IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF PATHOGENIC *Escherichia coli* IN DRINKING WATER SOURCES IN JOS, NIGERIA

BY

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ABSTRACT

Quality water is essential for the well-being of all people and one of the fundamental global advancement strategies of public health is accessibility to portable water. This study was aimed at identifying pathogenic *E. coli* and determining the antimicrobial susceptibility profile in drinking water sources in Jos Nigeria. Samples were collected from sixty (60) sites in Jos Metropolis. The coliform count was determined using the most probable number (MPN) technique. Samples were cultured using spread plate method. The multiplex polymerase chain reaction (MPCR) technique was used to identify the pathogenic *E. coli* strains. Fifteen (15) *Escherichia coli* were identified from the sample sites. The results revealed that borehole water had coliform counts ranging from 2 to 150 coliforms/100 mL, whereas, well water analyzed had coliform counts ranging from 2 to 1700 coliforms/100mL.Two (2) samples were identify to be pathogenic *E. coli* flagella in K12 from Dangara and Dahwak water samples. Results also revealed that 40 % of the water sources have no evidence of *E. coli* while 60% were contaminated with faecal coliforms. The results of susceptibility study revealed difference in the antibiogram profile among *E. coli* isolates. The high susceptibility of most bacterial isolates was observed with, gentamycin, ciprofloxacin, sulfamethoxazole, oxfloxacin and enrofloxacin. While high resistance was observed with cefoxitin and tetracycline. This present study revealed that water sources were contaminated with pathogenic *E. coli* and were susceptible to the antibiotics used. *Escherichia coli* flagellin K12 isolated from water samples suggests that water from these sources is not safe for drinking. People using these water sources are prone to enteric infections by *E. coli* flagellin K12. This study suggests that water should be properly treated before consumption to avoid risk of infection by these organisms.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Water makes up 70 % of the earth crust and it is the most valuable natural resources and all life forms including humans and microbes depends on its availability for survival (Oyedum *et al*., 2016). According to the World Health Organization (2017), access to potable drinking water is a basic human right which is essential to health and a component of effective policy for health protection. Water fit for human consumption is termed potable water (GARKUWA *et al*., 2019), and such can be used for various purposes without any risk of acquiring any water borne disease (Oyedum *et al*., 2016). The availability of potable water is essential for human survival and directly impacts the quality of human life across the planet. However, in third world countries of the world, Nigeria inclusive, the problem of potable water supply has posed a lot of challenges. Oyedum *et al*. (2016) reported that many people especially in the developing world depends on untreated surface and ground water sources for their daily water needs, and water from these sources is often faecally contaminated. *Escherichia coli* is an intestinal flora of warm-blooded animals, including humans, this is ubiquitous in the environment and it has been used as an indicator of fecal contamination to assess the safety and quality of water (Maal *et al*., 2015). Although most *E. coli* strains are harmless, certain strains are pathogenic and cause diseases such as watery diarrhea, bloody diarrhea, urinary tract infection, meningitis, and sepsis, which can lead to death (Isibor *et al*., 2013). The normally zoonotic bacterial pathogen has been responsible for waterborne outbreaks in humans through contaminated drinking and recreational water not only in developing countries, but also in industrialized countries (Isibor *et al*., 2013). Environmental water

sources are prone to bacterial pollution from both humans and animals. Possible human sources include discharge of wastewater, sewage leaks, and failing septic tanks, as well as municipal, residential, medical, and industrial waste facilities. Animal sources include runoffs from animal farms, land application of animal manure, pet wastes from parks, and wildlife such as raccoons and deer. Since surface waters are often used for recreational and drinking purposes, the presence of pathogenic *E. coli* in waterways may increase the likelihood of human infections after exposure to these water sources. The presence of *E. coli* O157 in drinking water offered to livestock contributes also to the prevalence of infection in animals and may lead to the contamination of meat products and the environment (Elder *et al*., 2000).

Following the application of polymerase chain reaction in the simultaneous amplification of multiple loci in the human dystrophin gene (Chamberlain *et al*., 1988), multiplex PCR has been firmly established as a general technique. To date, the application of multiplex PCR in pathogen identification, gender screening, linkage analysis, template quantification, and genetic disease diagnosis is widely established (Chehab and Wall, 1992; Kong *et al*., 2002). Identification of pathogen, PCR analysis of bacteria is advantageous, as the culturing and typing of some pathogens has proven difficult or lengthy. Bacterial multiplexes indicate a particular pathogen among others, or distinguish species or strains of the same genus. Multiplex assay is used to distinguish species of *Escherichia coli* from other genus members or associated bacteria Multiplex PCR has been found to be the most effective technology for DNA typing, as the likelihood of two people having identical allele decreases as the number of polymorphic loci investigated grows.

1.2 Statement of the Research Problem

Access to safe water supplies is a critical human need. However, consumption of contaminated water causes an estimated 88 percent of Disability-Adjusted Life Years (DALY) from diarrheal disease, and is responsible for 1.8 million deaths and 4.1 percent of total DALY global burden of diseases (GBD) (Fotadar *et al*., 2005). The Federal Ministry of Health in Nigeria rated diarrhea second after malaria as a disease of high prevalence, it accounts for 16% of under 5 children mortality. This indicates that Nigeria is not on the path to achieving Millennium Development goals except it redoubles her effort and intensifies the present reforms on water and sanitation (World Health Organisation, WHO, 2017).

Water is a vital natural resource because of its basic role to life, quality of life, the environment, food production, hygiene, industry, and power generation. With the rapid increase in world population and increased urbanization, there is a massive strain on the existing water supply and sanitation facilities (United Nations Department of Public Informations, UNDPI, 2005). In the developing world, poor access to safe water and inadequate sanitation continues to be a danger to human health (WHO, 2004). The scarcity of water does not only threaten food security, but also the production of energy and environmental integrity. This often results in water usage conflicts between different communities, and water contamination when humans and animals share the same source of water (Kusiluka *et al*., 2005). *Escherichia coli*, which normally resides in the intestinal flora of warm-blooded animals, including humans, is ubiquitous in the environment and has been used as an indicator of faecal contamination to assess the safety and quality of water (Adams and Moss, 2008). The source of water contamination responsible for the spread of infectious diseases is almost invariably faeces (Adams and Moss, 2008). In most developing countries, much of the water available for drinking is not only short in supply, but also unsafe for drinking due to

contamination mostly with human and animal faeces as a result of poor sanitation i.e. lack of improved excreta and solid waste disposal (Gimba, 2011).

Faecal contamination of water is globally recognized as one of the leading causes of water borne diseases. In Nigeria, cases of water related diseases abound. The most common waterborne disease in Nigeria includes cholera, dracunculiasis, hepatitis and typhoid (Adeyinka *et al*., 2014). Cases of water borne diseases linked to contaminations of drinking water with pathogens have been reported in several towns (Nwabor *et al*., 2015).

1.3 Aim and Objectives of the Study

The aim of this study was to determine the antimicrobial susceptibility and identification of pathogenic *E. coli* strains by multiplex polymerase chain reaction in drinking water sources from Jos metropolis.

The objectives of this study were to:

i. isolates and identify the pathogenic *E. coli* in drinking water sources.
ii. determine the physico-chemical properties of the drinking water from well and borehole.
iii. determine the antimicrobial susceptibility pattern of the *E. coli* isolates.

1.4 Justification for the Study

The major sources of water within Jos metropolis is the underground water and this underground water contains a myriad of microorganisms which are introduced into water body through open defecation. Most community in the study area source their drinking water from underground water source. Untreated water from these sources leads to waterborne diseases. The most common water borne disease of public health importance in developing countries is diarrhea whose causative agent is *Escherichia coli*. Diarrhea disease is the second leading cause of death in children under five years old. Each year diarrhea kills around 525 000 children under five, globally, there are nearly

1.7 billion cases of childhood diarrhea disease every year (WHO, 2017). This work will benefit the students, unsuspecting consumers, policy makers and health agencies on any health risk that might arise from this drinking water sources.

CHAPTER TWO

2. 0 LITERATURE REVIEW

2.1 Water Quality

Water is a universal solvent, which consist of hydrogen and oxygen atoms. Chemically, it could be defined as a chemical substance with two atoms of hydrogen and one atom of oxygen in each of its molecules; hence the molecular formula is H_2O (Onyeneke *et al*., 2020). It is formed by the direct reaction of hydrogen with oxygen; $2H_2 + O_2 \rightarrow 2H_2O$. Water is essential to sustain life, and a satisfactory supply must be made available to consumers. It is a critical requirement in the maintenance of metabolic functions and homeostasis (the ability to maintain stable body conditions) in living cells. The human body is composed of about 60% water by weight in adult males, 50% in females and 70% in new born infants (Svagzdiene *et al*., 2010). Water is one of the most important necessities to all forms of life on this planet (Owolabi *et al*., 2014). Therefore, adequate and safe water supply should be available to humans, plants, and animals in all parts of the world. The regular intake of adequate amounts of water is essential in the maintenance of good health and well-being. The approximate human dietary requirement of water is estimated to be two litres per day for an average adult. According to the World Health Organization every effort should be made to achieve a safe drinking water supply in every community of the world because it is known that improving access to safe drinking-water can result in significant benefits to health. The most important attribute of drinking water that has to be assured and maintained is its safety and quality for human consumption (Adeyinka *et al*., 2014). Drinking water must be free of harmful contaminants, such as pathogenic microorganisms, toxic substances, physical and chemical residues, undesirable organo-leptic properties like odour, colour, and taste of water is essential

for living things, both in the composition of their cells and in the environment surrounding them. Organisms are made up of between 60 and 95 per cent water by weight, and even inert (dormant) forms like spores and seeds have a significant water component. This dependence on water is a function of its unique properties, which in turn derive from its polar nature. To humans, water is indispensable. In the home, water is used for cooking, washing, bathing and other domestic uses. Industrially, water is the starting point of most processes (Exall, 2004).

2.2 Sources of Domestic Water

The most common sources of drinking water are streams, lakes, rivers, ponds, rainwater, and underground water (spring, wells, and boreholes). Because the ground serves as an excellent filter media, underground water is safer and purer for residential use than surface water (Aderibigbe *et al.,* 2008). Deep well and deep spring water absorb a lot of salts and other minerals, which is a major issue with underground water, and the water becomes salty, often too salty or "hard" for any use unless the salts are removed, which is costly (Adogo *et al*., 2016).

2.3 Sources of Water Pollution

Seventy to eighty percent of water pollution is attributed to domestic sewage. Industrial wastes such as waste from sugar, textiles, electroplating, insecticides, pulp and paper industry are also sources of water pollution (Kamble, 2014). Rivers that have been polluted have a foul odour and lesser plants and animals. Water security is a serious problem for 80 percent of the earth's population. Large amount of domestic sewage, the majority of which is untreated, is discharged into the river. Domestic sewage often comprises of faeces, oil, solid waste, plastic litter, and bacterial pollutants. The main source of water pollution is untreated industrial effluents discharged into rivers. (Owa, 2013). Hazardous material discharged from the industries is responsible for surface water and ground water contamination. Toxic metals enter in to water and

reduced the quality of water, 25 % pollution is caused by the industries and is more harmful. Increasing population is creating many issues but it also plays negative role in polluting the water. Increasing population leads to increase in solid waste generation. Solid and liquid waste is discharged in to rivers. Water is also contaminated by human excreta. In contaminated water, a large number of bacteria are also found which is harmful for human health (Jabeen *et al*., 2011). Polythene bag and plastic waste is a major source of pollution. Waste is thrown away by putting it in to plastic bags (Adogo *et al.,* 2016). In urban areas, it is estimated that three out of every four people defecate in the open, with 77 percent in use of a flushable latrines with 8 % using pit latrines (Adogo *et al.,* 2016). Many infectious diseases can be spread by urbanization. In metropolitan regions, overcrowding, unsanitary conditions, and contaminated drinking water are major health concerns. Disease affects one-quarter of the urban population. Pesticides are substances which eradicate pathogens such as pests and bacteria. Chemical components of these pesticides aid in pollution and reduction in quality of water directly. Pesticides that are used in excess or are not properly controlled endanger the agriculture ecology. In fertilizer application, only 60 % of it is used up in the soil while the other remaining 40 % is leached in the soil and pollute the water; cyanobacteria thrive in polluted water; and eutrophication is caused by excessive phosphate runoff. As a result of flooding, extreme rainfall, and over-irrigation, chemical residues combine with river water and enter the food chain. These compounds, which are harmful to living organisms, are found in many fruits and vegetables (Kamble, 2014). Pharmaceuticals in trace levels in water pollute the environment and endanger human health.

2.4 Effect of Water Pollution on Human Health

There is a greater association between pollution and health problem. Disease causing microorganisms are known as pathogens and these pathogens are spreading disease directly among humans. Some pathogens are worldwide some are found in well-defined area (Kamble, 2014). Many waters borne diseases are spreading man to man. Heavy rainfall and floods are related to extreme weather and creating different diseases for developed and developing countries (Kamble, 2014). Ten percent of the population depends on food and vegetables that are grown in contaminated water. Many waterborne infectious diseases are linked with faecal pollution of water sources and results in faecal- oral route of infection (Ahmed *et al.*, 2014). Health risk associated with polluted water includes different diseases such as respiratory disease, cancer, diarrheal disease, neurological disorder and cardiovascular disease (Angulo and Scallan, 2007). Nitrogenous chemicals are responsible for cancer and blue baby syndrome (Eisentein *et al*., 1987).

Mortality rate due to cancer is higher in rural areas than urban areas because urban inhabitants use treated water for drinking while rural people don't have facility of treated water and use unprocessed water. Poor people are at greater risk of disease due to improper sanitation, hygiene and water supply. Contaminated water has large negative effects in those women who are exposed to chemicals during pregnancy; it leads to the increased rate of low birth weight as a result foetal health is affected (Currie *et al*., 2013). Poor quality water destroys the crop production and infects our food which is hazardous for aquatic life and human life (Prakash, 2020). Pollutants disturb the food chain and heavy metals; especially iron affects the respiratory system of fishes. An iron clog in to fish gills and it is lethal to fishes, when these fishes are eaten by human leads to the major health issue. Metal contaminated water leads to hair loss, liver cirrhosis, renal failure and neural disorder (Ahmed *et al*., 2014). Untreated

drinking water with faecal contamination is the major cause of bacterial diseases and diarrheal, and the spread of *Campylobacter jejuni* from 4 % to 15 % worldwide (Bivins *et al*., 2017). The most common symptoms of diarrhoea include fever, stomach pain, nausea, and headache. Cholera is a disease caused by polluted water. This sickness is caused by *Vibrio cholerae*. In the digestive tracts, this bacterium creates poisons. Watery diarrhoea, nausea, and vomiting are symptoms of this disease, and watery diarrhoea can lead to dehydration and renal failure. To get rid of bacteria, antimicrobial therapy is employed. Shigellosis is a bacterial disease caused by Shigella bacteria. It affects the digestive tract of humans and damages the intestinal lining. Watery or bloody diarrhoea, abdominal cramps, vomiting and nausea are symptoms and it can be cured with antibiotics and good hygienic practice. Salmonellosis is infecting the intestinal tract. Salmonella bacteria are found in contaminated water and it results in inflammation of intestine and often death occurs.

2.5 Bacteriological Water Analysis

Bacteriological water analysis is a method of analyzing water to estimate the numbers of bacteria present and, if needed, to find out what sort of bacteria they are. It represents one aspect of water quality. It is an analytical procedure which uses samples of water and from these samples determines the concentration of bacteria. It is then possible to draw inferences about the suitability of the water for use from these concentrations (Adogo *et al*., 2016). The pathogenic organisms which may be present in water are numerous and identifying these organisms individually in practice (bacteria, protozoa and helminths) is difficult. As their presence is always linked to faecal pollution (except for guinea worm), it is preferable to look for organisms which are “indicators” of this pollution (Donnenberg and Whittam, 2001). The common features of all these routine screening procedures is that the primary analysis is for indicator organisms rather than the pathogens that might cause concern. Indicator

organisms are bacteria such as non-specific coli forms, *Escherichia coli* and Faecal Streptococci such as *Enterococcus faecalis* that are very commonly found in the human or animal gut and which, if detected, may suggest the presence of sewage. It is therefore reasonable to summarize that if indicator organism levels are low, then pathogen levels will be very much lower or absent. The count of those colonies which develop with a characteristic appearance gives the number of faecal coliforms in the sample of water. Analysis is usually performed using culture, biochemical and sometimes optical methods. When indicator organism's levels exceed pre-set triggers, specific analysis for pathogens may then be undertaken and these can be quickly detected (when suspected) using specific culture methods or molecular biology (Adogo *et al*., 2016).

2.6 Water Borne Diseases

Water-borne diseases are any illness caused by water people drink that is contaminated by animal or human faeces, which contain pathogenic microorganisms. Waterborne diseases are caused by pathogenic microorganisms that most commonly are transmitted in contaminated fresh water. Water should be harmless to health and have an appearance and taste acceptable to the population. Ideally the water supplied should meet the quality standard of the WHO. Quite a number of human pathogens find their way into a susceptible host through contaminated water. These pathogens often called waterborne pathogens, have the ability to survive at least for a short period in water and thus water may act as a route of transmission for them (Adogo *et al*., 2016). Waterborne diseases are posing a serious threat to health since the potential of contaminated water to transmit disease is very high. Often, they lead to epidemic. According to a WHO 2017 survey about 30,000 people die from water related diseases every day and that about 80 % of all illness in developing countries is water related.

2.6.1 Typhoid fever

This is caused by ingestion of *Salmonella* Typhi bacteria in food or water. Infection causes a sudden high fever, nausea, severe headache, and loss of appetite. It is sometimes accompanied by constipation or diarrhoea (Meerburg *et al.*, 2009).

2.6.2 Hepatitis A and E

This is caused by viral infection. Symptoms include yellowing of the skin and eyes (jaundice), dark urine, fatigue, nausea and vomiting. Two forms of the disease, hepatitis A and E, are primarily caused by ingestion of faecally contaminated drinking water (United Nations International Children's Emergency Fund, UNCIEF, 2008). Hepatitis A causes about 1.5 million infections each year (mostly in children), and can occur in epidemics. Hepatitis E is less common than hepatitis A, and occurs mainly in epidemics caused by monsoon rains, heavy flooding, contamination of well water, or massive uptake of untreated sewage into water bodies. No specific treatment exists for hepatitis A or E, but most (greater than 98 %) patients recover completely. Hepatitis can have more serious effects on older or immune-compromised people, and pregnant women are particularly vulnerable to hepatitis E, with approximately 20 % mortality rates (WHO, 2004)

2.6.3 Leptospirosis disease

Leptospirosis is a blood infection caused by the bacteria *Leptospira*. They are disease spread by contact with water polluted with infected urine from a variety of animals (principally the rodents) Signs and symptoms can range from none to mild (headaches, muscle pains, and fevers) to severe (bleeding in the lungs or meningitis). Efforts to prevent the disease include protective equipment to block contact when working with potentially infected animals, washing after contact, and reducing rodents in areas where people live and work (Meerburg *et al.*, 2009). It is estimated that one million severe cases of leptospirosis occur every year, causing about

58,900 deaths. The overall risk of death is from 5 to 10 %. However, when the lungs are involved, the risk of death increases to the range of 50 to 70 % (Meerburg *et al*., 2009).

2.6.4 Other diseases

Another category of water related diseases is those with an insect vector which develops in or lives near to the water, for example malaria, dengue and yellow fevers, and onchocerciasis (Bartram and Hunter, 2015). Besides these diseases, water is also involved in the transmission of "water- based" diseases (that is, diseases of which the causative agent passes part of its life cycle in an aquatic plant or animal): The different schistosomiasis or bilharziasis: diseases caused by helminths (worms) which are usually contracted by contact with infected water but sometimes also via the oral route. "Dracunculiasis" (Guinea worm), transmitted only by drinking infested water (Bartram and Hunter, 2015).

2.7 General Overview of *Escherichia coli* as a Pathogen

*Escherichia coli*are Gram-negative, facultative anaerobic, rod-shaped and highly motile bacteria. They are often classified under *"enterobacteriacea"* known to be *normal inhabitants of the* gastrointestinal tract of both animals and human beings but only some strains of *E. coli* have become highly adapted to cause diarrhoea and a range of extra- intestinal diseases (Kubitschek, 1990). *Escherichia coli* were first isolated by a German paediatrician, Theodore Esherich, in 1884 from faeces of human neonates (Kubitschek, 1990). The first confirmed isolation of *E. coli* O157:H7 in the United States of America was in 1975 from a Californian woman with bloody diarrhoea. It was since 1982 that EHEC have been recognized as an important aetiological agent of diarrhoeal diseases in man and animals. *E coli* O157 was described as a rare serotype (Karmali *et al*., 1983). Studies conducted between 1983 and 1985 in the United States and Canada, have linked EHEC infection to

haemorrhagic colitis (HC) and it had a close relation with the classical form of haemolytic uraemic syndrome (HUS) (Karmali *et al*., 1985). As a result of these and other studies, Karmali, (1989) re-examined isolates of *E. coli* belonging to the O157 serogroup that had been submitted to the International *Escherichia* and *Klebsiella* Centre. Three isolates were found that had the H7 antigen (Karmali, 1989). These three isolates were from the faeces of one animal out of a batch of 39 calves with "*colibacillosis*" in Argentina. It was also speculated that cattle might be the reservoir for these organisms.

2.7.1 Taxonomic classification of *Escherichia coli*

The comparative analysis of 5S and 16S ribosomal RNA sequences suggest that Escherichia and Salmonella diverged from a common ancestor between 120 and 160 million years ago, which coincides with the origin of mammals. *Escherichia* and *Shigella* have been historically separated into different genera within the "*Enterobacteriaceae*" family. DNA sequence analysis of their genomes reveals a high degree of sequence similarity and suggests that they should be considered a single species (Ochman and Wilson, 1987). There are several types of *E. coli* strains but these strain types can be divided into six groups or pathotypes based on the mechanism they cause disease: *Enteropathogenic E. coli E.* (EPEC), Attaching and effacing *E. coli* (A/EEC), *Enterotoxigenic E. coli* (ETEC), *Enteroinvasive* (EIEC),and *Enteroaggregative E. coli* (EAEC).*Escherichia coli* strains that produce the Stx toxins have been referred to as vero toxin-producing *E. coli* (VTEC), shiga-toxigenic *E. coli* (STEC) an enterohaemorrhagic *E. coli* (EHEC) (Nataro and Kaper, 1998). *Escherichia coli* are divided into pathogenic *E. coli* and non-pathogenic *E. coli*. The non- pathogenic strains of *E. coli* described as commensal *E. coli* are present in the normal microflora of intestine which is harmless, hinder the growth of harmful bacteria and produce vitamins (Beauchamp and Sofos, 2010). Pathogenic *E. coli* strains

can be further classified into intestinal diarrheagenic *E. coli* which causes diarrhea and extra intestinal *E. coli* (ExPEC) which causes wide range of illnesses in humans such as the neonatal meningitis, chronic urinary tract infections, septicemia and hemolytic uremic syndrome (Croxen and Finlay, 2010). Commensal *E. coli* In spite of the presence of highly diversified and complex microbiota in the gut, *E. coli* is highly adaptable to the gastrointestinal environment and play several important roles in humans, such as performing specific metabolic functions which are absent in humans, modulating the morphology and physiology in the gut as well as assisting in development of the immune system (Croxen and Finlay, 2010). Pathogenic *E. coli* Intestinal diarrheagenic *E. coli* strains are known to be the classes of diarrheagenic *E. coli*thatare identified, namely, en-terotoxigenic *E. coli (*ETEC), enterohaemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), *enteroinvasive E. coli* (EIEC) include *Shigella, enteroaggregative E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) (Jafari *et al*., 2012) and the recently emerged, adherent invasive *E. coli* (AIEC) (Allocati *et al*., 2013).

2.7.1.1 *Enteropathogenic Escherichia coli*

These organisms are a significant cause of infant diarrhea in developing nations. Enteropathogenic *E. coli* (EPEC) were historically recognized on the basis of serotypes such as O55:H6 and O127:H6. EPEC are an established etiological agent of human infantile diarrhea. In developing countries, Enteropathogenic *E. coli* (EPEC) is one of the most common pathogens (Xia *et al*., 2010).

2.7.1.2 *Enteroinvasive Escherichia coli*

These organisms often cause a broad spectrum of human's diseases. They are biochemically, genetically and pathogenetically closely related to *Shigella* species. Both characteristically cause an invasive inflammatory colitis, but either may also

elicit a watery diarrhea syndrome indistinguishable from that caused by other *E. coli* pathogens (Xia *et al*., 2010).

2.7.1.3 *Enterotoxigenic Escherichia coli*

They are a major cause of secretory diarrhea. enterotoxigenic (ETEC, causative agent of diarrhea in humans, pigs, sheeps, goats, cattle, dogs and horses. ETEC produce toxins which are heat-labile (LT) and/or heat-stable (STa and STb) that are also causing diarrhea (Allocati *et al*., 2013).

2.7.1.4 *Enteroaggregative Escherichia coli*

These strains of organism are defined by their distinctive adherence pattern on HEp-2 cells in culture (Nataro and Kaper, 1998). The essential element of the aggregative phenotype is the stacked brick pattern by lying side-by-side with an appreciable distinction of where one bacterium begins and another ends (Nataro and Kaper, 1998).

2.7.1.5 *Enterohaemorrhagic Escherichia coli*

The strains of these organisms are implicated in food-borne diseases principally due to ingestion of uncooked minced meat and raw milk. These strains produce shiga like toxin 1 (Stx1), shiga-like toxin 2 (Stx2). Serotype EHEC O157:H7 is the prototype of increasing importance and is associated with hemorrhagic colitis, bloody diarrhea and the hemolytic uremic syndrome (Cornick *et al*., 2000).

2.7.1.6 *Shiga-toxigenic Escherichia coli*

There are many serotypes in STEC and among them, the EHEC serotype O157:H7 is found to be highly virulent, responsible for causing outbreaks of bloody diarrhea and hemolytic uremic syndrome (HUS) around the globe. Shiga-toxin or Vero toxin producing (STEC/VTEC). *Enterohaemorrhagic Escherichia coli* (EHEC) is the most important pathotype in human diseases of all diarrheagenic *E. coli* identified (Donnenberg and Whittman 2001). Runminants are recognized as natural

reservoir host for *E. coli* O157:H7 (Muniesa *et al*., 2006). No treatment has yet been found for the infections caused by EHE (Muniesa *et al*., 2006). All STEC including serotype O157:H7 have the same morphology. They are Gram- negative, facultative anaerobic bacteria that belong to the "*Enterobacteriaceae*" family and the Escherichia genus (Xia, *et al*., 2010). *Escherichia coli* O157:H7 produces shiga toxin which is an important cause of food borne illness in humans and ruminant animals where they appear to be more frequently colonized by *E. coli* STEC than other animals, but the reason for this is unknown (Cornick *et al*., 2000).

2.8 Epidemiology of Pathogenic *Escherichia coli*

The epidemiology of each pathogenic *E. coli* was reported to vary according to different species and strains of *E. coli*. The presence of these pathogenic *E. coli* was found in various animal reservoirs and spread within and as well as to other animals (Ullah *et al*., 2014). Numerous epidemiology studies carried out found that various factors contribute to the shift of prevalence based on different geographical areas, population, age distribution, socioeconomic class and detection methods (Vickers, 2017). Researchers, especially on the detection of EHEC serotype, O157:H7, although other non-O157 strains are also major causes of many outbreaks in many regions including North America, Australia and Europe (Angulo and Scallan, 2007). Due to the severity of infections caused by EHEC, surveillance and control measures had taken place such as the establishment of specific program called PulseNET, which was created to provide information necessary in case of sudden outbreaks. Currently, Pulse NET network is available internationally.

2.9 Cell Structure and Physiology of *Escherichia coli*

Escherichia coli is the head of the large bacterial family, "*Enterobacteriaceae*", the enteric bacteria, which are facultative anaerobic and nonperforming bacilli having about 2 μm long and 0.5 μm in diameter with a cell volume of 0.6 to 0.7 μm3

(Kubitschek, 1990). They are approximately 0.5 μm in diameter and 1.0 to 3.0 μm in length. Within the periplasm is a single layer of peptidoglycan. The peptidoglycan has a typical subunit structure where the N-acetylmuramic acid is linked by an amide bond to a peptide consisting of L-alanine, D- glutamic acid, meso-diaminopimelic acid and D-alanine. *Escherichia coli* are commonly motile in liquid by means of peritrichous flagella. *Escherichia coli* are commonly fimbriated. The type 1 pili are the most common and are expressed in a phase switch on or off manner that leads to piliated and nonpiliated state (Eisenstein *et al*., 1987). One of the traits commonly encoded on the larger genetic islands of the different pathotypes of *E. coli* are additional pili (chaperone-usher and type IV pili families and non-pili adhesions (Schreiber *et al.*, 2002). Among *E. coli* isolates, there is considerable variation and many combinations of somatic (O and K) and flagellar (H) antigens. *Escherichia coli* are routinely characterized by serological identification of somatic O, flagellar H and capsular K antigens. However, while some serotypes correlate closely with certain clinical syndromes, differentiation of pathogenic strains from the normal flora depends on the identification of virulence characteristics. Among pathogenic strains, there are few patterns of these antigens and few phylogenetic groupings. For *E. coli*, there are over 150 antigenically unique O-antigens (Whitfield and Roberts, 1999). K type capsular material occurs in two or four forms on the basis of physical, biochemical and genetic criteria The epidemiology of EHEC has been of a major focus and deem important among the (Whitfield and Roberts, 1999). Over 80 serologically and chemically distinct capsular polysaccharides have been recognized. In addition, a slime layer, colonic acid extracellular polysaccharide, is common to many *E. coli* isolates and can be co-expressed with some K-type capsules. There are 53 H-antigen specificities among *E. coli* (Schreiber *et al.*, 2002). the periplasm is a single layer of peptidoglycan. The

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2.10 Growth Characteristic of *Escherichia coli*

Escherichia coli is a facultative anaerobe. Though most *E. coli* strains are capable of growing over a wide range in temperature (approximately 15 to 48 °C), the growth

rate is maximal in the narrow range of 37 to 42 °C. *Escherichia coli* can grow within a pH range of approximately 5.5 to 8.0 with best growth occurring at neutrality. Some diarrheagenic *E. coli* strains have the ability to tolerate exposure to pH 2.0. Such an acid shock mimics transit through the stomach and induces expression of sets of genes involved in survival and pathogenesis (Fotadar *et al*., 2005). It is capable of reducing nitrates to nitrites. When growing fermentatively on glucose or other carbohydrates, it produces acid and gas (mainly H_2 and CO_2). Clinical biochemical tests for *E. coli* confirms it positive for indole production and the methyl red test, most strains are oxidase, citrate, urease and hydrogen sulfide negative. The classic differential test to primarily separate *E. coli* from Shigella and Salmonella is the ability of *E. coli* to ferment lactose, which the latter two genera fail to do (Fotadar *et al*., 2005). Aside from lactose, most E. coli strains can also ferment D-mannitol, D-sorbitol, and Larabinose, maltose, D-xylose, trehalose and Dmannose. There are limited instances where pathogenic strains differ from the commensals in their metabolic abilities. For example, commensal *E. coli* strains generally use sorbitol, but *E. coli* O157:H7 does not. Most diarrheagenic strains cannot utilize D-serine as a carbon and nitrogen source, but uropathogenic and commensal fecal strains can use this enantiomer of serine (Roesch *et al*., 2003).

2.11 Biochemical Properties

Escherichia coli can be differentiated from other members of the *"Enterobacteriaceae"* on the basis of a number of sugar-fermentation and other biochemical tests. Classically an important group of tests used for this purpose are known by the acronym IMViC. These tested for the ability to produce: indole from tryptophan (I); sufficient acid to reduce the medium pH below 4.4, the break point of the indicator methyl red (M); acetoin (acetylmethyl carbinol) (V); and the ability to utilise citrate (C) (Adam and Moss, 2008). Despite *E. coli* can be identified with

a variety of biochemical reactions, the indole test remains the most useful method to differentiate *E. coli* from other members of the "*Enterobacteriaceae*" (Xia *et al.*, 2010).

2.12 Mode of Contamination and Transmission of *Escherichia coli*

A wide range of foods may be a vehicle for pathogenic *E. coli* in association with their respective ecologies. Food may be contaminated and/or cross-contaminated during growth and harvest (horticulture products), collection (milk) or slaughter (meat). Further contamination can occur during post-harvest handling, transport, processing and unhygienic food handling during preparation. Factors contributing to the persistence of *E. coli* in food systems include inadequate control of processing parameters (e.g. cooking temperature, pH value, water activity and storage at warm temperatures for sufficient time to allow growth). Examples of contaminated foods include: raw/under-processed meat (fermented meat, undercooked ground and beef), unpasteurised dairy products (cheese and milk), unpasteurised fruit juices and raw vegetables (sprouted seeds, lettuce, spinach, cantaloupe and mushrooms). Garba *et al.*, 2009).

2.12.1 Foodstuff of animal origin

Ruminant animals, mainly cattle, are recognized as the primary natural reservoir of STEC, and EHEC (O157:H7), in particular. Although some strains are known to cause diarrhoea in calves, the others appear to be commensal inhabitants of the gut of animals and do not cause clinical disease. Pigs and poultry have not been identified as major sources of STEC for human infection in Europe. Fresh meat and raw milk are, *Escherichia coli* shed by animals and humans in their faeces may enter crop agro ecosystems through manure, irrigation water, contaminated seeds, wildlife and insect pests, or nematode vectors. Consequently, contaminated fresh produce has become much more important as a cause of epidemics of human pathogenic bacteria,

including *E. coli* of different pathotypes. Survival and growth of *E. coli* populations on crop plants and in the soil of crop fields has been proven. *E. coli* have been shown to survive in contaminated soil for up to 20 months, and thus may remain an environmental contaminant for a prolonged period of time. What is more, survival on crop leaves and roots can be higher than in soil alone. Younger leaves tend to provide a better habitat than older leaves, and leaves with higher levels of nitrogen, damaged leaves and fruits are able to support faster multiplication and increased survival of *E. coli* (Hamad, 2012). Nevertheless, considered as common vehicles for *E. coli*, particularly for the EHEC (0157:H7) strain. Contamination of meat usually occurs during animal slaughter, as a result of poor slaughter practices, abattoir hygiene and animal handling practices. Therefore, slaughterhouse practices most likely to contaminate meat involve: removal of animal hide, spillage from the gut of animals and overall sanitary conditions on the abattoir grounds (Franz and Van Bruggen, 2008).

2.12.2 Processed food

Processed food can be contaminated by raw materials, unsanitary water treatment and handling, as well as by cross-contamination. The bacteria can continue to grow in food, unless relevant process parameters are controlled, such as pH value, water activity, temperature and time. Only a few bacterial cells surviving in food can be sufficient to cause illness (Hamad, 2012).

2.12.3 Fresh produce and sprouts

In recent years, the popularity of sprouted seeds has increased significantly owing to their nutritional value. However, reports of food borne outbreaks associated with such raw vegetable sprouts have raised concerns among public health agencies and consumers (Alegbeleye *et al*., 2018).

2.13 Control of Pathogenic *Escherichia coli* in Food and Water

Because the important sites of control differ depending on the pathotype, understanding local foodborne disease epidemiology is critical for developing an appropriate and effective food safety program. This requires a multidisciplinary approach that focuses on the interactions that take place among humans, animals and plants within their ecosystems (Franz and Van Bruggen, 2008). Major food safety risks, could be managed with sustainable crop production intensification through ecosystem approach. Control points along the food chain should be targeted that will ensure the greatest reduction of risk to public health. Risk mitigation steps ought to be taken by following recognized codes of good practice and relevant recommendations of the veterinary and public health services. For pathotypes, such as STEC/ EHEC, the farm-to-consumer pathway needs to be analysed. At the pre-harvest stage, such steps include minimizing colonization of cattle herds and prevention of manure contamination of crops, and at the post-harvest stage, they include slaughterhouse and milking shed hygiene and handling during packing of produce. Pathogenic *E. coli* behave similarly to generic *E. coli* and are able to persist and grow in many foods. They grow prolifically during sprouted seeds production, so that minimizing initial seed contamination and limiting subsequent growth are important controls. Some *E. coli* strains can elicit stress responses that enhance their growth and persistence, e.g. STEC may tolerate acid conditions in fruit juice and fermented grounds (Franz and Van Bruggen, 2008)

2.13.1 Pre-harvest interventions in farm animal production

Strategies that will reduce shedding in live animals offer methods to reduce pathogen populations in food animals before they enter the food chain. For example, abruptly switching cattle from a high grain ration to a high-quality hay-based diet has been shown to reduce generic *E. coli* and *E. coli* (O157:H7) populations. However, switching feed

lot cattle from grain-based to hay-based diets prior to slaughter may not be practical. The feeding of probiotic Lactobacillus acidophilus has been proven effective and has been adopted for the pre-harvest control for *E. coli* (O157:H7) in cattle (Newell and Ragione, 2018).Further research is needed to elucidate the mechanism (for example, competitive exclusion, physical removal, forage quality, tannins, lignin, other phenolics, etc.) by which forage-feeding impacts the microbial ecology of the bovine intestinal tract, including the ecology of *E. coli* and *E. coli* (O157:H7) populations, so that economically viable and practical dietary modifications can be implemented. At present, areas of investigation include feed and water hygiene, but also dietary supplements and vaccination. All these control measures are still in the experimental stages of development, although a vaccine against *E. coli* (O157:H7) is commercially available. Current research is aimed at improving the understanding of the factors that cause individual animals to shed high numbers of the pathogenic *E. coli* (super-shedders). The research also focuses on the identification of such animals and the farm holdings which are the source. This would allow more risk-based controls to be applied to limit the risk of contamination from such animals or holdings (Vogstad, 2012) Effective prevention and control of contamination in abattoirs requires the application of good hygiene practices, the application of Hazard Analysis and Critical Control Point (HACCP)-based management practices and risk-based meat inspection practices to minimize faecal contamination of carcasses. In an effort to improve quantity and quality of food, FAO is promoting good management practices in the dairy and beef sector often in collaboration with the private sector. Examples include the preparation of manuals such as the IDF/FAO Guide to Good Dairy Farming Practice or the development of training material and capacity building interventions in relation to hygienic milk handling and processing but also testing and quality control for the

meat sector, the FAO manual "Good practices for the meat industry" available in electronic format is highly recommended. Other documents related to slaughterhouse management and slaughterhouse cleaning and disinfection are also available. FAO is involved in projects to strengthen veterinary public health systems and services, pertaining to issues of veterinary supervision and inspection of animal slaughter, meat inspection and slaughter house hygiene (Howlett *et al*., 2005).

2.13.2 Pre-harvest strategies in fresh produce and sprout production

Appropriate on-farm manure storage and handling procedures with no run offs from farms are important. In addition, crop management can reduce some of the factors associated with *E. coli* populations and should reduce the risks of epidemics in humans. The findings that arise from ecosystem approaches suggest that it is possible to reduce the survival and growth of *E. coli* populations in crops by adopting good agricultural practices. These could include reducing the overuse of nitrogenous fertilizer, applying only treated or well-processed manure with a higher C/N ratio, applying compost, ensuring that seeds are not contaminated before planting, encouraging better animal and human hygiene in the field and irrigating with clean water. These practices, intended to reduce risks from *E. coli*, also support sustainable intensification of crop production. Save and Grow Systems emphasize careful monitoring of nitrogen levels so as to reduce risks from outbreaks of plant pathogens and pollution. This is one way to increase crop yields and reduce risks from *E. coli* and other human pathogenic enteric bacteria (Howlett *et al*., 2005). To prevent faecal contamination of carcasses, abattoirs must strictly adopt the management practices of Hazard Analysis and Critical Control Point (HACCP), risk- based meat inspection practices and appropriate hygiene methods. In addition, appropriate retail and consumer behaviours are also essential. To ensure that those who come directly or indirectly in contact with food are not likely to contaminate it with pathogenic *E.*

coli, food handlers should follow the recommended Codex Alimentarius Code of Practice (Codex, 2009).

2.13.3 Household and food service hygiene

The World Health Organization (WHO) guide, entitled Five Keys to Safer Food, provides an outline of good food hygiene practices that will help prevent disease transmission. Good food hygiene practice, as described in the WHO Five Keys to Safer Food, can prevent the transmission of pathogens responsible for many foodborne diseases and also protect against foodborne diseases caused by pathogenic *E. coli*. These recommendations should be followed at the household consumer level. With respect to best household hygiene practices, it is highly recommended to ensure that the food is "cook (ed) thoroughly" reaching a temperature of 70 °C (WHO, 2017).

2.14 Diagnosis of *Escherichia coli*

Detection of *E. coli* O157:H7 is based on phenotypic differences from most other serotypes: its inability to ferment sorbitol on MacConkey sorbitol agar and absence of b-glucuronidase activity in most strains. Presumptive *E. coli* O157:H7 from these tests must then be confirmed serologically for which a latex agglutination kit is commercially available (Adam and Moss, 2008). Identification of diarrhoeagenic *E. coli* can be based on detection of their associated virulence factors. For example, procedures are available to detect the ST and LT of ETEC serologically, and the LTI and Stx genes in ETEC and EHEC using gene probes and the polymerase chain reaction (PCR) (Adam and Moss, 2008).

2.15 Treatment of *Escherichia coli*

The use of antibiotics in the treatment of STEC infection is controversial (Collins and Green, 2010). Some authors reported that antibiotics may have beneficial effects in STEC infection and reduce the risk of STEC-associated complications, while others reported an increase in the level of shiga toxin production and a greater

risk of fatal complications following administration of antibiotics in STEC infection (Zhang *et al*., 2008). In vitro studies showing most strains are susceptible to various antibiotics, although certain antibiotics, at sub lethal concentrations may increase the release of Shiga-like toxin which has been associated with the development of HUS. No clinical studies have indicated that antibiotics are effective in reducing the duration of *E. coli* infection or duration of bloody diarrhea (Collins and Green, 2010). In vitro data have demonstrated that ciprofloxacin or sub inhibitory concentrations of trimethoprim- sulfamethoxazole induce shiga toxin production by *E. coli* O157:H7 (Besser *et al*., 1999). Treatment of HUS is supportive, with particular attention to the management of fluids and electrolytes. With meticulous care, the mortality rate for HUS is approximately 4 %. Numerous other treatment modalities have been tried but are of unproven efficacy. These include plasma infusion, plasma exchange, intravenous immunoglobulin, Shiga toxin inhibitors, prostacyclin, antithrombotic therapy, vitamin E, recombinant tissue plasminogen activator, and transfusion with P1-positive erythrocytes (Besser *et al*., 1999).

2.16 Antimicrobial resistance

In animal production antimicrobial drugs are used for therapy, prophylaxis and growth promotion. The use of such drugs causes a selective pressure to be imposed on bacterial populations and antimicrobial resistances are selected. The pool of resistance genes is thus spread in the environment (WHO, 2004). Drug resistance in food borne bacterial enteric pathogens is an almost inevitable consequence of the use of antimicrobial drugs in food-producing animals, and specifically in the developing countries by use of medicines in humans (Bogaard and Stobberingh, 2000). A major concern is that the high levels of antibiotic resistance are a result of the use of antibiotics in food animals. Over the last two decades, development of antimicrobial

resistance resulting from agricultural use of antibiotics that could impact on the treatment of diseases affecting the human population that require antibiotic intervention has become a significant global public health concern (Rahimi and Nayebpour, 2012). Different antibiotic resistance profiles have been detected in *E. coli* O157:H7 isolates from different sources, including humans, animals and foods (Rahimi and Nayebpour, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Jos metropolis which comprises of the capital city (Jos) and its environs. Plateau state is located between latitude 80°24'N and longitude 80°32' and 100°38' east. The altitude ranges from around 1,200 meters (about 4000 feet) to a peak of 1,829 meters above sea level in the Shere Hills range near Jos. With a higher altitude than most of the other parts of the country, Plateau State has a near temperate climate with an average temperature of between 18 and 22 °C. Harmattan winds cause the coldest weather between December and February. The warmest temperatures usually occur in the dry season months of March and April. The mean annual rainfall varies from 131.7cm in the southern part to 146 cm on the Plateau. The highest rainfall is recorded during the wet season months of July and August. Plateau State is subdivided into seventeen Local Government Areas (LGAs). This study was carried out specifically in Jos South metropolis (Figure 1). Jos south consists of 5 districts namely Gyel, Kuru, Zawan, Du and Vwang (popularly known as Vom), occupying a land area of 1037 square kilometre. It has a projected population of 311,371 from 1991 census figure. The main towns and villages are Bukuru, Giring, Hwoslshe, Vom, Kuru, Trade Center, Rantya and Du. Others are Shen, Anguldi, Zawan and Dadin Kowa

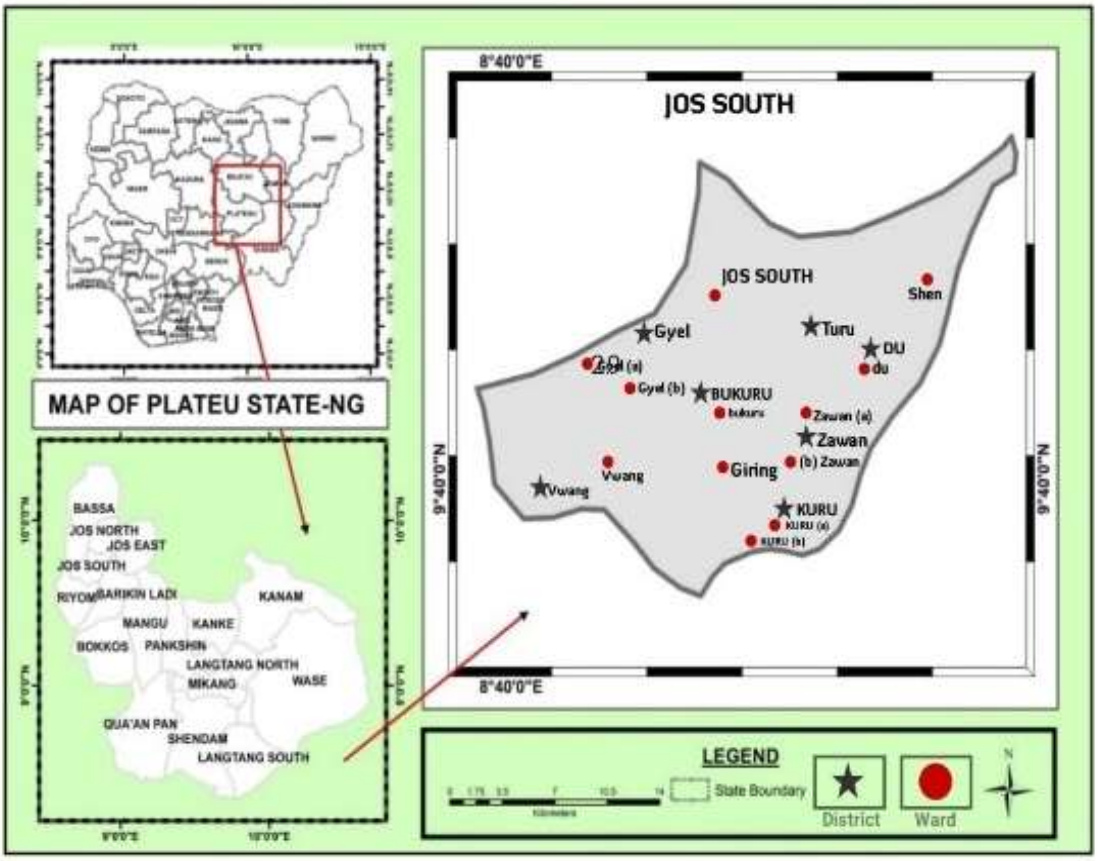

Figure 1: Map of Jos Metropolis

Source: (Kihupi *et al.*, 2016)

3.2 Sampling

Jos South comprises of five (5) districts which includes Gyel, Kuru, Zawan, Du and Vwang (popularly known as Vom). Sixty (60) water samples were randomly collected from these districts. In each districts samples were randomly collected from the wards under each district. from Kuru districts samples were collected from Dahwak, Kumbna, Dangara, Hwak, Dop, ASTC, Root Crop and Technical College. From Vwang district samples were collected from Dahwolgbo, Chugwi, Turu, Agwan Mission and Vet district. In Gyel district, samples were collected from Bukuru, Railway, DDzang, Rahwol, Kugiya, Yellowa water treatment and Bukuru Express. From Zawan district samples were collected from Angudi, Kwata, Police

Staff College, Grand cerals and Zawan. From Du district, samples were collected from Rayfield and Sot (Envuladu *et al.,* 2012).

3.3 Study Design

The study design was a laboratory based analytical study. Water samples were investigated for their values in eleven parameters known to be linked to water quality which are pH, hardness, turbidity, temperature, total dissolved solid, total suspended solid, Residual chlorine, electrical conductivity, biological oxygen demand, chemical oxygen demand and microbiological purity or quality.

3.4 Sample Collection and Processing

3.4.1 Borehole water

Borehole water samples were collected aseptically using 250 mL sterile sampling containers, the mouth and the outer parts of the pipes head were sterilized with the flame of a cigarette lighter, and allowed to cool by running the water for about 1 minute before water collection. Each water sample for analysis was collected using a clean 250 mL sterile glass container with a screw cap which was thoroughly washed with detergent and sterilized in an oven at 120 ^{o}C for 1hour. At the point of collection, the container was rinsed three times with the boreholes water sample prior to collection. All of the collected water samples was immediately transported to the laboratory and was stored in the refrigerator at 4 °C in the laboratory prior to analysis to avoid microbial action affecting their concentration (Bala, 2006; Kihupi *et al*., 2016).

3.4.2 Well water

Water source sampling from wells was carried out by drawing water using a bucket and taking 250 mL into a sterile container. At the point of collection, the container was rinsed three times with the boreholes water sample prior to collection (Bala, 2006; Too *et al*., 2016). This was considered to be more representative of what is actually being consumed by the household. The bottles were corked and transported to the

central diagnostic laboratory of National Veterinary Research Institute Vom for further analysis.

3.5 Sample Size

Sixty (60) water samples were randomly selected which include 30 well and 30 bore hole water sources was used as a representative sample for the analysis.

3.6 Microbiological Analysis

3.6.1 Enumeration of coliforms

The Most Probable Number (MPN) technique (multiple tube technique) was used to determine the number of coliform bacteria most probably present in 100 mL of the water sample according to the standard Methods for the Examination of Water and Waste Water (Bala, 2006).

3.6.1.1 *Presumptive test*

Fifteen test tubes were used for each sample. Five test tube containing 0.1mL of single strength of lactose broth was inoculated with 0.1mL of water sample, this was followed by 5 test tube of single strength of lactose broth and was inoculated with 1mL of water sample. Ten millilitres of double strength of lactose broth were inoculated with 10 mL of water sample. Durham tubes were placed in each test tube for the collection of gas produced after 48 hours incubation at 37 °C. Coliform most probably present in 100 mL of water samples analysed were ascertained based on the number of positive tubes in each using Mc Crady's Table (Bala, 2006).

3.6.1.2 *Confirmed test*

The above procedure (presumptive test) was repeated using 10 mL single strength lactose broth and 0.1mL water sample and 10mL double strength lactose incubated with 10 mL of water sample (Bala, 2006).

3.6.1.3 *Completed test*

A Positive sample from presumptive test was streaked onto MacConkey Agar (Oxoid CM7) (for easy identification of lactose fermenting organisms), and was incubated for 37 °C. Pure colony was then transfer unto a freshly prepared Eosin Methylene Blue Agar (Oxoid CM OO69) (for easy identification of the green metallic sheen appearance characteristic of *E. coli* colonies. Sorbitol MacConkey Agar (Oxoid, CM813) was used to selectively differentiate the non- sorbitol-fermenting *E. coli* strains (pathogenic) from other *E. coli* strains that ferment sorbitol (non-pathogenic). Further test such as biochemical and Gram staining techniques were carried out. Positive samples were then preserved in Nutrient agar for further molecular identification (Bala, 2006).

3.7 Gram Staining and Microscopy

A labelled clean glass slide was placed on a flat work bench and a drop of normal saline was put on it. The colonies that appeared as green metallic sheen on the Eosin methylene blue agar (EMB) were picked with the aid of a sterile wire loop and emulsified on the normal saline and spread out to make a thin layer. The smear was allowed to air dry and heat fixed by passing it over a Bunsen burner flame three times with the smear surface facing up. The slides were then placed on a clean rack and flooded with crystal violet (primary dye) and allowed to stand for 60 seconds, the slides were rinsed with water and Grams iodine a mordant was poured on the slides and allowed to stand for another 60 seconds, and rinsed with water .the slides were then flooded with acetone and washed off immediately, they were flooded with safranin for counter staining and allowed to stand for 60 seconds after which they were washed, back cleaned , and allowed to dry in slanting position at room temperature. A drop of oil immersion was placed on each dried stained slides and viewed under the microscope with the ×100 objective, the Gram reaction and morphology of the organism were

observed and recorded (Cheesebrough, 2000). Biochemical tests were performed on presumptive *E. coli* colonies and other isolates using standard techniques.

3.8 Biochemical Identification of Isolates

This involved carrying out the following tests: indole production, citrate utilization, gas production, methyl red and Vogesproskauer test.

3.8.1 Indole test

The organism was grown in 5 mL of peptone water in a test tube for 24 hours at 37 °C, three drops of Kovacs reagent was added to the turbid peptone water. A positive result was indicated by the development of a red colour in the reagent negative reaction, the indole reagent retained its yellow colour (Cheesbrough, 2000).

3.8.2 Citrate test

The isolates were inoculated on Simon citrate slant in a test tube. It was incubated at 37 °C for 24 hours. The development of a deep blue colour indicates a positive reaction and no colour change indicates a negative reaction (Cheesbrough, 2000).

3.8.3 Triple sugar iron test

The organism was inoculated into a freshly prepared triple sugar iron agar using a straight inoculation needle it was stabbed at the centre of the medium to the bottom of the tube and then streaked at the surface of the agar slant. It was incubated at 37 °C for 24 hours. The ability of the organism to form bubbles or cracks and change in colour from red to yellow in the agar indicate the production of gas (Hydrogen sulphide) and that indicates a positive reaction (Cheesbrough, 2000).

3.8.4 Methyl red test

Glucose phosphate broth was prepared, dispensed into test tubes and sterilized by autoclaving at 121 ^{o}C for 15 minutes. The sterile medium was inoculated with bacterial culture and incubated at 37 ^{o}C for 48 hours. Four drops of methyl red indicator

were added and gently mixed. Positive test was indicated by bright red colour while negative test was indicated by yellow colour (Cheesbrough, 2000).

3.8.5 Voges Proskauer test

Two millilitre (2 mL) of 40 % KOH and 3 mL of 5 % alpha–naphtol was added to the test organism in peptone water and incubated at 37 ^{o}C for 48 hours and shaken gently. Pinkish colorations indicated positive to Voges Proskauer test (Cheesbrough, 2000).

3.9 Molecular Identification

3.9.1 Genomic DNA extraction

A simple bacterial DNA extraction method was done involving centrifugation and boiling. The bacterial isolates grown at nutrient broth were harvested and centrifuged at 5000g for 10 minutes to pellet the cells. The cells were washed twice with 1ml PBS pH 7.4 and suspended in 200 µL PBS pH 7.4. Two hundred micro litre (200 µL) of 10 % chelex suspension was added to the suspended *E. coli* cells and was incubated at 57 °C for 15 minutes in a hot water bath. Seven hundred µL Lysis Buffer was added to the digest and mixed thoroughly by vortexing in a vortex mixer it was then incubated at 100 °C for 8 minutes in a water bath and immediately centrifuged at 13,000 rpm for 3 minutes in a micro centrifuge. The extracted DNA samples were stored at -20 °C until needed (Chamberlain *et al*., 1988).

3.9.2 Multiplex polymerase chain reaction

Two sets of primer mixtures were used to run the Multiplex PCR (Table 1). In general, the reagents mixture was as follows: 5 µL of genomic DNA extract was used as template 12.5 µL 2xPCR reaction mix (Taq DNA polymerase 0.05u/µL, reaction buffer, 4mM $MgCL_2$, 0.4 mM DNTP) (Fermentas®). Set A contained 0.2 µL E16S (0.1 µM), 1.2 µL HlyA; (0.6 µM) 1.25 µL Stx2;(1.5 µM) 0.8 µL of FliC;(0.4 µM), 1.0 µL of RfbE (0.91 µM) and 3.05 nuclease free water. Bringing the final reaction volume to 25

µL. PCR amplification was performed in GeneAmp 9700 (Applied Biosystems) with initial denaturation at 95 °C for 8 minutes. This was followed by 30 cycles of denaturation at 95 °C for 30 s; annealing at 58 °C for 30 s; and extension at 72 °C for 30 s. Final extension was at 72 °C for 7 min. Ten microlitres of the PCR product was electrophoresed in an agarose gel (1.5 %) containing 5µL of 10 mg/mL ethidium bromide at 80 volts for 60 minutes. 100 bp DNA marker (Fementas®) was used as molecular size marker. DNA amplifications were examined under U.V transilluninator (Sigma) and results documented using Gel Documentation System (Synegene®). Set B mixture was 0.5 µLStx1 (20 µM) 6.5µL nuclease free water, 12.5 µL 2xmaster mix. Polymerase chain reaction amplification was performed in GeneAmp 9700 (Applied Biosystems) with initial denaturation at 94 °C for 5 minutes. This was followed by 30 cycles of denaturation at 94 °C for 1 minute; annealing at 53 °C for 1 minute; and extension at 72 °C for 1 minute. Final extension was at 72 °C for 5 minutes. Ten microlitres of the PCR product was electrophoresed in an agarose gel (1.5 %) containing 5 µL of 10 mg/ml ethidium bromide at 80 volts for 60 minutes. 100 bp DNA marker (Fementas®) was used as molecular size marker. DNA amplifications were examined under U.V trans-illuminator (Sigma) (Wang *et al*., 2002).

Table 3.1: List of Primers Used in the Study

Primer set	Primer	Sequence (5' – 3')	Target gene	Size of amplicon (bp)
A1	HylA-F	AGCTGCAAGTGCGGGTCTG	EHEC hylA	569
A2	HylA-R	TACGGGTTATGCCTGCAAGTTCAC		
A 3	RfbE-F	CTACAGGTGAAGGTGGAATGG	rfbEO157	327
A4	RfbE-R	ATTCCTCTCTTTCCTCTGCGG		
A 5	FliC-F	TACCATCGCAAAAGCAACTCC	FliC	247
A 6	FliC-R	GTCGGCAACGTTAGTGATACC		
A7	Stx2-F	TTAACCACACCCACGGCAGT	Stx2	346
A8	Stx2-R	GCTCTGGATGCATCTCTGGT		
B1	E16S-F	CCCCCTGGACGAAGACTGAC	16S rRNA	401
B2	E16S-R	ACCGCTGGCAACAAAGGATA		
All 1	Stx1-F	GAAGAGTCCGTGGGATTACG	stx1	130
All 2	Stx1-R	AGCGATGCAGCTATTA		

(Pollard *et al.*, 1990

3.10 Physico-Chemical Analysis

The following physicochemical analyses were carried out in the department of biochemistry laboratory Federal College of Animal Health and Production Technology, Vom, Plateau state (Ayuba, 2017).

3.10.1 Determination of pH

The pH of the water samples was analyzed using the Wagtech pH meter. After standardizing the pH meter with a standard solution, 10 mL of each of the water samples was poured into a sterile beaker and the anode of the pH meter was dipped into it and readings was obtained when it is stable (Ayuba, 2017).

3.10.2 Determination of turbidity

Turbidity was measured using DR 2000 (HACH) spectrometer. Freshly prepared distilled water was filled into 25 mL sample cell and another containing sample water to be analyzed. The knob of the spectrophotometer was tuned to a programmed number of turbidity which is 750 and wavelength of 450 nm. The distilled water was inserted first into the cell holder and the lid closed. The knob was set at zero to standardize the instrument after which the sample was inserted into the cell holder and the "Read" button was pressed and results was displayed in Formazine Turbidity Unit (FTU=NTU) (Ayuba, 2017).

3.10.3 Determination of residual chlorine (RC)

Residual chlorine of samples was determined by taking 25 mL of samples and DPD total chlorine was added. Using 0.00564 N ferrous ethylene diammonium sulphate (FES) cartridges, it was titrated with 0.00564N FES to a colourless end point. After which RC was calculated thus; using Equation 1.

$$RC = \frac{\text{Digital reading}}{100} \qquad \text{(equation 1)}$$

3.10.4 Determination of total hardness

Total hardness was analyzed by titration of 50 mL water sample with standard Ethylenediamine tetracetic acid (EDTA). The EDTA was added in drops at pH 10 using Erichrome black T indicator until the colour changes into purple and the hardness was calculated by multiplying the average number of drops of EDTA used for the sample by the calibration factor of 20 (Mentley, 2012).

3.10.5 Determination of electrical conductivity

One hundred mills (100 mL) of distilled water and 100 mL of water sample was poured into two separate beakers. The conductivity meter was switched on and its sensor rod was dipped into the beaker containing distilled water to standardize it and then it was dipped into the second beaker containing water sample and readings was taken and reported in microSiemens/Cl (Mentley, 2012).

3.10.6 Determination of total suspended solid

This parameter was determined by pouring 100mL of water sample through a pre weighted filter (glass fibre) of specific pore size, the filter was weighed again after the drying process that will remove all the water from the filter. The gain in weight is a dry weight measure of the particulates present in the water samples. Results were reported in milligrams of solids per litre of water (mg/L) (Mentley, 2012).

3.10.7 Determination of temperature

Temperature of water samples was determined by a hand- h e l d thermometer. The thermometer was dipped in water sample and allowed to stay for 3 minutes after which the reading on the calibration was read and recorded (Mentley, 2012).

3.10.8 Determination of total dissolved solid

A clean and dry evaporating dish was weighed. 100 mL of sample was filtered through a filter paper and the filtrate was taken in a evaporating dish. The sample was evaporated on hot water bath. When the whole water had evaporated, the weight of the evaporating dish, the weight

of the evaporating dish after cooling in desiccator was noted and differences was calculated as thus: using equation 2 (Mentley, 2012).

$$\text{TDS (g/l)} = \frac{A-B}{V} \times 100 \qquad \text{(Equation 2)}$$

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

3.10.9 Determination of biological oxygen demand

Water samples was filled in an airtight bottle of specified size at a temperature (20 ^{o}C) and incubated for 5 days. The difference in the dissolved oxygen measured initially and after incubation gives the BOD of the sample (Mentley, 2012).

3.10.10 Determination of chemical oxygen demand

Fifteen mills (15 mL) of conc. sulphuric acid with 0.3 g of mercuric sulphate and a pinch of silver sulphate along with 5 mL of 0.025 M potassium dichromate was taken into a Nessler's tube. Ten millilitres of water sample (thoroughly shaken) were pipetted out into this mixture and kept for about 90 minutes on the hot plate for digestion. Forty milliliters of distilled water were added to the cooled mixture (to make up to 50 mL) and titrated against 0.25 M FAS using ferroin indicator, till the colour turns from blue green to wine red indicating the end point. A reagent blank was carried out using 10 mL of distilled water (Mentley, 2012).

3.11 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was done using the standard disc diffusion method on Mueller-Hinton agar (MH) (Conda, Madrid) as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2011). Fresh colonies (18 hours old) from nutrient agar culture plates were picked into test tubes containing 5 mL of sterile normal saline. The turbidity of the suspension was adjusted to 0.5 McFarland standards. Sterile swabs with bacterial suspensions were used to inoculate the MH agar plates by spreading uniformly on the surface of the agar. Selection of antimicrobials was based on the type of organism to be tested and source of the isolate (CLSI, 2011). Also, the antibiotics were selected as representatives of different

classes of antibacterial drugs, to better depict the behaviour of the examined strains against these molecules. The antimicrobial susceptibility test for *E. coli* isolates was determined using the following antibiotic discs as its common to the research area: gentamicin (10 µg), Enrofloxacin (5 µg), tetracycline (30 µg), cefoxitin (30 µg), ciprofloxacin (5 µg), streptomycin (10 µg), sulfamethoxazole (30 µg), oxfloaxicin (5 µg) and Erythromycin (15 µg). The pre-determined antimicrobial discs were dispensed onto surface of the inoculated agar plate using antibiotic dispenser (Oxoid, Antimicrobial Susceptibility Test System, UK). After 24 hours of incubation at 30 to 32 °C, diameter of inhibition zones was measured to the nearest millimetre. The result was interpreted using CLSI, (2011) guidelines.

3.12 Data Analysis

Results were expressed as the mean values ± standard error of mean (SEM) by measuring three independent replicates. Means were compared by using independent sample t-test, one-way analysis of variance (ANOVA) and Duncan's test was performed to test the significance difference between means values obtained among the treatments at the 5 % level of significance using SPSS software (version 21, IBM SPSS). Differences were considered significant at $p<0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Coliform Count

The coliform counts for bore holes and well water source is presented in the Tables below.

4.1.1.1 *Coliform counts on borehole water source from different sites*

Results reveal the coliform count of borehole water samples in the different locations in Jos South. The coliform counts of the borehole water samples ranges from 2 to 150 coliform/100 mL. The water sample of Dahwak and Kushe had the least coliform count followed by samples from Rayfield, Rantya, and Zawan. The water sample of Kwata had the highest coliform count followed by the coli form count of the water samples of Dangara and Bukuru.While the water sample of Rahwol, Dahwolgbo, Turu, Vwang, Jenta, Kufang, Kumbuna and Sot had no coliform growth (Table 4.1).

4.1.1.2 *Coliform count on well water source from different sites*

The result reveals the coliform count of well water samples in the different locations in Jos south. The coliform counts of well water samples ranges from 2 to1700 coliform/100 mL.The water sample of Chugwi, Rayfield, Bukuru, Chwol and Jenta had the least coliform count followed by samples from Kumbna, Rahwol, Augudi and Dakan. The water sample of Agwan Mission had the highest coliform count followed by samples of Gyel while Rantya, Dahwolgbo, Turu, Sot and Dakan had no coliform growth (Table 4.2).

Table 4.1: Coliform Count of Borehole Water Source from Different Sites

SAMPLE SITES	MNP/100 mL
Zawan	5
Agwan Mission	72
Rahwol	NCG
Kwata	150
Chugwi	45
Dahwak	2
Kuru	7
Dahwolgbo	NCG
DOP	2
Turu	NCG
Angudi	23
DDZang	7
Vwang	NCG
Dashe	5
Kushe	2
Gangare	72
DU	7
Dakan	14
Kogom	35
Chwol	29
Jenta	NCG
Kufang	NCG
Rayfield	4
Kumbna	NCG
Vwang	72
Bukuru	45
Rantya	4
Dangara	35-50
Kugiya	7-9
SOT	NCG

Key: NCG; no coliform growth, WHO Standard 0 coliform per 100ml of water

Table 4.2 Coliform Count on Well Water Source from Different Sites

SAMPLE SITES	MPN/100mL
Chugwi	2
Rayfield	2
DDzang	40-55
Kumbna	4
Agwan mission	1350-1700
Vwang	200-280
Bukuru	2
Rantaya	NCG
Dangara	2
Gyel	450-660
Kwata	45
Rahawol	4
Angudi	5
Zawan	9
Dahwak	18-22
Dahwolgbo	NCG
Turu	NCG
Hawk	18
SOT	NCG
DOP	14
Kugiya	18
Dashe	18-22
Kushe	40-55
Gangare	200-280
Du	4
Dakan	NCG
Kogwom	18
Chwol	2
Jenta	2

Kufang	NCG

Key: NCG; no coliform growth, WHO Standard 0 coliform per 100ml of water

4.1.1.3 *Coliform count from water sources*

This result reveals the comparison of coliform count of well wáter to that of Borehole. The coliform count of well wáter was higher than that of Borehole. Statistically, there is a significant difference in the coliform count of well source at ($p<0.05$) and for that of coliform count of borehole at ($p<0.05$) when comparing with the P-value of 0.201 (Table 4.3).

Table 4.3: Coliform Count from Water Sources

Source	Mean MPN/100 mL	P-value
Borehole	30.80 ± 8.34	0.201
Well	119.30 ± 69.29	

Results shows mean values ± standard error of mean of triplicate determination.

4.1.2 Biochemical characteristics and identities of isolate in water samples

The biochemical characteristics and identities of the isolates in water sample are shown in Table 4.4. The result revealed that the morphological and biochemical characterization of the organism to be *Escherichia coli*. *Escherichia coli* are Gram negative short rod and it utilizes citrate, produces gas and utilizes indole. *Escherichia coli* show a green metallic sheen on Eosine methyl blue. Pathogenic *E. coli* strain does not ferment Sorbitol MacConkey Agar.

Table 4.4: Morphological Characteristics and Identities of Isolate

SC	GR	SH	TSI	CI	IN	MR	VP	GEMB	GSMCA
Azw	-	Rod	AAg	-	+	+	-	+	+
Azb	-	Rod	AA	-	+	+	-	+	-
Amw	-	Rod	AAg	-	+	+	-	+	-
Amb	-	Rod	AAg	-	+	+	-	+	-
Buw	-	Rod	AAg	-	+	+	-	+	-
Cgw	-	Rod	AAg	-	+	+	-	+	-
Cgb	-	Rod	AAg	-	+	+	-	+	-
Dkw	-	Rod	AAg	+	+	+	-	+	-
Dgb	-	Rod	AAg	-	+	+	-	+	-
Dgw	-	Rod	AAg	-	+	+	-	+	-
Zb	-	Rod	AAg	-	-	-	-	-	-
Kbw	-	Rod	AA	+	+	+	-	+	+
Kww	-	Rod	AA	-	+	+	-	+	-
Kwb	-	Rod	AAg	-	+	+	-	+	-
Kb	-	Rod	AA	-	+	+	-	+	-

GR: Gram's reaction, SH: shape, SC: sample code, +: positive, -: negative, CI: Citrate utilization, IN: Indole, MR: Methyl red, TSI: triple sugar iron test, VP: Voges proskauer, GEMB: growth on eosine methylene blue agar, GSMCA: growth on sorbitol macConkey Azw: Augudi Zawan well, Azb: Augudi Zawan borehole, Amw: Agwan mission well, Amb: Agwan mission borehole, Buw: Bukuru well, Cgw: Chugwi well, Cgb: Chugwi borehole, Dkw: Dahwak well, Dgb: Dankara borehole, Dgb: Dankara borehole, Kbw: Kumbna well, Kww: Kwata well, Kwb: Kwata borehole, Zb: Zawan borehole, Kb: Kuru borehole.

4.1.3 Molecular characteristics of isolates in water samples

Multiplex PCR analysis was carried out on fifteen presumptive *E. coli*isolates, isolated from the sixty (60) water samples (Plate 1). Six (6) set of primers were used according to Wang *et al* (2002) in which the reaction conditions were empirically determined and optimized to give optimum results was used in this study. Two set of Primers used were to target the virulence genes mostly associated with pathogenic *E. coli*. They include E165s, flic (flagellin *E. coli* strain k12), hlyA (Alpha hemollysis), RfbE (Perosamine synthetase 0157: H39), stx2 (shiga toxin type 0157:H7) and stx2 (shiga toxin 2). The result revealed that lane 2 and lane 7 were positive for fliC virulence gene. These water sample where from Dankara and Dahwak respectively.

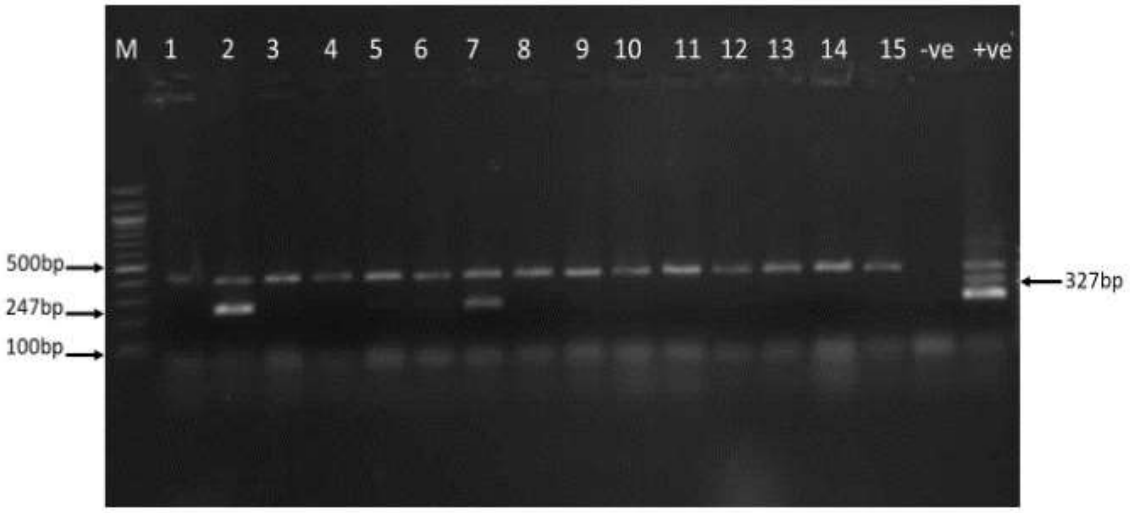

Plate 1: Gel Electrophorsis of Isolates in Water Sample

Multiplex PCR of virulence genes in water samples. Lane M – 100bp marker (Fermentas®); lanes 1-15 water samples from different sources. HlyA-F and HlyA-R primers set amplified HlyA gene fragment (569bp); FliC-F and FliC-R primers set amplified FliC gene fragment (247bp); Stx2d- F and Stx2d-R primers set amplified Stx2d gene fragment (175bp). Lane 18 – negative control (nuclease free water).

4.1.4 Physicochemical Properties of Water Samples

The physicochemical properties of the borehole and well water are presented in the

following section

4.1.4.1 Physiochemical properties of well water samples

The physicochemical properties of the various well water samples based on location are shown in Table 4.5. The findings reveals that the temperature from the different location is significantly different from each other. The value of temperature ranges from 27 to 29 °C. Water sample from Agwan mission, Dankara, Dashe, Dop, Kushe, Rahwol and Vwang have the least temperature followed by Augudi, Chugwi, Dahwak, Dahwolgbo, Du, Ganagara, Gyel, Kuru, Jenta, Kogwom, Kumbma, Kwata, Rantaya, Sot, Turu and Zawan while the water sample from Kufang, Bukuru, Chwol, Kugiya, Kwata and Rayfield recorded the highest.

The pH of the water samples are significantly different at ($p<0.05$). The value of pH ranges from 5.6-7.0 with Zawan having the least value of followed by Augudi and Rantaya, with a value 5.7 respectively followed by the water sources from Bukuru, Kugiya and Turu with a value of 5.8, 5.87 and 6.0 respectively. The water source from Chwol, Dahwak, Dahwolgbo, Dakan, Dankara, Dashe, Du, Kohggwom, Kumbna, Kushe and Zawan are not significantly different while the samples from Agwan mission, Dop, Gely, Hwak, Kufang, Kuru and Sot show the highest value for pH with a value of 7.05 respectively there are not significantly different from each other followed by the water sample from Jenta.

Water sample from Dakan, Dankara, Dashe, Du, Gangara and Kushe shows the least value of total hardness and are not significantly different from each other followed by the water sample of Augudi and was followed by Chwol, Kogom and Vwang while the water samples from Agwam Mission, Dop, Jenta and Rantaya shows the highest value for total hardness and this was followed by water sample from Bukur, Dahwak, Dahwolbho, Kugiya and Turu and are not significantly different from each other.

The Resdiual chlorine ranges from 0.51 mg/L- 0.9 mg/L. The residual chlorine of Rahwol

and Sot are the least followed by Dahwak, Dahwolgbo, Dakan, Dankara, Dashe, Gangara and Kushe.Water sampled from Kwata with a value 0.9 mg/L is the highest in terms of residual chloride followed by Ratfield and Bukuru with a value of 0.8 mg/L and 0.625 mg/L respectively. The results of Electrical conductivity for all well water samples from different locations are statistically different at ($p<0.05$) with a range value of 6.03 – 6.45 μSmg/L. Augudi, Kwata, Rayfield, Turu and Vwang had the least followed by Chwol, Dahwolgbo, Dop, Du, Kugiya and Zawan. The well water from Agwan Mission, Jenta, Rahwol and Sot are not siginifiacntly different from each other. Well water sample from Dankara, Dakan and Du had the highest electrical conductivity value followed by Bukuru, Dashe, Gyel, Kugiya, kushe and Kumbna with a value of 6.3 μSmg/L respectively and are not significantly different from each other. The turbidity result of Jenta, Kufang and Kumbna are not significantly different and they show the least value of turbidity in this study. Followed by the turbidity value of Rahwol, Kuru and Kwata. The turbidity value of Augudi, Chwol, Kogom, Rafield, Vwang and Zawan are not significantly different from each other. Agwan Mission, Dop and Rantaya shows the highest followed by Chugwi, Dahwak, Dahwollgbo, Dakan, Dankara, Dashe, Du, Gangara, gyel, Hwak, Kushe, Sot and Turu. The result of the total suspended solid reveals that at there is significant difference at ($p<0.05$) among the well water sources from different location. The TSS value of the well water sampled from Agwan Mission, Agudi, Chugwkwi, Dop, Kufang, Rantaya, Turu and Vwang had the least followed by Chwol, Dhwak, Dahwolbo, Gngara, Hwak, Kwata and Zwan. The water sample of Kugiya and Rayfield had the highest then followed Bukuru and Dankara. The water sample from Kumbna and Kuru were no significantly different from each other, but were higher than that of Dahwak, Zawan, Angwan Mission and Chungwi. The water sample of Dahwak had the highest total dissolved solid (TDS) followed by Dankara, the TDS of water samples of Agwan mission and Chuwgwi were not significantly different from each other but

higher than those of Bukuru, Kumbna, Kuru and Kwata while the water sample of Augudi and Zawan had the least TDS. The result of the biological oxygen demand of well water sample from different location reveals that, the water sample from Gyel and Hwak had the least BOD followed by Augudi and Vwang while Dakan, Dankara, Dashe, Du, Kushe, Rayfield and Zawan were not significantly different from each other but higher Jenta, Kogom, Kufang, Kumbna and Turu. The well water sample from Dop, Agwan Mission, Rantaya had the highest followed by Kwata but still higher than Bukuru, Chugwi, Kugiya and Kuru.

Table 4.5: Physicochemical Parameter of Well Water Base on Location

Sample site	Temp (°C)	PH	TH (mg/L)	RC (mg/L)	EC(µSmg/L)	TUB (5NTU)	TSS (mg/L)	TDS (mg/L)	BOD (mg/L)	COD (mg/L)
Agwan misión	27±0.0^{a}	7.05±0.05^{g}	1.255±0.005^{i}	0.66±0.0^{efg}	6.2±0.0^{bcd}	10.32±0.01^{g}	0.02±0.0^{a}	0.12±0.0^{g}	11.25±0.25^{gh}	0.21±0.0^{efg}
Aungudi	28±1^{ab}	5.7±0.0^{ab}	0.71±0.01^{b}	0.675±0.005^{fgh}	6.03±0.0^{a}	7±0.0^{d}	0.02±0.0^{a}	0.03±0.005^{a}	7.5±0.0^{b}	0.2±0.0^{def}
Bukuru	29±0.0^{c}	5.8±0.1^{abc}	1.215±0.005^{h}	0.625±0.005^{cdef}	6.3±0.0^{cde}	8.45±0.005^{f}	0.065±0.005^{de}	0.095±0.005^{f}	10.2±0.1^{e}	0.26±0.01^{jk}
Chugwi	28±0.0^{ab}	6±0.0^{cd}	0.965±0.015^{de}	0.7±0.0^{h}	6.05±0.05^{ab}	7.99±0.01^{e}	0.01±0.0^{a}	0.145±0.005^{g}	10.05±0.05^{e}	0.155±0.005^{a}
Chwol	29±0.0^{c}	6.4±0.0^{e}	0.73±0.01^{bc}	0.58±0.0^{bcd}	6.15±0.05^{abc}	7±0.0^{d}	0.039±0.001^{b}	0.03±0.0^{a}	7.375±0.025^{ab}	0.21±0.0^{efg}
Dahwak	28±0.0^{ab}	6.45±0.05^{e}	1.225±0.005^{h}	0.515±0.005^{ab}	6.1±0.0^{ab}	8±0.0^{e}	0.039±0.001^{b}	0.16±0.0^{ij}	7.2±0.2^{ab}	0.23±0.0^{ghi}
Dahwolgbo	28±0.0^{ab}	6.5±0.0^{e}	1.22±0.01^{h}	0.515±0.005^{ab}	6.15±0.05^{abc}	8±0.0^{e}	0.04±0.0^{b}	0.155±0.005^{ij}	7.2±0.0^{ab}	0.235±0.005^{gh}
Dakan	27±1^{a}	6.5±0.0^{e}	0.455±0.005^{a}	0.535±0.005^{ab}	6.4±0.0^{e}	8±0.0^{e}	0.0595±0.0005^{cde}	0.12±0.0^{g}	7.85±0.005^{c}	0.17±0.0^{ab}
Dankara	27±0.0^{a}	6.45±0.05^{e}	0.465±0.005^{a}	0.54±0.0^{ab}	6.4±0.0^{e}	8±0.0^{e}	0.06±0.0^{cde}	0.165±0.005^{j}	7.8±0.1^{c}	0.165±0.005^{ab}
Dashe	27±0.0^{a}	6.45±0.05^{e}	0.455±0.005^{a}	0.535±0.005^{ab}	6.35±0.05^{de}	8±0.0^{e}	0.06±0.02^{cde}	0.155±0.005^{ij}	8.005±0.005^{c}	0.165±0.005^{ab}
Dop	27±0.0^{a}	7.05±0.05^{g}	1.27±0.01^{i}	0.67±0.01^{efgh}	6.15±0.05^{abc}	10.165±0.165^{g}	0.02±0.0^{a}	0.115±0.005^{g}	11.4±0.1^{h}	0.205±0.005^{ef}
Du	27.5±0.5^{ab}	6.5±0.0^{e}	0.465±0.005^{a}	0.545±0.005^{ab}	6.45±0.05^{e}	8±0.0^{e}	0.06±0.0^{cde}	0.165±0.0^{j}	7.85±0.05^{c}	0.265±0.005^{jk}
Gangara	28±0.0^{ab}	6.55±0.05^{e}	0.465±0.005^{a}	0.54±0.0^{ab}	6.4±0.0^{e}	8±0.0^{e}	0.06±0.0cde	0.16±0.0^{ij}	10.5±0.0^{f}	0.175±0.005^{abc}
Gyel	28±0.0^{ab}	7±0.0^{g}	1.17±0.01^{g}	0.615±0.005^{cde}	6.3±0.0^{cde}	8±0.0^{e}	0.04±0.0^{b}	0.069±0.001^{de}	7.15±0.05^{a}	0.205±0.005^{ef}
Hwak	28±0.0^{ab}	7±0.0^{g}	1.175±0.0^{g}	0.625±0.005^{cdefg}	6.3±0.0^{cde}	8±0.0^{e}	0.039±0.001^{b}	0.07±0.0^{de}	7.15±0.0^{a}	0.21±0.0^{efg}
Jenta	28±0.0^{ab}	6.75±0.25^{f}	1.26±0.0^{i}	0.575±0.005^{abc}	6.2±0.1^{bcd}	5±0.0^{a}	0.065±0.005^{de}	0.06±0.0^{cd}	9.4±0.0^{d}	0.165±0.005^{ab}
Kogwom	28±0.0^{ab}	7±0.0^{g}	0.735±0.005^{c}	0.7±0.0^{g}	6.02±0.001^{a}	7±0.0^{d}	0.06±0.0^{cde}	0.06±0.0^{cd}	9.25±0.15^{d}	0.17±0.02^{ab}
Kufang	29±0.0^{c}	6.4±0.0^{e}	0.73±0.01^{bc}	0.575±0.005^{abc}	6.15±0.005^{abc}	5±0.0^{a}	0.02±0.0^{a}	0.059±0.001^{c}	9.45±0.05^{d}	0.175±0.005^{abc}
Kugiya	29±0.0^{c}	5.875±0.02^{bcd}	1.22±0.0^{h}	0.635±0.015^{cdef}	6.3±0.0^{cde}	8.5±0.0^{f}	0.069±0.001^{e}	0.095±0.005^{de}	10.15±0.15^{e}	0.27±0.02^{k}
Kumbna	28±0.0^{ab}	6.45±0.05^{e}	0.74±0.0	0.575±0.005^{abc}	6.3±0.0^{cde}	5±0.0^{a}	0.0585±0.001^{cde}	0.06±0.0^{cd}	9.4±0.0^{d}	0.17±0.0^{ab}
Kuru	28±0.0^{ab}	7±0.0^{g}	0.975±0.005^{e}	0.645±0.005^{efg}	6.04±0.01^{ab}	6.5±0.0^{c}	0.05±0.0^{bc}	0.07±0.0^{de}	10±0.0^{e}	0.245±0.005^{ij}
Kushe	27±0.0^{a}	6.45±0.05^{e}	0.455±0.005^{a}	0.535±0.005^{ab}	6.325±0.075^{de}	7.975±0.025^{e}	0.06±0.0^{cde}	0.155±0.005^{ij}	7.85±0.05^{c}	0.17±0.0^{ab}
Kwata	29±2^{c}	6.05±0.05^{d}	1.17±0.01^{g}	0.9±0.0^{i}	6.005±0.005^{a}	6.5±0.0^{c}	0.04±0.0^{b}	0.0775±0.0025e	11.05±0.05^{g}	0.22±0.01^{fg}
Rahwol	27±0.0^{a}	6.05±0.05^{d}	0.945±0.005^{d}	0.51±0.01^{a}	6.2±0.1^{bcd}	6.3±0.1^{b}	0.01±0.0^{a}	0.15±0.0^{gh}	8.005±0.005^{c}	0.18±0.01^{bcd}
Rantaya	28±0.0^{ab}	5.7±0.1^{ab}	1.27±0.01^{i}	0.665±0.005^{efgh}	6.1±0.1^{ab}	10.165±0.165^{g}	0.02±0.0^{a}	0.12±0.0^{g}	11.25±0.25^{gh}	0.205±0.005^{ef}
Rayfield	29±0.0^{c}	5.8±0.0^{abc}	1.055±0.015^{f}	0.8±0.1^{i}	6.03±0.0^{a}	6.845±0.0^{d}	0.07±0.0^{e}	0.07±0.0^{de}	8.01±0.0^{c}	0.26±0.01^{jk}
SOT	28±0.0^{ab}	7±0.0^{g}	1.06±0.01^{f}	0.51±0.01^{a}	6.2±0.01^{bcd}	8±0.0^{e}	0.055±0.005^{cd}	0.07±0.0^{de}	7.25±0.05^{ab}	0.21±0.0^{efg}
Turu	28±0.0^{ab}	6±0.0^{cd}	1.21±0.01^{h}	0.61±0.01^{cde}	6.03±0.0^{a}	7.9±0.1^{e}	0.01±0.0^{a}	0.155±0.005^{ij}	9.55±0.05^{d}	0.16±0.0^{ab}
Vwang	27±0.0^{a}	5.65±0.05^{a}	0.72±0.0^{bc}	0.68±0.0^{fg}	6.015±0.015^{a}	7±0.0^{d}	0.02±0.0^{a}	0.03±0.0^{a}	7.5±0.0^{b}	0.2±0.0^{def}
Zawan	28±0.0^{ab}	6.4±0.0^{e}	1.07±0.0^{f}	0.64±0.0^{defg}	6.15±0.05^{abc}	7±0.0^{d}	0.04±0.0^{b}	0.04±0.0^{b}	8.02±0.01^{c}	0.195±0.005^{cde}
WHO	40	6.5-8.5	150	250	400	5	1000	1500	6	10

Results shows mean values ± standard error of mean of triplicate determination. Values with the same superscript letters in the same column are not significantly different at $p<0.05$. pH: hydrogen ion concentration, RCL: residual chlorine, EC: electrical conductivity, TSS: total soluble solid, TDS: total dissolved solids, BOD: biological oxygen demand, COD: chemical oxygen demand.

4.1.4.2 *Physico-chemical Parameter of Borehole Water Source Base on Location*

The physicochemical properties of the various borehole water samples based on location are shown in Table 4.6. The temperature from the different location are significantly different from each other. The value of temperature ranges from 27to 29 °C with Agwan Mission, Dakan, Dankara, Dashe, Dop, Gangara, Kushe and Rahwol having the least temperature. The water sample from Augudi, Chugwi, Dahwolgbo, Dahwak, Gyel, Hwak, Jenta, Kogwom, Kumbna, Kuru, Rantaya, Rafield, Sot, Turu, Vwang and Zawan are not significiantly different from each other with the borehole sample from Bukuru, Chwol, Kufang, Kugiya and Kwata having the highest temperature value.

The pH of the water sample are significantly different at ($p<0.05$) with Angudi having the least value followed by Chugwi and Bukuru. Water sample from Dahwak, Dankara and Kumbna are not significantly different from each other at ($p<0.05$) while Kuru has the highest pH value in this study.

The total hardness of the borehole water sample are significantly different from each other with the water sample of Dakan having the least followed by the water sample of Dankara, Dashe, Gangara, Kushe, Rahwol and Kumbna. However, the water sample of Chwol, Kogom, Kuru and Kwata were not significantly different from each other. While the borehole source of Dahwaqk, Dhwologbo, Kugiya, Rantaya and Turu had the highest followed by Chwol but higher than Agwan mission, Hwak, Rayfield and Sot.

The Residual chlorine of borehole water sample of Dahwolgbo and Rahwol has the least followed by Dahwak and Sot. However, Dakan, Dankara, Dashe, Du are not significantly different from each other but are lower than Dop. Agwan mission and Kogom had the highest followed by Bukuru, Chugwi and Rayfield but higher than Augudi, Kwata, Rantaya, Vwang and Zawan.

The result of Electrical conductivity for all water sample from different locations are significantly different from each other at ($p<0.05$) with borehole water sample of Augudi and Dashe having the least followed by Agwan mission, Chugwi, Dahwak, Dahwologbo, Kufang, Kuru, kwata, Rayfield, Turu and Vwang but lower in value than Bukuru, Dop, Gyel, Hwak, Jenta, Kogom, Kugiya, Kumbna, Rahwol and Sot while the borehole water of Dakan and Gangara had the highest followed by Dankara and Zawan.

The turbidity result of the borehole water sample of Agwan mission, Kufang, Kumbna and Zawan were not significantly different and they showed the least value of turbidity in this study followed by Jenta and Rahwol and they are lower than the turbidity value of water sample from Augudi, Chwol, Kogom, Kuru, Kwata, Ryfield and Vwang. Water samples of Rantaya had the highest turbidity value followed by Bukuru and Dop but higher Chugwi, Dahwak, Dahwologbo, Dakan, Dankara, Du, Gangara, Gyel, Hwak, Kushe, Sot and Turu.

The result of the total suspended solid revealed that there is no significant difference at ($p<0.05$) among the water sources from different location. The TSS value of the borehole water sampled from Agwan mission, Augudi, Chugwi, Dop, Kufang, Rahwol, Rantaya, Turu and Vwang had least followed by Dakan. However, the borehole water source from Bukuru, Chwol, Dahwak, Dahwolgbo, Dashe, Gangara, Gyel, Hwak and Zawan were not significantly different from each and higher than that of Dakan. While the TSS value of Jenta, Kugiya, Kwata and Sot had the highest value followed by Kushe but higher than Dankara, Du and Kumbna.

The borehole water sample of Augudi, Chugwi, Chwol and Vwang had the least total dissolved solid (TDS) followed by Turu and Zawan. The TDS of the borehole water sample from Agwan mission and kuru are not significantly different and are lower than

that of Kubmbna, Gyel, Kogom, Kufang, Kwata and Sot. While the TDS value of Dahwolgbo, Dankara, Du and Gangara had the highest followed by Rahwol and are higher than Kushe. Dashe and Dop.

The result of the biological oxygen demand of borehole water sample from different location reveals that, the water sample from Augudi had the least followed by Chugwi and Kuru, however, the water samples of Dop and Rantaya were not significantly different but higher than Hwak. Water sample of Kwata had the highest BOD followed by Jenta and still strictly higher than Kogom, Kumbna, Kufang and Kugiya.

The Chemical oxygen demand of all water sampled from different location are significantly different from each other at ($p<0.05$) with borehole water sample fro Chugwi, Dankara, Dashe, Gangara, Kogom having the least COD followed by Kumbna, Turu, Dakan, Jenta, Kufang, Kushe and Zawan while Kugiya had the highest followed by Bukuru, Du and Kuru.

Table 4.6: Physicochemical Parameter of Borehole Water Source Base on Location

Sample site	Temp(0c)	pH	TH (mg/L)	RC (mg/L)	EC (µslmL)	TUB (5NTU)	TSS(mg/L)	TDS(mg/L)	BOD (mg/L)	COD (mg/L)
Agwan mission	27±0.0^{a}	6.35±0.05^{ef}	1.065±0.015^{i}	0.77±0.01^{k}	6.05±0.05^{ab}	5±0.0^{a}	0.02±0.0^{a}	0.045±0.005^{b}	7.2±0.0^{de}	0.24±0.0^{fg}
Aungudi	28±0.0^{ab}	5.4±0.1^{a}	0.69±0.01^{de}	0.68±0.0^{ghi}	6.01±0.01^{a}	7±0.0^{d}	0.02±0.0^{a}	0.04±0.0^{a}	4.5±0.0^{a}	0.2±0.0^{cde}
Bukuru	29±0.0^{b}	6.5±0.0^{efg}	1.12±0.0^{j}	0.71±0.01^{j}	6.3±0.0^{bcd}	8.5±0.0^{g}	0.039±0.001^{bc}	0.09±0.0^{e}	8.15±0.15^{hi}	0.27±0.0^{gh}
Chugwi	28±0.0^{ab}	6±0.0^{cd}	0.655±0.005^{d}	0.7±0.0^{j}	6.05±0.05^{ab}	7.945±0.055^{ef}	0.01±0.0^{a}	0.03±0.0^{a}	5.1±0.0^{b}	0.16±0.0^{a}
Chwol	29±0.0^{b}	6.3±0.0^{ef}	0.925±0.015^{h}	0.575±0.005^{bcdef}	6.1±0.0^{abc}	7±0.0^{d}	0.045±0.005^{bc}	0.03±0.0^{a}	7.4±0.0^{ed}	0.21±0.0^{de}
Dahwak	28±0.0^{ab}	6.4±0.1^{efg}	1.23±0.0^{k}	0.52±0.0^{ab}	6.05±0.05^{ab}	8±0.0^{ef}	0.04±0.0^{bc}	0.11±0.0^{f}	7.15±0.05^{ede}	0.22±0.01^{def}
Dahwolgbo	28±0.0^{ab}	6.45±0.05^{efg}	1.225±0.005^{k}	0.51±0.01^{a}	6.05±0.05^{ab}	8±0.0^{ef}	0.04±0.0^{bc}	0.155±0.0^{i}	7.15±0.05^{ed}	0.225±0.005^{efg}
Dakan	27±0.0^{a}	6.55±0.05^{efg}	0.06±0.0^{a}	0.545±0.005^{abc}	6.4±0.0^{d}	8±0.0^{ef}	0.0375±0.0025^{b}	0.12±0.0^{fg}	7.8±0.1^{g}	0.18±0.01^{abc}
Dankara	27±1^{a}	6.5±0.0^{efg}	0.46±0.0^{b}	0.54±0.0^{abc}	6.35±0.05^{cd}	8±0.0^{ef}	0.059±0.001^{de}	0.16±0.0^{i}	7.875±0.025^{gh}	0.165±0.005^{a}
Dashe	27±0.0^{a}	6.5±0.0^{efg}	0.46±0.0^{b}	0.545±0.005^{abc}	6.005±0.005^{a}	7.795±0.205^{e}	0.0475±0.0025^{bc}	0.12±0.0^{fg}	8.005±0.005^{gh}	0.165±0.005^{a}
Dop	27±0.0^{a}	7.05±0.05^{i}	1.06±0.0^{i}	0.55±0.05^{abcd}	6.15±0.05^{abcd}	10.165±0.165^{g}	0.02±0.0^{a}	0.12±0.0^{fg}	5.45±0.05^{c}	0.215±0.0^{def}
Du	27±0.0^{a}	6.6±0.1^{fg}	0.8±0.01^{g}	0.53±0.01^{abc}	6.3±0.1^{bcd}	8±0.0^{ef}	0.06±0.0^{de}	0.165±0.005^{i}	7.825±0.075^{gh}	0.275±0.005^{gh}
Gangara	28±2^{a}	6.5±0.0^{efg}	0.45±0.01^{b}	0.61±0.01^{defg}	6.4±0.0^{d}	8±0.0^{ef}	0.039±0.001^{bc}	0.16±0.0^{i}	8.3±0.2^{ij}	0.1645±0.005^{a}
Gyel	28±0.0^{ab}	7±0.0^{hi}	1.08±0.0^{ij}	0.625±0.005^{efgh}	6.2±0.1^{abcd}	8±0.0^{ef}	0.04±0.0^{bc}	0.0655±0.0045^{cd}	7.2±0.0^{de}	0.21±0.0^{de}
Hwak	28±0.0^{ab}	6.7±0.3^{gh}	1.29±0.01^{l}	0.61±0.01^{defg}	6.155±0.145^{abcd}	8±0.0^{ef}	0.04±0.0^{bc}	0.069±0.001^{d}	7.1±0.1^{d}	0.195±0.015^{bcd}
Jenta	28±0.0^{ab}	7±0.0^{hi}	1.06±0.0^{ij}	0.57±0.01^{bcde}	6.2±0.1^{abcd}	5.5±0.5^{b}	0.08±0.0^{f}	0.07±0.0^{d}	9.55±0.15^{l}	0.18±0.01^{abc}
Kogwom	28±0.0^{ab}	6.9±0.1^{hi}	0.95±0.05^{h}	0.775±0.075^{k}	6.265±0.235^{abcd}	7.1±0.1^{d}	0.075±0.015^{f}	0.065±0.005^{cd}	9.25±0.15^{k}	0.16±0.01^{a}
Kogwom	29±1^{b}	6.4±0.0^{efg}	0.745±0.005^{f}	0.63±0.01^{fghi}	6.05±0.05^{ab}	5.025±0.025^{a}	0.02±0.0^{a}	0.065±0.005^{cd}	9.45±0.05^{kl}	0.18±0.01^{abc}
Kugiya	29±0.0^{b}	5.8±0.1^{bc}	1.235±0.015^{k}	0.61±0.01^{defg}	6.2±0.1^{abcd}	8.3±0.0^{fg}	0.0775±0.002^{f}	0.095±0.005^{e}	9.35±0.05^{kl}	0.28±0.01^{i}
Kumbna	28±0.0^{ab}	6.35±0.05^{ef}	0.53±0.01^{c}	0.565±0.015^{abcde}	6.3±0.0^{bcd}	5±0.0^{a}	0.06±0.0^{de}	0.057±0.003^{c}	9.225±0.175^{k}	0.17±0.0^{ab}
Kuru	28±0.0^{ab}	9.65±0.15^{j}	0.97±0.01^{h}	0.61±0.01^{defg}	6.055±0.025^{ab}	7±0.0^{d}	0.05±0.0^{cd}	0.045±0.005^{b}	5±0.0^{b}	0.25±0.0^{gh}
Kushe	27±0.0^{a}	6.4±0.1^{efg}	0.475±0.015^{b}	0.59±0.01^{cdef}	6.1±0.1^{abc}	8±0.0^{ef}	0.065±0.005^{e}	0.13±0.0^{g}	7.95±0.05^{gh}	0.18±0.01^{abc}
Kwata	29±0.0^{b}	6.7±0.1^{gh}	0.945±0.035^{h}	0.66±0.02^{ghi}	6.045±0.035^{ab}	6.9±0.1^{d}	0.075±0.005^{f}	0.065±0.005^{cd}	10.1±0.1^{m}	0.225±0.015^{efg}
Rahwol	27±0.0^{a}	5.95±0.15^{cd}	0.46±0.0^{b}	0.51±0.01^{a}	6.2±0.1^{abcd}	6.3±0.1^{c}	0.01±0.0^{a}	0.14±0.01^{h}	8.04±0.03^{gh}	0.195±0.005^{bcd}
Rantaya	28±0.0^{ab}	5.55±0.05^{ab}	1.235±0.005^{k}	0.665±0.005^{ghij}	6.15±0.05^{abcd}	10.215±0.115^{h}	0.02±0.0^{a}	0.11±0.0^{f}	5.35±0.15^{c}	0.215±0.005^{def}
Rayfield	28±1^{ab}	5.95±0.05^{cd}	1.065±0.005^{i}	0.69±0.01^{ij}	6.035±0.005^{ab}	7±0.0^{d}	0.05±0.0^{cd}	0.07±0.0^{d}	8.005±0.005^{gh}	0.2645±0.005^{hi}

SOT	28±0.0^{abb}	7±0.0^{ab}	1.045±0.025^{i}	0.52±0.0^{ab}	6.15±0.15^{abcd}	8±0.0^{ef}	0.08±0.0^{f}	0.065±0.005^{cd}	7.25±0.05^{def}	0.195±0.015^{bcd}
Turu	28±0.0^{ab}	6±0.0^{cd}	1.21±0.01^{k}	0.61±0.01^{defg}	6.015±0.015^{ab}	8±0.0^{ef}	0.01±0.0^{a}	0.04±0.0^{ab}	8.5±0.0^{i}	0.17±0.01^{ab}
Vwang	27.5±0.5^{ab}	5.6±0.1^{ab}	0.725±0.005^{ef}	0.67±0.01^{ghij}	6.02±0.01^{ab}	7.1±0.1^{d}	0.019±0.001^{a}	0.03±0.0^{a}	7.5±0.0^{f}	0.2±0.0^{cde}
Zawan	28±0.0^{ab}	6.25±0.15^{de}	1.08±0.01^{ij}	0.66±0.02^{ghij}	6.35±0.15^{cd}	5±0.0^{a}	0.04±0.0^{bc}	0.04±0.0^{ab}	8.005±0.005^{gh}	0.175±0.015^{abc}
WHO	40	6.5-8.5	150	250	400	5	1000	1500	6	10

Results shows mean values ± standard error of mean of triplicate determination. Values with the same superscript letters in the same column are not significantly differen at p<0.05. pH: hydrogen ion concentration, RCL: residual chlorine, EC: electrical conductivity, TSS: total soluble solid, TDS: total dissolved solids, BOD: biological oxygen demand, COD: chemical oxygen deman

4.1.4.3 *Physicochemical parameter base on water source*

The result of the physiochemical parameter based on water sources shown in Table 4.7 Reveals that, all the physiochemical parameters studied are not significantly different at ($p<0.05$) when comparing well and borehole sources.

Table 4.7: Physicochemical Parameter Base on Water Source

Parameter	Borehole	Well	P-value
Temp	27.8833±0.1038	27.9167±0.107	0.709
pH	6.4767±0.0955	6.3992±0.0584	0.281
TH	0.8767±0.0404	0.9285±0.0388	0.022
RC	0.6118±0.0098	0.6143±0.0116	0.786
EC	6.1568±0.0193	6.1932±0.0185	0.045
TUB	7.3948±0.1703	7.5870±0.1696	0.161
TSS	0.0430±0.0028	0.0426±0.0025	0.858
TDS	0.0856±0.0055	0.1009±0.0060	0.001
BOD	7.6243±0.1812	8.8205±0.1844	0.000
COD	0.2045±0.0047	0.2018±0.0044	0.158

4.1.5 Antimicrobial susceptibility testing of isolates from water source

The result of the antibacterial susceptibility of *E. coli* isolated from different water location shown in Table 4.7 revealed that *E. coli* isolate from Dankara well shows the least sensitivity to gentamicin followed by *E. coli* isolated from Dahwak well. The susceptibility of *E. coli* isolated from Bukuru well, Chugwi well and Agwan mission borehole is not significantly different from each other. However, the isolate from Agwan mission well, Kwata well and Chugwi borehole shows the highest susceptibility to gentamicin followed by that isolates from Zawan well.

The *E. coli* isolates from Chugwi well shows the least susceptibility to Enrofloxacin followed by isolates from Agwan mission well and Kwata borehole. However, isolates from Dahwak, Dankara well, Dankara borehole and Agwan mission borehole are not significantly different. While *E. coil* isolate from Augudi well, Bukuru well, Zawan well, Kumbna well, Aungudi borehole and Chugwi borehole shows the highest susceptibility to Enrofloxacin.

E. coli isolates from Dahwak well shows the least susceptibility to ciprofloxacin followed by Dankara well and Kuru borehole. However, isolates from Aungudi well and Bukuru well, are not significantly different from each other. While Agwan mission well, Dankara well, Agwan mission borehole and Chugwi borehole shows the highest susceptibility to ciprofloxacin followed by Chugwi well, Zawan well, Kumbna well, Kwata well, Kwata borehole and Augudi borehole.

Isolate from Kwata well and Kuru borehole shows the least susceptibility to streptomycin followed by Dahwak well. *E. coil* isolates from Agwan mission well, Bukuru well, Dankara well, Zawan well, Kumbna well and Dankara borehole are not significantly different from each other. While *E. coli* isolated from Augudi well, Chugwi

well, Kwata borehole, Augudi borehole and Chugwi borehole shows the highest susceptibility to streptomycin. All *E. coli* isolates from all water sources shows no susceptibility to cefoxitin.

The antibacterial susceptibility of *E. coli* isolated from Chugwi well, Kumbna borehole and Chugwi borehole shows the least susceptibility to tetracycline followed by Bukuru well, Dankara well, Kumbna well, Kwata well and Dankara borehole while the *E. coli* isolates from Kwata borehole had the highest susceptibility to tetracycline followed by Augudi well, Agwan mission well, Dahwak well and Agwan mission borehole.

The *E. coli* isolate from Dahwak well shows least susceptibility to oxfloxacin followed by Dankara well and Kumbuna well. However, isolate from Augudi well, Agwan mission well, Bukuru well and Kwata well are not significantly different from each other. While *E. coli* from Chugwi well and Chugwi borehole shows the highest susceptibility to Oxfloxacin.

The antibacterial susceptibility of *E. coli* isolated from Dahwak well, Kumbna well, Kwata well and Kwata bore shows the least susceptibility followed by Agwan mission well and Kuru borehole. However, *E. coli* isolate from Augudi well, Bukuru well, Chugwi well, Dankara well and Agwan mission borehole shows the highest susceptibility to erythromycin followed by Augudi borehole and Zawan well.

Table 4.8: Antibacterial Susceptibility of *Escherichia coli* isolated from different water locations

Sample	Gentamicin	Enrofla	Cipro	Strep	Cefo	Tetra	Sulf	Oxfla	Erythromy
[illegible]	21±0^{cde}	29±5^{bcde}	20.5±2.5^{bc}	22±0^{cd}	0±0	19±1^{d}	27±1de	25±0^{bc}	18±0^{c}
[illegible]	31±1^{e}	25±2^{b}	26±0^{d}	10±1^{c}	0±0	20±0^{d}	26±0^{cde}	28±1^{bc}	10±1^{b}
[illegible]	16±2^{bc}	32±0de	20±1^{bc}	20±0^{c}	0±0	12±1^{b}	25±0^{cde}	25±1^{bc}	17±0^{c}
[illegible]	16±0^{bc}	0±0^{a}	26±4^{bcd}	23±1^{cd}	0±0	0±0^{a}	10±1^{b}	32±2^{e}	17.5±0.5^{c}
[illegible]	15±0^{b}	25±3^{bcd}	0±0^{a}	8.5±0.5^{b}	0±0	19±0^{d}	0±0^{a}	0±0^{a}	0±0^{a}
[illegible]	0±0^{a}	24.5±0.5^{bcd}	26±2^{d}	26±3^{c}	0±0	23±0^{b}	30±0^{e}	22±0^{b}	18±2^{c}
[illegible]	24±0^{c}	30±0^{bcde}	23±0^{bcd}	20±1^{c}	0±0	11±0^{b}	20±0^{c}	26±3^{bcd}	16±1^{ed}
Kwanbori well	18.5±0.5^{bcd}	26±2^{bcde}	26±1^{bcd}	20.5±0.5^{c}	0±0	11±0^{b}	29±1^{e}	22±0^{b}	0±0^{a}
Kwari well	32±0^{f}	0±0^{a}	24.5±0.5^{bcd}	0±0^{a}	0±0	11±2^{b}	24±2^{bcd}	25±0^{bc}	0±0^{a}
Kwata bore	20±2^{bcde}	24±1^{b}	23±0^{bcd}	22±0^{cd}	0±0	23±2^{c}	21±0^{cd}	27±2^{cd}	0±0^{a}
Kuru bore	22±0de	29.5±0.5^{bcde}	19.5±0.5^{b}	0±0^{a}	0±0	0±0^{a}	25±2^{cde}	28±0^{bcd}	10±0^{b}
Auugudi borehole	33±0de	31.5±0.5^{cde}	23±0^{bcd}	23±0^{cd}	0±0	14.5±0.5^{c}	22±1^{cd}	30.5±0.5^{de}	16±0de
Dangara borehole	20±2^{bcde}	33±0^{bcd}	25±2^{cd}	20±0^{c}	0±0	11±0^{b}	30±5^{e}	26±3^{bcd}	14±2^{cd}
Agwan mission borehole	16±3^{bc}	23±2^{bcd}	20±0^{d}	21±0^{c}	0±0	23±0^{d}	24±0^{cd}	25±0^{cde}	17±0^{c}
Chugwi borehole	30±0^{f}	29±5^{bcde}	21±0^{d}	25±0^{cd}	0±0	0±0^{a}	27±4de	30±0^{e}	12±0^{bc}

Results shows mean values ± standard error of mean of triplicate determination. Values with the same superscript letters in the same column are not significantly different at $p<0.05$

4.1.5.1 *Susceptibility and resistance patterns of Escherichia coli isolates*

The antibiotics susceptibility and resistance pattern in percentage presented in Table 4.9 Revealed that, the highest resistance was shown to cefoxitin and tetracycline with 15 and 8 isolates representing 100 % and 53.3 % respectively of the isolates resistance to the antibiotic. The highest susceptibility was shown to ciprofloxacin, and Enrofloxacin with 14 (93.3 %) and 8(86.6 %) isolates susceptible to these antibiotics respectively, similarly,14(93.3 %), 14 (40 %) and 13(86.6 %) susceptibility was recorded for gentamycin, tetracycline and sulfamethoxazole respectively. However, intermediate resistance was showed to 8(53.3 %) and 3(20 %) for Erythromycin and streptomycin respectively.

Table 4.9: Susceptibility and Resistance Patterns of *Escherichia coli* Isolates

Antibiotics	**Disc code**	**Resistant %**	**Intermediate %**	**Susceptible %**
GENTAMICIN	GM	1(6.6)	0	14(93.3)
ENROFLOXACIN	En	2(13.3)	0	13(86.6)
CIPROFLOXACIN	Cip-5	1(6.6)	0	14(93.3)
STREPTOMYCIN	S-10	4(26.6)	3(20)	8(53.3)
CEFOXITIN	FOX	15(100)	0	0
TETRACYCLINE	Te-30	8(53.33)	1(6.6)	6(40)
SULFAMETHOXAZOLE	STX	2(13.3)	0	13(86.6)
OXFOLAXICIN	OFX	7(46.6)	0	14(93.3)
ERYTHROMYCIN	E	7(46.6)	8(53.3)	0

4.1.5.2 *Antibacterial sensitivity of Escherichia coli isolated from different water source*

The result presented below revealed the comparison of antibiotics to *E. coli* isolates from different water source. The result further showed that *E. coli* from well water sample had no significant difference at ($p<0.05$) to the *E. coli* isolated from borehole in terms of the different antibiotics used (Table 4.10).

Table 4.10: Antibacterial sensitivity of *Escherichia coli* isolated from different water Source

Source	Well	Borehole	P-Value
GENTAMICIN	20.5±2.085	20.8±1.529	0.402
ENROFLOXACIN	25.062±2.55	26.2±1.593	0.573
CIPROFLOXACIN	19.875±2.047	22.2±0.583	0.284
STREPTOMYCIN	15.5±2.373	20.8±1.019	0.427
CEFOXITINE	0±0.0	0±0.0	0
TETRACYCLINE	11.93±2.027	18.2±2.93	0.921
SULFAMETH	22.06±2.448	21.2±2.03	0.094
OXFOLAXICIN	23.18±2.37	26.2±0.73	0.174
ERYTHROMYCIN	9.93±1.88	9.8±4.00	0.425

4.2 Discussion

4.2.1 Coliform counts on water sources

Sixty (60) sites of the different well and borehole water sampled were analyzed for total coliform. Out of the sites samples of water taken for bacteriological examination, only 14 (23.33 %) samples met the WHO recommended value for total coliform. This is as result of water sites been situated far from septic tank, dump sites and less industrial activities in the sample area. Whereas 46 (76.66 %) did not met the WHO standard which is 0 cfc/100 mL. Although total coliform count is not an indicator of faecal contamination, it is primarily used for assessment of the general sanitary quality of finally water treated and disinfected drinking water.

World Health Organisation (WHO) guideline stipulates that there should be no coliform bacteria per 100 mL in either treated water entering distribution system or in the distribution system. The well water records the highest coliform counts in this studies the reason for the high presence of coliform is as a result of poor hygienic practice by community members and lack of good water treatment method. This result is in agreement with the work done by Envuladu *et al*. (2012) who recorded a high level of coliform contamination in the bore hole water samples from Jos metropolis. Other investigators have reported the microbial contamination of well and bore hole water in Nigeria (Bala, 2006; Garba *et al*., 2009; Jagaba *et al*., 2020,). This investigator reported high level of coliform in the drinking water sources from their sampling sites. Among the factors contributing to high contamination of these wells are that, a good number of wells were not lined with concrete, aprons and covers were lacking in some of the wells while others had no permanent vessels for drawing water and the vessels are often kept on bare floor. These poor practices are possible sources of contamination.

Nwanta *et al*. (2010) reported that a total of 194 kg of solid waste is generated daily in Nsukka metropolitan abattoir, without any hygienic disposal and/or management system.

Further studies on the waste raised serious public health concerns, as bacteria such as *E. coli*, *Bacillus* sp, and *Staphylococcus* sp. were frequently detected. More so Galadima *et al*. (2011) in a review reported the various sources of water contaminations to be as situation of water sources close to pit latrines, septic tanks, agricultural practices and children throwing dirt into water sources.

4.2.2 Biochemical characteristics and identities of isolate in water sample

Fiftteen (15) *E. coli* was isolated from sixty water samples (about 25 %) through stand procedure in this present study. This result is in agreement with the result of Ayuba (2017) who identified coliform bacteria from all the underground water sampled from parts of Bukuru in Jos metropolis and Garba *et al.* (2009) who identify 63(35 %) of *E. coli* in some public water sources in Gusau municipal, North Western Nigeria. *Escherichia coli* were found to be dominant in the water sampled.

4.2.3 Molecular characteristics of isolates in water samples

Waterborne diseases have been regarded as a major global health problem throughout history. Microbial contamination remains a critical risk factor in water in many parts of the world, municipal sewage become the conduits for the passage of pathogens into surface water (Irvine *et al*.,1995), *Escherichia coli* cause diarrhoea in humans through diverse mechanisms. However, the pathogenic and non-pathogenic *E. coli* cannot be differentiated by the conventional diagnostic methods due to the lack of distinct phenotypic differences. Thus this study employed the method of multiplex because it has the capacity to simultaneously detect a number of sequences from the target organism (Osek, 2003; Duris *et al*., 2009) to detect several pathogenic species in a single sample (Kong *et al*., 2002) or to detect critical virulence factors, e.g. stx1, stx2, FliC, hlyA and rfbE. In the present study the result of the amplification of multiplex are shown in plate1. The PCR product sizes obtained for the various genes (h l y A -569bp, fliC -247bp, rfbE - 327bp, stx2 346, E16s 401 and stx1 130) were the same with the ones obtained by

Wang *et al*. (2002). The amplification process with reagents mix after optimization and Reaction conditions gave the same amplification pattern, which indicates the reproducibility of the protocol. Results of amplification revealed that *E. coli* E16S rRNA gene was amplified in all the sample. This is an indication of high copy number of 16SrRNA genes in the genome. In the first set of primers used, lane two and seven shows very clear bands seize for fliC while others showed negative for other primers used. However, the second set of primers which were the stx1 and stx2 run individually showed negative for the entire presumptive *E. coli*. This result is in line with El-shatoury *et al*. (2015) who used same set of primers expect Eae which was not used in this study to identify 1(5 %) of FliC in El-Rahawydrain water, in his report other virulence gene such as stx1, stx2, eae, rfbE and hylA were present which were not found in this study. More so, the result is agreement but with small modification with the study carried out by Ferasyi *et al*. (2020) who reported that 3 out of 72 faecal samples from cattle showed the presence of fliC and rfbE. However, rfbE was not present in any of the isolate used in this study.

Similarly, worked carried out by Franz and Van Bruggen, (2008) who also used same set of prime to detected six virulence gene in 0157:H7 isolated from different water source revealed that twenty-nine out of 44(66 %) *E. coli* O157: H7 isolate carried fliC, other genes such as stx1, stx2, eae and rfbE were present but were not present in any of the isolate tested in this study. Possible explanation for this may be that the tested isolates did not carry this gene. Another explanation could be that the amplification process requires further modification and optimization.

4.2.4 Physiochemical properties of water samples

The results of the physiochemical analysis carried out in this study reveals that the Temperature which is the measure of the degree of hotness or coldness of water which

was taken as soon as water sampling was conducted to investigate the presence of thermal input in water varies from 27 to 29 °C. There is no definite temperature value speculated by WHO for drinking water, all values obtained surpassed standards room temperature of 20 to 22 °C this may be due to climate of the area at the time of sampling. This finding is similar to the finding by Onoja *et al.* 2017 and Jagaba *et al.* (2020) who found out that no heat effect is associated with water sampled exceeding room temperature. The turbidity and pH of the well and borehole water samples, shows that all water sample from different location had turbidity above 5 NTU expect four samples from borehole from Agwan mission, Kufang, Kumbna and Zawan and 3 samples from well which are Jenta, Kufang and Kumbna which has a value of 5 NTU. All Water samples had pH within the WHO recommended value of (pH value of 6.5 to 8.5) expect for borehole water sample from Kuru in which the pH exceed this standard. This could be as a result of underground sulphide mineralization. This difference could also be that water from that locations are not been treated with chlorine and it could also be as a result of broken pipes in the distribution channels. Turbidity and pH affect chlorination of water, the higher the turbidity and pH the more the chlorine that is required and the longer the contact period before adequate disinfection.

Total hardness (TH): The principal hardness causing ions are calcium and magnesium. For hand dug wells high value of hardness could result from regular addition of large quantities of detergent used by residents which later drains into water bodies. The hard water can cause indigestion problem, it leads to deposit of scaling in pipelines, reduce water treatment efficiency, increase the cost of soap used for domestic and industrial purpose. It affects the taste of water as well as influences its lathering ability when used for washing. Long term consumption of this hard water may also lead to incidence of

prenatal mortality, cardiovascular disorder and some types of cancer. Very low hardness can however cause corrosiveness and can dissolved heavy metals. In this study, hardness values for borehole water sampled ranges from 0.06 mg/L to 1.29 mg/L and for well water samples 0.455 mg/L to 1.27 mg/L. Borehole water sample from Dakan and Hwak recorded the minimum and maximum values respectively while well water samples from Dakan, Dankara, Dashe, Kushe, Rantaya and Dop recorded minimum and Maximum value. All the values recorded in this study were within the permissible limit of 150 mg/L set by WHO. The relatively lower value recorded in this study may be due to the presence of lower calcium and magnesium in the various source of water sampled. Since water falls below standard, they will not result into skin itching and would not require much soap to form lather.

Residual chloride: the concentration of chloride is an indicator of sewage pollution and also imparts laxative effect. Atmospheric sources or sea water contamination is the reason for bulk of the chloride concentration in water which may exceed due to base exchange phenomena, high temperature, domestic effluents, septic tanks and low rainfall. Porosity soil and permeability also plays a key role in building up the chlorides concentration. The residual chloride content of the studied water samples was within the permissible limit of 250 mg/L prescribed by WHO. Electrical conductivity (Ec): This is the quantitative measure of the ability of water to pass electric current. It is used to estimate the number of dissolved minerals. Result from the sampling locations presented in this studies revealed that all water samples examined were below 1000µs/cm which is the WHO permissible Ec level for drinking water. This indicates that contamination due to dissolving ion is relatively low thus, the water are suitable for drinking purpose from the Ec point of view.

Total suspended solid (TSS): The TSS values of water sample indicate the quality of non-filterable particles contained in it. The permissible value recommended by WHO is 1000 mg/L. TSS range of well water source evaluated in these study ranges from 0.01 mg/L to 0.07 mg/L and for borehole 0.01 mg/L to 0.08 mg/L. The result for all water samples were within the recommended range for WHO standard. This implies that all water source examined in this study are suitable for drinking in terms of TSS. Total dissolved solid (TDS). Total dissolved solid is an indicator for water quality because it directly affects the aesthetic value of the water by increasing turbidity. High concentrations of TDS reduce the suitability of water as a drinking source and irrigation purpose. The acceptable range of TDS by WHO is 1500 mg/L. In this study all water examined fall within the range expect borehole and well from water sources of Du with a mean value 0.165 mg/L. This is as a result of the sewage, septic tanks, chemical discharge, urban and agricultural runoff in the sampled location. The lowest value of 0.03 mg/L obtained from both well and borehole source of Augudi, Bukuru, Chugwi and Vwang indicates less influence of domestic and industrial waste in the ground water in the study area.

Biological oxygen demand (BOD): This test measured the number of microorganisms in a water sample and the nutrients available to them. The permissible value stipulated by WHO is 6 mg/L, all the water sources sampled falls above the standards expect borehole water source from Augudi, Bukuru, Dop, Kuru and Rantya that falls below standard. This condition implies that each of the water sampled had high organic pollution and may constitute aquatic that cannot support the existence of marine organism. This result does not agree with the work done by Jagaba *et al*. (2020). Who reported that the BOD sampled from hand dug wells in Rafin Zurfi, Bauchi State was within the WHO permissible value. The reason for this disagreement could have been that the water sampled from the study area could have be highly polluted with industrial effluents and

also the wells are sited near damp sites in which microorganisms are leached into water bodies.

Chemical oxygen demand (COD): The results of all samples were within the acceptable value recommended by WHO which is 10 mg/L. This implies that there is less presence of strong organic contaminant concentration in the water sample within the study locations

4.2.5 Antimicrobial susceptibility testing of isolates from water source

Antibiotics play very important role in decreasing diseases, illness and/or death associated with bacterial infections in humans and animals (Onwa *et al.,* 2019). The results of antibiotic study revealed noticeable differences among *E. coli*i solates in their susceptibility and resistance patterns to antibiotics. The high susceptibility of most bacterial isolates was observed with, gentamycin, ciprofloxacin, sulfamethoxazole, oxfloxacin and enrofloxacin. The reason for the high susceptibility observed in this study is due to the less use of these antibiotics in clinical practice. This data is in agreement with a report from Onwa *et al.* (2019) who reported that gentamycine was highly susceptible to *E. coli* isolated from underground water in Abakaliki metropolis in Ebonyi State. More so the study is in line with that of Howlett *et al*. (2005) who reported that *E. coli* isolated from cattle and pig shows high susceptibility to Ciprofloxacin and Sulfamethoxazole. The higher levels of resistance to Cefoxitin, tetracycline and Enrofloxacine that was observed in the isolates suggests that the isolates have acquired resistant genes to the tested antibiotics, probably due to exposure to sub-lethal doses in the environment or possession of intrinsic genes by the isolates. The rising trend of resistance in most of the isolates in this study from water source affirms the fact that disposed antibiotics may have been washed down the water sources and accumulated downstream especially during the rainy season accounting for the high resistance (Onwa *et al.,* 2019).

CHAPTER FIVE

5.0 CONCLUSSION AND RECOMMENDATIONS

5.1 Conclusion

The water used by the resident of Jos South metropolis is unsafe for drinking, because none of the waters sampled met the international standards for drinking water. However only 14(23.33 %) of borehole and well water sampled from Sot, Kumbna, Jenta, Vwang, Rahwol, Dahwolgbo, Turu, Dakan and Kufang met the international standards in terms of coliform count. All the various sources of water were prone to contamination. However, borehole water was slightly contaminated and thus, safer for drinking when compared to well water sources.

The water sample from Dankara and Dahwak are heavily contaminated with the FliC virulence gene of *Escherichia coli* therefore resident from these locations are prone to water related infections such as diarrheal, cholera and typhoid.

All the water sampled failed to meet WHO recommendation in terms of BOD, Turbidity and the total dissolved solid of water source of Du.

The best antibiotics that can be used to treat the likely illness associated with the *E. coli* from this study is gentamicin, ciprofloxacin and oxofloxacin

5.2 Recommendations

Based on the findings of this study, the following recommendations are made

1. There is the need to develop cheap and effective small-scale water disinfection methods for making well water safe for drinking, thus the appropriateness of water storage, solar water disinfection, boiling and chlorination as points of use (small-scale) methods for making well water safe for drinking is recommended.

2. It was observed that majority of the resident source there drinking water from wells and bore holes, hence the state government should come to their aid by providing more bore holes, reconstruct the old wells to meet the WHO criteria and provide pipe borne water for use.
3. Waste Management Board in conjunction with Urban Development Board and Ministry of Housing should provide tactical and logistic support for proper waste management and housing.
4. State and local governments should embark on continuous monitoring of water quality and undertake repairs of leaking/rusted pipes.
5. Further studies should be embarked upon by researchers to know the real source of *E. coli* contamination whether it's from animals or human's source

5.3 Contribution to knowledge

The research revealed that borehole water and well water had coliform counts ranging from 2 to 150 coliforms/100 ml and 2 to 1700 coliforms/100 ml respectively. Fifteen (15) *Escherichia coli* were identified from the sample sites and the multiplex polymerase chain reaction (MPCR) technique was used to identify the pathogenic E. coli strains. Two (2) strains were identified to be pathogenic *E. coli* flagellin K12 from Dangara and Dahwak water samples. This study thus concludes that 40 % of the water sources had no evidence of E. coli while 60 % were contaminated with fecal coliform and that high susceptibility of most bacterial isolates was observed with, Gentamycin, Ciprofloxacin, Sulfamethoxazole, Oxfloxacin and Enrofloxacin.

This work will benefit the students immensely by broaden their knowledge on water contaminations matters, the community who uses the water on the danger of consuming contaminated water. The policy makers and health agencies on how to make policies concerning waste management, continues monitoring of water quality, sanitation practice, sitting of wells and bore holes and other relevant laws guiding safe drinking water.

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IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF PATHOGENIC *Escherichia coli* IN DRINKING WATER SOURCES IN JOS, NIGERIA

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