this study. This could be due to the fact that there are several kinds of β-lactamase genes such asbla_{CMY},bla_{SHV}, $bla_{PSE\text{-}1}$ gene or bla_{CTX} genes which were not covered in this study whichcould be present but have not been determined (Chen *et al.*, 2004; Momtaz *et al*., 2012; Zhang *et al.,* 2009).

Resistance gene analysis of the resistant isolates showed no gene was confirmed for ciprofloxacin resistance and agrees with the findings of Abdullahi *et al.,* (2013). The presence of Nalidixic acid resistance gene, *gyr*B was seen in 100% (15 / 15) of the tested isolates with *S.*Arizonae having 60%(9/15) while *S.*Pullorum had 40% (6/15). Though nalidixic acid and ciprofloxacin are from the same group (quinolones) higher frequency of resistance to Nalidixic acid were noted which could be as a result of selective pressure. Nalidixic acid resistance is due to mutation (s) in *gyr B* gene. The genetic change may be acquired by the transfer of resistance genes located in plasmids or transposons from one bacterium to another (Denyer *et al.,* 2011). This finding is in line with the findings of Asma *et al.,* 2005 and Abdullahi *et al.,* 2013 where they reported the presence of antibiotic resistance genes among *Salmonella* spp isolates The presence of resistance genes observed in the isolates could be as a result of changes in the cell envelope; down regulation of membrane porins; increased Lipopolysaccharide (LPS) component of the outer membrane; quorum sensing and biofilm formation. The phenotypic resistance observed by the Kirby Bauer disc diffusion method was seen to be genetically confirmed by PCR in some of the isolates. However, some isolates showed antibacterial drug resistance phenotypically but not genetically.These discrepancies between the phenotypes and genotypes is not surprising, since several genes may confer a given phenotype and some isolates must have contained other genes that were not tested for in this study. Other reasons might be due to inoculum effect and substrate specificity which may affect the enzyme in an uninduced state at the time of testing with the disc diffusion test (Asma *et al.,* 2005).

CHAPTER SIX

6.0 CONCLUSION AND RECOMENDATION

6.1 Conclusion

The findings of this studydemonstrated that the physicochemical parameters for Turbidity, Hardness and Chemical Oxygen Demand exceeded the permissible limits for all the locations and months in both dry and wet seasons. .Turbidity ranged from 20.18±26.17(Shika dam) to 37.39±35.69) NTU (Galma), hardness ranged from 233.46±82.10 Galma to 238.55±104.31mg/l Shika and COD ranged from 238.80±48.95mg/l Galma to 274.25±97.90mg/l Kubanni dam.

The study established a high mesophilic aerobic bacterial count with a mean of $1.09\times10^{8}\pm7.20\times10^{6}$ for Shika Dam, $9.60\times10^{7}\pm6.76\times10^{6}$ CFU/ml for Kubanni Dam and $9.98\times10^{7}\pm6.87\times10^{6}$ CFU/ml for Galma Dam during the dry season while that of wet season was ($1.53\times10^{8}\pm2.71\times10^{6}$ CFU/ml) for Shika Dam, $1.43\times10^{8}\pm3.62\times10^{6}$CFU/ml for Kubanni Dam and $1.52\times10^{8}\pm1.85\times10^{6}$ CFU/ml for Galma Dam.The results were statistical significant with P-value < 0.05 implying they were continually being polluted by the population of heterotrophic bacteria present in the raw water.

The study revealed the presence of *Salmonella* spp in dam water samples analyzed with an overall isolation rate of 9.5%. A prevalence of 6.0%, 3.1% and 0.4% was obtained for *S.* Arizonae*, S.* Pullorum*,* and *S.* Choleraesuis respectively.

All the *Salmonella* spp isolates were resistant to at least one of the antibiotics used in the study, indicating multiple resistance due to possible transfer of resistance genes. All the 43 isolates (100%) were resistant to ampicillin, nalidixic acid and augmentin. They were also resistant to cefoxitin and cotrimoxazole (95.3%) . Intermediate resistance occurred for chloramphenicol (60.5%).The antibiotics effective against all the *Salmonella* isolates tested were ceftriaxone and imipenem (100%), Gentamicin (93%) and ciprofloxacin (74.4%),

All the *Salmonella* spp isolates harboured the *gyr B* gene, for Nalidixic acid. The antibiotic resistant genes *catP, sul2, Tem,* and *gyrB* demonstrated 9(60.0%), 2(13.3%), 3(20.0%), 2(13.3%) and 15(100%) respectively of *Salmonella* spp*.* Multiple resistance genes were detected in this study. A combination of four different resistance genes were detected in 2(13.3%) isolates while three different genes were observed in only one isolate. All the *Salmonella* spp isolate harboured at least one of the antibiotics resistance gene.

6.2 Recommendation

It was evident during the course of this study that the quality of the water in the dams were polluted due to increased human population, industrialization, use of fertilizers in the agriculture and man-made activity.The following are therefore recommended: -

1.That there is a need for routine monitoring of the water quality of the dams to ensure that these parameters are within the acceptable limits.

2. That farming and the use of inorganic fertilizers should be discouraged or restricted around these reservoirs.Proper sewage treatment and river quality monitoring are needed to warn against hazards to public health and vulnerable water resources.

3.Infections due to multidrug resistant *Salmonella* can be eliminated not by massive use of antibiotics but by improvement in the conditions of animal husbandry and reduction in the opportunities for the initiation and spread of the disease

4.Attention should be focussed by both the public and government on putting in place antibiotic resistance surveillance programs in the country.

5.Resistance genes responsible for cefoxitin should be investigated as well as other resistance genes that were not included in this study.

6.Vaccines for *Salmonella* spp infections should be encouraged and produced locally to reduce cost.

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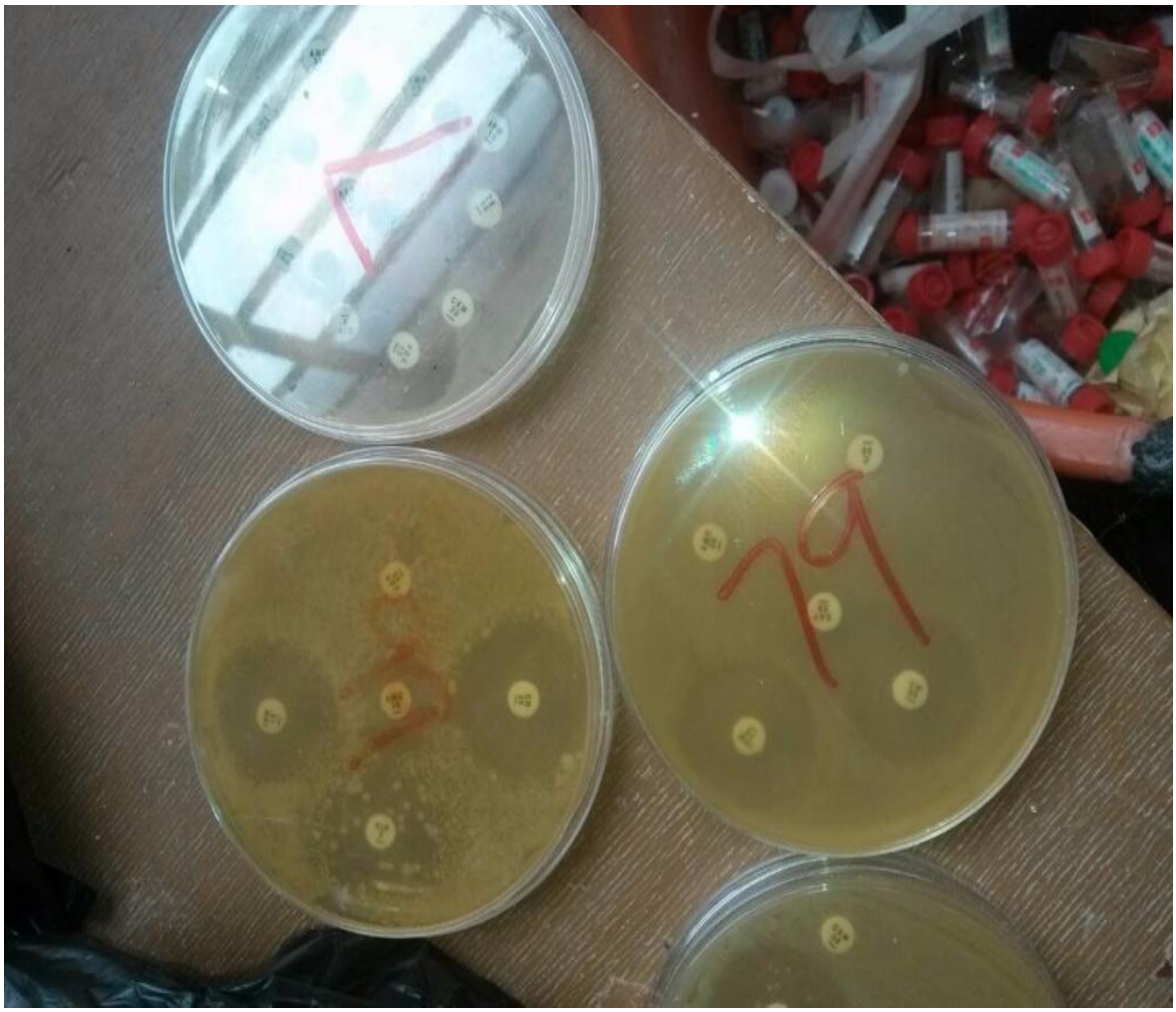
APPENDIX

APPENDIX I: Images from Microgen Identification Test Kits, Presumptive *Salmonella* Isolates



APPENDIX II: Images from Antibiotic resistance and susceptibility (P1,P2& P3) by Isolates

P1: *S.* Arizonae; **P2 & P3**: *S.* Pullorum and *S.* Choleraesuis

APPENDIX III: Identification of *Salmonella* spp using Microgen™

Enterobacteriaceae GNA- ID System

Sample code	Lysine	Orn	H_2S	Glu	Mann	Xylose	ONPG	Indole	Urease	VP	Citrate	TDA	%	OC
$KUB_{20}SP$	+	+	+	+	+	+	+	-	+	-	+	+	83.51	7753
$KUB_{4}SP$	+	+	+	+	+	+	+	-	+	-	-	+	51.81	7751
$KUB_{64}SA$	+	+	+	+	+	+	+	-	-	-	+	+	98.11	7743
$KUB_{5}SP$	+	+	+	+	+	+	+	-	+	-	-	+	97.11	7701
$MAR_{70}SA$	+	+	+	+	+	+	+	+	-	-	+	+	83.51	7753
$GAL_{50}SA$	+	+	+	+	+	+	+	+	-	-	+	+	83.51	7753
$MAR_{11}SA$	+	+	+	+	+	+	+	+	+	-	+	+	83.10	7773
$GAL_{35}SA$	+	+	+	+	+	+	-	-	-	-	+	-	80.89	7702
$GAL_{114}SA$	+	+	+	+	+	+	-	-	+	-	+	-	97.71	7712
$GAL_{68}SA$	+	-	+	+	+	+	+	-	-	-	+	-	91.79	5742
$MAR_{15}SP$	+	+	+	+	+	-	-	-	-	-	+	-	90.87	7602
$MAR_{69}SP$	+	+	+	+	+	+	-	-	-	-	-	+	98.26	7701
$GAL_{25}SA$	+	+	+	+	+	+	+	+	-	-	+	-	98.69	7762
$MAR_{134}SP$	+	+	+	+	+	+	-	-	-	-	-	+	97.11	7701
$MAR_{65}SP$	+	+	+	+	+	+	-	-	+	-	-	+	93.84	7711
$MAR_{13}SA$	+	+	+	+	+	+	+	-	+	-	+	+	83.51	7753
$MAR_{116}SP$	+	+	+	+	+	+	+	+	-	-	-	-	91.92	7760
$KUB_{79}SC$	+	+	-	+	+	+	-	-	-	-	-	+	76.10	6701
$MAR_{14}SA$	+	+	+	+	+	+	+	+	-	-	-	-	98.69	7763
$GAL_{106}SA$	+	+	+	+	+	+	+	-	+	-	+	+	83.51	7753
$GAL_{6}SA$	+	+	+	+	+	+	+	-	+	-	+	+	83.51	7753

$GAL_{38}SA$	+	+	+	+	+	+	+	-	+	-	-	+	51.81	7751
$KUB_{124}SA$	+	+	+	+	+	+	+	-	+	-	+	+	83.51	7753
$GAL_{123}SC$	+	+	+	+	+	+	+	+	-	-	+	-	76.10	6701
$GAL_{36}SA$	+	+	+	+	+	+	+	-	+	-	+	+	83.51	7753
$GAL_{34}SA$	+	+	+	+	+	+	+	+	-	-	+	+	98.69	7763
$MAR_{77}SA$	+	+	+	+	+	+	-	-	-	+	-	+	83.51	7753
$MAR_{80}SA$	+	-	+	+	+	+	-	-	-	-	+	+	91.63	5743
$MAR_{113}SP$	+	+	+	+	+	+	-	-	-	-	-	+	66.15	7701
$MAR_{46}SA$	+	+	+	+	+	+	+	-	-	-	+	+	98.11	7743
GAL_{32} SA	+	+	+	+	+	+	+	-	+	-	-	+	51.81	7751
$MAR_{99}SA$	+	+	+	+	+	+	+	-	-	-	+	-	97.71	7742
$MAR_{97}SP$	+	-	+	+	+	+	+	-	-	-	+	-	91.79	5742
$ABU_{76}SA$	+	+	+	+	+	-	-	-	-	-	+	+	98.11	7743
$MAR_{31}SP$	+	+	+	+	+	-	-	+	-	-	-	-	98.26	7721
$GAL_{21}SA$	+	+	+	+	+	+	+	-	+	-	-	+	98.06	7751
$KUB_{35}SA$	+	+	+	+	+	+	+	+	-	-	+	+	83.51	7753
$KUB_{79}SP$	+	+	-	+	+	+	-	-	-	-	-	+	76.10	6701
$KUB_{91}SP$	+	+	+	+	+	-	+	-	-	-	-	+	98.26	7721
$MAR_{90}SA$	+	+	+	+	+	+	+	-	-	-	+	+	98.11	7743
$MAR_{96}SA$	+	+	+	+	+	+	-	+	+	-	+	+	98.69	7763
$MAR_{66}SP$	+	+	+	+	+	+	+	+	+	-	+	-	84.66	7772
$MAR_{98}SA$	+	+	+	+	+	+	+	-	+	-	+	+	98.69	7763
$GAL_{38}SA$	+	+	+	+	+	+	+	-	+	-	-	+	51.81	7751
$GAL_{86}SP$	+	+	+	+	+	+	-	-	+	-	-	+	89.04	7711

GNAwells

KEY:

MAR=Markwa Dam, GAL=Galma Dam, KUB=Kubanni Dam, SA= *S.* Arizonae.SP = *S.* Pullorum, SC= *S.* Choleraesuis, ONPG=Ortho-Nitrophenyl-β-galactosidase, TDA=Triptophan deaminase, H_2S=Hydrogen sulphide, VP=Voges-Proskauer, OC**=** Octal Code, S= *Salmonella*, %=Percentage probability