BACTERIOLOGICAL AND PHYSICOCHEMICAL QUALITY ASSESSMENT OF PACKAGED AND OTHER DRINKING WATER SOURCES IN ZARIA, KADUNA STATE, NIGERIA

BY

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BY

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES, AHMADU BELLO UNIVERSITY ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY,

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ZARIA, NIGERIA.

MAY, 2016.

DECLARATION

I hereby declare that the work in this dissertation entitled **"Bacteriological and Physicochemical Quality Assessment of Packaged and other Drinking Water Sources in Zaria, Kaduna State, Nigeria"** was performed by me in the Department of Microbiology under the supervision of Prof. S.E Yakubu and Prof. S.A. Ado. The information derived from the literature has been dully acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

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Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled "BACTERIOLOGICAL AND PHYSICOCHEMICAL QUALITY ASSESSMENT OF PACKAGED AND OTHER DRINKING WATER SOURCES IN ZARIA, KADUNA STATE, NIGERIA" by Rufus Precious ENENYA meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to the Almighty God for his divine providence, to my parents and to my brothers and sisters.

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ABSTRACT

Continuous increase in the sale and indiscriminate consumption of packaged drinking water and untreated water types such as borehole and well waters in Nigeria is of public health significance. Three hundred samples comprising 120 sachets, 60 bottled water brands, 60 borehole and 60 well water samples from five sampling sites in Zaria, North Western Nigeria were analysed microbiologically and physicochemically using standard procedures. Membrane filter technique was used to detect the presence of bacterial indicators of water quality as well as specific pathogens such as *Escherichia coli* O157:H7 and *Enterococcus* spp. The research was conducted between June 2014 to February 2015 during the wet and dry seasons. Isolated pathogens were characterized conventionally, using Microgen^{TM} ID Kits and molecularly by Polymerase Chain Reactions (PCR). The nitrate level in samples of the wells (100%) and borehole (40%) water were above the United States Environmental Protection Agency (USEPA) minimum contamination level of 10mg/l. Cadmium (Cd) and Lead (Pb) contents of boreholes, wells, and brands of sachet waters were above the minimum permissible limits set by NAFDAC (0.003mg/l and 0.01mg/l respectively). However, Lead (Pb) was not detected in the bottled water brands sampled. Total coliforms and *Escherichia coli* were detected in sachet water brands (80%), bottled water brands (20%), borehole (100%) and well water (100%). Enterococci were recovered from sachet water brands (70%), borehole (100%) and well water (100%). There were no statistically significant differences ($P \leq 0.05$) between the total coliform counts of the sachet water brands and borehole water in Zaria, therefore, the purity of sachet water as claimed by the manufacturers is doubtful. Bacteriological counts were higher during the wet season than dry season. Antibiogram of *Escherichia coli* O157:H7 (80%) and *Enterococcus* spp (37.6%) isolates showed multiple antibiotics resistance (MAR) with MAR indices of 0.3 and above. Polymerase chain reaction (PCR) amplified some housekeeping genes such as *tuf* gene of *Enterococcus* genus and antibiotics resistance genes such as glycopeptides Vancomycin and Teicoplanin resistance gene VANR. *Escherichia coli* O157:H7 extended spectrum β-lactamase genes: bla-TEM and bla-CTX-M genes. Most of the sachet water brands fell below NAFDAC and WHO drinking water standards and are therefore of doubtful quality and need strict adherence to standards. Efforts need to be intensified in the monitoring of activities in this rapidly expanding industry with a view to raising standards while government at all levels in Nigeria should take the issue of supply of adequately treated water to the public as an essential public service.

CHAPTER ONE

1.0 INTRODUCTION

Water is a simple molecule, consisting of two hydrogen atoms bonded to an oxygen atom with molecular formula H_2O (Bassem, 2013). It is one of the most important chemical substances for the sustenance of life and is vital for all known forms of life on Earth; It constitutes about 75% of the Earth`s surface (UN, 2005; EL-Jakee *et al.*, 2009; Hongyue *et al.*, 2013). In terms of sheer volume, About 97.5% of all the water on Earth is salt water, only 2.5% of all the water on Earth is fresh water and 98.8% of that fresh water is frozen in Antarctica and Greenland icecaps or lies too deep underground to be accessible; only 1.2% of the Earth's freshwater is available for withdrawal and human use (Shiklomanov, 2000; UN, 2005; Bassem, 2013). However, this is continually being polluted by various anthropogenic activities thereby further reducing the available freshwater for human use. The drinking water of most communities including Zaria in Nigeria is obtained from various sources: boreholes, rivers, streams, and well waters. Source water contamination poses a risk to public health and increases the cost of drinking water treatment.

The threats posed by deteriorating water quality caused by among other things, the contamination of potable water sources have led the public to seek for alternative potable water sources. Although access to safe and reliable sources of drinking water is a global challenge, it is particularly acute in developing countries, including Nigeria (Ivey *et al.*, 2006).

The production, sale and consumption of plastic bagged drinking water has grown tremendously over the years in many developing countries such as Nigeria. The plastic bagged drinking water was introduced into the Nigerian market as a less expensive means of accessing drinking water than bottled water.

1.1 Statement of Research Problem

Estimate of the global burden of water associated human diseases provide a simple index hiding a complex reality. World Health Organization (WHO) estimates that worldwide some 2.2 million people die each year from diarrhoeal related diseases. For an estimated 88% of diarrhoeal cause, the underlying cause is unsafe water, inadequate sanitation and poor hygiene (WHO, 2008; Mosier *et al.,* 2012). Water borne diseases continue to be one of the major health problems in developing nations, including Nigeria especially on the issues of safe drinking water quality (Mead *et al.,* 1999). Water borne diseases account for one third of the intestinal infection worldwide (Hunter and Syed, 2001; Offre *et al*., 2011).

The high prevalence of diseases such as diarrhoea, typhoid fever, cholera, and bacillary dysentery among the populace have been linked to the consumption of unsafe water and unhygienic drinking water production practices (Mead *et al.,* 1999; Park *et al.*, 2010). Pathogens such as *Salmonella* species, *Shigella* species, *Vibrio cholerae* and *E. coli* being seen in human and animal faeces ultimately find their way into water supply through seepage of improperly treated sewage into ground water, (Dipaola, 1998; Wei *et al*., 2011).

1.2 Justification for the Study

Most bottle water manufacturers in Nigeria also engage in sachet water packaging and obtain their raw water mostly from local, municipal piped or well water, however, adherence to production and analytical standards are doubtful as most of the factories are observed to lack the appropriate technology for achieving these (Oyedeji *et al.,* 2013). Diseases related to contamination of drinking-water constitute a major burden on human health and interventions to improve the quality of drinking-water provide significant benefits to health (UNDP, 2006). The potential health consequences of microbial contaminations are such that its control must always be of paramount importance and must never be compromised (Mead *et al.,* 1999).

1.3 Research Hypothesis

H_O: Packaged and other drinking water sources in Zaria do not meet the bacteriological quality standard.

H_O: Drinking water sources do not contain *Escherichia coli* O157:H7.

H_O: The target pathogenic bacteria are not resistant to the antibiotics tested.

H_O: *Escherichia coli* O157:H7 and *Enterococcus* spp isolated do not possess the virulence shigatoxin genes (Stx1 and Stx2) and antibiotics resistance genes (CTX-M, TEM) and vanr.

1.4 Aim

The aim of this study was to carry out bacteriological and physicochemical quality assessment of packaged and other drinking water sources in Zaria, Kaduna State, Nigeria.

1.5 Objectives were to:

1. Determine the bacteriological quality and physicochemical properties of packaged and other drinking water sources.
2. Isolate and characterize *Escherichia coli* O157:H7 and *Enterococcus* spp in the water samples
3. Determine the antibiotic susceptibility patterns of the isolates to commonly used antibiotics and screen for the presence of multiple antibiotic resistance strains.
4. Screen the *Escherichia coli* O157:H7 for the possession of extended spectrum β-lactamases genes (bla-CTX-M and bla-TEM) and virulence shigatoxin genes (Stx1 and Stx2) using the polymerase chain Reaction (PCR) technique.
5. Confirm *Enterococcus* using genus specific primer and detect the Vancomycin resistance gene (VANR) in *Enterococcus* spp. isolated.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Physicochemical Quality of Drinking Water

A number of chemical contaminants have been shown to cause adverse health effects in humans as a consequence of prolonged exposure through drinking-water (WHO, 2008; Dunn *et al*., 2014). There are many chemicals that may occur in drinking-water; however, only a few are of immediate health concern in any given circumstance, examples include fluoride, arsenic, lead, and nitrate (WHO, 2008). Exposure to high levels of fluoride, which occurs naturally, can lead to mottling of teeth and, in severe cases, crippling skeletal fluorosis. Similarly, arsenic may occur naturally, and excess exposure to arsenic in drinking-water may result in a significant risk of cancer and skin lesions. Iron and manganese are of widespread significance because of their effects on acceptability.

2.1.1 Total Dissolved Solids (TDS)

Total Dissolved Solids **(**TDS) comprise inorganic salts, principally calcium, magnesium, potassium, sodium, bicarbonates, chlorides and sulfates, and small amounts of organic matter that are dissolved in water (Grayson *et al*., 2012).

2.1.2 pH

The pH is a measure of the acidity or alkalinity of a solution and could also be defined as the negative of the logarithm to the base 10 of the hydrogen ion concentration (Sarker and Sathasivan, 2012).

2.1.3 Electrical conductivity (EC): Electrical conductivity is a function of ions concentration in a water sample. It is a measure of how much total salts are present in the water. It can serve as an indicator of water quality (Sarker and Sathasivan, 2012).

2.1.4 Chlorine and chloride

Chloride in drinking-water originates from natural sources, sewage and industrial effluents. Excessive chloride concentrations increase rates of corrosion of metals in the distribution system, depending on the alkalinity of the water (WHO, 2008). This can lead to increased concentrations of metals in the supply. In water, chlorine reacts to form hypochlorous acid and hypochlorites. However, it is normal practice to supply water with a chlorine residual of a few tenths of a milligram per litre to act as a preservative only during distribution.

2.1.5 Nitrate and nitrite

The nitrate concentration in groundwater and surface water is normally low but can reach high levels as a result of leaching or runoff from agricultural land or contamination from human or animal wastes as a consequence of the oxidation of ammonia and similar sources (WHO, 2008). Toxicologically, the primary health concern regarding nitrate and nitrite is the formation of methaemoglobinaemia, so-called “blue-baby syndrome.” Nitrate is reduced to nitrite in the stomach of infants, and nitrite is able to oxidize haemoglobin (Hb) to methaemoglobin (metHb), which is unable to transport oxygen around the body. The reduced oxygen transport becomes clinically manifest when metHb concentrations reach 10% or more of normal Hb concentrations; the condition, called methaemoglobinaemia, causes cyanosis and, at higher concentrations, asphyxia (WHO, 2008).

2.1.6 Heavy metals

Heavy metals could be defined as elements with atomic number greater than 22 and a density greater than 5g/ml (Tewari and Vivekanand, 2013). Sources of Heavy

metal contamination in water include discharge from industrial by-products such as fertilizer production plants and mining activities (Tewari and Vivekanand, 2013).

2.1.7 Cadmium

Cadmium is released to the environment through wastewater, and diffuse pollution is caused by contamination from fertilizers and local air pollution. Contamination in drinking-water may also be caused by impurities in the zinc of galvanized pipes and solders and some metal fittings (Kumar, 2012). Toxicologically, Cadmium accumulates primarily in the kidneys and has a long biological half-life (10–35 years) in humans (Kumar, 2012).

2.1.8 Lead

Lead is toxic to the central and peripheral nervous systems, inducing subencephalopathic neurological and behavioural effects. There is electrophysiological evidence of effects on the nervous system in children with blood lead levels well below 30mg/dl (WHO, 2003). The intake of lead from drinking-water constitutes a greater proportion of total lead (Pb) intake ('Water meters meet lead-free rules', 2011).

2.1.9 Iron

Iron is one of the most abundant metals in the Earth’s crust. It is found in natural fresh waters at levels ranging from 0.5 to 50 mg/litre (Allred, 2011). Iron may also be present in drinking-water as a result of the use of iron coagulants or the corrosion of steel and cast iron pipes during water distribution. Iron is an essential element in human nutrition.

2.1.10 Chromium

Chromium is widely distributed in the Earth's crust. It can exist in valences of +2 to +6. In general, food appears to be the major source of intake (He *et al*., 2014). The Provisional guideline value is 0.05 mg/litre for total chromium (WHO, 2008).

2.2 Microbiological Quality of Drinking Water

Public and environmental health protection requires safe drinking water, which means that it must be free of pathogenic microorganisms (Mwambete, 2013). Among the pathogens disseminated in water sources, enteric pathogens are the ones most frequently encountered (McFadyen, 2014). As a consequence, sources of faecal pollution in waters devoted to human activity must be strictly controlled.

The determination of the microbiological quality of water is essential in testing for the overall quality of water, which involves the enumeration of bacteria of faecal origin. Most coliforms are present in large numbers among the intestinal flora of humans and other warm-blooded animals, and are thus found in faecal wastes. As a consequence, coliforms detected in higher concentrations than pathogenic bacteria are used as an index of the potential presence of enteric pathogens in water environments.

2.2.1 Coliforms (total and faecal coliforms)

The coliform group include a broad diversity in terms of genus and species, they belong to the Enterobacteriaceae family. Most definitions of coliforms are essentially based on common biochemical characteristics (McFeters *et al*., 1986). In Standard Methods for the Examination of Water and Wastewater (APHA *et al*., 1998), coliform group members are described as: all aerobic and facultative anaerobic, Gram negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48 h at 35 ^{o}C.

The definition of members of the coliform group has recently been extended to include other characteristics, such as β-D-galactosidase-positive reactions and the cytochrome-oxidase test which is also used as a confirmation test to eliminate some

bacteria of the Aeromonas or Pseudomonas genera that would ferment lactose (Fricker *et al.,* 2010).

The French Standardization Association (Association Francaise de Normalisation) (AFNOR, 1990)., which can be considered as a representative model for European legislation, defines total coliforms (TC) as: rod-shaped, non-spore-forming, Gram-negative, oxidase-negative, aerobic or facultative anaerobic bacteria that are able to grow in the presence of bile salts or other replacement surface active agents having an analogous growth inhibitory effect and that ferment lactose with gas and acid (or aldehyde) production within 48 h at 37 ± 1 ^{o}C. AFNOR (1990) goes further by defining other coliform groups, including the thermotolerant coliforms (also called faecal coliforms, FC) and, more specifically, *E. coli*: thermotolerant coliforms have the same fermentation properties as total coliforms (TC) but at a temperature of 44 ± 0.5 ^{o}C. *E. coli* is a thermotolerant coliform which, among other things, produces indole from tryptophane at a temperature of 44 ± 0.5^{o}C, gives a positive methyl red test result, is unable to produce acetyl–methyl carbinol and does not use citrate as its sole carbon source.

2.3 Antibiotics Resistance in Bacteria

Antibiotics resistance is a natural phenomenon whereby microorganisms develop or acquire resistance to antimicrobial agent as a consequence of exposure to sub lethal amount of antimicrobials in its environment (Carrottoli, 2009). Mobile genetic elements such as plasmids, transposons and integrons disseminate antibiotics resistance genes by horizontal or vertical gene transfer which could lead to the development of multidrug resistance genes in Gram-negative and Gram-positive bacteria (Pitout *et al.,* 2004). Infection by resistant bacterial strains can occur as a result of consumption of food and water with resistant bacteria (UN, 2010).

2.4 Water Sources in Zaria

Zaria community water systems are obtained from two sources: surface water and ground water. Surface water is water that collects on the ground or in a stream, river, lake, reservoir. Ground water is water located below the ground surface in pores and spaces in the rock. Ground water is obtained by drilling wells and boreholes. Most homes in sub-Saharan African countries including Nigeria lacks the infrastructural and financial capacity to undertake adequate water treatment resulting in high level patronage of generally perceived pure packaged water.

2.5 Production of Packaged Drinking Water

Borehole water is collected into a reservoir and is treated with chlorine. The water is then pumped through graded sand bed filter and activated carbon into another tank. From here, the water is then passed through three sets of microfilters with pore sizes of 5µ, 2 µ and 0.5µ respectively into another tank usually PVC or stainless steel tank. The water then passes through a stainless steel ultra violet (UV) steriliser before finally passing through a packaging machine where it is automatically packed into sachets (500ml) (NAFDAC, 2015). The bags used for packaging factory produced plastic bag drinking water are made of high-density polyethylene (HDPE), which is very strong and has higher tensile strength difficult to elongate, and can withstand higher temperatures (NAFDAC, 2015).

2.6 Microorganisms Associated With Packaged and other Drinking Water Sources

Most waterborne pathogens are introduced into drinking-water supplies through human or animal faeces, most of which do not grow in water. However, *Legionella*, atypical mycobacteria, *Burkholderia pseudomallei* and *Naegleria fowleri* are

environmental organisms that can grow in water and soil. Of all the waterborne pathogens, the helminth *Dracunculus medinensis* is unique in that it is the only pathogen that is solely transmitted through drinking water (Ogbeibu *et al*., 2013).

2.6.1 *Escherichia coli*

Escherichia coli (*E. coli*) are Gram-negative, non-spore-forming, rod shaped bacteria which are capable of aerobic and facultative anaerobic growth in the presence of bile-salts or other surface-active agents with similar growth-inhibiting properties . They usually ferment lactose at 37 °C within 48 hours, possess the enzymes β-glucuronidase and β-galactosidase and are oxidase-negative (Anon, 1997). *Escherichia coli* is present in large numbers in the normal intestinal flora of humans and animals, where it generally causes no harm. However, in other parts of the body, *E. coli* can cause serious disease, such as urinary tract infections, bacteraemia and meningitis. A limited number of *E. coli* strains can cause acute diarrhoea.

2.6.1.1 *Classification of Escherichia coli strains associated with diarrhoeal disease:*

Several classes of *E. coli* have been identified on the basis of different virulence factors and clinical features.

2.6.1.1.1 EHEC (Enterohaemorrhagic *E. coli*): causes life-threatening haemorrhagic diarrhoea (colitis) in all ages, without pus cells, and often without fever. It can progress to haemolytic uraemic syndrome with renal failure. EHEC has been reported mainly from Europe and North America. Outbreaks have also occurred in refugee camps in Mozambique, Swaziland and Malawi. EHEC is due to cytotoxins damaging vascular endothelial cells, and is mainly associated with the serogroup 0157:H7. It is sometimes referred to as VTEC (verocytotoxin producing *E. coli*, because it is toxic to vero monkey cells in culture). Infection occurs by ingesting contaminated meat products, unpasteurized milk and dairy products (Cheesbrough, 2006).

2.6.1.1.2 EPEC (Enteropathogenic *E. coli*): causes vomiting, fever, and prolonged diarrhoea mainly in infants (less than 2 y). Due to bacteria adhering to epithelial cells, multiplying and causing lesions. Many serogroups are involved. EPEC is a major problem in developing countries (Cheesbrough, 2006).

2.6.1.1.3 ETEC (Enterotoxigenic *E. coli*): causes watery (secretory) diarrhoea due to the production of plasmidmediated toxins (LT, ST) in infants and adults, particularly in developing countries. It is often referred to as traveller's diarrhoea. Many serogroups are involved (Cheesbrough, 2006).

2.6.1.1.4 EIEC (Enteroinvasive *E. coli*): causes dysentery (similar to shigellosis), fever and colitis, with blood, mucus, and many pus cells in faecal specimens. Due to bacteria invading and multiplying in epithelial cells. Many serogroups are involved (Cheesbrough, 2006).

2.6.1.1.5 EaggEC (Enteroaggregative *E. coli*): causes chronic watery diarrhoea and vomiting, mainly in children. Due to the bacteria adhering to tissue cells often in stacks (aggregates) (Cheesbrough, 2006).

2.6.1.2 *Source and occurrence of Escherichia coli*

Enteropathogenic *E. coli* are enteric organisms, and humans are the major reservoir, particularly of EPEC, ETEC and EIEC strains. Livestock, such as cattle and sheep and, to a lesser extent, goats, pigs and chickens, are a major source of EHEC strains. The latter have also been associated with raw vegetables, such as bean sprouts. The pathogens have been detected in a variety of water environments (Nataro and Kaper, 1998).

2.6.1.3 *Significance of pathogenic Escherichia coli in drinking-water*

Waterborne transmission of pathogenic *E. coli* has been well documented for recreational waters and contaminated drinking-water **(**Nataro and Kaper, 1998; O'Connor, 2002). A well publicized waterborne outbreak of illness caused by *E. coli* O157:H7 (and *Campylobacter jejuni*) occurred in the farming community of Walkerton in Ontario, Canada. The outbreak took place in May 2000 and led to 7 deaths and more than 2300 illnesses (Clark *et al*., 2003; Ali, 2004). The drinking-water supply was contaminated by rainwater runoff containing cattle excreta (Ali, 2004). Control measures that can be applied to manage potential risk from enteropathogenic *E. coli* include protection of raw water supplies from animal and human waste, adequate treatment and protection of water during distribution. There is no indication that the response of enteropathogenic strains of *E. coli* to water treatment and disinfection procedures differs from that of other *E. coli*. Hence, conventional testing for *E. coli* (or, alternatively, thermotolerant coliform bacteria) provides an appropriate index for the enteropathogenic serotypes in drinking-water.

2.6.2 Enterococci

Enterococci are Gram-positive bacteria that grow over a wide temperature range, 10–45 ºC. at pH 9.6, survive at 60°C for 30 minutes and in 6.5% sodium chloride (NaCl), and the ability to reduce 0.1% methylene blue and a positive reaction with Lancefield's Group D antisera (Ashbolt *et al.*, 2001; Cheesbrough, 2006). Enterococci, a term used in the USA, include all species described as members of the genus *Enterococcus (*Junco, 2001). In practice, the terms faecal streptococci, enterococci, intestinal enterococci and *Enterococcus* group may refer to the same bacteria (WHO, 2003). However, the subgroup intestinal enterococci consists of the species *Enterococcus faecalis, E. faecium, E. durans and E. hirae.* This group was separated

from the rest of the faecal streptococci because they possess Lancefield Group D antigen as also some streptococci but the Group D antigen of enterococci is unlike that of other Lancefield groups which is not extracted by traditional acid extraction techniques but rather, Enzyme techniques based on Maxted's enzyme are often required (Cheesbrough, 2006). *E. faecalis*, however, is usually identified culturally, biochemically and are relatively specific for faecal pollution. However, some intestinal enterococci isolated from water may occasionally also originate from other habitats, including soil, in the absence of faecal pollution (WHO, 2008).

2.6.2.1 *Indicator value of* enterococci

Intestinal enterococci group can be used as an index of faecal pollution (WHO, 2008). Most species do not multiply in water environments hence bears direct relationship to the exact amount of pollution in water. The numbers of intestinal enterococci in human faeces are generally about an order of magnitude lower than those of *E. coli*. Important advantages of this group are that they tend to survive longer in water environments than *E. coli*; they are more resistant to chlorination (Pinto, 1999). Intestinal enterococci have been used in testing raw water as an index of faecal pathogens that survive longer than *E. coli* and in drinking-water to augment testing for *E. coli*. In addition they have been used to test water quality after repairs of distribution systems or after new mains have been laid (Ashbolt *et al.,* 2001).

2.6.2.2 *Significance of* enterococci *in drinking-water*

The presence of intestinal enterococci provides evidence of recent faecal contamination, and detection should lead to consideration of further action, which could include further sampling and investigation of potential sources such as inadequate treatment or breaches in distribution system integrity **(**Pinto, 1999).

2.6.3 Enteric viruses

The viruses referred to here are a combined group of those that infect the human gastrointestinal tract and are predominantly transmitted by the faecal–oral route. Well known members of this group include the enteroviruses, astroviruses, enteric adenoviruses, orthoreoviruses, rotaviruses, caliciviruses and hepatitis A and E viruses (WHO, 2008). The enteric viruses cover a wide spectrum of viruses, members of which are a major cause of morbidity and mortality worldwide. Members of the group of enteric viruses differ with regard to structure, composition, nucleic acid and morphology. There are also differences in the numbers and frequency of excretion, survival in the environment and resistance to water treatment processes. Enteric viruses have robust capsids that enable them to survive unfavourable conditions in the environment as well as allowing passage through the acidic and proteolytic conditions in the stomach on their way to the duodenum, where they infect susceptible epithelial cells (Williamson *et al.,* 2011).

2.6.3.1 *Significance of Enteric viruses in drinking-water*: The presence of any enteric viruses in drinking-water should be regarded as an index for the potential presence of other enteric viruses, is conclusive evidence of faecal pollution and also provides evidence of shortcomings in water treatment and disinfection processes (Grabow *et al.,* 2001).

2.7 Current Methods and Emerging Approaches for the Detection and Enumeration of Coliforms in Drinking Water.

Since drinking waters constitute oligotrophic systems, the lack of sensitivity of cultivation methods in the detection of stressed and starved bacterial cells can generate serious limitations due to contamination-level underestimation (Rompré *et al.*, 2002). There exist methods which may be used for coliform detection. These includes:

classical methods such as Multiple-tube Fermentation Technique and Membrane Filter (MF) Technique, Enzymatic Methods such as presence/absence Techniques and Enumeration by Multi-Tube Techniques Using Enzymatic Methods, MF Technique Conjugated to Enzymatic Detection of Coliforms, Direct Determination of Enzymatic Activity by Fluorimetry and Detection of Coliforms by Enzymatic Methods Using Solid-Phase Cytometry, Molecular Methods such as Immunological Methods, Nucleic Acid-Based Methods which includes Polymerase Chain Reaction (PCR) Methods and *In situ* Hybridization Techniques (Rompré *et al.*, 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS.

3.1 The Study Area

Zaria is a town in Kaduna State, in the North-Western part of Nigeria and is located on latitude $11^{o}04$N and longitude $7^{0}43$E and about 660M above sea level (National Population Census, 2010; Oladimeji and Ojibo, 2012).

3.2 Sampling Sites

Five sampling sites were selected in Zaria for this study. These sites include; Samaru , Wusasa, Tudunwada , Zaria city and Sabongari. The map of Zaria is shown in Figure 3.1.

3.3 Study Design: This study was a cross-sectional study.

3.4 Sample Size: The sample size for this study was determined using the following formula as described by (Naing *et al.*, 2006).

$$N = \frac{Z^2 p(1-p)}{d^2}$$

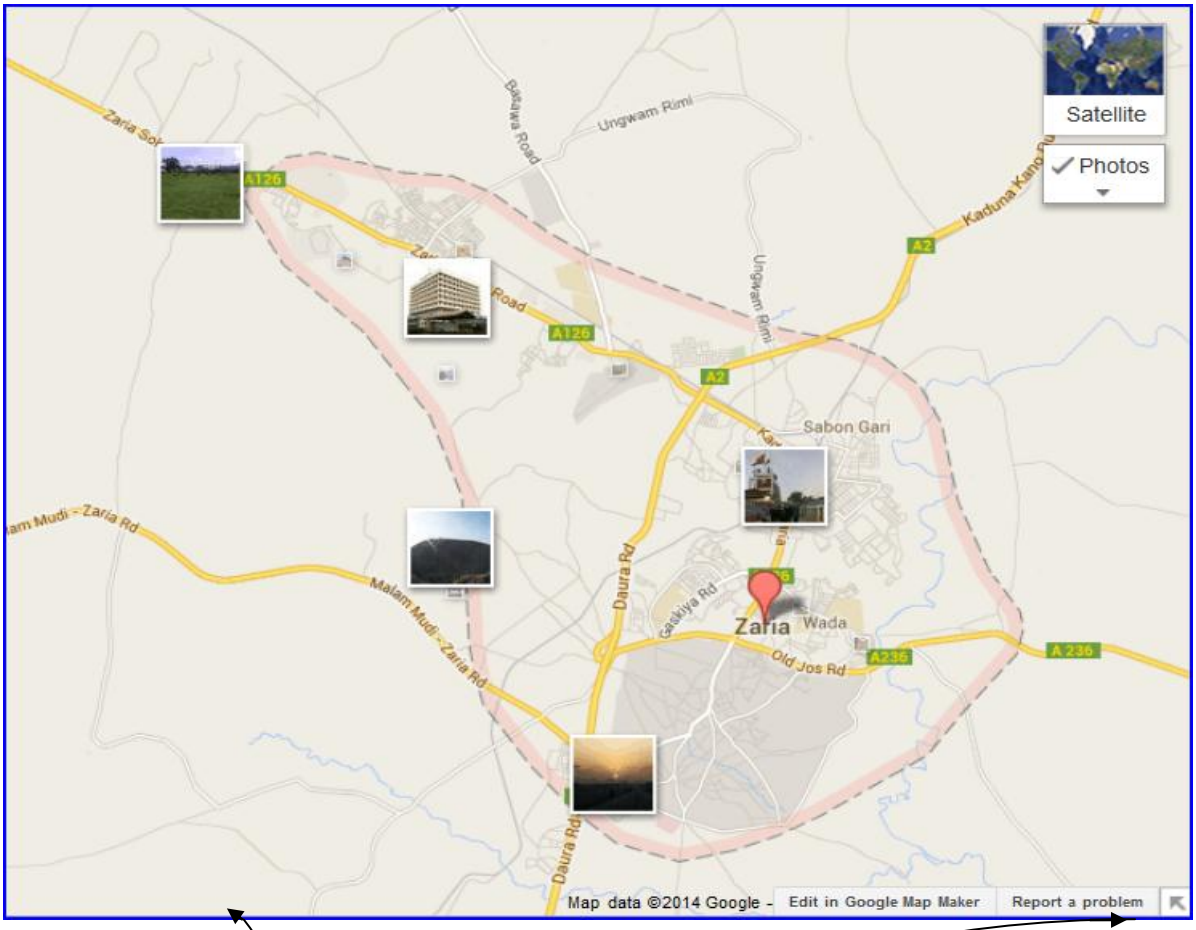
Where N= *sample* size or Number of sample.

Z= Standard normal distribution at 95% confidence interval=1.96

P= Prevalence rate of contamination of previous study = 23.3% (Oyedeji *et.al.*, 2013). d= Absolute desire precision of 5% = 0.05. Therefore,

$$N = \frac{1.96^2 \times 0.23(1-0.23)}{0.05^2}$$

N = 273. However, a total of 300 samples were collected for this study.



Map of Nigeria Showing Kaduna State



Figure 3.1: Map of Zaria showing sampling locations
Source: GIS Imagery; **July 2015**

3.5 Collection of Samples

Water samples were collected in the rainy and dry seasons. Water samples were collected according to the procedure recommended by the American Public Health Association (APHA, 1998). From the selected districts in the sampling area, well water and borehole water samples were collected in sterile screw capped 1000ml capacity bottles, while the plastic bagged sachet drinking water and plastic bottle water were purchased using purposive sampling from different manufacturers and vendors in Zaria. Samples were packed in an ice packed cooled box during the transportation to the laboratory (Food and Industrial/Environmental laboratory, Department of Microbiology and the Department of Water Resources and Environmental Engineering Ahmadu Bello University, Zaria) for analysis.

3.6 Physicochemical Analysis of Water Samples

The physicochemical analysis was carried out to determine the physicochemical quality of the water sources in accordance to the methods of the American Public Health Association (APHA, 1998). The physicochemical parameters analyzed include; pH, temperature, electrical conductivity, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), sulphate, Chloride, nitrate and phosphate at the Department of Water Resources and Environmental Engineering Ahmadu Bello University, Zaria. While the heavy metals; Lead (Pb), Iron (Fe) and Cadmium (Cd) were determined using fast sequential atomic absorption spectrophotometer model AA240FS at the multipurpose laboratory, Department of Chemistry, Ahmadu Bello University, Zaria.

3.6.1 Temperature, pH, electrical conductivity and total dissolved solids

The pH, temperature, electrical conductivity, and total dissolved solids of the water samples were determined at the point of sample collection using the HANNA combo

(H198130) instrument (A tester that gives high accuracy of temperature, pH, EC, and TDS in a single test). Following the manufacturers instructions, the electrodes connected to each meter was submerged in a clean beaker containing the sample. Selecting a specific test via the mode buttons, the values read for each parameter by the tester was observed and recorded.

3.6.2 Dissolved oxygen (DO) and biological oxygen demand (BOD)

The Hanna instrument (HANNA 3200) was used to determine the Dissolved Oxygen (DO) and Biological Oxygen Demand (BOD). The instrument was inserted into the sample to determine the DO at the point of collection. The BOD was obtained after the sample was collected, transferred to a BOD bottle and allowed to stand for 5days at room temperature ($28^oC \pm 2^oC$). The BOD was determined by inserting the Hanna instrument into the incubated sample and then subtracted the initial DO from the final DO value to obtain the BOD_5 (APHA, 1998).

3.6.3 Nitrate

This was carried out by the method described by APHA, (1998). The sample was neutralized at pH 7.0. One hundred millilitres (100ml) of the sample was placed in a beaker and allow to evaporate to dryness on a water bath. The residue was dissolved using a glass rod with 2ml disulphuric acid reagent and then transferred to Nesslers tube. Six millilitres (6ml) of ammonium hydroxide (NH_4OH) and EDTA was added drop-wise till the residue dissolved. A blank was also prepared in the same way using distilled water instead of the sample. The colour development was read at 410nm with a light path of 1cm. The nitrate concentration was calculated using the formula bellow:

$$\text{Mg/L nitrate} = \frac{\text{mg nitrate N} \times 1000}{\text{ml sample taken}}$$

The nitrogen concentration was estimated by comparing the reading with a standard curve previously prepared.

3.6.4 Sulphate

One hundred millilitre (100ml) of the sample was dispensed in a 250ml capacity Erlenmeyer flask , 5ml conditioning reagent was added and mixed using a magnetic stirrer. A spoonful of barium chloride crystals was added while the solution was still being stirred, the turbudity was measured at 30seconds after stirring at interval of 4minutes. A blank was prepared with no barium chloride added and the sulphate concentration was calculated using the formula:

$$\text{mg/L } SO_4 = \frac{\text{mg } SO_4 \times 1000}{\text{ml sample taken}}$$

the sample was estimated by coparing the turbidity reading with a standard curve (APHA, 1998).

3.6.5 Determination of heavy metals in the water samples.

The concentration of heavy metals (Lead, Iron and Cadmium) in the water samples were analyzed using the fast sequential atomic absorption spectrophotometer (AAS 240FS) (Agilent Technology California USA). The instruments settings and operational conditions were done in accordance to the manufacturers specifications by calibrating with analytical grade metal standard stock solution.

One hundred millilitre of each water sample was digested in a beaker by adding 20ml of the prepared aquariga (which was prepared by mixing hydrochloric acid and nitric acid in the ratio of 1:3 12.5ml of hydrochloric acid mixed with 37.5ml of nitric acid). Heated to almost dryness and topped up to 100ml with distilled water. The digested samples

were filtered to remove any insoluble materials that could clog the atomizer. The filtrate was then analyzed for heavy metals using the Atomic Absorption Spectrophotometer (AAS) (APHA, 2002**).**

3.7 Bacteriological Analysis of Water Samples Using Membrane Filter (MF) Technique

3.7.1 Enumeration of *Escherichia coli* using membrane lactose glucuronide agar (MLGA)

MLGA is a medium for the differentiation and enumeration of *Escherichia coli* and other coliforms by a single membrane filtration technique. Organisms are isolated on a membrane filter and placed onto MLGA. The medium contains lauryl sulphate to inhibit Gram-positive organisms. Identification of *Escherichia coli* and coliforms is facilitated through two biochemical reactions within the medium. Lactose fermentation is detected by the dye phenol red which gives yellow colonies when acid is produced. The chromogenic substrate, 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide (BCIG) is cleaved by the enzyme glucuronidase and produces a blue chromophore which builds up in the *Escherichia* coli cells.

Coliforms other than *Escherichia coli* are lactose-positive so colonies will be yellow; *Escherichia coli* is both lactose-positive and possesses glucuronidase so will appear as green colonies. A Membrane Filtration Technique was used for the detection and enumeration of *Escherichia coli* according to standard procedures (APHA, 2002**).** Membrane lactose glucuronide agar (MLGA) was prepared by measuring 22g in 250ml of distilled water in a flask, allowed to dissolve and autoclaved at 121°C for 15 minutes. The medium was cooled to 50°C and pour into sterile Petri dishes. Hundred milliliters (100ml) of different brands of sachet and bottle water while 10ml each of well and borehole water samples were filtered in triplicate through white, grid marked 47mm

diameter, Millipore HA-type cellulose membrane filters (Millipore, India) with pore size of 0.45 μm. Samples were filtered using a vacuum pump at a pressure of 65kpa (500mmHg) and a triple stainless steel filtration unit (Millipore, Bedford, UK). The membrane filters were placed on MLGA in Petri dishes using flame sterilized forceps ensuring that no air-bubbles are trapped under the membrane. The plates were incubated at 30°C for 4 hours, then at 37°C for 14 hours. All yellow and green colonies were counted using a colony counter. The yellow colonies were presumptive non-*Escherichia coli* coliform bacteria and the green colonies are *Escherichia coli.* The combined count can be regarded as the number of coliform bacteria. Results are expressed in colony forming units per volume of sample.

3.7.2 Enumeration of total coliforms

Total coliforms were estimated using the Membrane Filtration Technique (MF) according to standard procedures (APHA, 2002**).** The procedure was as described for *Escherichia coli* but the combined count of the yellow colonies and the green colonies can be regarded as the number of total coliform bacteria in the water sample.

3.7.3 Enumeration of faecal coliforms

Faecal coliforms were estimated using the Membrane Filtration Technique (MF) according to standard procedures (APHA, 2002**).** The procedure was as described for Total Coliforms but Petri dishes were inverted and incubated at 44°C for 24 hours. Membrane Filter on Petri dishes showing yellow colonies and green colonies can be regarded as the number of faecal coliform bacteria in the water sample and counted using a colony counter and expressed as Colony Forming Units (cfu) per 100ml.

3.7.4 Enumeration of thermotolerant (faecal) *Escherichia coli.* The procedure was as described for faecal coliforms but only thermotolerant green colonies were identified as positive faecal *Escherichia coli* and counted using a colony counter and expressed as Colony Forming Units (cfu) per 100ml.

3.7.5 Enumeration of Enterococci

A Membrane Filtration Technique was used in the detection and isolation of enterococci according to Anon, (1997). Hundred milliliters of water samples were filtered through 47 mm diameter Corning membrane filter with the pore size of 0.45 µm. Slanetz and Bartley agar were prepared by measuring 10.9g in 250ml distilled water and brought to boil and then poured into sterile Petri dishes and allowed to cool to solidify. It needed no autoclaving. Filters were placed on Slanetz and Bartley agar on Petri dishes using flamed sterilized forceps. The Petri dishes were incubated at 37°C for 4 hours and then transferred to 44°C for 44 hours. Red, maroon or pink colonies were counted using a colony counter and expressed as Colony Forming Units (cfu) per 100ml.

3.7.6 Enumeration of total heterotrophic bacteria or total viable count (TVC)

The total heterotrophic bacteria plate counts (HPC) in the water samples were obtained using the pour plate technique according to Anon (1997). Dilutions of 10^{-1} – 10^{-6} of water samples were prepared in sterile distill water and triplicate 1ml aliquots of 10^{-1}, 10^{-3} and 10^{-6} dilution inoculated into Petri dishes, 10 ml molten standard plate count agar were poured into the Petri dishes containing the 1ml aliquots of 10^{-1}, 10^{-3} and 10^{-6} dilution, After swirling gently to mix it was allowed to solidify and the Petri dishes

were incubated for 24 hours at 35±2°C. Discrete colonies were counted using a colony counter and the results expressed as the number of viable bacteria colonies per milliliter.

3.7.7 Isolation of *Escherichia coli* O157:H7 from water samples

Using sorbitol MacConkey agar (oxoid) supplemented with cefixime-tellurite (oxoid) preceded with 6h resuscitation on Tryptone Soya agar (oxoid), A Membrane Filtration Technique was used for the detection and isolation of *Escherichia coli* O157:H7 according to standard procedures (APHA, 2002). One hundred millilitre of water samples were filtered through 47 mm diameter track-etched polycarbonate membrane filter with the pore size of 0.45 μm. Sorbitol MacConkey Agar (SMAC) was prepared by measuring 25.75g in 500ml distilled water and brought to boil to dissolve completely and autoclaved at 121°C for 15 minutes. One vial of cefixime-tellurite supplement (oxoid) was reconstituted with 2ml of sterile distilled water and the contents aseptically added to 500ml autoclaved Sorbitol MacConkey Agar (SMAC) cooled to approximately 50°C, it was mixed well and 15ml poured into each of the sterile Petri dishes and allowed to cool and solidify. Tryptone Soya Agar (TSA) was prepared by measuring 10g in 250ml distilled water and brought to boil to dissolve completely and autoclaved at 121°C for 15 minutes and then poured into new sterile Petri dishes and allowed to cool to solidify. Filters were placed on the surface of TSA on Petri dishes using flamed sterilized forceps. The Petri dishes were incubated at 37°C for 6h for full resuscitation of stressed *Escherichia coli* O157:H7, and then using flamed sterilized forceps, the membrane was aseptically transferred to the surface of SMAC supplemented with Cefixime-Tellurite (CT) and then further incubated at 37°C for 18h . *E.coli* O157 does not ferment sorbitol and therefore, produces colourless colonies. In contrast, most *E. coli* strains and other coliforms ferment sorbitol and form pink

colonies. potassium tellurite selects serogroup O157 from other *E. coli* serogroups and inhibits *Providencia* and *Aeromonas* species. Cefixime is inhibitory to *Proteus* spp.

3.8 Biochemical Characterization of Isolates by Conventional Methods

3.8.1 Indole test

Two to three presumptive *E. coli* colonies were inoculated into test tubes containing peptone water, which contains amino acid, tryptophan, and incubated at 37^{o}C for 24h. Following incubation two drops of Kovac's reagent were added. Kovac's reagent consists of para-dimethyl aminobenzaldehyde, isoamyl alcohol and conc. HCl. Formation of a red or pink coloured ring at the top is taken as positive. *Escherichia coli* is Positive (Cheesbrough, 2006).

3.8.2 Methyl red-voges Proskaeur (MR-VP) test:

Two to three colonies of the isolates were inoculated into 5ml methyl red Voges-Proskauer broth (MR-VP) and incubated for 48h at 35^{o}C. After incubation, 1ml of broth was transferred into a sterile tube and two drops of methyl red indicator was added. red colour was indicative of a positive MR result. To 2.5ml of the remaining culture broth, 0.6ml of 5% α-naphthol solution and 0.2ml of 40% potassium hydroxide was added and shaken properly. Formation of a pink-red within 5-15min indicates a positive (acid) result (Cheesbrough, 2006).

3.8.3 Citrate test

The isolates were inoculated onto Simon's citrate agar using a wire loop. Growth indicated by blue colour in Simon's citrate agar from green colour indicates a positive result. This means that citrate has been utilized as sole source of carbon) and no

colour change indicate negative. *Escherichia coli* is negative for citrate test (Cheesbrough, 2006).

3.8.4 Litmus milk decolorization test to identify enterococci

Using a sterile loop, heavy inoculum of the presumptive enterococci isolates was inoculated into test tubes containing 0.5 ml of sterile litmus milk medium and then incubated at 37 ^{o}C for up to 4 hours while examining at half hour interval for a reduction reaction as shown by a change in colour from mauve to white or pale yellow. White or pale yellow colour is indicative of *Enterococcus* while no change or a pink colour is negative (Cheesbrough, 2006).

3.8.5 Aesculin hydrolysis test to identify enterococci

Aesculin hydrolysis test was performed by incubating the enterococci isolates on bile aesculin agar. A positive test is indicated by the agar and the colonies around it turning black/grey while a negative test result has no change in colour of the medium (Cheesbrough, 2006).

3.8.6 Oxidase test

Isolates were tested for oxidase production. Smear of test isolates was made on oxidase test strips (oxoid) and observed for violet/purple colour change. Colour change to deep violet/purple at the area of the smear with the test organism within 2-3 seconds (maximum 1 minute) was recorded as positive reaction, while smears of test isolates with no colour change indicate negative reaction (Cheesbrough, 2006).

3.9 Biochemical Identification of Presumptive *Escherichia coli* Isolates Using Microgen^{TM} GnA-ID Kit.

The microgen GnA-ID system is a standardized micro-substrate system design to simulate conventional biochemical substrates for the identification of enterobacteriaceae. It was further used to confirm conventional biochemical

characterisation of isolates following manufacturer`s instructions. Organism identification by microgenTM GnA ID system was based on pH change and substrate utilization as established by published reference methodologies. The microgenTM GnA solid microplate format made up of 12 substrates was used for further biochemical characterisation of presumptive *Escherichia coli* isolates. From a 24h culture, isolated colony was picked and emulsified in a 5ml of sterile saline solution and mixed thoroughly to obtain a homogeneous bacterial suspension. A 100µl of the bacteria suspension was dispensed into each well of microbact identification system and incubated at 37^{o}C for 24h. Reactions were evaluated as positive or negative by comparing its colour with the colour chart provided by the manufacturer. Results were recorded in the report form provided which generated an octal coding system. Each group of three reactions provided a single digit of the code. Using the result obtained, the indices of the positive reactions were indicated. The sum of these indices in each group of three reactions formed the code number. The code was entered into the computer software package which then generated percentage score against the organism name as share of the probability for that organism as a part of the total probabilities of all choices.

3.10 Biochemical Identification of Presumptive *Enterococcus* Species Isolates Using Microgen^{TM} STREP-ID.

The Microgen^{TM} STREP-ID system was used to identify members of the genus *Enterococcus* and *Streptococcus.* The Microgen^{TM} STREP-ID system are as described above for GnA-ID except that the Microgen^{TM} STREP-ID system comprises of 15 biochemical tests of which 12 are performed in the standardized 12 microwell test strip, a hippurate test (provided) and 2 based on observations of the colonial morphology of

isolates on blood agar medium and also a heavy inoculum corresponding to 2.0 MacFarland standard was used.

3.11 Serological Identification of Presumptive *E. coli* O157

All biochemically confirmed sorbitol negative *E. coli* isolates were serologically identified using *E. coli* O157 latex test kit (oxoid, UK) according to manufacturer's instructions; latex reagents were brought to room temperature and a drop of saline was placed on the circle distant from the latex. Using a sterile wire loop, a colony of presumptive *E. coli* O157 on CT-SMAC agar plate was emulsified in the saline drop on a portion of the circle on the card. The test latex reagent was added and mixed together with the emulsified organism using wire loop. The card was rocked in a circular motion observing for agglutination within one minute. Agglutination of the test latex within one minute was recorded as positive results. This serves as a positive serological confirmation of *E. coli* O157 strains possessing the O157 sero-group antigen (Cheesbrough, 2006).

3.12 Antibiotics Susceptibility Testing

The antibiotics susceptibility testing of the isolates were carried out using the Kirby- Bauer disk diffusion technique according to the methods recommended by the Clinical Laboratory and Standards Institute (CLSI, 2014) to determine susceptibility or resistance profiles of the isolates, discrete colonies of the isolates were Inoculated into 5ml of normal saline standardized with 0.5 MacFarland standard suspensions. Sterile cotton swab was used for the inoculation of the bacterial suspension to freshly prepared pre-dried Mueller-Hinton agar plates prepared according to manufacturer's instructions. The antibiotic sensitivity discs were aseptically and spaciously placed on the inoculated Mueller-Hinton agar plates. The antibiotic discs used for *E. coli* O157 isolates were Amoxicillin(10µg), Ampicillin (10µg), Cefoxitin (30µg), Gentamicin (l0µg),

Tetracycline (30µg), Ciprofloxacin (5µg), Trimethoprim Sulphamethozazol (25µg), Chloramphenicol (30µg) (Oxoid, UK). While the antibiotic discs used for the *Enterococcus* species were Ampicillin (10µg), Vancomycin (30µg), Erythromycin (15µg), Tetracyclin(30µg), Ciprofloxacin(5µ), Chloramphenicol (30µg), Gentamicin (30µg) and Tigecyclin(15µg) (Oxoid, UK). The reference standard strains used as control in this study include *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 for *Escherichia coli* O157 and *Enterococcus* species respectively. It was obtained from the National Institute for Pharmaceutical Research and Development, Idu. Abuja, Nigeria.

3.12.1 Measurement of inhibition zone diameter

After incubation, the test plates were examined for confluent growth and zone of inhibition. The diameter of each zone of inhibition was measured in millimetre (mm) using a ruler on the underside of the plate. The interpretation of the measurement as sensitive, intermediate and resistant was made according to Clinical Laboratory Standards Institute (CLSI, 2014) manual.

3.12.2 Multi-drug resistance index

The multiple-antibiotic resistance (MAR) index was determined for each isolate by dividing the number of antibiotics to which the isolate was resistant to, by the total number of antibiotics tested (Olayinka *et al.*, 2004).

$$\text{MDR index} = \frac{\text{Number of antibiotics the isolate was resistant to}}{\text{Total number of antibiotics tested.}}$$

3.13 Molecular Characterization of *Escherichia coli* O157:H7 and *Enterococcus* species

The identities of the *Escherichia coli* O157:H7 isolates were further confirmed through the amplification of the Stx1 and Stx2 gene fragments and characterised

through the amplification of antibiotics resistance genes: CTX-M and TEM which are extended spectrum β-lactamases gene fragments. The primers, polymerase chain reaction (PCR) preparations and conditions used are presented in Table 3.1.

The identities of the *Enterococcus* species isolates were further confirmed through the amplification of the conserved region (tuf gene) of the members of the genus *Enterococcus* using genus specific primer ENT1 (Danbing *et al.*,1999). Also, confirmed *Enterococcus* species isolates were further characterised using antibiotics resistance gene (VANR).

3.13.1 DNA Extraction using extraction kit

Pure colonies of *Escherichia coli* O157:H7 and *Enterococcus* species were inoculated into each 3ml of Luria-Bertani (LB) broth medium and incubated at 37^{o}C overnight. One ml of the overnight culture was centrifuged for 2min at 16000 x g. pelleted bacterial cells were resuspended in 200µl of sterile distilled water thoroughly by votexing before being subjected to DNA extraction using Zymoresearch^{TM} Genomic DNA extraction kit (Irvin,CA, USA). By following the manufacturer's instructions.

3.13.2 DNA Quantification Using NanoDrop

The NanoDrop ND-1000 spectrophotometer was used following the manufacturer's instructions. This machine uses a patented sample retention system that holds 1µl of sample without the need for traditional containment devices such as cuvettes and capillaries. A sample volume of 1µl is dispensed onto the lower optical surface and the instrument lever arm is lowered, the upper optical surface engages with the sample, forming a liquid column with the path length defined by the gap between the two optical surfaces. During each measurement, the sample is assessed at both A_{260} and A_{280} wavelength and DNA concentration and purity calculated.

3.13.3 DNA amplification and Detection

A total of 8 *Escherichia coli* O157:H7 isolates identified in this study was subjected to polymerase chain reaction (PCR) for the detection and amplification of Stx1, Stx2, CTX-M and TEM genes. While a total of 9 representative out of the 20 *Enterococcus* species isolated and identified in this study were subjected to polymerase chain reaction (PCR) for the detection and amplification of conserved region of the members of the genus *Enterococcus* using the primer ENT1 (Danbing *et al*.,1999). Also, *Enterococcus* species isolates were further characterised using antibiotics resistance gene (VANR). The primers, polymerase chain reaction (PCR) preparations and conditions used for *Escherichia coli* O157:H7 and *Enterococcus* species are presented in Tables 3.1. and 3.2 respectively.

3.13.4 Electrophoresis of PCR amplicons.

The PCR products (amplicons) were separated by electrophoresis on a 2%(w/v) agarose gel containing 2µl florosafe. Electrophoresis was run at 100v for 30mins for detection of the genes and visualised on UV transilluminator gel imaging system (gel documentation unit). The bands were system photographed and band positions were determined and compared to molecular weight markers.

3.14 Statistical Analysis

Analysis of variance (ANOVA) was used to analyze the data through the SPSS software version 19.

Table 3.1: Primer sequences, PCR preparations and conditions used in molecular characterization of *E.coli* O157:H7 in the study.

Target	Primer sequence (5′-3′)	PCR preparation (25 µl)	PCR conditions	Reference (s)
Stx1-F	Stx1F-614bp ACACTGGATGATCTCAGTGG Stx1R-614bp CTGAATCCCCCTCCATTATG Stx1F-779bp	12.5 µl Dream Taq mastermix.8.5 µl nuclease free water, 3 µl template DNA and 0.5 µl of each primer mix.	5mins at 94°C prior to 35cycles of 1min at 94 °C, Annealing temperature at 53 °C for 1min, extension at 72 °C for 1min and a final extension of 10mins at 72 °C.	Gannon *et al*., (1992)
Stx2	Stx1F-779bp CCATGACAACGGACAGCAGTT Stx1R-779bp CCTGTCAACTGAGCACTTTGPCR	12.5 µl Dream Taq mastermix.8.5 µl nuclease free water, 3 µl template DNA and 0.5 µl of each primer mix.	5mins at 94°C prior to 35cycles of 1min at 94 °C, Annealing temperature at 53 °C for 1min, extension at 72 °C for 1min and a final extension of 10mins at 72 °C.	Gannon *et al*., (1992)
CTX-M	Ctx-m1F-896 GACGATGTCACTGGCTGAGC CTX-m1R-896 AGCCGCCGACGCTAATACA	12.5 µl Dream Taq mastermix.8.5 µl nuclease free water, 3 µl template DNA and 0.5 µl of each primer mix.	5mins at 94°C prior to 40cycles of 1min at 94 °C, Annealing temperature at 55 °C for 1min, extension at 72 °C for 1min and a final extension of 10mins at 72 °C.	Pitout *et al*., (2004)
TEM-1	Tem1F-198 CATTTCCGTGTCGCCCTTATTC Tem1R-198 CGTTCATCCATAGTTGCCTGAC	12.5 µl Dream Taq mastermix.8.5 µl nuclease free water, 3 µl template DNA and 0.5 µl of each primer mix.	10mins at 94°C prior to 30cycles of 40s at 94 °C, Annealing temperature at 60 °C for 40s, extension at 72 °C for 1min and a final extension of 7mins at 72 °C.	Dallenne *et al*.,(2010)

Key: Stx1, Stx2 = Shiga toxin1 and 2 gene

CTX-M = β-lactamase gene

TEM-1 = β-lactamase gene

Table 3.2: Primer sequences, PCR preparations and conditions used in molecular characterization of *Enterococci* in the study.

Target	Primer sequence (5′-3′)	PCR preparation (25 µl)	PCR conditions	Reference (s)
Ent1	Ent1F-112bp TACTGACAAACCATTCATGATG Ent1R-112bp AACTTCGTCACCAACGCGAAC	12.5 µl Dream Taq mastermix.8.5 µl nuclease free water, 3 µl template DNA and 0.5 µl of each primer mix.	3mins at 96°C prior to 40cycles of 1min at 95 °C, Annealing temperature at 55 °C for 1min, extension at 72 °C for 1min and a final extension of 10mins at 72 °C.	Danbing *et al*., (1999)
VANR	VANRF-645bp AGCGATAAAATACTTATTGTGGA VANRR-645bp CGGATTATCAATGGTGTCGTT	12.5 µl Dream Taq mastermix.8.5 µl nuclease free water, 3 µl template DNA and 0.5 µl of each primer mix.	3mins at 94°C prior to 40cycles of 30s at 94 °C, Annealing temperature at 62 °C for 2min, extension at 72 °C for 2min and a final extension of 6mins at 72 °C.	Miele *et al*., (1995)

Key: Ent1 = *Enterococcus* Specific Primer

VANR = Vancomycin resistance gene

CHAPTER FOUR

4.0 RESULTS

4.1 Physicochemical Properties of the Packaged and other Drinking Water Sources in Zaria, Kaduna State, Nigeria.

Table 4.1 shows the mean physicochemical properties of the well water samples from the five sampling sites, Samaru (W1), Tudun-wada (W2), Wusasa (W3), Zaria city (W4), and Sabon-gari (W5). The result shows that there was no significant difference in pH of the well water samples collected from the different sampling sites. However, the pH of all the well water samples were bellow the (6.5-8.5) recommended limits of NAFDAC except sampling site W4 with a mean ±SE value of 6.55±0.14. The result also revealed that there were significant differences in turbidity of the well water samples collected from the different sampling sites. These differences in order of increasing turbidity were W3<W1<W5<W2<W4. However, the turbidity of the well water samples from sampling sites W2, W4, and W5 with mean ±SE values of 8.32±0.12NTU, 13.87±0.22NTU, and 6.95±0.01NTU were above the permissible limit (≤5NTU) of NAFDAC.

Total dissolved solids and electrical conductivity of all the well water samples were within the permissible limits (≤500mg/l and ≤1000µS/cm) of NAFDAC except well water samples from sampling sites W2 and W4 with mean ±SE values of 513.60±91.92mg/l and 539±141.63mg/l, 1026.80±183.94µS/cm and 1078.20±283.22 respectively. There was significant differences (P≤0.05) observed in the alkalinity, dissolved oxygen, biological oxygen demand, Nitrate, phosphate, and chloride but all were within the permissible limits of NAFDAC. However, the highest nitrate level occurred in sampling site Zaria city (W4) with mean ±SE value of 39.22±0.40mg/l.

Table 4.1: Comparison of Mean Values of Physicochemical Quality Parameters of Well Water Samples in Zaria.

Physicochemical Parameters	Sampling Sites					P value	Permissible Limit (NAFDAC)
	Samaru	Tudun Wada	Wusasa	Zaria City	Sabon Gari		
pH	6.10±0.08^{ab}	6.24±0.14^{ab}	6.13±0.09^{ab}	6.55±0.14^{a}	5.87±0.26^{b}	0.068ns	6.5-8.5
Turbidity(NTU)	2.69±0.01^{d}	8.32±0.12^{b}	2.48±0.02^{d}	13.87±0.22^{a}	6.95±0.01^{c}	0.000**	≤5
Temperature(^{0}C)	27.76±0.02^{a}	26.16±0.34^{a}	26.64±0.76^{a}	28.34±1.22^{a}	26.92±0.68^{a}	0.254ns	20-26
TDS(mg/l)	390.60±58.14^{a}	513.60±91.92^{a}	450.80±55.97^{a}	539.00±141.63^{a}	336.40±32.95^{a}	0.440ns	≤500
EC(µS/cm)	782.20±116.22^{a}	1026.80±183.94^{a}	902.80±113.05^{a}	1078.20±283.22^{a}	673.20±65.97^{a}	0.443ns	≤1000
Alkalinity(mg/l)	39.30±0.18^{a}	15.94±0.41^{c}	14.84±0.41^{d}	31.86±0.39^{b}	7.92±0.42^{e}	0.000**	≤100
DO(mg/l)	1.44±0.07b^{c}	1.34±0.06^{c}	1.63±0.06^{b}	2.03±0.07^{a}	1.55±0.06^{bc}	0.000**	ND
BOD(mg/l)	0.79±0.00^{b}	0.19±0.00^{e}	0.59±0.00^{c}	1.09±0.01^{a}	0.49±0.00^{d}	0.000**	ND
Nitrate(mg/l)	21.54±0.42^{d}	37.54±0.42^{b}	26.50±0.41^{c}	39.22±0.40^{a}	21.02±0.40^{d}	0.000**	≤50
Phosphate(mg/l)	12.20±0.67^{a}	9.83±0.53b^{c}	11.18±0.56^{ab}	10.11±0.61b^{c}	9.28±0.56^{d}	0.017*	≤30
Chloride(mg/l)	43.94±0.62^{b}	28.70±0.31^{e}	57.15±0.31^{a}	34.77±0.36^{d}	36.39±0.87^{c}	0.000**	≤200

KEY: means with different superscripted alphabets across the row are significantly (P≤0.05) different. Values are expressed as means±SE (Standard error of means). NAFDAC=National Agency for Food and Drug Administration and Control, NTU=Nephelometric Turbidity Unit, mg/l=Milligram per litre, µS/cm=Micro Siemens per Centimetre, TDS= Total Dissolved Solids, EC=Electrical Conductivity, DO=Dissolved oxygen, BOD=Biological Oxygen Demand. *significant at P≤0.05, **significant at P≤0.01, ns=not significant. ND=Not defined.

Table 4.2 shows the mean physicochemical properties of borehole water samples from the five sampling sites; Samaru (H1), Tudun-wada (H2), Wusasa (H3), Zaria city (H4) and Sabon gari (H5). The result showed that there was no significant difference in the pH of all the borehole water in the sampling sites. However, the observed mean±SE values in order of decreasing pH of the sites were H5<H2<H1<H4<H3. The slightly acidic pH of all the borehole water samples from various sampling sites was below the recommended limits (6.5-8.5) of NAFDAC. There were significant differences in the turbidity, alkalinity, dissolved oxygen, biological oxygen demand, nitrate, phosphate and chloride while there was no significant difference in temperature, total dissolved solids and electrical conductivity which were all within the NAFDAC permissible limits except temperature. Table 4.3 shows the physicochemical properties of the ten sampled brands of sachet water ; Dansil table water (A), Basako table water (B), Sa`a table water (C), Lifespan table water (D), IBM table water (E), Makoye table water (F), Napri table water (G), Shika table water (H), Alternative table water (I), and El-khalil table water (J) Sold in Zaria, Kaduna state, Nigeria. The results revealed that there was no significant difference in pH and temperature of the sampled sachet water brands collected. However, there was significant differences in turbidity of the samples collected from the different brands. These differences in order of increasing turbidity were D<G<E<I<A<J<C<F<B<H. However, Total dissolved solids (TDS), electrical conductivity (EC), alkalinity, dissolved oxygen, biological oxygen demand, phosphate, turbidity, chloride and nitrate clearly revealed that they were within the permissible limits of NAFDAC, However, significant differences observed in nitrate of the packaged sachet water brands were in the increasing order D<G<J<I<F<B<A=H<C=E. However, temperature of all the sampled brands were above the recommended limit (20-26^{o}C) by NAFDAC.

Table 4.2: Comparison of Mean Values of Physicochemical Quality Parameters of Borehole Water Samples in Zaria

Physicochemical Parameters	Sampling Sites					P value	Permissible Limit (NAFDAC)
	Samaru	Tudun Wada	Wusasa	Zaria City	Sabon Gari		
pH	5.91±0.18^{a}	5.93±0.19^{a}	5.82±0.18^{a}	5.90±0.13^{a}	6.11±0.12^{a}	0.784ns	6.5-8.5
Turbidity(NTU)	1.58±0.02^{b}	3.58±0.03^{a}	0.71±0.02^{c}	0.45±0.02^{d}	0.67±0.02^{c}	0.000**	≤5
Temperature(^{0}C)	29.72±51.32^{a}	27.81±0.57^{a}	28.59±0.57^{a}	28.14±0.43^{a}	28.86±0.29^{a}	0.430ns	20-26
TDS(mg/l)	221.40±87.79^{a}	159.00±66.37^{a}	262.00±58.22^{a}	274.60±71.35^{a}	411.40±105.11^{a}	0.280ns	≤500
EC(µS/cm)	443.20±175.71^{a}	318.40±132.78^{a}	524.80±116.90^{a}	549.20±142.60^{a}	823.40±210.02^{a}	0.280ns	≤1000
Alkalinity(mg/l)	39.54±0.20^{a}	28.42±0.17^{c}	20.78±0.32^{e}	23.54±0.22^{d}	32.66±0.27^{b}	0.000**	≤100
DO(mg/l)	1.85±0.06^{b}	1.45±0.06^{c}	2.45±0.06^{a}	1.87±0.07^{b}	0.85±0.06^{d}	0.000**	ND
BOD(mg/l)	0.19±0.00^{d}	0.39±0.00^{c}	1.19±0.00^{a}	0.59±0.01^{b}	0.19±0.00^{d}	0.000**	ND
Nitrate(mg/l)	10.89±0.16^{a}	8.89±0.16^{c}	9.07±0.15^{c}	10.62±0.15^{a}	9.75±0.10^{b}	0.000**	≤50
Phosphate(mg/l)	2.78±0.03^{a}	2.58±0.03^{b}	1.78±0.03^{d}	2.48±0.03^{c}	1.18±0.03^{e}	0.000**	≤30
Chloride(mg/l)	76.83±0.40^{a}	45.88±0.37^{d}	48.39±0.36^{c}	38.84±0.39^{e}	66.40±0.35^{b}	0.000**	≤200

KEY: means with different superscripted alphabets across the row are significantly (P≤0.05) different when compared with NAFDAC. Values are expressed as means±SE (Standard error of means). NAFDAC=National Agency for Food and Drug Administration and Control, NTU=Nephelometric Turbidity Unit, mg/l=Milligram per litre, µS/cm=Micro Siemens per Centimetre, TDS= Total Dissolved Solids, EC=Electrical Conductivity, DO=Dissolved oxygen, BOD=Biological Oxygen Demand. **significant at P≤0.01, ns=not significant.ND=Not defined.

Table 4.3: Comparison of Mean Values of Physicochemical Quality Parameters of Sachet Water Samples in Zaria

Physicochemical Parameters	Brands										P value	Permissible Limit (NAFDAG)
	A	B	C	D	E	F	G	H	I	J		
pH	6.89±0.16ab	7.12±0.16ab	6.84±0.20ab	7.45±0.02a	7.00±0.19ab	7.30±0.02ab	7.11±0.40ab	6.97±0.00ab	6.93±0.19ab	6.75±0.29b	**0.383ns**	6.5-8.5
Turbidity(NTU)	0.81±0.02e	1.60±0.02b	1.08±0.02c	0.55±0.02h	0.64±0.02g	1.11±0.02c	0.57±0.02gh	1.71±0.02a	0.71±0.02f	1.00±0.02d	**0.000****	≤5
Temperature(^{0}C)	27.96±0.42a	28.90±1.26a	28.82±1.17a	27.98±0.32a	28.04±0.28a	27.86±0.31a	28.12±0.10a	27.80±0.20a	29.32±0.96a	29.50±1.15a	**0.683ns**	20-26
TDS(mg/l)	117.80±0.49a	46.00±1.64d	43.20±0.49d	83.00±0.63b	45.20±0.92d	35.80±6.21e	58.00±0.00c	33.00±0.00e	55.20±0.20c	34.60±0.40e	**0.000****	≤500
EC(µS/cm)	235.60±0.98a	92.00±3.30d	86.00±1.00def	130.40±15.66b	90.60±1.89de	71.80±12.35ef	116.00±0.00bc	67.00±0.00f	110.60±0.60c	69.40±0.98f	**0.000****	≤1000
Alkalinity(mg/l)	20.52±0.21h	37.52±0.21b	27.52±0.21d	25.44±0.19f	25.40±0.21f	22.46±0.19g	27.00±0.00e	38.50±0.21a	30.00±0.00c	25.28±0.17f	**0.000****	≤100
DO(mg/l)	3.05±0.06a	2.94±0.06ab	2.85±0.06bc	2.75±0.06cd	2.75±0.06cd	2.95±0.06ab	1.90±0.00e	2.85±0.06bc	2.61±0.00d	2.04±0.06e	**0.000****	ND
BOD(mg/l)	0.79±0.00f	0.79±0.00f	1.07±0.01c	1.28±0.01a	0.68±0.01g	1.29±0.01a	0.99±0.01d	1.19±0.00b	0.89±0.00e	0.99±0.00d	**0.000****	ND
Nitrate(mg/l)	3.89±0.16a	3.09±0.16b	3.99±0.16a	0.32±0.01f	3.99±0.16a	1.89±0.16c	1.25±0.03e	3.89±0.16a	1.71±0.00cd	1.41±0.00de	**0.000****	≤50
Phosphate(mg/l)	0.63±0.03a	0.43±0.03de	0.60±0.03ab	0.18±0.03f	0.53±0.03bc	0.38±0.03e	0.60±0.00ab	0.48±0.03cd	0.15±0.00f	0.53±0.03bc	**0.000****	≤30
Chloride(mg/l)	9.32±0.34b	10.83±0.34a	5.83±0.34d	7.33±0.34c	10.82±0.34a	5.82±0.34d	3.50±0.01e	5.83±0.34d	5.49±0.00d	4.32±0.34e	**0.000****	≤200

KEY: means with different superscripted alphabets across the row are significantly (P≤0.05) different when compared with NAFDAC. Values are expressed as means±SE (Standard error of means). NAFDAC=National Agency for Food and Drug Administration and Control, NTU=Nephelometric Turbidity Unit, mg/l=Milligram per liter, µS/cm=Micro Siemens per Centimeter, TDS= Total Dissolved Solids, EC=Electrical Conductivity, DO=Dissolved oxygen, BOD=Biological Oxygen Demand. **significant at P≤0.01, ns=not significant. A,B,C,D,E,F,G,H,I and J= Dansil, Basako, Sa`a, Lifespan, IBM, Makoye, Napri, Shika, Alternative and EL-Khalil sachet-packaged table water respectively. ND= Not Defined.

Table 4.4 shows the physicochemical properties of bottled water samples sold in Zaria; Swan bottled water (T1), Faro bottled water (T2), Meyer bottled water (T3), Banquets bottled water (T4), and Green field bottled water (T5). The data indicated that there were significant differences in pH of the various sampled brands. These differences in order of increasing pH were T3<T5<T4<T2<T1. The acidic pH of brands T2, T3, T4 and T5 with mean±SE value of 5.70±0.00, 4.58±0.00, 5.26±0.00 and 4.85±0.00 respectively were bellow the recommended limits (6.5-8.5) of NAFDAC.

However, the values of turbidity, temperature, total dissolved solids, electrical conductivity, nitrate, phosphate and chloride clearly revealed that they were within the permissible limits of NAFDAC.

Table 4.5 shows the comparison of mean values of physicochemical quality parameters of both well, borehole, packaged sachet table water and bottled table water samples in Zaria. The data indicated that there was significant differences (p=0.000) in pH of the various sample category. These difference in order of increasing pH were bottled water <borehole water <well water < sachet water. However, the pH of the bottle water (5.48±0.29), borehole water (5.93±0.07) and well water (6.18±0.08) were bellow the permissible limit (6.5-8.5) of NAFDAC. The result also revealed significant differences (p=0.000) in the turbidity of the various sample type (Table 4.5). well water sample had the highest turbidity with a mean ±SE values of 6.86±0.86 NTU which was higher than the minimum permissible limit (≤5NTU) of NAFDAC. There was also significant differences (p=0.003) in the alkalinity of the samples with borehole water sample having the highest with a mean ±SE value of 28.99±1.36mg/l. however, the results of the total dissolved solids, electrical conductivity, alkalinity, dissolved oxygen, biological oxygen demand, nitrate, phosphate and chloride varied significantly across sample category (Table 4.5).

Table 4.4: Comparison of Mean Values of Physicochemical Quality Parameters of Bottled Water Samples in Zaria

Physicochemical Parameters	Sampled Brands					P value	Permissible Limit (NAFDAC)
	T1	T2	T3	T4	T5		
pH	7.02±0.00 ^{a}	5.70±0.00^{b}	4.58±0.00^{cf}	5.26±0.00 ^{cd}	4.85±0.00^{ce}	0.012*	6.5-8.5
Turbidity(NTU)	0.20±0.00^{a}	0.20±0.00^{a}	0.35±0.05^{b}	0.40±0.00^{ab}	0.47±0.01^{a}	0.001**	5
Temperature(^{0}C)	26.70±0.00^{bc}	27.50±0.40^{a}	26.90±0.00^{ab}	26.60±0.00^{bc}	26.20±0.00^{c}	0.027*	20-26
TDS(mg/l)	87.00±0.00 ^{a}	9.00±0.00 ^{b}	0.50±0.00^{d}	5.00±0.00 ^{c}	1.00±0.00^{d}	0.00*	≤500
EC(µS/cm)	175.00±0.00 ^{a}	18.00±0.00 ^{b}	1.00±0.00 ^{d}	10.00±0.00 ^{c}	2.00±0.00 ^{d}	0.00*	≤1000
Nitrate(mg/l)	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.15±0.05^{a}	0.017*	≤50
Phosphate(mg/l)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00		≤30
Chloride(mg/l)	7.00±0.00^{c}	7.25±0.05^{c}	19.00±1.00^{a}	20.00±0.00^{a}	12.00±0.00^{b}	0.000**	≤200

KEY: means with different superscripted alphabets across the row are significantly (P≤0.05) different. Values are expressed as means±SE (Standard error of means). NAFDAC=National Agency for Food and Drug Administration and Control, NTU=Nephelometric Turbidity Unit, mg/l=Milligram per litre, µS/cm=Micro Siemens per Centimetre, TDS= Total Dissolved Solids, EC=Electrical Conductivity, DO=Dissolved oxygen, BOD=Biological Oxygen Demand. T1,T2,T3,T4 and T5= Swan, Faro, Myer, Banquet and Greenfield bottled table water respectively. *significant at P≤0.05, **significant at P≤0.01.

Table 4.5: Comparison of Mean Values of Physicochemical Quality Parameters of all Well, Borehole, Sachet and Bottled Water Samples in Zaria.

Physicochemical Parameters	Sample Type/Category				Permissible Limit (NAFDAC)	P value
	Well	Borehole	Sachet Water	Bottle Water		
pH	6.18±0.08[b]	5.93±0.07[b]	7.04±0.06[a]	5.48±0.29[c]	6.5-8.5	**0.000****
Turbidity(NTU)	6.86±0.86[a]	1.40±0.24[b]	0.98±0.06[b]	0.32±0.04[b]	≤5	**0.000****
Temperature(^{0}C)	27.16±0.34[a]	28.63±10.27[a]	28.43±0.23[a]	26.78±0.15[a]	20-26	**0.289ns**
TDS(mg/l)	446.08±37.94[a]	265.68±36.65[b]	55.18±3.64[c]	20.50±11.13[c]	≤500	**0.000****
EC(µS/cm)	892.64±75.92[a]	531.80±73.33[b]	106.94±7.00[c]	41.20±22.39[c]	≤1000	**0.000****
Alkalinity(mg/l)	21.97±2.39[b]	28.99±1.36[a]	27.96±0.80[a]		≤100	**0.003****
DO(mg/l)	1.60±0.06[b]	1.70±0.11[b]	2.67±0.06[a]		ND	**0.000****
BOD(mg/l)	0.63±0.06[b]	0.51±0.08[b]	1.00±0.03[a]		ND	**0.000****
Nitrate(mg/l)	29.16±1.60[a]	9.85±0.17[b]	2.54±0.19[c]	0.03±0.02[d]	≤50	**0.000****
Phosphate(mg/l)	10.52±0.32[a]	2.16±0.12[b]	0.45±0.02[c]	0.00±0.00[c]	≤30	**0.000****
Chloride(mg/l)	40.19±2.01[b]	55.27±2.88[a]	6.91±0.36[c]	13.05±1.86[d]	≤200	**0.000****

KEY: means with different superscripted alphabets across the row are significantly (P≤0.05) different. Values are expressed as means±SE (Standard error of means). NAFDAC=National Agency for Food and Drug Administration and Control, NTU=Nephelometric Turbidity Unit, mg/l=Milligram per litre, µS/cm=Micro Siemens per Centimetre, TDS= Total Dissolved Solids, EC=Electrical Conductivity, DO=Dissolved oxygen, BOD=Biological Oxygen Demand. **significant at P≤0.001, ns=not significant. ND=Not Defined.

Table 4.6 shows the results of the effect of seasonal variations on the mean values of physicochemical quality parameters of well water, borehole water and packaged sachet water samples in Zaria. The data revealed that there was significant differences (p=0.014) in dissolved oxygen of well water samples with wet and dry season mean±SE values 1.44±0.08mg/l and 1.70±0.06mg/l respectively, Also, there was significant differences (p=0.000) in phosphate values in well water samples with wet and dry season mean±SE values of 9.08±0.32mg/l and 11.48±0.30mg/l respectively. Highest values was also recorded in the dry season for alkalinity, nitrate and chloride.

However, these values were not statistically significant p=0.775, p=0.618 and p=0.806 respectively. The highest values observed in well water samples during the wet season were turbidity, total dissolved solids, electrical conductivity and biological oxygen demand (Table 4.6). However, in borehole water samples, there was no significant difference between the mean physicochemical parameters in the wet and dry season (Table 4.6). In packaged sachet water samples, there was significant differences (p=0.025) in the phosphate level with wet and dry season mean ±SE value of 0.39±0.04mg/l and 0.50±0.03mg/l respectively. There was significant differences (p=0.004) in the temperature with wet and dry season mean ±SE value of 28.96±0.07^{o}C and 27.64±0.35 ^{o}C respectively.

Parameters such as pH with wet and dry mean values of 6.98±0.13 and 7.07±0.07, turbidity 0.92±0.09NTU and 1.01±0.07NTU, total dissolved solids 56.70±5.59 (mg/l) and 54.17±4.86 (mg/l), electrical conductivity 108.60±10.49 (mg/l) and 105.83±9.48 (mg/l), Alkalinity 27.60±1.28 (mg/l) and 28.21±1.04 (mg/l), dissolved oxygene, biological oxygene demand, nitrate and chloride were observed not to be significantly different between the wet and dry seasons respectively in the sachet water brands sampled.

Table 4.6: Effect of Seasonal Variation on the Mean Value of Physicochemical Quality Parameters of Well, Borehole and Sachet Water Samples in Zaria

Physicochemical Parameters	Season	Sample Type/Category			Permissible Limit (NAFDAC)
		Well	Borehole	Sachet Water	
pH	Dry	6.25±0.10	5.94±0.10	7.07±0.07	6.5-8.5
	Wet	6.07±0.12	5.92±0.08	6.98±0.13	6.5-8.6
	P value	0.269ns	0.870ns	0.469ns	
Turbidity(NTU)	Dry	6.78±1.11	1.43±0.31	1.01±0.07	≤5
	Wet	6.99±1.43	1.34±0.38	0.92±0.09	≤5
	P value	0.905ns	0.857ns	0.423ns	
Temperature(^{0}C)	Dry	26.29±0.43	27.85±17.10	27.64±0.35	20-26
	Wet	27.74±0.43	28.58±0.28	28.96±0.07	20-26
	P value	0.034*	0.413ns	0.004**	
TDS(mg/l)	Dry	441.00±52.42	218.60±45.99	54.17±4.86	≤500
	Wet	453.70±56.35	336.30±55.66	56.70±5.59	≤500
	P value	0.874ns	0.118ns	0.737ns	
EC(µS/cm)	Dry	882.27±104.86	437.60±92.04	105.83±9.48	≤1000
	Wet	908.20±112.79	673.10±111.29	108.60±10.49	≤1000
	P value	0.871ns	0.117ns	0.849ns	
Alkalinity(mg/l)	Dry	22.55±3.09	29.37±1.77	28.21±1.04	≤100
	Wet	21.11±3.96	28.41±2.24	27.60±1.28	≤100
	P value	0.775ns	0.737ns	0.715ns	
DO(mg/l)	Dry	1.70±0.06	1.80±0.14	2.75±0.07	ND
	Wet	1.44±0.08	1.54±0.18	2.55±0.08	ND
	P value	0.014*	0.263ns	0.073ns	
BOD(mg/l)	Dry	0.62±0.08	0.50±0.10	0.99±0.04	ND
	Wet	0.64±0.10	0.52±0.12	1.01±0.05	ND
	P value	0.871ns	0.920ns	0.685ns	
Nitrate(mg/l)	Dry	29.83±2.08	10.08±0.21	2.70±0.26	≤50
	Wet	28.16±2.59	9.49±0.27	2.30±0.27	≤50
	P value	0.618ns	0.096ns	0.303ns	
Phosphate(mg/l)	Dry	11.48±0.30	2.21±0.16	0.50±0.03	≤30
	Wet	9.08±0.32	2.08±0.20	0.39±0.04	≤30
	P value	0.000**	0.608ns	0.025*	
Chloride(mg/l)	Dry	40.61±2.65	55.88±3.77	7.36±0.48	≤200
	Wet	39.57±3.23	54.35±4.70	6.24±0.53	≤200
	P value	0.806ns	0.801ns	0.132ns	

KEY: means with different superscripted alphabets across the row are significantly (P≤0.05) different. Values are expressed as means±SE (Standard error of means). NAFDAC=National Agency for Food and Drug Administration and Control, NTU=Nephelometric Turbidity Unit, mg/l=Milligram per litre, µS/cm=Micro Siemens per Centimetre, TDS= Total Dissolved Solids, EC=Electrical Conductivity, DO=Dissolved oxygen, BOD=Biological Oxygen Demand. *significant at P≤0.05, **significant at P≤0.01, ns=not significant. ND=Not defined

4.2 Heavy Metal Content of the Drinking Water Sources and Packaged Water Sold in Zaria, Kaduna State.

Table 4.7 shows the results of the heavy metal contents of the well water samples in Zaria. The results revealed that the cadmium concentrations at the five sampling sites; Samaru(0.023mg/l), Tudun wada (0.022mg/l), wusasa(0.014mg/l), Zaria city(0.027mg/l) and sabon gari(0.014mg/l) varied significantly (p=0.000) and were all above the standard limit(0.003mg/l) of NAFDAC. It was also observed that the lead concentrations of all the well water samples were above the minimum permissible limit (0.01mg/l) of NAFDAC. However, Iron concentrations at sampling sites Samaru (3.546mg/l), Tudun Wada (5.77mg/l) and Zaria city (3.477mg/l) were higher compared to the standard limit (0.3mg/l) of NAFDAC.

Table 4.8 shows the heavy metal contents of the borehole water samples in Zaria, the result indicated significant difference (p=0.000) in the cadmium, lead and Iron concentrations across the five sampling sites. It was observed that sampling site Zaria city had the highest cadmium (0.032mg/l) and lead (0.150mg/l) concentrations. The result also shows that the cadmium, lead and Iron concentrations were all above the permissible limits (0.003mg/l), (0.01mg/l) and (0.3mg/l) of NAFDAC.

Among the sachet water brands sampled, the result clearly revealed that the cadmium and Lead concentrations of all the brands were above the permissible limits (0.003mg/l) and (0.01mg/l) respectively of NAFDAC. Also, the Iron concentration in sample brands; A(1.109mg/l), B(1.817mg/l), C(1.158mg/l), D(1.003mg/l), G(0.856mg/l) and I(1.483mg/l) were higher compare to the standard permissible limit (0.3mg/l) of NAFDAC (Table 4.9).

Table 4.7: Comparison of Mean Values of Heavy Metal Contents of Well Water Samples in Zaria

Heavy Metals	Sampling Sites					P value	Permissible Limits (NAFDAC)
	Samaru	Tudun Wada	Wusasa	Zaria City	Sabon Gari		
Cadmium (mg/l)	0.023±0.000[b]	0.022±0.000[c]	0.014±0.000[d]	0.027±0.000[a]	0.014±0.001[d]	**0.000****	≤0.003
Lead (mg/l)	0.290±0.000[a]	0.171±0.000[b]	0.054±0.000[d]	0.130±0.000[c]	0.044±0.001[e]	**0.000****	≤0.01
Iron (mg/l)	3.546±0.000[b]	5.770±0.000[a]	0.062±0.000[e]	3.477±0.000[c]	0.074±0.001[d]	**0.000****	≤0.3

KEY: means with different superscripted alphabets across the row are significantly (P≤0.05) different. Values are expressed as means±SE (Standard error of means). NAFDAC=National Agency for Food and Drug Administration and Control, mg/l=Milligram per litre, **significant at P≤0.01.

Table 4.8: Comparison of Mean Values of Heavy Metal Content of Borehole Water Samples in Zaria

Heavy Metals	Sampling Sites					P value	Permissible Limits (NAFDAC)
	Samaru	Tudun Wada	Wusasa	Zaria City	Sabon Gari		
Cadmium (mg/l)	0.025 ± 0.000^{c}	0.027 ± 0.000^{b}	0.014 ± 0.001^{e}	0.032 ± 0.000^{a}	0.016 ± 0.000^{d}	**0.000****	≤0.003
Lead (mg/l)	0.110 ± 0.000^{d}	0.135 ± 0.000^{b}	0.067 ± 0.001^{e}	0.150 ± 0.000^{a}	0.122 ± 0.000^{c}	**0.000****	≤0.01
Iron (mg/l)	3.235 ± 0.000^{c}	4.204 ± 0.000^{b}	1.222 ± 0.000^{e}	3.217 ± 0.000^{d}	4.352 ± 0.001^{a}	**0.000****	≤0.3

KEY: means with different superscripted alphabets across the row are significantly (P≤0.05) different. Values are expressed as means±SE (Standard error of means). NAFDAC=National Agency for Food and Drug Administration and Control, mg/l=Milligram per liter, **significant at P≤0.01.

Table 4.9: Comparison of Mean Values of Heavy Metal Contents of Sachet Water

Sampling Brands	Heavy Metals		
	Cadmium (mg/l)	**Lead (mg/l)**	**Iron (mg/l)**
A	0.007 ± 0.000^{f}	0.050 ± 0.000^{e}	1.109 ± 0.000^{d}
B	0.010 ± 0.000^{c}	0.057 ± 0.000^{d}	1.817 ± 0.000^{a}
C	0.009 ± 0.000^{d}	0.043 ± 0.000^{g}	1.158 ± 0.000^{c}
D	0.009 ± 0.000^{d}	0.063 ± 0.000^{b}	1.003 ± 0.000^{e}
E	0.008 ± 0.000^{e}	0.026 ± 0.000^{i}	0.000 ± 0.000^{j}
F	0.011 ± 0.000^{b}	0.047 ± 0.000^{f}	0.102 ± 0.000^{i}
G	0.012 ± 0.000^{a}	0.059 ± 0.000^{c}	0.856 ± 0.000^{f}
H	0.011 ± 0.000^{b}	0.041 ± 0.000^{h}	0.212 ± 0.000^{g}
I	0.011 ± 0.000^{b}	0.079 ± 0.000^{a}	1.483 ± 0.000^{b}
J	0.012 ± 0.001^{b}	0.025 ± 0.001^{j}	0.103 ± 0.001^{h}
P value	**0.000****	**0.000****	**0.000****
Permissible Limit (NAFDAC)	≤0.003	≤0.01	≤0.3

KEY: means with different superscripted alphabets across the row are significantly (P≤0.05) different. Values are expressed as means±SE (Standard error of means). NAFDAC=National Agency for Food and Drug Administration and Control, mg/l=Milligram per litre, **significant at P≤0.01. A,B,C,D,E,F,G,H,I and J= Dansil, Basako, Sa`a, Lifespan, IBM, Makoye, Napri, Shika, Alternative and EL-Khalil sachet-packaged table water respectively.

Table 4.10 shows the Comparison of Mean Values of Heavy Metal Contents of Bottled Water Sampled in Zaria, Kaduna State Nigeria. The result clearly showed that all the bottled water brands had no lead (0.00mg/l) contaminant. However, sample brands T3 (0.007MG/L), T4 (0.013MG/L) and T5 (0.007mg/l) had slightly higher cadmium concentration compare to the standard limit (0.003mg/l) of NAFDAC. Sample brand T1 (4.209mg/l) had the highest Iron concentration while T3 (0.00mg/l) had the lowest. Table 4.11 shows the result of the comparative analysis of the mean values of heavy metal contents of well water, borehole water, sachet-packaged water and bottled water brands sampled in Zaria. The data indicated that there was no significant difference between the lead (Pb) concentrations in well water and borehole water in Zaria but significant differences exist between well and sachet-packaged water brands (P=0.00). Also, significant differences (P=0.00) exist between well water and bottled water brands, while there was significant difference (P=0.00) between the lead content of the borehole and that of the sachet-packaged water, there was no statistically significant differences between the Cadmium (Cd) contents of borehole water and sachet-packaged water brands in Zaria and also, there was no statistically significant differences between the Cadmium (Cd) contents of the well water and that of the sachet-packaged waters. However, significant differences exist between the Cadmium (Cd) contents of these three categories (well, borehole and sachet-packaged) and the bottled water brands in Zaria, Kaduna State Nigeria. Iron (Fe) varied significantly (P=0.00) across each sample types or category (well, borehole, sachet-packaged and bottled waters) in this study.

4.3 Bacteriological Quality of Packaged and other Drinking Water Types in Zaria.

Plate I shows the Membrane Lactose Glucuronide Agar (MLGA) plate of sachet water Brand B.

Table 4.10: Comparison of Mean Values of Heavy Metal Contents of Bottled Water Samples in Zaria

Heavy Metals	Brands					P value	Permissible Limits (NAFDAC)
	T1	T2	T3	T4	T5		
Cadmium (mg/l)	0.002±0.000^{d}	0.003±0.000^{c}	0.007±0.000^{b}	0.013±0.000^{a}	0.007±0.001^{b}	**0.000****	≤0.003
Lead (mg/l)	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		≤0.01
Iron (mg/l)	4.209±0.000^{a}	0.335±0.000^{d}	0.000±0.000^{e}	1.696±0.000^{c}	2.454±0.001^{b}	**0.000****	≤0.3

KEY: means with different superscripted alphabets across the row are significantly (P≤0.05) different. Values are expressed as means±SE (Standard error of means). NAFDAC=National Agency for Food and Drug Administration and Control, mg/l=Milligram per litre, **significant at P≤0.01. T1,T2,T3,T4 and T5= Swan, Faro, Myer, Banquet and Greenfield bottled table water respectively.

Table 4.11: Comparative Analysis of Mean Values of Heavy Metal Contents of Well, Borehole, Sachet and Bottle Water Samples in Zaria

Heavy Metals	Sample Category/Type				P value	Permissible Limits (NAFDAC)
	Well	Borehole	Sachet Water	Bottle Water		
Cadmium (mg/l)	0.020±0.002[a]	0.023±0.002[a]	0.010±0.000[a]	0.006±0.001[b]	**0.000****	≤0.003
Lead (mg/l)	0.138±0.030[a]	0.117±0.009[a]	0.049±0.004[b]	0.000±0.000[c]	**0.000****	≤0.01
Iron (mg/l)	2.586±0.738a[b]	3.246±0.372[a]	0.784±0.140[c]	1.739±0.508[bc]	**0.000****	≤0.3

KEY: means with different superscripted alphabets across the row are significantly (P≤0.05) different. Values are expressed as means±SE (Standard error of means). NAFDAC=National Agency for Food and Drug Administration and Control, mg/l=Milligram per litre, **significant at P≤0.01.

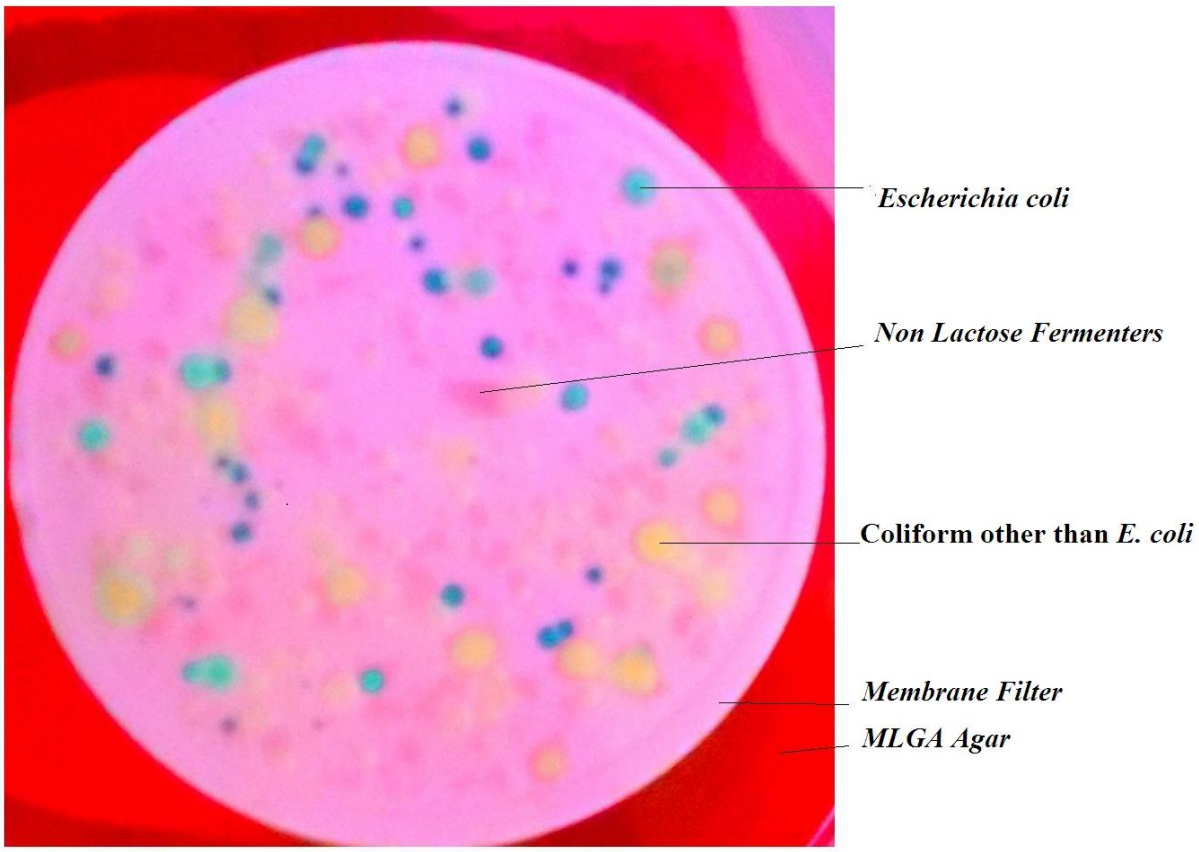


Plate I: Membrane Lactose Glucuronide Agar Plate of Sachet water brand B.

Table 4.12 shows the mean and range of total coliform counts (TCC) from the packaged and other drinking water sources in Zaria. The results showed that the sachet water brands investigated had total coliform counts (TCC) that ranged between 0.00 and 5.25×10^{3} cfu/100ml. with mean TCC which varied from the lowest in brand G(1.50±1.00cfu/100ml) to the highest in D($8.44\times10^{2}\pm6.30\times10^{2}$ cfu/100ml). TCC was detected in only one of the five brands of the bottled water investigated with mean TCC of 5.25±4.15cfu/100ml. The borehole water samples had TCC which ranged between 0.00 and 1.60×10^{3} cfu/100ml with mean TCC which varied from the lowest in site H3:Wusasa($1.01\times10^{2}\pm3.39\times10^{1}$ cfu/100ml) to the highest in H5:Sabongari ($4.20\times10^{2}\pm 1.94\times10^{2}$cfu/100ml). All the well water samples from the five sampling sites were found to contain total coliforms that ranged between 2.50×10^{1} and 6.42×10^{4}cfu/100ml. with mean TCC which varied between the lowest count in sampling site W1:Samaru($1.96\times10^{3}\pm1.12\times10^{2}$ cfu/100ml) to the highest count in W3:Wusasa ($9.58\times10^{3}\pm7.82\times10^{3}$cfu/100ml). There was no significant difference in the TCC between the Well water sampled from the various sampling sites in Zaria. Table 4.13 shows the mean and rang of the total *Escherichia coli* counts (TEC) from various brands of packaged and other drinking water sources in Zaria. The results revealed that the sachet-packaged drinking water contained total *Escherichia coli* which ranged between 0.00 and 5.44×10^{2}cfu/100ml. mean TEC varied significantly (P=0.037) from the lowest in brand G(0.00±0.00) and I(0.00±0.00)cfu/100ml to the highest in brand C($1.09\times10^{2}\pm7.00\times10^{2}$cfu/100ml). TEC was detected in only one brand T5(1.00±0.13 cfu/100ml) out of the five bottled water brands investigated in this study.

In the borehole water samples, TEC ranged between 0.00 and 2.80×10^{1}cfu/100ml. mean TEC varied from the lowest in H5:Sabon-gari(4.13±1.99) to the highest in site H2:Tudun wada($1.01\times10^{1}\pm4.52$ cfu/100ml). However, all the well water samples were found to be contaminated with TEC.

Table 4.12: Mean and Range of Total Coliform Counts from Packaged and other Drinking Water Sources in Zaria.

Sample type	Mean TCC count/100ml±SE	Range of TCC/100ml
Sachet water brands		
A	$3.88\times10^{2}\pm4.20\times10^{1}$	$2.73\times10^{2} - 6.61\times10^{2}$
B	$3.01\times10^{2}\pm5.57\times10^{1}$	$1.39\times10^{2} - 5.11\times10^{2}$
C	$4.86\times10^{2}\pm2.59\times10^{2}$	$2.10\times10^{2} - 2.09\times10^{2}$
D	$8.44\times10^{2}\pm6.30\times10^{2}$	$7.20\times10^{2} - 5.25\times10^{3}$
E	$2.48\times10^{2}\pm6.0\times10^{1}$	$1.19\times10^{2} - 6.34\times10^{2}$
F	$2.52\times10^{2}\pm2.56\times10^{1}$	$1.70\times10^{2} - 3.61\times10^{2}$
G	1.50±1.00	0.00– 5.00
H	$4.13\times10^{2}\pm9.5\times10^{2}$	$1.47\times10^{2} - 7.61\times10^{2}$
I	1.63±1.00	0.00 – 7.00
J	$1.51\times10^{1}\pm3.99$	$1.00 - 3.20\times10^{1}$
Bottled water Brands		
T1	0.00±0.00	0 .00– 0.00
T2	0.00±0.00	0 .00– 0.00
T3	0.00±0.00	0.00 – 0.00
T4	0.00±0.00	0.00 – 0.00
T5	5.25±4.15	$0 .00 - 3.4\times10^{1}$
Borehole water		
H1	$1.47\times10^{2}\pm5.01\times10^{1}$	$4.6\times10^{1} - 4.80\times10^{2}$
H2	$3.47\times10^{2}\pm1.17\times10^{2}$	$2.4\times10^{1} - 8.25\times10^{2}$
H3	$1.01\times10^{2}\pm3.39\times10^{1}$	$2.6\times10^{1} - 2.66\times10^{2}$
H4	$2.88\times10^{2}\pm1.64\times10^{2}$	$0.00 - 1.38\times10^{3}$
H5	$4.20\times10^{2}\pm1.94\times10^{2}$	$3.9\times10^{1} - 1.60\times10^{3}$
Well water		
W1	$1.96\times10^{3}\pm1.12\times10^{2}$	$2.50\times10^{1} - 9.72\times10^{3}$
W2	$3.56\times10^{3}\pm2.50\times10^{3}$	$5.55\times10^{2} - 2.10\times10^{4}$
W3	$9.58\times10^{3}\pm7.82\times10^{3}$	$6.52\times10^{2} - 6.42\times10^{4}$
W4	$2.70\times10^{3}\pm1.73\times10^{3}$	$1.52\times10^{2} - 1.46\times10^{4}$
W5	$3.59\times10^{3}\pm2.00\times10^{3}$	$2.59\times10^{2} - 1.62\times10^{4}$

KEY: TCC= Total coliform count, SE= Standard error of mean, A,B,C,D,E,F,G,H,I and J= Dansil, Basako, Sa`a, Lifespan, IBM, Makoye, Napri, Shika, Alternative and EL-Khalil sachet table water respectively. T1,T2,T3,T4 and T5= Swan, Faro, Myer, Banquet and Greenfield bottled table water respectively. H1,H2,H3,H4,and H5= Samaru, Tudun-wada, wusasa, Zaria-city, and Sabon-gari sampled Borehole water locations respectively. W1,W2,W3,W4 and W5= Samaru, Tudun-wada, wusasa, Zaria-city and Sabon-gari sampled Well water locations respectively. NAFDAC permissible limit=1cfu/100ml.

Table 4.13: Mean and Range of Total *Escherichia coli* Counts from Packaged and other Drinking Water Sources in Zaria.

Sample type	Mean count TEC/100ml±SE	Range of TEC/100ml
Sachet water brands		
A	6.88 ± 1.85^{b}	$3.00 - 1.9\times10^{1}$
B	5.00 ± 3.33^{b}	$0.00 - 2.8\times10^{1}$
C	$1.09\times10^{2}\pm7.00\times10^{2a}$	$7.00 - 5.44\times10^{2}$
D	6.13 ± 1.00^{b}	3.00 – 9.00
E	$1.90\times10^{1}\pm4.37^{b}$	$9.00 - 4.50\times10^{1}$
F	$2.36\times10^{1}\pm9.45^{b}$	$0.00 - 6.00\times10^{1}$
G	0.00 ± 0.00^{b}	0.00 – 0.00
H	$1.15\times10^{1}\pm2.76^{b}$	$0.00 - 2.10\times10^{1}$
I	0.00 ± 0.00^{b}	0.00 – 0.00
J	1.08 ± 1.00^{b}	0.00 – 4.00
	P-value: 0.037*	
Bottled water Brands		
T1	0.00±0.00	0.00 - 0.00
T2	0.00±0.00	0.00 - 0.00
T3	0.00±0.00	0.00 - 0.00
T4	0.00±0.00	0.00 - 0.00
T5	1.00±0.13	0.00 - 1.00
Borehole water		
H1	5.13±3.37	$0.00 - 2.20\times10^{1}$
H2	$1.01\times10^{1}\pm4.52$	$0.00 - 2.80\times10^{1}$
H3	6.25±3.53	$0.00 - 2.40\times10^{1}$
H4	4.75±1.93	$0.00 - 1.40\times10^{1}$
H5	4.13±1.99	$0.00 - 1.40\times10^{1}$
Well water		
W1	$3.43\times10^{2}\pm2.77\times10^{2}$	$5.00 - 2.28\times10^{3}$
W2	$4.68\times10^{1}\pm3.97\times10^{2}$	$1.90\times10^{1} - 3.24\times10^{3}$
W3	$5.82\times10^{3}\pm5.75\times10^{3}$	$2.00 - 4.61\times10^{4}$
W4	$1.46\times10^{2}\pm6.99\times10^{1}$	$1.90\times10^{1} - 6.00\times10^{2}$
W5	$6.99\times10^{2}\pm6.05\times10^{2}$	$1.80\times10^{1} - 4.92\times10^{3}$

KEY: TEC = Total *Escherichia coli* counts, SE= Standard error of mean, A,B,C,D,E,F,G,H,I and J= Dansil, Basako, Sa`a, Lifespan, IBM, Makoye, Napri, Shika, Alternative and EL-Khalil sachet table water respectively. T1,T2,T3,T4 and T5= Swan, Faro, Myer, Banquet and Greenfield bottled table water respectively. H1,H2,H3,H4,and H5= Samaru, Tudun-wada, wusasa, Zaria-city, and Sabon-gari sampled Borehole water locations respectively. W1,W2,W3,W4,and W5= Samaru, Tudun-wada, wusasa, Zaria-city and Sabon-gari sampled Well water locations respectively. *significant at $P\leq0.05$, in each category, values with different superscripted alphabets are significantly different. NAFDAC permissible limit=0cfu/100ml.

Table 4.14 shows the mean and range of the faecal coliform counts (FCC) from the packaged and other drinking water sources in Zaria. The results showed that the various brands of sachet-packaged drinking water were contaminated with faecal coliform whose counts ranged between 0.00 and 1.58×10^{3}cfu/100ml. mean FCC varied significantly(p=0.007) amongst brands from the lowest in brands G and I(0.00±0.00) to the highest in brand C($3.42\times10^{2}\pm1.99\times10^{2}$cfu/100ml). FCC was however not found in any of the bottled water brands analysed. In the borehole water samples, FCC was recorded and varied significantly (P=0.016) with highest counts in site H5:Sabon-gari($2.54\times10^{2}\pm8.08\times10^{1}$) while H4:Zaria city ($3.51\times10^{1}\pm1.06\times10^{1}$cfu/100ml) had the lowest counts. More so, the well water samples had FCC which ranged between 8.00 and 4.79×10^{4} cfu/100ml. mean FCC varied from the lowest in W4:Zaria city ($1.28\times10^{3}\pm7.90\times10^{2}$ cfu/ml) to the highest in W3:Wusasa ($6.59\times10^{3}\pm5.90\times10^{3}$cfu/ml).

Table 4.15 shows the results of the faecal Escherichia coli count. It revealed that there was statistically significant difference (P=0.020) in the FEC counts among the various brands of sachet-packaged water investigated in this study. The highest mean counts was observed in brand C($4.86\times10^{1}\pm2.94\times10^{1}$)cfu/ml while the lowest was in brands G and I(0.00)cfu/ml which recorded zero FEC (20%). Similarly, FEC was not found in any of the five brands of the bottled drinking water. However, in borehole water samples, faecal *E. coli* ranged between 0.00 and 2.4×10^{1} cfu/ml with mean counts that varied from the lowest in sites H1 and H5(2.00±1.32)cfu/ml to the highest counts in site H2(8.50±3.81) cfu/100ml. Also, in the well water samples, the highest faecal *E. coli* counts was found in site W3($4.11\times10^{3}\pm4.08\times10^{3}$ cfu/ml) while the lowest in site W4($7.40\times10^{1}\pm4.31\times10^{1}$) cfu/100ml. however, this observed differences was not statistically significant across the sampling sites.

Table 4.14: Mean and Range of Faecal Coliform Counts from Packaged and other Drinking Water Sources in Zaria.

Sample type	Mean count FCC/100ml±SE	Range of FCC/100ml
Sachet water brands		
A	$2.87\times10^{2}\pm5.39\times10^{1ab}$	$1.25\times10^{2} - 6.13\times10^{2}$
B	$1.54\times10^{2}\pm2.77\times10^{1abc}$	$5.20\times10^{1} - 2.49\times10^{2}$
C	$3.42\times10^{2}\pm1.99\times10^{2a}$	$1.10\times10^{1} - 1.58\times10^{3}$
D	$1.85\times10^{2}\pm5.88\times10^{1abc}$	$2.00\times10^{1} - 5.50\times10^{2}$
E	$1.74\times10^{2}\pm4.86\times10^{1abc}$	$3.90\times10^{1} - 4.67\times10^{2}$
F	$6.21\times10^{1}\pm6.63^{bc}$	$2.10\times10^{1} - 8.4\times10^{1}$
G	0.00 ± 0.00^{c}	0.00 – 0.00
H	$1.68\times10^{2}\pm3.04\times10^{1abc}$	$5.70\times10^{1} - 2.73\times10^{2}$
I	0.00 ± 0.00^{c}	0.00 – 0.00
J	$1.35\times10^{1}\pm3.80^{c}$	$0.00 - 2.90\times10^{1}$
	P-value: 0.007**	
Bottled water Brands		
T1	0.00±0.00	0.00 – 0.00
T2	0.00±0.00	0.00 - 0.00
T3	0.00±0.00	0.00 - 0.00
T4	0.00±0.00	0.00 - 0.00
T5	0.00±0.00	0.00 - 0.00
Borehole water		
H1	$1.00\times10^{2}\pm4.60\times10^{1b}$	$2.2\times10^{1} - 4.15\times10^{2}$
H2	$8.41\times10^{1}\pm4.64\times10^{1b}$	$5.00 - 4.00\times10^{2}$
H3	$3.91\times10^{1}\pm1.72\times10^{1b}$	$2.00 - 1.48\times10^{2}$
H4	$3.51\times10^{1}\pm1.06\times10^{1b}$	$0.00 - 9.4\times10^{1}$
H5	$2.54\times10^{2}\pm8.08\times10^{1a}$	$1.1\times10^{1} - 6.08\times10^{2}$
	P-value:0.016*	
Well water		
W1	$1.32\times10^{3}\pm9.47\times10^{2}$	$8.00 - 7.92\times10^{3}$
W2	$1.62\times10^{3}\pm1.33\times10^{3}$	$1.80\times10^{2} - 1.09\times10^{4}$
W3	$6.59\times10^{3}\pm5.90\times10^{3}$	$2.99\times10^{2} - 4.79\times10^{4}$
W4	$1.28\times10^{3}\pm7.90\times10^{2}$	$9.2\times10^{1} - 6.72\times10^{3}$
W5	$2.43\times10^{3}\pm1.55\times10^{3}$	$1.74\times10^{2} - 1.28\times10^{4}$

KEY: FCC = Faecal coliform counts, SE= Standard error of mean, A,B,C,D,E,F,G,H,I and J= Dansil, Basako, Sa`a, Lifespan, IBM, Makoye, Napri, Shika, Alternative and EL-Khalil sachet table water respectively. T1,T2,T3,T4 and T5= Swan, Faro, Myer, Banquet and Greenfield bottled table water respectively. H1,H2,H3,H4,and H5= Samaru, Tudun-wada, wusasa, Zaria-city, and Sabon-gari sampled Borehole water locations respectively. W1,W2,W3,W4,and W5= Samaru, Tudun-wada, wusasa, Zaria-city and Sabon-gari sampled Well water locations respectively. *significant at $P\leq0.05$, **significant at $P\leq0.01$, in each category, values with different superscripted alphabets are significantly different. NAFDAC permissible limit=0cfu/100ml.

Table 4.15: Mean and Range of Faecal *Escherichia coli* Counts from Packaged and other Drinking Water Sources in Zaria.

Sample type	Mean count FEC/100ml±SE	Range of FEC/100ml
Sachet water brands		
A	3.25 ± 0.37^{b}	2.00 – 5.00
B	2.75 ± 1.39^{b}	$0.00 – 1.20\times10^{1}$
C	$4.86\times10^{1}\pm2.94\times10^{1a}$	$2.00 – 2.16\times10^{2}$
D	4.00 ± 1.00^{b}	1.00 – 7.00
E	8.25 ± 1.67^{b}	$5.00 – 1.90\times10^{1}$
F	$1.28\times10^{1}\pm5.05^{b}$	$0.00- 3.30\times10^{1}$
G	0.00 ± 0.00^{b}	0.00 – 0.00
H	4.63 ± 1.95^{b}	$0.00 – 1.3\times10^{1}$
I	0.00 ± 0.00^{b}	0.00 – 0.00
J	1.00 ± 0.16^{b}	0.00 – 1.00
	P-value: 0.020*	
Bottled water Brands		
T1	0.00±0.00	0.00 - 0.00
T2	0.00±0.00	0.00 - 0.00
T3	0.00±0.00	0.00 - 0.00
T4	0.00±0.00	0.00 - 0.00
T5	0.00±0.00	0.00 - 0.00
Borehole water		
H1	2.00±1.32	0.00 - 9.00
H2	8.50±3.81	$0.00 – 2.4\times10^{1}$
H3	2.50±1.45	$0.00 – 1.00\times10^{1}$
H4	2.25±1.00	0.00 - 6.00
H5	2.00±1.32	$0.00 – 1.10\times10^{1}$
Well water		
W1	$2.65\times10^{2}\pm2.19\times10^{2}$	$4.00 – 1.80\times10^{3}$
W2	$2.92\times10^{2}\pm2.67\times10^{2}$	$0.00 – 2.16\times10^{3}$
W3	$4.11\times10^{3}\pm4.08\times10^{3}$	$0.00 – 3.26\times10^{4}$
W4	$7.40\times10^{1}\pm4.31\times10^{1}$	$1.1\times10^{1} – 3.60\times10^{2}$
W5	$5.47\times10^{2}\pm4.72\times10^{2}$	$1.1\times10^{1} – 3.84\times10^{3}$

KEY: FEC = Faecal *Escherichia coli* counts, SE= Standard error of mean, A,B,C,D,E,F,G,H,I and J= Dansil, Basako, Sa`a, Lifespan, IBM, Makoye, Napri, Shika, Alternative and EL-Khalil sachet table water respectively. T1,T2,T3,T4 and T5= Swan, Faro, Myer, Banquet and Greenfield bottled table water respectively. H1,H2,H3,H4,and H5= Samaru, Tudun-wada, wusasa, Zaria-city, and Sabon-gari sampled Borehole water locations respectively. W1,W2,W3,W4,and W5= Samaru, Tudun-wada, wusasa, Zaria-city and Sabon-gari sampled Well water locations respectively. *significant at $P\leq0.05$, in each category, values with different superscripted alphabets are significantly different. NAFDAC permissible limit=0cfu/100ml.

Table 4.16 shows the mean Enterococci counts of the packaged and other drinking water types investigated in this study. The data revealed that in sachet-packaged water, Enterococci counts ranged between 0.00 and 5.00cfu/100ml. mean Enterococci counts varied from the lowest in G, H and I (0.00) to the highest in C(1.38±0.53)cfu/ml. However, Enterococci was not detected in 3(30%) out of the ten brands of sachet-packaged drinking water sampled. More so, Enterococci were not found in all the five brands of the bottled drinking water investigated in this study. On the contrary, the borehole water samples had Enterococci cunts which ranged between 0.00 and 7.6×10^{1}cfu/100ml with mean Enterococci counts which varied from the lowest in site H1(1.00cfu/100ml) to the highest in site H2($1.25\times10^{1}\pm9.11$)cfu/ml. The results revealed that all the well water samples had Enterococci counts which ranged between 3.00 and 7.30×10^{3} cfu/100ml.

Table 4.17 shows the mean and range of the total viable counts (TVC) or heterotrophic plate counts(HPC) of the packaged and other drinking water sources in Zaria. In the sachet-packaged drinking water samples, TVC ranged between 3.00 and 3.46×10^{5}cfu/ml. mean total viable counts varied significantly (P=0.001) from the highest in brand D($1.06\times10^{5}\pm5.13\times10^{4}$)cfu/ml to the lowest in brand I ($2.41\times10^{1}\pm1.09\times10^{1}$)cfu/ml. Among the bottled water brands sampled, T5($5.9\times10^{1}\pm2.75\times10^{1}$)cfu/ml had the highest counts while the rest had counts which were not significantly different . Borehole water samples had counts which ranged between 7.23×10^{2} and 7.60×10^{4} cfu/ml with mean total viable counts that varied from the highest in sampling site H2($1.96\times10^{4}\pm1.02\times10^{4}$) cfu/ml. while the well water samples had counts which ranged between 2.00×10^{3} and 3.46×10^{5} cfu/ml. with mean total viable counts that varied from the highest in sampling site W2($9.48\times10^{4}\pm4.38\times10^{4}$) cfu/ml.

Table 4.16: Mean and Range of Enterococci Counts from Packaged and other Drinking Water Sources in Zaria.

Sample type	Mean count ECC/100ml±SE	Range of ECC/100ml
Sachet water brands		
A	1.00±0.41	0.00 - 3.00
B	1.25±0.53	0.00 - 4.00
C	1.38±0.53	0.00 - 4.00
D	1.00±0.26	0.00 - 2.00
E	1.00±0.18	0.00 - 1.00
F	1.00±0.63	0.00 - 5.00
G	0.00±0.00	0.00 - 0.00
H	0.00±0.00	0.00 - 0.00
I	0.00±0.00	0.00 - 0.00
J	1.00±0.26	0.00 - 2.00
Bottled water Brands		
T1	0.00±0.00	0.00 - 0.00
T2	0.00±0.00	0.00 - 0.00
T3	0.00±0.00	0.00- 0.00
T4	0.00±0.00	0.00 - 0.00
T5	0.00±0.00	0.00 - 0.00
Borehole water		
H1	1.00±0.38	0.00 - 3.00
H2	$1.25\times10^{1}\pm9.11$	$1.00 - 7.6\times10^{1}$
H3	2.63±2.20	$0.00 - 1.8\times10^{1}$
H4	1.00±0.52	0.00 – 4.00
H5	$1.21\times10^{1}\pm5.36$	$0.00 - 4.0\times10^{1}$
Well water		
W1	$2.28\times10^{2}\pm4.97\times10^{1}$	$1.9\times10^{1} - 4.26\times10^{2}$
W2	$1.16\times10^{2}\pm7.44$	$6.7\times10^{1} - 1.34\times10^{2}$
W3	$1.07\times10^{3}\pm8.92\times10^{2}$	$3.00 - 7.30\times10^{3}$
W4	$1.53\times10^{2}\pm3.70\times10^{1}$	$3.5\times10^{1} - 3.20\times10^{2}$
W5	$1.17\times10^{2}\pm3.02\times10^{1}$	$3.1\times10^{1} - 3.20\times10^{2}$

KEY: ECC = Enterococci counts, SE= Standard error of mean, A,B,C,D,E,F,G,H,I and J= Dansil, Basako, Sa`a, Lifespan, IBM, Makoye, Napri, Shika, Alternative and EL-Khalil sachet table water respectively. T1,T2,T3,T4 and T5= Swan, Faro, Myer, Banquet and Greenfield bottled table water respectively. H1,H2,H3,H4,and H5= Samaru, Tudun-wada, wusasa, Zaria-city, and Sabon-gari sampled Borehole water locations respectively. W1,W2,W3,W4,and W5= Samaru, Tudun-wada, wusasa, Zaria-city and Sabon-gari sampled Well water locations respectively. NAFDAC permissible limit=0cfu/100ml.

Table 4.17: Mean and Range of Total Viable Counts from Packaged and other Drinking Water Sources in Zaria.

Sample type	Mean TVC count/ml±SE	Range of TVC/ml
Sachet water brands		
A	$1.94\times10^{4}\pm8.38\times10^{3b}$	$3.94\times10^{3} - 6.24\times10^{4}$
B	$1.25\times10^{4}\pm5.30\times10^{3b}$	$1.25\times10^{3} - 3.72\times10^{4}$
C	$8.96\times10^{3}\pm4.03\times10^{3b}$	$9.81\times10^{2} - 2.80\times10^{4}$
D	$1.06\times10^{5}\pm5.13\times10^{4a}$	$1.1\times10^{3} - 3.46\times10^{5}$
E	$9.56\times10^{3}\pm4.21\times10^{3b}$	$2.05\times10^{3} - 3.40\times10^{4}$
F	$4.66\times10^{3}\pm3.34\times10^{3b}$	$3.56\times10^{3} - 6.40\times10^{3}$
G	$2.91\times10^{1}\pm1.33\times10^{1c}$	$4.00 - 9.50\times10^{1}$
H	$3.72\times10^{3}\pm1.65\times10^{3b}$	$3.68\times10^{2} - 1.15\times10^{4}$
I	$2.41\times10^{1}\pm1.09\times10^{1c}$	$3.00 - 9.8\times10^{1}$
J	$1.46\times10^{2}\pm4.24\times10^{1b}$	$1.2\times10^{1} - 3.92\times10^{2}$
	P-value: 0.001**	
Bottled water Brands		
T1	2.75 ± 1.11^{b}	0.00 – 8.00
T2	1.38 ± 0.26^{b}	0 .00- 2.00
T3	4.75 ± 0.96^{b}	1.00 – 9.00
T4	2.00 ± 1.09^{b}	0.00 – 8.00
T5	$5.9\times10^{1}\pm2.75\times10^{1a}$	$1.00 - 2.14\times10^{2}$
	P-value: 0.007**	
Borehole water		
H1	$1.20\times10^{4}\pm6.23\times10^{3}$	$8.43\times10^{2} - 4.40\times10^{4}$
H2	$1.96\times10^{4}\pm1.02\times10^{4}$	$1.12\times10^{3} - 7.60\times10^{4}$
H3	$4.32\times10^{3}\pm1.74\times10^{3}$	$7.23\times10^{2} - 1.30\times10^{4}$
H4	$1.84\times10^{4}\pm9.68\times10^{3}$	$2.13\times10^{3} - 7.60\times10^{4}$
H5	$8.26\times10^{3}\pm3.33\times10^{3}$	$1.93\times10^{3} - 2.70\times10^{4}$
Well water		
W1	$6.79\times10^{4}\pm2.53\times10^{4}$	$8.00\times10^{3} - 2.30\times10^{5}$
W2	$9.48\times10^{4}\pm4.38\times10^{4}$	$2.00\times10^{3} - 3.07\times10^{5}$
W3	$8.31\times10^{4}\pm4.19\times10^{4}$	$1.90\times10^{4} - 3.46\times10^{5}$
W4	$6.49\times10^{4}\pm3.59\times10^{4}$	$2.00\times10^{3} - 2.80\times10^{5}$
W5	$4.69\times10^{4}\pm1.81\times10^{4}$	$2.73\times10^{3} - 1.24\times10^{5}$

KEY: TVC = Total Viable counts, SE= Standard error of mean, A,B,C,D,E,F,G,H,I and J= Dansil, Basako, Sa`a, Lifespan, IBM, Makoye, Napri, Shika, Alternative and EL-Khalil sachet table water respectively. T1,T2,T3,T4 and T5= Swan, Faro, Myer, Banquet and Greenfield bottled table water respectively. H1,H2,H3,H4,and H5= Samaru, Tudun-wada, wusasa, Zaria-city, and Sabon-gari sampled Borehole water locations respectively. W1,W2,W3,W4,and W5= Samaru, Tudun-wada, wusasa, Zaria-city and Sabon-gari sampled Well water locations respectively. **significant at $P\leq0.01$, in each category, values with different superscripted alphabets are significantly different. NAFDAC permissible limit=100cfu/100ml.

Table 4.18 shows the pooled mean total and faecal coliform counts based on the sample category or type. The data revealed that there was statistically significant differences (P=0.000) in the total coliform counts across the different drinking water category or type (sachet, bottled, borehole and well waters). Well water samples had the highest significant mean total coliform counts of $4.28\times10^{3}\pm1.70\times10^{3a}$cfu/100ml. however, the mean total coliform counts of the sachet-packaged drinking water ($2.95\times10^{2}\pm7.11\times10^{1b}$cfu/100ml) was not significantly different from the mean total coliform counts of the borehole water ($2.61\times10^{2}\pm5.75\times10^{1b}$ cfu/100ml). The pooled mean total coliform counts of the bottled water brands was significantly (P=0.000) the lowest with a mean value of 1.05 ± 0.56^{c}cfu/100ml which was different from the sachet, borehole and well waters. The pooled mean faecal coliform counts (Table 4.18) showed that there was no significant difference (P =0.001) between the faecal coliform counts of the borehole water and that of the Sachet-packaged water.

The pooled mean total *Escherichia coli* counts (Table 4.19), showed that there was no significant difference between the total *Escherichia coli* counts of the borehole water and that of the sachet-packaged water and likewise, the pooled mean faecal *Escherichia coli* counts.

More so, pooled mean Enterococci counts and pooled mean total viable counts of the four category of drinking waters in this study, showed similar pattern of bacteria load variations across sample category or type as expressed above and shown in Tables 4.20.

However it was observed that sachet-packaged water had higher bacterial load compared to borehole waters in Zaria, Kaduna State Nigeria.

Table 4.18: Comparison of Mean Values of Total and Faecal coliform counts of various sample types

Sample type	Mean TCC/100ml±SE	Mean FCC/100ml±SE
Sachet Water	$2.95\times10^{2}\pm7.11\times10^{1b}$	$1.38\times10^{2}\pm2.46\times10^{1b}$
Bottled Water	1.05 ± 0.56^{c}	0.00 ± 0.00^{c}
Borehole Water	$2.61\times10^{2}\pm5.75\times10^{1b}$	$1.02\times10^{2}\pm2.38\times10^{1b}$
Well Water	$4.28\times10^{3}\pm1.70\times10^{3a}$	$2.65\times10^{3}\pm1.25\times10^{3a}$
P value	**0.000****	**0.001****
permissible limit (NAFDAC)	1cfu/100ml	0cfu/100ml

KEY: TCC= Total coliform count, FCC = Faecal coliform counts, SE= Standard error of mean, **significant at $P\leq0.01$. In each category, values with different superscripted alphabets are significantly different. cfu= colony forming unit.

Table 4.19: Comparison of Mean Values of Total *Escherichia coli* and Faecal *Escherichia coli* counts of various water sample sources in Zaria.

Sample type	Mean Total *Escherichia coli*/100ml±SE	Mean Faecal *Escherichia coli* /100ml±SE
Sachet Water	$1.82\times10^{1}\pm7.59^{b}$	8.45 ± 3.23^{b}
Bottled Water	1.00 ± 0.03^{c}	0.00 ± 0.00^{c}
Borehole Water	6.07 ± 1.41^{b}	3.45 ± 0.96^{b}
Well Water	$1.49\times10^{3}\pm1.15\times10^{3a}$	$1.06\times10^{3}\pm8.18\times10^{3a}$
P value	**0.087ns**	**0.087ns**
Permissible limit (NAFDAC)	0cfu/100ml	0cfu/100ml

KEY: SE= Standard error of mean, ns= not significant at $P\leq0.05$, in each category, values with different superscripted alphabets are significantly different. cfu= colony forming unit.NAFDAC= National agency for Food and Drug Administration and control.

Table 4.20: Comparison of Mean Values of Enterococci counts and Total viable counts of Various Water Sample Sources in Zaria.

Sample type	Mean Enterococci counts/100ml±SE	Mean Total viable counts /100ml±SE
Sachet Water	1.00 ± 0.12^{b}	$1.65\times10^{4}\pm6.02\times10^{3b}$
Bottled Water	0.00 ± 0.00^{c}	$1.38\times10^{1}\pm6.36^{c}$
Borehole Water	5.75 ± 2.22^{b}	$1.25\times10^{4}\pm3.14\times10^{3b}$
Well Water	$3.37\times10^{2}\pm1.79\times10^{2a}$	$7.15\times10^{4}\pm1.48\times10^{4a}$
P value	**0.004****	**0.000****
Permissible limit (NAFDAC)	0cfu/100ml	100cfu/100ml

KEY: SE= Standard error of mean, **significant at P≤0.01. in each category, values with different superscripted alphabets are significantly different. cfu= colony forming unit.

4.3.1 Effect of seasonal variations on the bacteriological quality of the packaged and other drinking water sources in Zaria.

Table 4.21 shows the effect of seasonal variations on the mean total coliform counts from packaged and other drinking water sources in Zaria. The results showed that four (40%) of the sachet-packaged drinking water brands had significantly (P=0.00) higher total coliform counts in the wet season than in the dry season. the results also revealed that 2(20%) of the sachet brands had significantly higher total coliform counts in the dry season than in the wet season while 4(40%) of the sachet brands had observed higher total coliform counts in the wet season than in the dry season which were not statistically significant. However, in the bottled water brands, observed difference occurred in only one out of the five brands sampled. In the borehole water and well water samples, the observed differences in total coliform counts were not statistically significant. 3(30%) of the sachet packaged water brands had significantly (P=0.002) higher total Escherichia coli counts in the wet season than in the dry season (Table 4.22). These were observed in brands E($2.78\times10^{1}\pm6.14^{a}$ and $1.03\times10^{1}\pm0.63^{b}$)cfu/100ml, F($4.63\times10^{1}\pm8.66^{a}$ and 1.00 ± 0.41^{b})cfu/100ml and H($1.68\times10^{1}\pm2.36^{a}$ and 6.25 ± 3.42^{b})cfu/ml. in wet and dry season respectively. Significant (P=0.008) difference also occurred in borehole water sample site H2 which recorded higher counts in the wet season ($2.03\times10^{1}\pm5.19^{a)}$cfu/100ml than in the dry season (0.00). However, there was no significant difference in the total Escherichia coli counts in all the well water sampled in this study. Faecal coliform counts occurred significantly (P=0.000) higher in the wet season (Table 4.23). Eight (80%) of the sachet-packaged water brands sampled had higher faecal coliform counts in the wet season, although only 4(40%) brands were significant. In borehole and well waters, faecal coliform counts were also higher in the wet season (Table 4.23).

Table 4.21: Effect of Seasonal Variations on Mean Total Coliform Counts from Packaged and other Drinking Water Sources in Zaria.

	Seasons		
	Wet Season	Dry Season	
Sample type	Mean TCC/100ml±SE	Mean TCC/100ml±SE	P-value
Sachet water brands			
A	$4.34\times10^{2}\pm8.21\times10^{1}$	$3.42\times10^{2}\pm2.11\times10^{1}$	**0.321ns**
B	$4.39\times10^{2}\pm3.95\times10^{1a}$	$1.62\times10^{2}\pm1.01\times10^{1b}$	**0.000****
C	$8.01\times10^{2}\pm4.96\times10^{2}$	$1.71\times10^{2}\pm1.06\times10^{1}$	**0.251ns**
D	$1.46\times10^{3}\pm1.26\times10^{3}$	$2.30\times10^{2}\pm1.42\times10^{1}$	**0.369ns**
E	$3.57\times10^{2}\pm9.34\times10^{1}$	$1.38\times10^{2}\pm8.67$	**0.058ns**
F	$1.89\times10^{1}\pm7.22^{b}$	$3.15\times10^{2}\pm1.94\times10^{1a}$	**0.001****
G	3.00 ± 1.15^{a}	0.00 ± 0.00^{b}	**0.041***
H	$6.61\times10^{2}\pm3.69\times10^{1a}$	$1.65\times10^{2}\pm9.27^{b}$	**0.000****
I	3.25 ± 1.49^{a}	0.00 ± 0.00^{b}	**0.040***
J	$2.48\times10^{1}\pm3.20^{a}$	6.75 ± 3.15^{b}	**0.007***
Bottled water Brands			
T1	0.00±0.00	0.00±0.00	
T2	0.00±0.00	0.00±0.00	
T3	0.00±0.00	0.00±0.00	
T4	0.00±0.00	0.00±0.00	
T5	9.00±8.35	1.50±1.19	**0.408ns**
Borehole water			
H1	$1.56\times10^{2}\pm1.08\times10^{2}$	$1.39\times10^{2}\pm1.16\times10^{1}$	**0.879ns**
H2	$3.54\times10^{2}\pm1.94\times10^{2}$	$3.39\times10^{2}\pm1.66\times10^{1}$	**0.956ns**
H3	$1.05\times10^{2}\pm5.44\times10^{1}$	$9.65\times10^{1}\pm4.92\times10^{1}$	**0.914ns**
H4	$8.48\times10^{1}\pm3.47\times10^{1}$	$4.92\times10^{2}\pm3.11\times10^{2}$	**0.241ns**
H5	$6.03\times10^{2}\pm3.69\times10^{2}$	$2.37\times10^{2}\pm1.27\times10^{2}$	**0.384ns**
Well water			
W1	$2.98\times10^{3}\pm2.26\times10^{3}$	$9.39\times10^{2}\pm1.85\times10^{2}$	**0.403ns**
W2	$6.05\times10^{3}\pm4.99\times10^{3}$	$1.07\times10^{3}\pm2.66\times10^{2}$	**0.358ns**
W3	$1.68\times10^{4}\pm1.58\times10^{4}$	$2.34\times10^{4}\pm9.69\times10^{2}$	**0.395ns**
W4	$4.73\times10^{4}\pm3.34\times10^{4}$	$6.61\times10^{2}\pm4.07\times10^{1}$	**0.268ns**
W5	$6.36\times10^{3}\pm368\times10^{3}$	$8.08\times10^{2}\pm4.97\times10^{1}$	**0.182ns**

KEY: TCC= Total coliform count, SE= Standard error of mean, A,B,C,D,E,F,G,H,I and J= Dansil, Basako, Sa`a, Lifespan, IBM, Makoye, Napri, Shika, Alternative and EL-Khalil sachet table water respectively. T1,T2,T3,T4 and T5= Swan, Faro, Myer, Banquet and Greenfield bottled table water respectively. H1,H2,H3,H4,and H5= Samaru, Tudun-wada, wusasa, Zaria-city, and Sabon-gari sampled Borehole water locations respectively. W1,W2,W3,W4,and W5= Samaru, Tudun-wada, wusasa, Zaria-city and Sabon-gari sampled Well water locations respectively. *significant at P≤0.05, **significant at P≤0.01, ns= not significant at P≤0.05. in each category, values with different superscripted alphabets are significantly different. NAFDAC permissible limit=1cfu/100ml.

Table 4.22: Effect of Seasonal Variations on Mean Total *Escherichia coli* Counts from Packaged and other Drinking Water Sources in Zaria.

	Seasons		
	Wet Season	Dry Season	
Sample type	Mean TEC/100ml±SE	Mean TEC/100ml±SE	P-value
Sachet water brands			
A	9.75±3.20	4.00±0.41	**0.125ns**
B	9.50±6.17	1.00±0.29	**0.195ns**
C	$2.11\times10^{2}\pm1.28\times10^{2}$	7.75±0.48	**0.162ns**
D	6.25±1.38	6.00±1.22	**0.897ns**
E	$2.78\times10^{1}\pm6.14^{a}$	$1.03\times10^{1}\pm0.63^{b}$	**0.030***
F	$4.63\times10^{1}\pm8.66^{a}$	1.00 ± 0.41^{b}	**0.002****
G	0.00±0.00	0.00±0.00	
H	$1.68\times10^{1}\pm2.36^{a}$	6.25 ± 3.42^{b}	**0.045***
I	0.00±0.00	0.00±0.00	
J	1.50±0.96	1.00±0.25	**0.253ns**
Bottled water Brands			
T1	0.00±0.00	0.00±0.00	
T2	0.00±0.00	0.00±0.00	
T3	0.00±0.00	0.00±0.00	
T4	0.00±0.00	0.00±0.00	
T5	1.00±0.25	0.00±0.00	**0.356ns**
Borehole water			
H1	$1.03\times10^{1}\pm5.95$	0.00±0.00	**0.136ns**
H2	$2.03\times10^{1}\pm5.19^{a}$	0.00 ± 0.00^{b}	**0.008****
H3	$1.25\times10^{1}\pm5.68$	0.00±0.00	**0.070ns**
H4	3.75±2.17	5.75±3.47	**0.643ns**
H5	5.00±3.08	3.25±2.93	**0.695ns**
Well water			
W1	$6.45\times10^{2}\pm5.46\times10^{2}$	$4.23\times10^{1}\pm7.12$	**0.313ns**
W2	$9.11\times10^{2}\pm7.77\times10^{2}$	$2.40\times10^{1}\pm2.35$	**0.297ns**
W3	$1.16\times10^{4}\pm1.15\times10^{4}$	$5.80\times10^{1}\pm9.49$	**0.355ns**
W4	$2.37\times10^{2}\pm1.30\times10^{2}$	$5.43\times10^{1}\pm1.95\times10^{1}$	**0.215ns**
W5	$1.37\times10^{3}\pm1.19\times10^{2}$	$3.08\times10^{1}\pm5.12$	**0.304ns**

KEY: TEC = Total *Escherichia coli* counts, SE= Standard error of mean, A,B,C,D,E,F,G,H,I and J= Dansil, Basako, Sa`a, Lifespan, IBM, Makoye, Napri, Shika, Alternative and EL-Khalil sachet table water respectively. T1,T2,T3,T4 and T5= Swan, Faro, Myer, Banquet and Greenfield bottled table water respectively. H1,H2,H3,H4,and H5= Samaru, Tudun-wada, wusasa, Zaria-city, and Sabon-gari sampled Borehole water locations respectively. W1,W2,W3,W4,and W5= Samaru, Tudun-wada, wusasa, Zaria-city and Sabon-gari sampled Well water locations respectively. *significant at $P\leq0.05$, **significant at $P\leq0.01$, ns= not significant at $P\leq0.05$. in each category, values with different superscripted alphabets are significantly different. NAFDAC permissible limit=0cfu/100ml.

Table 4.23: Effect of Seasonal Variations on Mean Faecal Coliform Counts from Packaged and other Drinking Water Sources in Zaria.

	Seasons		
	Wet Season	Dry Season	
Sample type	Mean FCC/100ml±SE	Mean FCC/100ml±SE	P-value
Sachet water brands			
A	$3.26\times10^{2}\pm1.09\times10^{2}$	$2.48\times10^{2}\pm2.82\times10^{1}$	**0.513ns**
B	$2.25\times10^{2}\pm9.17^{a}$	$8.23\times10^{1}\pm1.03\times10^{1b}$	**0.000****
C	$5.99\times10^{2}\pm3.75\times10^{2}$	$8.45\times10^{1}\pm2.12\times10^{1}$	**0.220ns**
D	$2.56\times10^{2}\pm1.09\times10^{2}$	$1.14\times10^{2}\pm2.76\times10^{1}$	**0.256ns**
E	$2.66\times10^{2}\pm6.92\times10^{1a}$	$8.13\times10^{1}\pm2.36\times10^{1b}$	**0.045***
F	$7.30\times10^{1}\pm4.92$	$5.13\times10^{1}\pm1.01\times10^{1}$	**0.101ns**
G	0.00±0.00	0.00±0.00	
H	$2.47\times10^{2}\pm1.00\times10^{1a}$	$9.00\times10^{1}\pm1.12\times10^{1b}$	**0.000****
I	0.00±0.00	0.00±0.00	
J	$2.23\times10^{1}\pm2.93^{a}$	4.75 ± 2.81^{b}	**0.005****
Bottled water Brands			
T1	0.00±0.00	0.00±0.00	
T2	0.00±0.00	0.00±0.00	
T3	0.00±0.00	0.00±0.00	
T4	0.00±0.00	0.00±0.00	
T5	0.00±0.00	0.00±0.00	
Borehole water			
H1	$1.31\times10^{2}\pm9.47\times10^{2}$	$6.93\times10^{1}\pm1.66\times10^{1}$	
H2	$1.40\times10^{2}\pm8.87\times10^{1}$	$2.80\times10^{1}\pm9.86$	**0.255ns**
H3	$6.50\times10^{1}\pm2.99\times10^{1}$	$1.33\times10^{1}\pm5.50$	**0.141ns**
H4	$3.38\times10^{1}\pm1.15\times10^{1}$	$3.65\times10^{1}\pm1.99\times10^{1}$	**0.909ns**
H5	$3.05\times10^{2}\pm1.69\times10^{2}$	$2.03\times10^{2}\pm1.29\times10^{1}$	**0.568ns**
Well water			
W1	$2.24\times10^{4}\pm1.90\times10^{3}$	$4.05\times10^{2}\pm1.03\times10^{2}$	**0.373ns**
W2	$3.03\times10^{3}\pm2.63\times10^{3}$	$2.14\times10^{2}\pm1.44\times10^{1}$	**0.325ns**
W3	$1.23\times10^{4}\pm1.19\times10^{3}$	$8.84\times10^{2}\pm3.38\times10^{2}$	**0.372ns**
W4	$2.26\times10^{3}\pm1.51\times10^{3}$	$3.00\times10^{2}\pm2.34\times10^{1}$	**0.242ns**
W5	$4.48\times10^{3}\pm2.91\times10^{3}$	$3.82\times10^{2}\pm2.95\times10^{1}$	**0.209ns**

KEY: FCC = Faecal coliform counts, SE= Standard error of mean, A,B,C,D,E,F,G,H,I and J= Dansil, Basako, Sa`a, Lifespan, IBM, Makoye, Napri, Shika, Alternative and EL-Khalil sachet table water respectively. T1,T2,T3,T4 and T5= Swan, Faro, Myer, Banquet and Greenfield bottled table water respectively. H1,H2,H3,H4,and H5= Samaru, Tudun-wada, wusasa, Zaria-city, and Sabon-gari sampled Borehole water locations respectively. W1,W2,W3,W4,and W5= Samaru, Tudun-wada, wusasa, Zaria-city and Sabon-gari sampled Well water locations respectively. *significant at $P\leq0.05$, **significant at $P\leq0.01$, ns= not significant at $P\leq0.05$. in each category, values with different superscripted alphabets are significantly different. NAFDAC permissible limit=0cfu/100ml.

Two (20%) brands of the sachet-packaged water had significantly (P=0.002) higher faecal *Escherichia coli* counts in wet season than in the dry season (Table 4.24). All the borehole water samples had higher faecal *Escherichia coli* counts in the wet season than in dry season. Although only sampling site H2 was statistically significant(P=0.009). However, all the well water samples recorded higher faecal *Escherichia coli* counts in the wet season. Table 4.25 shows the effects of seasonal variations on the mean Enterococci counts of the various water sample sources, the results showed that there was no statistically significant difference in the mean Enterococci counts of all the various water sample sources. Although, the observed counts occurred higher in the wet season than in the dry season except in sampling site H4,W2, and W3 which had higher mean Enterococci counts in the dry season (Table 4.25). Table 4.26 shows the effect of seasonal variations on the mean total viable counts from the various water sample sources, the result revealed higher mean total heterotrophic counts in the wet season than in the dry season across all the various sample categories in this study.

4.4 Biochemical Characterization and Identification of Isolates

Appendix I shows the results of biochemical characterization and identification of presumptive isolates of *Escherichia coli* O1517:H7 using the microgene® GnA-ID system. Appendix II shows the results of biochemical characterization and identification of *Enterococcus* spp. Isolated from water samples in Zaria using the microgene® Strep-ID system. Using the microgene Strep-ID system for the biochemical characterization and identification of *Enterococcus* spp isolated from water samples in Zaria, Figure 4.1 showed different species of the bacteria as; 2(13%) *Enterococcus faecalis, 4(25%) Enterococcus gallinarum*, 7(44%) *Enterococcus faecium*, while 1(6.3%) each for *Enterococcus durans*, *Enterococcus mundtii* and *Enterococcus casseliflavis* respectively (Figure 4.1).

Table 4.24: Effect of Seasonal Variations on Mean Faecal *Escherichia coli* Counts from Packaged and other Drinking Water Sources in Zaria.

	Seasons		
	Wet Season	Dry Season	
Sample type	Mean FEC/100ml±SE	Mean FEC/100ml±SE	P-value
Sachet water brands			
A	3.75±0.48	2.75±0.48	**0.190ns**
B	5.00±2.35	1.00±0.29	**0.106ns**
C	$9.43\times10^{1}\pm5.14\times10^{1}$	3.00±0.41	**0.126ns**
D	4.50±1.26	3.50±0.96	**0.550ns**
E	$1.10\times10^{1}\pm2.80$	5.50±0.29	**0.098ns**
F	$2.48\times10^{2}\pm4.77^{a}$	1.05 ± 0.48^{b}	**0.002***
G	0.00±0.00	0.00±0.00	
H	8.75 ± 2.50^{a}	1.00 ± 0.29^{b}	**0.017***
I	0.00±0.00	0.00±0.00	
J	1.00±0.25	1.00±0.25	**1.000ns**
Bottled water Brands			
T1	0.00±0.00	0.00±0.00	
T2	0.00±0.00	0.00±0.00	
T3	0.00±0.00	0.00±0.00	
T4	0.00±0.00	0.00±0.00	
T5	0.00±0.00	0.00±0.00	
Borehole water			
H1	4.00±2.35	0.00±0.00	**0.139ns**
H2	$1.70\times10^{1}\pm4.43^{a}$	0.00 ± 0.00^{b}	**0.009***
H3	5.00±2.38	0.00±0.00	**0.080ns**
H4	2.75±1.60	1.75±1.03	**0.618ns**
H5	3.50±2.53	1.00±0.50	**0.289ns**
Well water			
W1	$4.93\times10^{2}\pm4.36\times10^{1}$	$3.60\times10^{1}\pm4.71$	**0.333ns**
W2	$5.70\times10^{2}\pm5.30\times10^{1}$	$1.45\times10^{1}\pm0.96$	**0.335ns**
W3	$8.19\times10^{3}\pm8.15\times10^{2}$	$4.13\times10^{1}\pm7.97$	**0.356ns**
W4	$1.34\times10^{2}\pm7.89\times10^{1}$	$1.38\times10^{1}\pm1.55$	**0.178ns**
W5	$1.06\times10^{3}\pm9.29\times10^{2}$	$3.63\times10^{1}\pm1.09\times10^{1}$	**0.314ns**

KEY: FEC = Faecal *Escherichia coli* counts, SE= Standard error of mean, A,B,C,D,E,F,G,H,I and J= Dansil, Basako, Sa`a, Lifespan, IBM, Makoye, Napri, Shika, Alternative and EL-Khalil sachet table water respectively. T1,T2,T3,T4 and T5= Swan, Faro, Myer, Banquet and Greenfield bottled table water respectively. H1,H2,H3,H4,and H5= Samaru, Tudun-wada, wusasa, Zaria-city, and Sabon-gari sampled Borehole water locations respectively. W1,W2,W3,W4,and W5= Samaru, Tudun-wada, wusasa, Zaria-city and Sabon-gari sampled Well water locations respectively. *significant at $P\leq0.05$, ns= not significant at $P\leq0.05$. in each category, values with different superscripted alphabets are significantly different. NAFDAC permissible limit=0cfu/100ml.

Table 4.25: Effect of Seasonal Variations on Mean Enterococci Counts from Packaged and other Drinking Water Sources in Zaria.

	Seasons		
	Wet Season	Dry Season	
Sample type	Mean ECC/100ml±SE	Mean ECC/100ml±SE	P-value
Sachet water brands			
A	1.25±0.75	1.00±0.25	**0.253ns**
B	1.50±0.87	1.00±0.71	**0.670ns**
C	2.00±0.91	1.00±0.48	**0.271ns**
D	1.05±0.48	1.00±0.29	**0.670ns**
E	1.05±0.25	1.00±0.29	**0.537ns**
F	1.25±1.25	1.00±0.48	**0.722ns**
G	0.00±0.00	0.00±0.00	
H	0.00±0.00	0.00±0.00	
I	0.00±0.00	0.00±0.00	
J	1.00±0.41	1.00±0.25	**0.168ns**
Bottled water Brands			
T1	0.00±0.00	0.00±0.00	
T2	0.00±0.00	0.00±0.00	
T3	0.00±0.00	0.00±0.00	
T4	0.00±0.00	0.00±0.00	
T5	0.00±0.00	0.00±0.00	
Borehole water			
H1	1.00±0.25	1.00±0.71	**0.356ns**
H2	$2.13\times10^{1}\pm1.83\times10^{1}$	3.75±1.25	**0.377ns**
H3	4.75±4.42	1.00±0.29	**0.375ns**
H4	0.00±0.00	2.00±0.85	**0.086ns**
H5	$1.80\times10^{1}\pm1.03\times10^{1}$	6.25±2.32	**0.307ns**
Well water			
W1	$2.42\times10^{2}\pm1.05\times10^{2}$	$2.14\times10^{2}\pm1.95\times10^{1}$	**0.800ns**
W2	$1.08\times10^{2}\pm1.45\times10^{1}$	$1.24\times10^{2}\pm2.31$	**0.309ns**
W3	$3.90\times10^{1}\pm2.47\times10^{1}$	$2.11\times10^{3}\pm1.73\times10^{3}$	**0.278ns**
W4	$1.96\times10^{2}\pm6.60\times10^{1}$	$1.10\times10^{2}\pm2.85\times10^{1}$	**0.279ns**
W5	$1.40\times10^{2}\pm6.27\times10^{1}$	$9.48\times10^{1}\pm0.48$	**0.497ns**

KEY: ECC = Enterococci counts, SE= Standard error of mean, A,B,C,D,E,F,G,H,I and J= Dansil, Basako, Sa`a, Lifespan, IBM, Makoye, Napri, Shika, Alternative and EL-Khalil sachet table water respectively. T1,T2,T3,T4 and T5= Swan, Faro, Myer, Banquet and Greenfield bottled table water respectively. H1,H2,H3,H4,and H5= Samaru, Tudun-wada, wusasa, Zaria-city, and Sabon-gari sampled Borehole water locations respectively. W1,W2,W3,W4,and W5= Samaru, Tudun-wada, wusasa, Zaria-city and Sabon-gari sampled Well water locations respectively. ns= not significant at $P\leq0.05$. NAFDAC permissible limit=0cfu/100ml.

Table 4.26: Effect of Seasonal Variations on Mean Total Viable Counts from Packaged and other Drinking Water Sources in Zaria.

	Seasons		
	Wet Season	Dry Season	
Sample type	Mean TVC/ml±SE	Mean TVC/ml±SE	P-value
Sachet water brands			
A	$3.43\times10^{4}\pm1.34\times10^{4}$	$4.57\times10^{3}\pm2.81\times10^{3}$	0.069ns
B	$2.35\times10^{4}\pm7.05\times10^{3a}$	$1.43\times10^{3}\pm8.28\times10^{2b}$	0.020*
C	$1.68\times10^{4}\pm5.91\times10^{3a}$	$1.12\times10^{3}\pm6.56\times10^{1b}$	0.038*
D	$2.12\times10^{5}\pm7.05\times10^{4a}$	$1.28\times10^{3}\pm8.02\times10^{1b}$	0.025*
E	$1.68\times10^{4}\pm6.94\times10^{3}$	$2.37\times10^{3}\pm1.45\times10^{2}$	0.084ns
F	$5.20\times10^{3}\pm5.16\times10^{2}$	$4.13\times10^{3}\pm2.54\times10^{2}$	0.112ns
G	$5.28\times10^{1}\pm2.14\times10^{1}$	5.50±1.00	0.069ns
H	$7.02\times10^{3}\pm2.35\times10^{3a}$	$4.28\times10^{2}\pm2.66\times1^{b}$	0.031*
I	$3.98\times10^{1}\pm1.95\times10^{1}$	8.50±3.66	0.167ns
J	$2.16\times10^{2}\pm6.05\times10^{1}$	$7.50\times10^{1}\pm3.74\times10^{1}$	0.095ns
Bottled water Brands			
T1	2.75±1.80	2.75±1.60	1.000ns
T2	1.00±0.25	1.00±1.00	0.670ns
T3	4.75±1.65	4.75±1.25	1.000ns
T4	2.00±2.00	2.00±1.22	1.000ns
T5	$7.33\times10^{1}\pm4.74\times10^{1}$	$4.45\times10^{1}\pm3.39\times10^{1}$	0.639ns
Borehole water			
H1	$2.30\times10^{4}\pm1.00\times10^{4}$	$1.01\times10^{3}\pm8.48\times10^{2}$	0.070ns
H2	$3.78\times10^{4}\pm1.62\times10^{4}$	$1.42\times10^{3}\pm1.34\times10^{2}$	0.066ns
H3	$7.80\times10^{4}\pm2.46\times10^{3a}$	$8.47\times10^{2}\pm5.41\times10^{1b}$	0.030*
H4	$3.39\times10^{4}\pm1.66\times10^{4}$	$2.84\times10^{3}\pm3.27\times10^{2}$	0.110ns
H5	$1.39\times10^{4}\pm5.55\times10^{3}$	$2.66\times10^{3}\pm3.07\times10^{2}$	0.091ns
Well water			
W1	$1.03\times10^{5}\pm4.65\times10^{4}$	$3.34\times10^{4}\pm5.99\times10^{3}$	0.191ns
W2	$1.67\times10^{5}\pm7.29\times10^{4}$	$2.27\times10^{4}\pm1.31\times10^{4}$	0.100ns
W3	$1.43\times10^{5}\pm7.61\times10^{4}$	$2.28\times10^{4}\pm1.69\times10^{3}$	0.164ns
W4	$1.23\times10^{5}\pm6.14\times10^{4}$	$7.04\times10^{3}\pm1.15\times10^{3}$	0.109ns
W5	$8.67\times10^{4}\pm2.15\times10^{4a}$	$7.12\times10^{3}\pm3.96\times10^{3b}$	0.011*

KEY: TVC = Total Viable counts, SE= Standard error of mean, A,B,C,D,E,F,G,H,I and J= Dansil, Basako, Sa`a, Lifespan, IBM, Makoye, Napri, Shika, Alternative and EL-Khalil sachet table water respectively. T1,T2,T3,T4 and T5= Swan, Faro, Myer, Banquet and Greenfield bottled table water respectively. H1,H2,H3,H4,and H5= Samaru, Tudun-wada, wusasa, Zaria-city, and Sabon-gari sampled Borehole water locations respectively. W1,W2,W3,W4,and W5= Samaru, Tudun-wada, wusasa, Zaria-city and Sabon-gari sampled Well water locations respectively. *significant at $P\leq0.05$, ns= not significant at $P\leq0.05$. in each category, values with different superscripted alphabets are significantly different. NAFDAC permissible limit=100cfu/100ml.

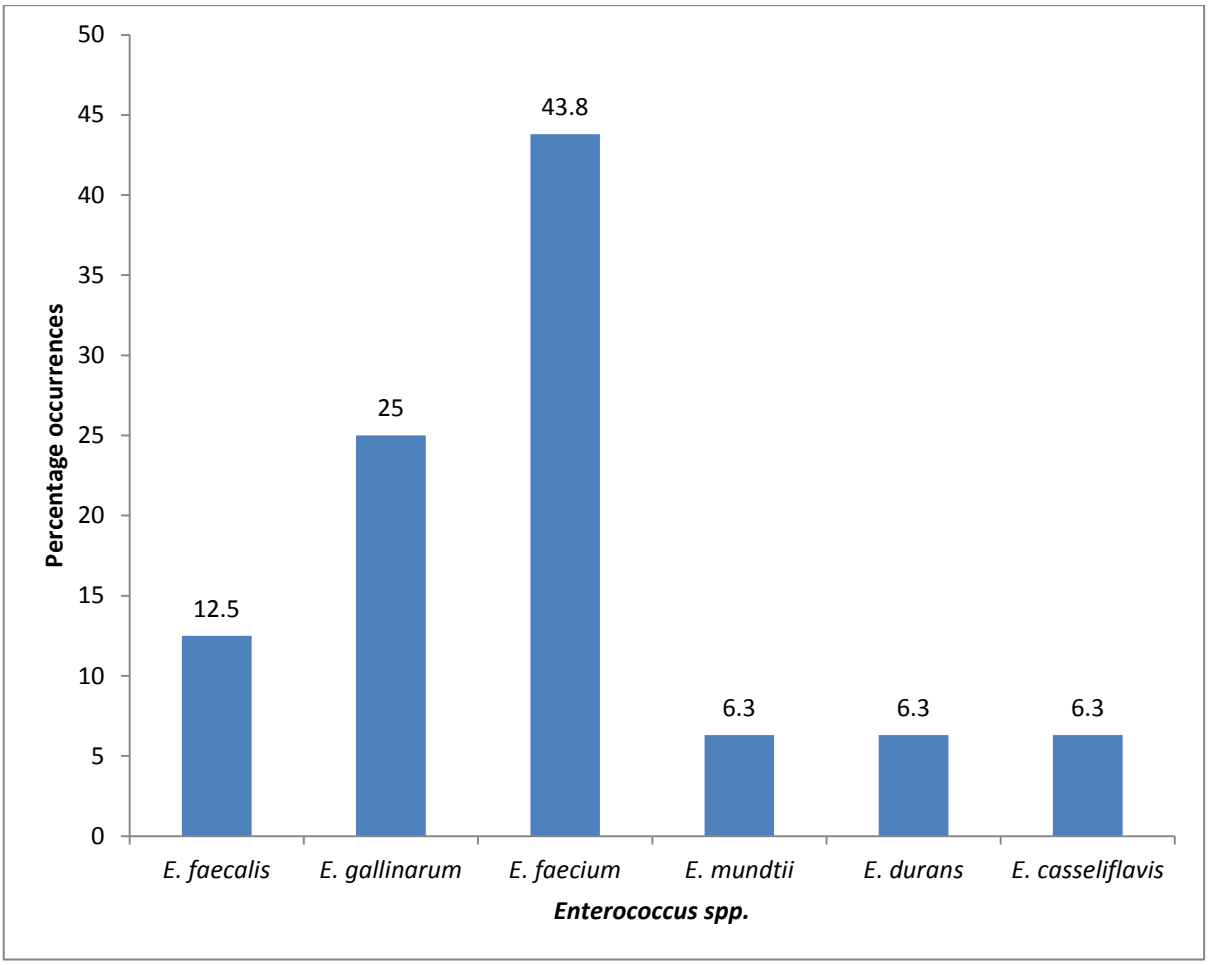


Figure 4.1: Percentage Occurrence of *Enterococcus* spp Isolated from Water Samples in Zaria, Kaduna State.

4.5 Antibacterial Susceptibility Profile of Bacteria Isolates

Table 4.27 shows the results of antibacterial susceptibility profile of 10 *Escherichia coli* O157:H7 isolates from water samples. Eight commonly used antibiotics in the study area were used for the susceptibility test. All the isolates were found to be susceptible to Ciprofloxacin 10(100%) while 9(90%) each were found susceptible to Gentamicin and Chloramphenicol respectively. Out of the 10 *Escherichia coli* O157:H7isolates obtained, 7(70%) were resistant to Ampicillin while 6(60%) were resistant to Trimethoprim Sulfamethoxazol. Only 6(60%) isolates were resistant to Amoxicillin, 1(10%) showed intermediate susceptibility, while 3(30%) were sensitive to this drug. Therefore, Amoxicillin and Ampicillin were the least active. Table 4.28 shows the results of Multidrug resistance (MDR) patterns of the 10 isolates tested for susceptibility to antibiotics. This showed that 8(80%) were resistant to two or more antibiotics viz; 2(20%) were resistant to 2 antibiotics, 5(50%) to four antibiotics, 1(10%) to five antibiotics, the remaining 1(10%) and 1(10%) were resistant to only Ampicillin and Cefotaxim respectively.

No resistance phenotype was found with the three, six, seven and eight antibiotics resistance combinations. Highest frequency of five (50%) was found in combinations of four antibiotics. Multidrug resistance is regarded as resistance to four or more classes of antibiotics. Three (30%) of the *E. coli* O157:H7 isolates exhibited multidrug resistance. Figure.4.2 shows the multiple antibiotic resistance index (MARI) of the *E. coli* O157:H7 isolates. Multiple antibiotic resistance index (MARI) gives an indirect indication or suggestion of the probable source of an organism. MARI greater than 0.2 indicates that an organism must have originated from an environment where antibiotics are often used (Olayinka *et al*., 2004). Among the isolated *E. coli* O157:H7 from water samples, 8(80%) of the tested bacteria had MAR index greater than 0.2.

Table 4.27: Antibacterial Susceptibility Profile of *Escherichia coli* O157:H7 Isolates.

			Susceptibility n=10		
Antibacterial agent	symbol	Disc content (μg)	R	I	S
Ampicillin	AMP	10	7(70)	0(0)	3(30)
Cefotaxim	FOX	30	5(50)	3(30)	2(20)
Gentamicin	CN	10	0(0)	1(10)	9(90)
Tetracycline	TE	30	5(50)	1(10)	4(40)
Ciprofloxacin	CIP	5	0(0)	0(0)	10(100)
Trimethroprim/ Sulfomethoxazol	SXT	25	6(60)	0(0)	4(40)
Chloramphenicol	C	30	1(10)	0(0)	9(90)
Amoxicillin	AML	10	6(60)	1(10)	3(30)

KEY: n=Number of isolates tested, R=resistanc, I=intermediate, S=Susceptible. AMP= Ampicillin, FOX= Cefotaxim, CN= Gentamicin, TE= Tetracycline, CIP= Ciprofloxacin, SXT= Trimethroprim/ Sulfomethoxazol, C= Chloramphenicol, AML= Amoxicillin.

Table 4.28: Multiple Antibiotics Resistance Pattern of *Escherichia coli O157:H7* Isolates Obtained from Water Samples in Zaria, Kaduna State Nigeria.

NO. of antibiotics combination	Resistance phenotype combination	No. of isolates with pattern (%)	MARI
1R	FOX; AMP	2(20%)	0.13
2R	AMP, AML; AMP,FOX	2(20%)	0.3
3R	NIL	NIL	NIL
4R	AMP, AML, TE, SXT; AMP, AML,TE,SXT; AMP, FOX,SXT, C; AML, FOX, TE, SXT; AMP, AML, TE, SXT.	5(50%)	0.5
5R	AMP, AML, FOX, TE, SXT	1(10%)	0.63
6R	NIL	NIL	NIL
7R	NIL	NIL	NIL
8R	NIL	NIL	NIL

MARI=Multiple antibiotic resistance index, R=Resistance, AMP= Ampicillin, FOX= Cefotaxim, CN= Gentamicin, TE= Tetracycline, CIP= Ciprofloxacin, SXT= Trimethroprim/ Sulfomethoxazol, C= Chloramphenicol, AML= Amoxicillin.

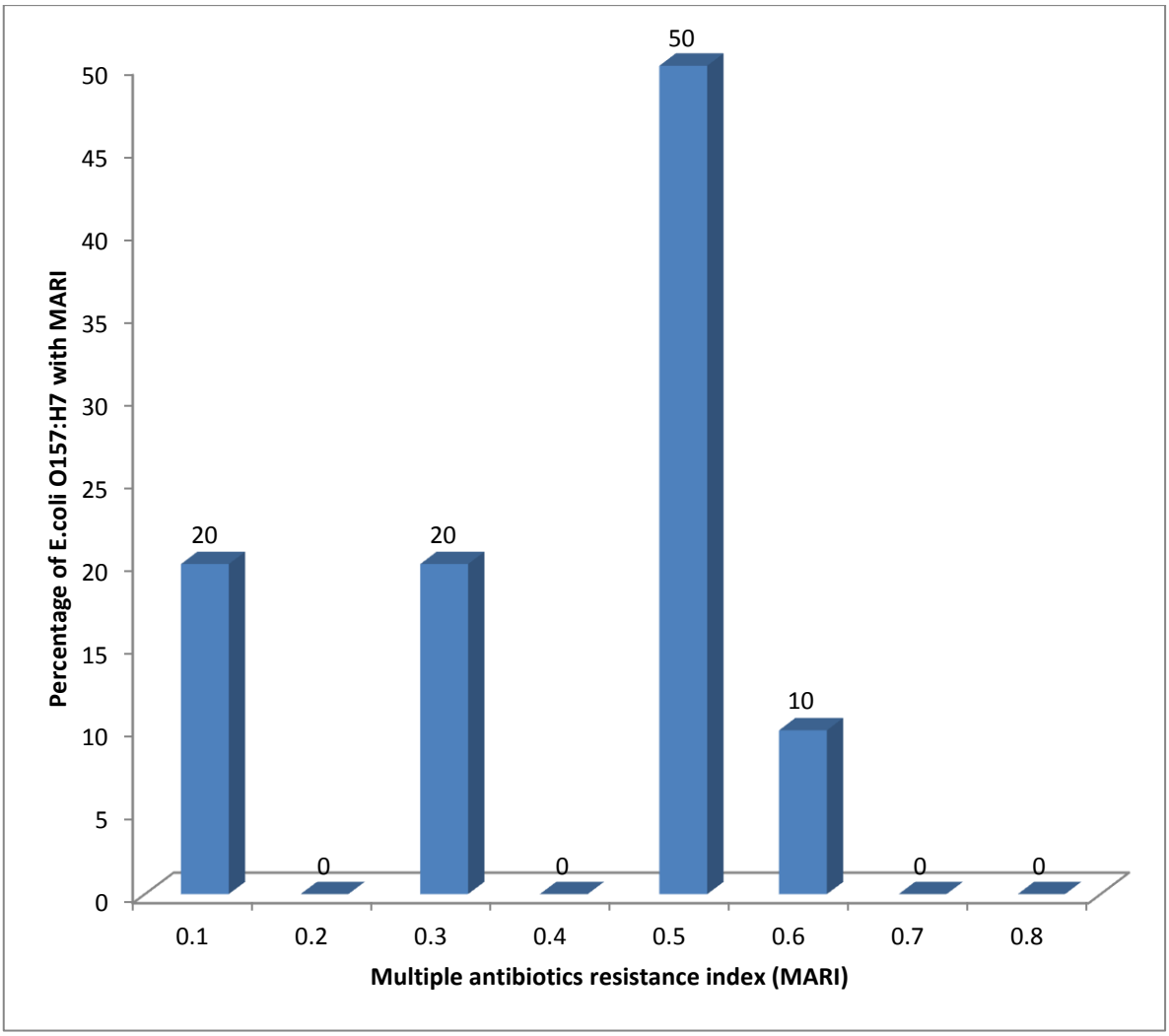


Figure 4.2: Multiple Antibiotics Resistance Indices of *E. coli* O157:H7 Isolated from Water Samples in Zaria, Kaduna state.

Table 4.29 shows the results of antibacterial susceptibility profile of 16 isolates of *Enterococcus* spp from water samples in Zaria. Seven commonly used antibiotics in the study area were used for the susceptibility test. Of all the antibiotics used, Chloramphenicol 14(87.5%), Ciprofloxacin 13(81.25%), Tetracycline 12(75%), Vancomycin 11(68.75%) were active. All the *Enterococcus faecalis* isolates *were* susceptible to Vancomycin 2(100%), Ciprofloxacin 2(100%) and Chloramphenicol 2(100%) while all the *Enterococcus faecium* and *Enterococcus gallinarum* were susceptible to Chloramphenicol 7(100%) and Ciprofloxacin 4(100%) respectively. Also, the *Enterococcus mundtii*, *Enterococcus durans* and *Enterococcus cassiflavis* isolated were all susceptible to Ciprofloxacin 3(100%) therefore, in terms of single antibiotic activity, Ciprofloxacin and Chloramphenicol were the most active in this study. In terms of combined antibiotic effect, a Synergy between Ampicillin, or Vancomycin and an aminoglycoside was predicted for *Enterococcus* by using a high-level aminoglycoside (Gentamicin 120µg) screening test (Table 4.29). This was in line with CLSI guideline 2014. All the isolates were susceptible to high-level Gentamicin 16(100%). Of all the isolates tested, only 1(6.25%) each showed resistance to Tetracycline, Ciprofloxacin and Chloramphenicol respectively while 4(25%) each showed resistance to Erythromycin and Vancomycin respectively. Ampicillin was the least active with 12(75%) resistance.

Figure 4.3 shows the multiple antibiotics resistance index of *Enterococci* spp isolated from water samples in Zaria. The multidrug resistance (MDR) pattern (Table 4.30). Of the sixteen *Enterococcus* spp isolated, it was revealed that 6(37.6%) were resistant to two or more antibiotics. that is, 2(12.6%) were resistant to two antibiotics, 4(25%) were resistant to three antibiotics. However, 8(50%) showed resistance to only one antibiotics: Ampicillin. While 2(12.5%) were susceptible.

Table 4.29: Antibiotics Susceptibility Profile of *Enterococcus spp* Isolated from Water Samples in Zaria, Kaduna State, Nigeria.

Antibiotics (Disc content)	Symbol	*Enterococcus faecalis* (n=2)			*Enterococcus faecium* (n=7)			*Enterococcus gallinarum* (n=4)			*Enterococcus durans, Enterococcus mundtii, Enterococcus casseliflavis* (n=3)			Number of Resistant Isolates (%)
		R	I	S	R	I	S	R	I	S	R	I	S	
Ampicillin(10µg)	AMP	2(100)	-	0(0)	6(85.7)	0(0)	1(14.3)	2(50)	-	2(50)	2(66.7)	-	1(33.3)	12(75)
Vancomycin(30µg)	VA	0(0)	0(0)	2(100)	2(28.6)	0(0)	5(71.4)	1(25)	1(25)	2(50)	1(33.3)	0(0)	2(66.7)	4(25)
Erythromycin(15µg)	E	0(0)	1(50)	1(50)	2(28.6)	1(14.3)	4(57.1)	1(25)	1(25)	2(50)	1(33.3)	0(0)	2(66.7)	4(25)
Tetracycline (30µg)	TE	0(0)	1(50)	1(50)	1(14.3)	0(0)	6(87.7)	0(0)	1(25)	3(75)	0(0)	1(33.3)	2(66.7)	1(6.25)
Ciprofloxacin(5µg)	CIP	0(0)	0(0)	2(100)	1(14.3)	2(28.6)	4(57.1)	0(0)	0(0)	4(100)	0(0)	0(0)	3(100)	1(6.25)
Chloramphenicol(30ug)	C	0(0)	0(0)	2(100)	0(0)	0(0)	7(100)	1(25)	0(0)	3(75)	0(0)	1(33.3)	2(66.7)	1(6.25)
Gentamicin(120µg)	CN	0(0)	0(0)	2(100)	0(0)	0(0)	7(100)	0(0)	0(0)	4(100)	0(0)	0(0)	3(100)	0(0)

KEY: n=Number of isolates tested, R=resistance, I=intermediate, S=Susceptible.

Table 4.30: Multiple Antibiotics Resistance Phenotype of *Enterococcus* spp Isolated from Water Samples in Zaria, Kaduna State, Nigeria.

Number of antibiotic combination	Isolates with pattern	Resistance phenotype	MARI
1	8(50%)	AMP	0.14
2	1(6.3%)	VA, E	0.29
	1(6.3%)	AMP, C	0.29
3	1(6.3%)	AMP, TE, CIP.	0.43
	3(18.8%)	AMP, VA, E.	0.43
4	NIL	AMP, VA, E.	NIL
5	NIL	NIL	NIL
6	NIL	NIL	NIL
7	NIL	NIL	NIL

MARI=Multiple antibiotic resistance index, AMP= Ampicillin, VA= Vancomycin, E= Erythromycin, TE= Tetracycline, C= Chloramphenicol , CIP=Ciprofloxacin.

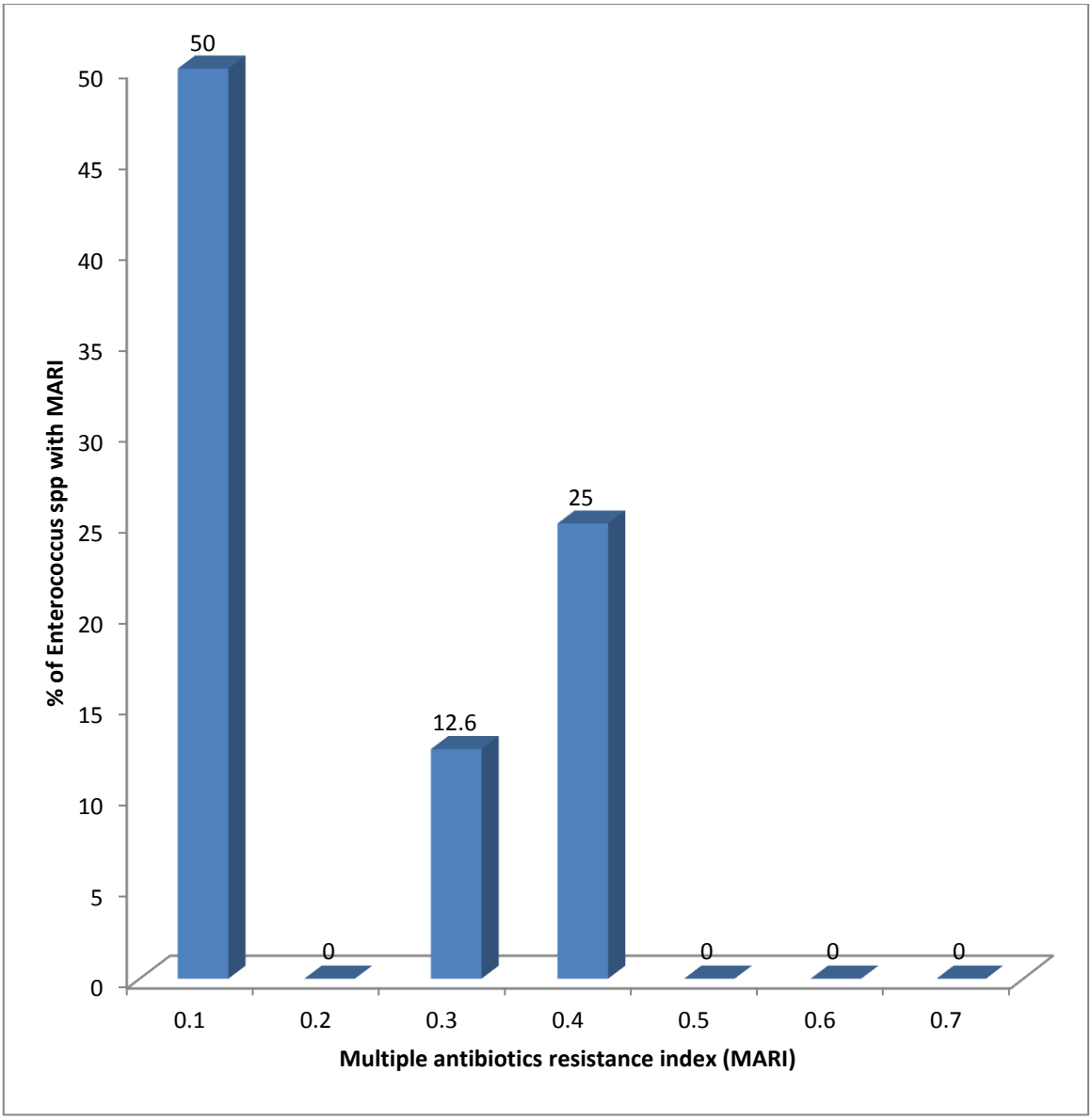
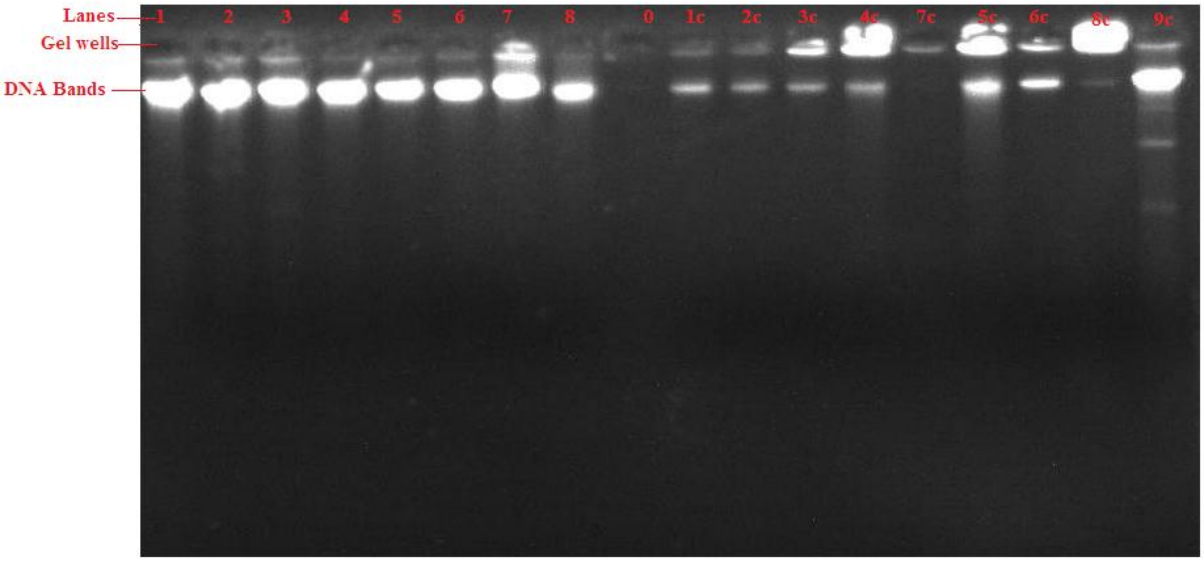


Figure 4.3: Multiple Antibiotics Resistance Indices of *Enterococcus* spp. Isolated from Water Samples in Zaria, Kaduna state.

4.6 Molecular Characterization of Isolates

Following DNA extraction using commercially available kit, Plate II shows the results of agarose gel electrophoresis of the extracted DNA. This revealed the presence of expected DNA bands. Table 4.31 shows the results of the NanoDrop spectrophotometric analysis; to quantitatively determine the concentration and purity of the extracted DNA from *Escherichia coli* O157:H7 and *Enterococcus spp* isolated from water samples in Zaria. Samples 1-8 is for *Escherichia coli* O157:H7 while samples 1C-9C is for *Enterococcus spp* with an average nucleic acid (DNA) concentration of 81.1ng/µl and 71.4ng/µl which ranges from 63.5-110.3ng/µl and 10.4-159.5ng/µl respectively. The sample purity was determined using the 260nm:280nm calculation. A pure DNA sample will yield an A260/280 of approximately 1.0-2.0 these ratios are commonly used to assess the amount of protein contamination that is left from the nucleic acid isolation process since proteins absorb at 280nm. In this study, 16(94%) samples were shown to contain ultrapure DNA with an A260/280 range of 1.03-2.03. *Enterococcus spp* assayed for the presence of a conserved region in the genus *Enterococcus* using the genus specific primer ENT1 showed amplification of the expected 112bp (Plate III). Vancomycin resistance in the *Enterococcus* spp isolated was not detected with primer amplification of VANR gene which is a member of the Vancomycin resitance gene cluster with the expected band size of 645bp (Plate IV).

Multidrug resistance in the *Escherichia coli* O157:H7 isolates were further confirmed with primer amplification of the extended spectrum β-lactamase genes CTX-M and TEM-1 conferring multidrug resistance to penicillins cephalosporins and some carbapenems (Plate V and VI) with band sizes of 894bp and 198bp respectively. However, no amplification was obtained for the shiga toxin genes (Plate VII and VIII).



Key; Lanes 1-8: *Escherichia coli* O157:H7 Isolates.
Lane 0: negative control.
Lanes 1c-9c : *Enterococcus* spp. Isolates.

Plate II: Agarose gel electrophoresis plate of the extracted DNA

Table 4.31: NanoDrop Spectrophotometric Analysis Results of the Extracted DNA.

Sample ID	Nucleic Acid (ng/µl)	A260(Abs)	A280(Abs)	260/280
Sample 1	98.3	1.966	1.15	1.71
Sample 2	74	1.48	0.886	1.67
Sample 3	88.1	1.763	1.009	1.75
Sample 4	71.2	1.423	1.071	1.33
Sample 5	74.2	1.484	0.865	1.72
Sample 6	69.4	1.387	1.541	0.9
Sample 7	63.5	1.271	0.808	1.57
Sample 8	110.3	2.207	1.191	1.85
Sample 1C	31.8	0.636	0.313	2.03
Sample 2C	22.7	0.453	0.259	1.75
Sample 3C	74.3	1.487	1.138	1.31
Sample 4C	159.5	3.189	2.42	1.32
Sample 5C	46	0.919	0.764	1.2
Sample 6C	125.5	2.51	1.907	1.32
Sample 7C	10.4	0.209	0.198	1.06
Sample 8C	154.1	3.081	2.276	1.35
Sample 9C	18.2	0.365	0.355	1.03

KEY: ID=Identification, ng/ µl= Nanogram per micro liter, Abs=Absorbance

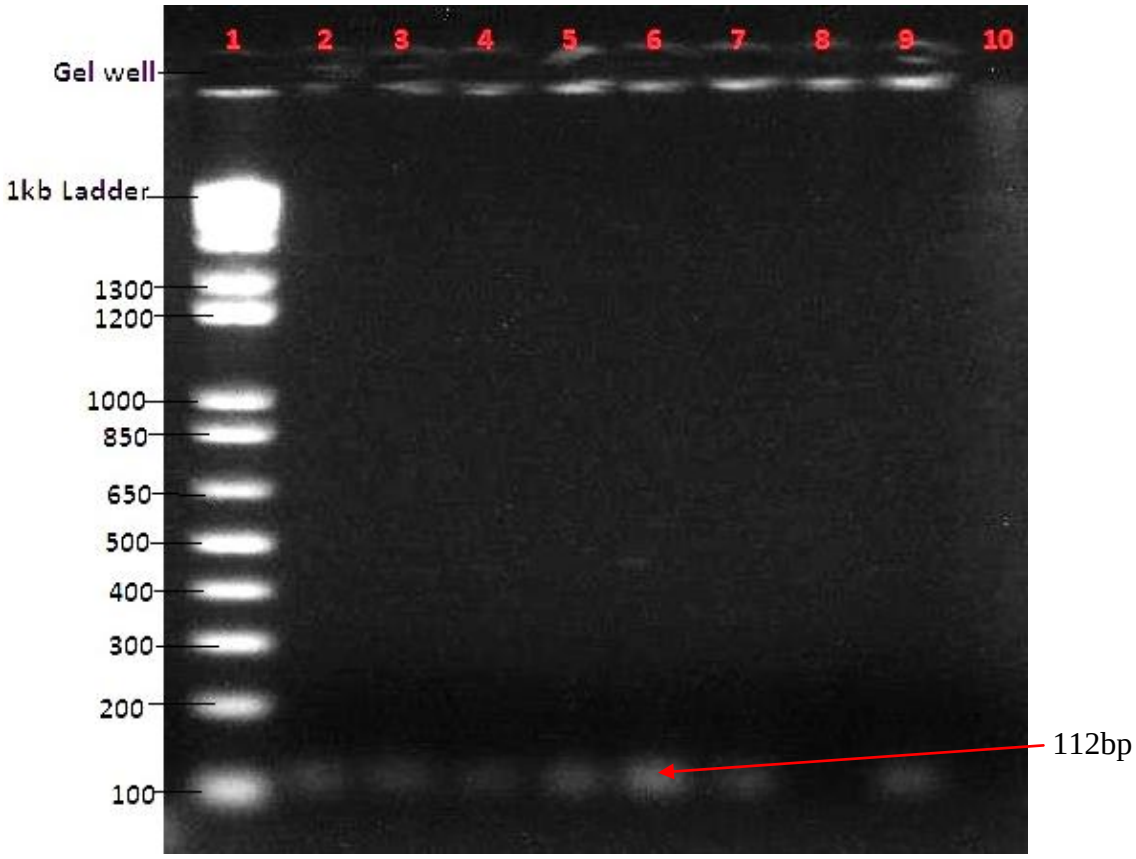


Plate III: Agarose gel electrophoresis plate showing the presence of *tuf* gene: ENT1 conserved region of the genus *Enterococcus*.

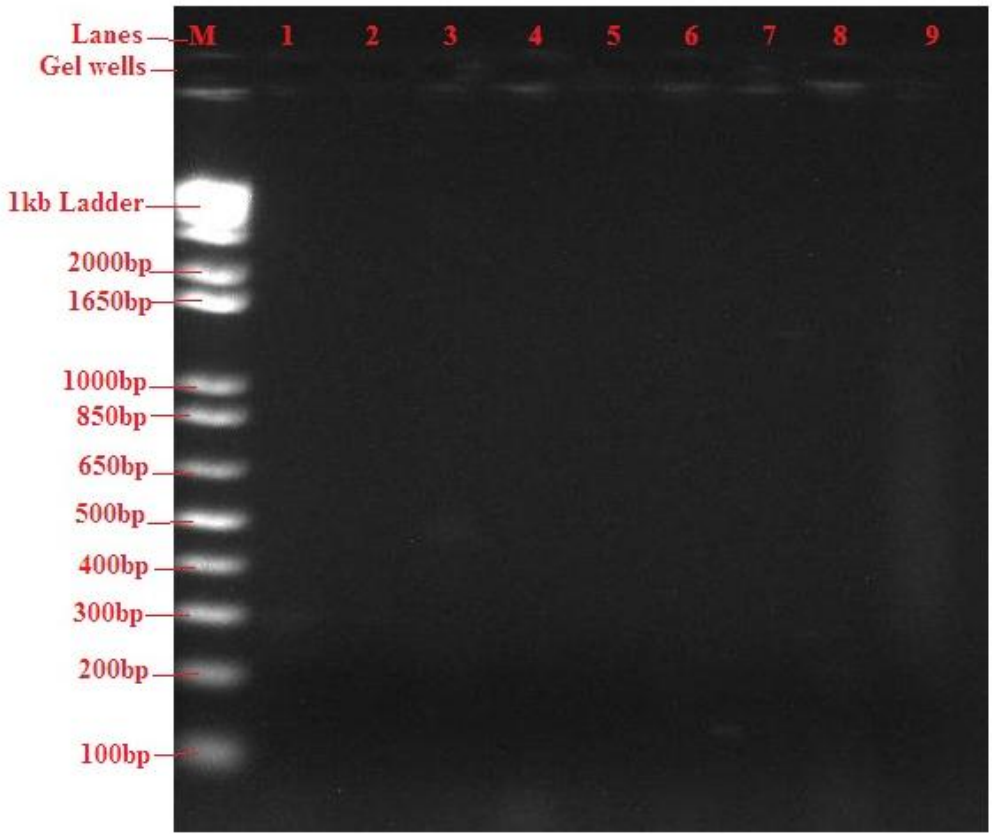


Plate IV: Agarose gel electrophoresis plate of VANR gene in *Enterococcus* spp.

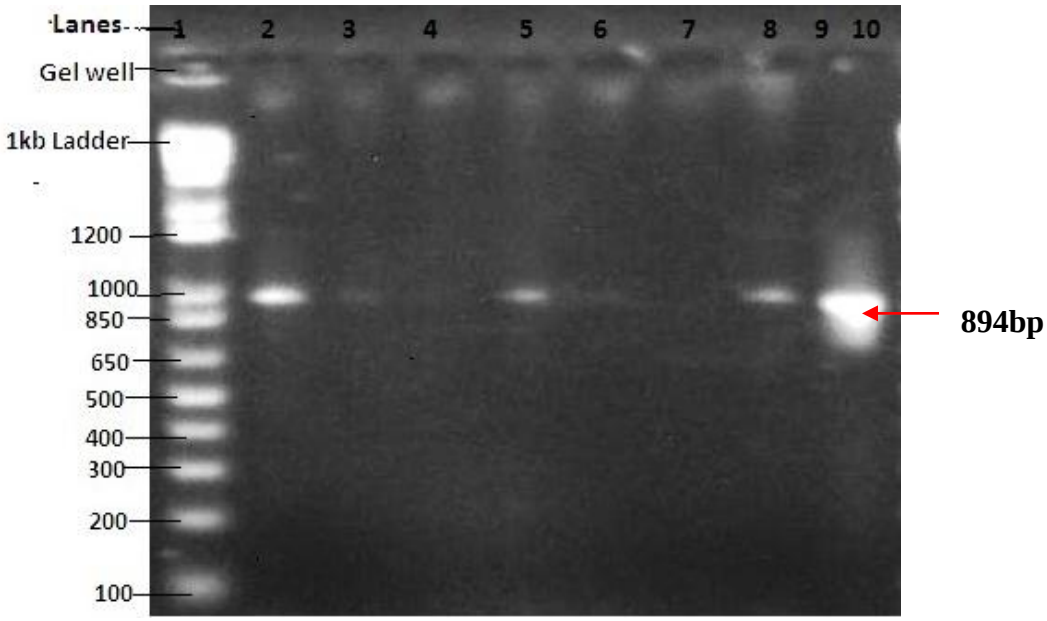


Plate V: Agarose gel electrophoresis plate showing the presence of extended-spectrum β-lactamase CTX-M gene in *Escherichia coli* O157:H7. 894bp

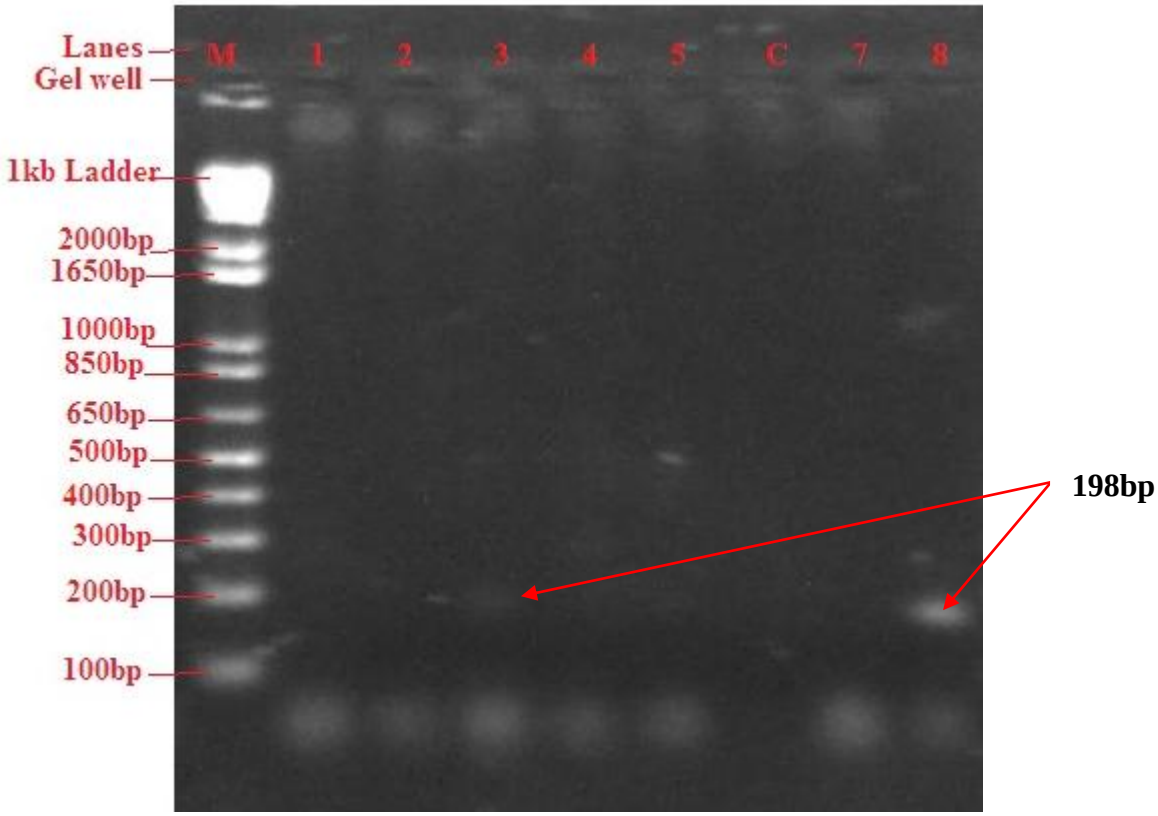


Plate VI: Agarose gel electrophoresis plate showing the presence of extended-spectrum β-lactamase TEM gene in *Escherichia coli* O157:H7. 198bp

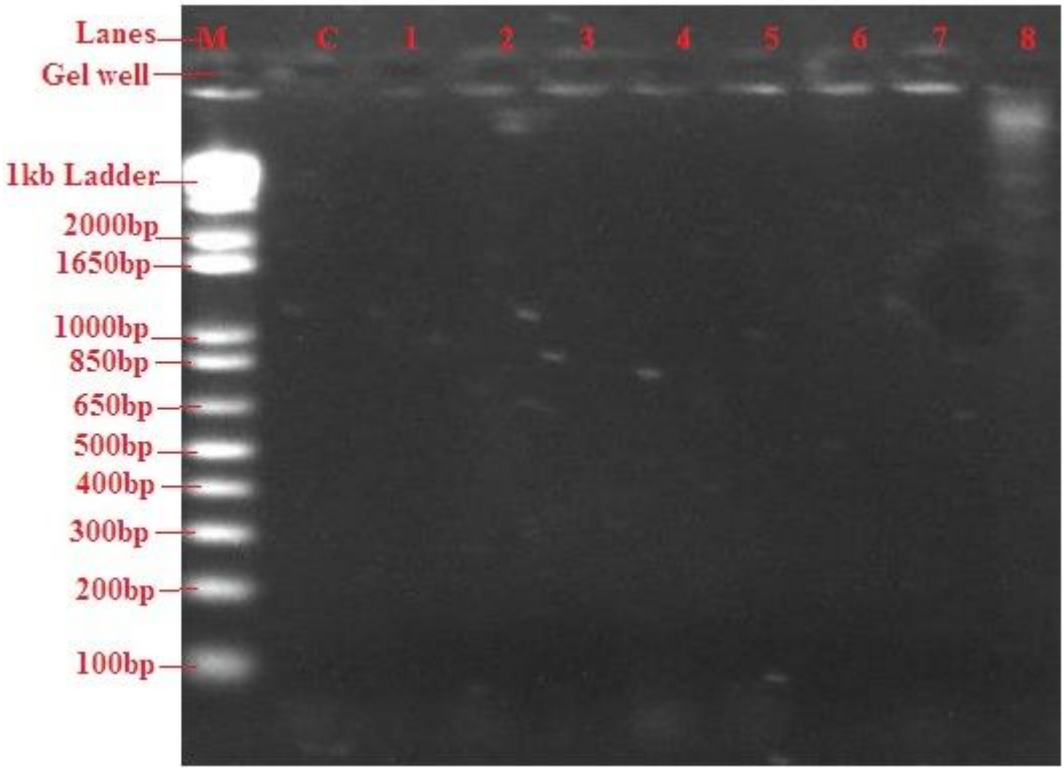


Plate VII: Agarose gel electrophoresis plate of *Escherichia coli* O157:H7 shiga toxin STX1 gene.

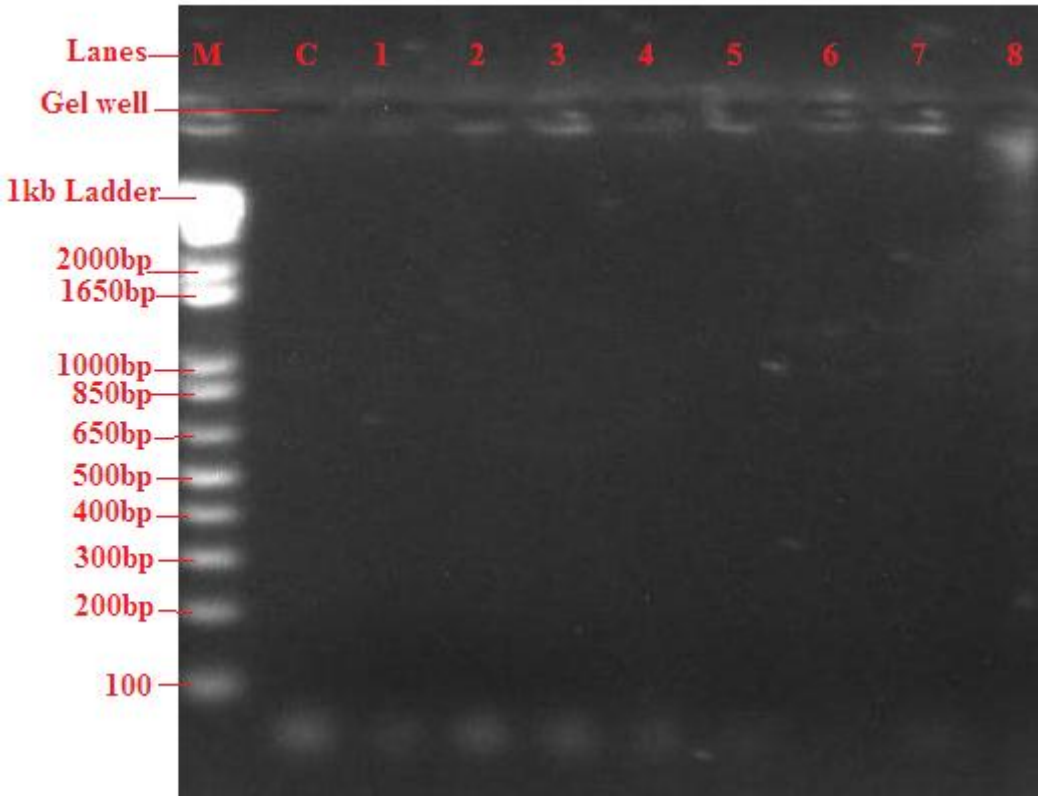


Plate VIII: Agarose gel electrophoresis plate of *Escherichia coli* O157:H7 shiga toxin STX2 gene.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physicochemical Parameters of the Packaged and other Drinking Water Sources in Zaria.

The results of the Physicochemical parameters obtained in this study showed that the borehole, well, sachet-packaged and bottled waters in Zaria exhibited various characteristics in terms of their physicochemical quality parameters. In the well water sampled from five different sampling sites in Zaria, the turbidity values of three (60%) of the sampling sites were above the permissible limits (5NTU) of National Agency for Food and Drug Administration and Control (NAFDAC). This could be due to the presence of organic and inorganic particulate matter which could protect microorganisms from the effects of disinfection and can stimulate bacterial growth. This is similar to the report of Ezeribe *et al*. (2012), who reported high turbidity in 75% of well water sampled in Bauchi. Also, the total dissolved solids and electrical conductivity which ranged from 336.40 to 539mg/l and 673.20 to 1078μS/cm respectively were observed in two (40%) sampling sites to be above the minimum recommended standard (500mg/l) and (1000mg/l) of NAFDAC. This could be attributed to the sepage of leachate from the nearby dumpsites and soakaways. Electrical conductivity is related to the ionic content of the sample which is in turn a function of the total dissolved (ionisable) solids concentration. This agrees with the findings of Aminu and Amadi (2014).

Also, the pH values of four (80%) of the sampling sites were bellow the recommended range (6.5-8.5) of NAFDAC. this may probably be attributed to acid rainfall due to atmospheric carbon dioxide and other airborne pollutants, runoff from

mining sites, and decomposition of organic materials. This is in agreement with the findings of Amadi *et al.* (2014), who reported a pH mean value of 6.35 in well waters. However, the alkalinity, dissolved oxygen, biological oxygen demand, nitrate, phosphate and chloride levels were within the acceptable limits of NAFDAC. This is similar to the report of Amadi *et al.* (2014). However, the high values of nitrate across the well water sampling sites in Zaria which ranged from 21.02 to 39.22mg/l were above the minimum contamination level (10mg/l) of United States Environmental Protection Agency (USEPA). This could be attributed to runoff from fertilizer use, leaching from septic tanks and sewage, erosion of natural deposits, improper disposal of animal and human wastes. Infants below the age of six months who drink water containing nitrate in excess of the Maximum Contaminant Level could become seriously ill and, if untreated, may die. Symptoms include shortness of breath and blue-baby syndrome (Methemoglobinemia) (USEPA, 2014). This is in agreement with the findings of of Ezeribe *et al*. (2012).

In the borehole water samples, the pH values of the five (100%) sampling sites were below the standard recommended range (6.5-8.5) of NAFDAC. This could be attributed to the presence of dissolved minerals, leaching or corrosion of sink pipes. This is very significant because acidic borehole water generally are very aggressive to copper and could Cause corrosion. Besides, the solubility of lead increases markedly as the pH is reduced below 8 because of the substantial decrease in the equilibrium carbonate concentration. This is similar to the findings of Oladipo *et al*. (2011), who also reported borehole water quality with low pH in Lapai. Also, the total dissolved solids (411mg/l) and electrical conductivity (823.40µS/cm) of borehole water sampling site sabon-gari were the highest. However, nitrate levels in two (40%) of the sampling sites H1 (10.89mg/l) and H4 (10.62mg/l) were above the minimum contamination level

(10mg/l) of USEPA. This could be due to leachates from waste disposal sites and sanitary landfills or shallow nature the boreholes. This is in agreement with the findings of Oladipo *et al*. (2011). Among all the sachet-packaged and bottled water brands sampled in this study, the turbidity, total dissolved solids, electrical conductivity, alkalinity, dissolved oxygen, biological oxygen demand, nitrate, phosphate and chloride were within the acceptable limits set by NAFDAC. This could be due to low contamination levels of these parameters in source water. These findings are in agreement with the report of Ashaye *et al.* (2001). However, the pH of four (80%) sampled brands of the bottled table water were bellow the recommended range (6.5-8.5) of NAFDAC. This could be due to due to the high levels of CO_2 which may consequently affect the bacterial counts (Balogun *et al*., 2014). This is in agreement with the work of Balogun *et al*. (2014).

The comparison of the mean values of the physicochemical quality parameters of all the water types (bottled, sachet-packaged, borehole and well waters), revealed that there was no significant difference between the pH value of the borehole water (5.93) and well water(6.18) in Zaria. This could be attributed to the exposure of these waters to similar composition of the rocks and sediments that surround the travel pathway of the recharge water (acid rain) infiltrating to the ground water, this could cause an increase in the concentration of dissolved contaminants due to acidic waters. This could be treated by increasing the pH of the water with limestone or marble chips. However, there was significant difference between the pH values of the sachet-packaged water (7.04) and bottled water brands (5.48) this could probably be due to excessive incorporation of high levels of CO_2 in bottled water leading to the formation of carbonic acid (H_2CO_3). There was significant difference between the nitrate levels in sachet-packaged water (2.54mg/l) and bottled water (0.03mg/l). This could be attributed

to treatment ineffectiveness or lack of appropriate technology by sachet-packaged water manufacturers in Zaria.

Also, there was no significant difference in the turbidity and alkalinity values of the borehole water and sachet-packaged drinking water. This may be attributed to the use of ordinary boiling of borehole water sources without exclusion of particles through sterilized filtration materials by the sachet-packaged water manufacturers. This agrees with the reports of Oyedeji *et al.* (2013). Also, in this study, the effects of seasonal variations on the mean values of physicochemical quality parameters of borehole, well, sachet-packaged and bottled drinking waters in Zaria showed that there was no significant difference between the physicochemical parameters in borehole likewise sachet-packaged water during the wet and dry season except phosphate of the sachet-packaged water with dry and wet mean values of 0.50mg/l and 0.39mg/l respectively. However, in the well water, significant difference was recorded in the dissolved oxygen, temperature and phosphate where the dry season recorded the highest values. This could be due to concentration effects of constituent minerals which may be more concentrated per unit volume during the dry season. This is similar to the report of Akoteyon (2014), who reported mean values of all the physicochemical parameters of well water being higher in dry season compared to the wet season except for pH.

5.2 Heavy Metal Content of the Packaged and other Drinking Water Sources in Zaria

In this study, the Cadmium (Cd), Lead (Pb), and Iron (Fe) contents of the various water samples were determined. In the well water sampled, the Cd, Pb, and Fe recorded across all the well water sampling sites ranged between (0.014 to 0.027mg/l); (0.044 to 0.290mg/l) and (0.062 to 5.77mg/l) respectively. These levels of Cd, Pb, and

Fe were above the acceptable limits set by NAFDAC. Also in the borehole water samples analysed, the Cadmium, lead, and Iron contents of the various borehole samples in Zaria were above the minimum contamination levels set by NAFDAC (Table 4.8). These could be attributed to Surface runoffs, leaching from sewage, erosion of natural deposits, improper disposal of batteries and electronic wastes (e-wastes). These could predispose consumers to health risks due to the consumption of water high in Cadmium concentrations which causes Kidney damage. This is in agreement with the findings of Abiola (2010), who reported lead contamination of underground water. In addition, all the sachet-packaged water sampled recorded high levels of Cadmium (Cd) and Lead (Pb) which were above the minimum permissible limits established by NAFDAC.

These could be due to treatment ineffectiveness or lack of appropriate facilities to achieve effective treatment. However, the Fe content of four (40%) of the sachet-packaged water brands were below the (0.3mg/l) of NAFDAC. In the bottled water brands, lead was not detected in all the five brands sampled while three (60%) of the bottled water brands had slightly higher Cadmium level compared to the (0.003mg/l) of NAFDAC. Comparing the mean heavy metal contents of borehole, well, sachet-packaged and bottled waters revealed that the Cadmium content of well water, borehole water and sachet-packaged water were not statistically significantly different. This could be a proof that most manufacturers of packaged drinking water in Zaria obtain their raw water mostly from local, municipal pipe-borne water, borehole water or well water without adherence to production and analytical standards. This is in agreement with the reports of Oyedeji *et al.* (2013). There was a significant difference in the lead (Pb) and Iron across different water types.

5.3 Bacteriological Quality of the Packaged and other Drinking Water Sources in Zaria.

Results obtained in this study showed that the sachet, bottled, borehole and well waters in Zaria Kaduna State exhibited variable characteristics in terms of their bacteriological quality. Most of the sachet water brands studied was contaminated with coliform bacteria although to varying degrees per 100ml of drinking water. However, two (20%) of the sachet water brands recorded zero coliform count in two to three occasions throughout the period of sampling. The average total coliform counts (TCC) vary from 1.50cfu/100ml in brand G to 8.44×10^2 cfu/100ml in brand D. this variation in total coliform counts across the different brands of sachet-packaged water could be attributed to the use of different treatment processes by manufacturers and contaminations from various environmental sources which reflects the fact that the sachet water brands are not always bagged under scrutinized sanitary conditions. This result is in agreement with Oyedeji *et al.* (2013), who reported 100% of sachet water brands with elevated total coliform counts in Ile-Ife, Southwest Nigeria. Only one of the five brands (20%) of the bottled water was contaminated with total coliform with a mean TCC of 5.25 per 100ml of drinking water. Therefore, the bottled water brands were of relatively better microbiological quality. This is similar to the report of Ajayi *et al*. (2008), who reported in an earlier study of packaged drinking waters in Ibadan, Nigeria in which larger proportions of sachet water were found to show positive coliform counts compared to bottled waters. All the borehole and well waters sampled from five different sampling sites were contaminated with total coliform bacteria although to a varying levels of counts per 100ml of water. However, in one accession, borehole water from sampling site H4 recorded zero TCC.

These contaminations could be attributed to surface runoffs and anthropogenic activities of man which include domestic, agricultural or uncontrolled industrial discharge of untreated effluents to the soil, stream and rivers, indiscriminate dumping of garbage and faeces which could lead to underground water contamination (Erah *et al.,* 2002). Anaele (2004), reported extensive contamination of residential wells and boreholes by sewage from the numerous septic tanks, latrines and soak away pits often sited near them. Total *Escherichia coli* (TEC) was detected in eight (80%) of ten brands of sachet water and one (20%) of the five bottled water brands. This could be due to the high quantitative efficiency of membrane filter technique, high specificity and sensitivity of the membrane lactose glucuronide agar (Oxoid UK) used in this study. This is contrary to the findings of Onifade and Ilori (2008), who reported 6.7% *E. coli* in sachet water brands. All (100%) of the borehole and well waters recorded TEC.

This provides an evidence of recent environmental contamination of well water, inadequate treatment of the packaged water or breaches in distribution system integrity of the borehole water. This agrees with the report of Stoler *et al*. (2012). Faecal coliform and thermotolerant *Escherichia coli* was detected in eight (80%) of the sachet drinking water but however not detected in all the brands of the bottled water investigated. The absence of faecal indicator bacteria in most brands of bottled drinking water could be attributed to better hygienic practices observed in the industry compared to the sachet water producing industry. These include use of protective sealed caps on bottles, improved and hygienic filling system and use of non-returnable plastic containers (Oyedeji *et al*., 2013). Human-water contacts during packaging unlike the fully automated system used in bottled water factories could also be a factor. Faecal coliform and thermotolerant *Escherichia coli* was detected in all the borehole and well water sampled in Zaria. This could be attributed to unhygienic situation of borehole

close to pit latrines and poor sanitary habits which may predispose the underground water to contamination. Also, shallowness of the wells in combination with a compromise of their sanitary integrity, presence of roaming, domestic and grazing animals close to the wells could contribute to the severity of faecal coliform contaminations. This is in agreement with the report of Ukpong and Okon (2013).

Enterococci were detected in seven (70%) of the sachet water brands but however not detected in all the brands of the bottled drinking water. Enterococci was also detected in all the borehole water sampled with sampling sites Tudun-wada (H2) and Sabongari (H5) with mean enterococci counts of 1.25×10^{1} and 1.21×10^{1}cfu/100ml respectively. This could be due to inadequate construction or deficient protection of the boreholes in these areas. All the well water sampled were contaminated with enterococci. However the highest counts were recorded in sampling site Wusasa (W3) with a mean enterococci count of 1.07×10^{3}cfu/100ml of water. This could be attributed to lack of proper drainage system, practice of open defecations and indiscriminate dumping of human and animal wastes in this area. The total viable counts (TVC) or heterothrophic plate counts (HPC) were very high in various brands of the sachet water with eight of the ten brands having mean HPC counts above 100 cfu/ml. All the bottled water brands had HPC bellow 100cfu/ml while all the borehole and well waters had HPC counts above 100cfu/ml, however, there was no significant difference between HPC in sachet water brands and the borehole water sampled in this study (Table 4.20). According to World Health Organization, 2002 report, a high HPC concentration does not itself present a risk to human health. Nevertheless HPCs are used as good indicators of the overall quality of production (Ferreira *et al.*, 1994; Obiri-Danso *et al*., 2003). These may therefore be used in assessing the cleanliness of the different brands of bottle and sachet drinking waters sold in the selected areas of study Oyedeji *et al.* (2013).

Comparative bacteriological data analysis showed that there were no significant differences between the total coliform counts of the sachet water and borehole water in Zaria. However, the sachet-packaged water were found to be more contaminated with pooled mean total coliform counts of 2.95×10^2 cfu/100ml while borehole water had 2.61×10^2 cfu/100ml. also, the sachet water brands had a higher mean faecal coliform counts than borehole with mean FCC of 1.38×10^2 cfu/100ml and 1.02×10^2 cfu/100ml respectively. Similarly, the sachet water also had higher counts of total and thermotolerant *Escherichia coli* with mean values of 1.82×10^1 cfu/100ml and 8.45cfu/100ml respectively while borehole water with lower counts of 6.07 and 3.45cfu/100ml respectively. These could be attributed to improper treatment applications or post treatment contaminations due to biofilm formation in treatment facilities or contamination from factory workers prior to packaging of the sachet-packaged water.

Therefore, the borehole water was of relatively better microbiological quality than the sachet-packaged water sold in Zaria. This is in agreement with the report of Stoler *et al.* (2012). Effects of seasonal variations on mean Total coliform counts, Total *Escherichia coli*, Faecal coliform counts, Faecal *Escherichia coli*, enterococci counts and Total viable counts (TVC) or heterothrophic plate counts (HPC) showed that higher bacteriological counts were recorded during the wet season than in the dry season. This could be due to increased run-off from faecally polluted dry soils. Similarly, water in the aquifer is "topped up" more rapidly after heavy rains and both the horizontal and vertical migrations of water are accelerated at such times. Bacteria held in the water are also carried through greater distances after periods of rain Obiri-Danso *et al*. (2003). These are in agreement with several researchers who reported seasonal variations in water quality works in: north west England (Obiri-Danso and Jones, 2000), Nigeria

(Blum *et al*., 1987), Gambia (Barrel and Rowland, 1987) and Kenya (Muhammed and Morrison, 1975). Have all shown higher microbial counts in water sources during the wet season or after periods of rainfall compared to the dry season. However, few exceptional cases of enterococci counts in borehole and well waters were higher in the dry season. This could be attributed to dilution effect of rain during the wet season and the ability of most members of the genus *Enterococcus* to withstand stress and survive various harsh environmental conditions.

5.4 Antibacterial Susceptibility Profile of the Bacteria Isolates

Development of resistance to first line antimicrobial agents increases the healthcare costs of managing gastrointestinal infections due to *Escherichia coli* which are the major source of haemolytic colitis, diarrhoea and death associated with contaminated water in the developing countries (Lalzampuia *et al.*, 2014). Ciprofloxacin (100%), Gentamicin (90%) and Chloramphenicol (90%) were the most active antibiotics against *Escherichia coli* O157*:*H7 isolates in this study. This is in agreement with the report of Yah and Eghafona (2007), who reported that Ciprofloxacin, is effective against diarrhoeagenic *Escherichia coli*. The high level resistance of these isolates to Ampicillin (70%) agrees with the findings of Yah and Eghafona (2007), who reported 100% resistance of *Escherichia coli* O157*:*H7 isolates to Ampicillin. The *Escherichia coli* O157*:*H7 isolates also recorded resistance against Trimethoprim-Sulfamethoxazol (60%) and Amoxicillin (60%). This could be attributed to the inexpensive and availability nature of these drugs across the counter and could be easily abused by the study population. The resistance of *Escherichia coli* O157*:*H7 isolates to Tetracycline (50%) is contrary to the findings of Chigor *et al.* (2010), who reported 100% resistance of *Escherichia coli* O157*:*H7 isolates to Tetracycline. This could however be due to dilution effects of water from where these bacteria were isolated.

Although there is evidence indicating that Tetracycline survives longer in the environment than other antibiotics which may be critical in maintaining the level of Tetracycline resistance at a high level (Yah and Eghafona, 2007). Amoxicillin and Ampicillin were the least active antibiotics against the isolated *Escherichia coli* O157:H7 in this study. The high susceptibility or zero resistance of these bacteria to the quinolones (Ciprofloxacin) may be because they are relatively new antibiotics and are more expensive than the Ampicillin and Amoxicillin and as such, may not be commonly purchased hence less used which reduces the presence of its residue in water and the environment.

The resistance of *Enterococcus* isolates to Ampicillin (75%) could be due to the fact that Ampicillin is readily available across the counter and it is relatively cheap and could be easily abused by the study population. However, the resistance of *Enterococcus* isolates to Vancomycin (25%) and Erythromycin (25%) presents an emerging challenge and a public health issue to the Microbiologists and the medical community because Vancomycin is the drug of choice for the treatment of severe infections caused by Gram-positive bacteria especially *Staphylococcus* and *Enterococcus.*

Multiple antibiotic resistance index MARI is a tool that reveals the spread of bacteria resistance in a given study population (Olayinka *et al*., 2004). MAR index greater than 0.2 implies that the strains of such bacteria originated from an environment where several antibiotics are used (Olayinka *et al.*, 2004). *Escherichia coli* O157:H7 (80%) and *Enterococcus* (37.6%) isolates in this study showed MAR indices of 0.3 and above. These reflect the high level of antibiotics resistance in water and its environment. The MAR indices obtained could be a result of extensive use of antibiotics by man and in agriculture which eventually find its way into the drinking water sources in Zaria.

The consumption of packaged-drinking water and other untreated borehole and well waters with these bacteria without any further treatment is worrisome and presents a potential public health risk. This could elevate the resistance gene pool in the human gut microflora.

5.5 Molecular Characterization of Isolates by the Polymerase Chain Reaction (PCR) Technique.

The PCR showed amplification of the expected 112bp tuf gene which encodes elongation factor *tuf*-gene in the genus *Enterococcus*. This is in agreement with the report of Danbing *et al.* (1999). And could be used for the rapid detection of *Enterococcus* in water and clinical samples thereby removing the 48h required for a culture based detection of *Enterococcus* in samples. Vancomycin resistance gene cluster consist of VANR gene which was not detected in this study. This result is contrary with the report of Miele *et al.* (1995), who reported that Vancomycin resistance in *Enterococcus* has been increasing over the last several years. Although, 4(25%) of the *Enterococcus* exhibited phenotypic resistance to Vancomycin. This is critically important due to the fact that glycopeptides: Vancomycin and teicoplanin constitute an important class of antibiotics for the treatment of severe infections caused by gram-positive bacteria, particularly *Staphylococcus* and *Enterococcus* (Miele *et al*., 1995). Multidrug resistance in *Escherichia coli* O157:H7 isolates were further confirmed with primer amplification of the extended spectrum β-lactamase genes; (bla-CTX-M and bla TEM-1) which codes for multidrug resistances penicillins, cephalosporins and carbapenems (Dallenne *et al.*, 2010). This is an emerging challenge and a confirmation of multidrug resistant bacteria in drinking water sources in Zaria.

However, the presence of these resistance genes and the observed reduced susceptibility of isolates to clinically relevant antibiotics demonstrate that drinking water sources may serve as a vehicle for the transmission of antimicrobial resistance in the study area and even across the nations. The inability to detect the shigatoxin Stx1 and Stx2 genes from the *Escherichia coli* O157:H7 could be due to the long period of preservation of the isolates as studies revealed that some verotoxin factors are plasmid encoded and the plasmid could be lost during the many subcultures of the bacteria strains (Abakpa *et al*., 2008; Clemont *et al.,* 2011).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The results obtained in this study showed that the borehole, well, sachet-packaged and bottled waters in Zaria are of variable physicochemical quality. However, turbidity (60%), total dissolved solids (40%), and electrical conductivity (40%) of the well water sampled were above the minimum recommended standard by NAFDAC and WHO while nitrate (100%) of all the well water sampled were above the USEPA recommended minimum contamination limits. This is critical because Infants below the age of six months who drink water containing nitrate in excess of the maximum contaminant level could become seriously ill and, if untreated, may die of blue-baby syndrome (Methaemoglobinemia). Also, the pH of (80%) of the sampled well water were below the recommended range (6.5-8.5) of NAFDAC and WHO. In the borehole water sampled, the pH of five (100%) sampled sites were below the standard recommended range (6.5-8.5) of NAFDAC and WHO. nitrate levels in (40%) of the sampled borehole water were above the minimum contamination level (10mg/l) of USEPA. There was no significant difference between the pH value of the borehole water (5.93) and well water (6.18) in Zaria. Also, there was no significant difference in the turbidity and alkalinity values of the borehole water and sachet-packaged drinking water. The sachet-packaged and bottled water brands sampled in this study were of good physicochemical qualities since values were within the acceptable limits set by NAFDAC. There was no significant difference between the physicochemical parameters in borehole likewise sachet-packaged water during the wet and dry season

except phosphate of the sachet-packaged water with higher mean value during the dry season. In the well water, significant difference was recorded in the dissolved oxygen, temperature and phosphate where the dry season recorded the highest values. In the borehole and well water samples analysed, the Cadmium (Cd), Lead (Pb), and Iron (Fe) contents of the various sampling sites in Zaria were above the minimum contamination levels set by NAFDAC. All the sachet-packaged water sampled recorded high levels of Cadmium (Cd) and Lead (Pb) which were above the minimum permissible limits established by NAFDAC. In the bottled water brands, Lead was not detected in all the five brands sampled. The Cadmium content of well water, borehole water and sachet-packaged water were not statistically significantly different. However, there was a significant difference in the Lead (Pb) and Iron across different water types studied.

Results of the bacteriological quality assessment obtained in this study showed that total coliform and *Escherichia coli* was detected in (80%) of the brands of sachet-packaged water and (20%) in the bottled water brands. Faecal coliform and thermotholerant *Escherichia coli* was detected in eight (80%) of the sachet drinking water but however not detected in all the brands of the bottled water investigated. All the borehole and well waters sampled from five different sampling sites were contaminated with total coliform, faecal coliform, thermotolerant *Escherichia coli* and enterococci except in very few occasions. There were no statistically significant differences between the total coliform counts of the sachet water and borehole water in Zaria. However, the sachet-packaged water was found to be more contaminated with total coliform, faecal coliform and *Escherichia coli.* Therefore, the borehole water was of relatively better microbiological quality than the sachet-packaged water sold in Zaria. Higher bacteriological counts were recorded during the wet season than in the dry season across all sample types. Ciprofloxacin (100%), Gentamicin (90%) and

Chloramphenicol (90%) were the most active antibiotics against *Escherichia coli* O157:H7 isolates in this study. There was high level resistance of *Escherichia coli* O157:H7 (80%) and *Enterococcus spp* (37.6%) to commonly used antibiotics with MAR indices of 0.3 and above. Rapid detection of *Enterococcus* was carried out using the genus specific primer ENT1 which showed amplification of the expected 112bp tuf gene through the PCR technique. VANR gene was not detected in *Enterococcus.* Although, 4(25%) of the *Enterococcus* exhibited phenotypic resistance to Vancomycin. This provides further indication of the emergence of Vancomycin resistance *Enterococcus* in the environment. the Stx1 and Stx2 genes from the *Escherichia coli* O157:H7 could not be detected in this study however, Multidrug resistance *Escherichia coli* O157:H7 isolates were confirmed with primer amplification of the extended spectrum β-lactamase genes; (bla-CTX-M and bla TEM-1) which codes for multidrug resistances penicillins, cephalosporins and carbapenems. This is an emerging challenge and a confirmation of multidrug resistant bacteria in drinking water sources in Zaria.

6.2 Conclusion

The nitrate level in samples of the wells (100%) and borehole (40%) water were above the United States environmental protection agency (USEPA) minimum contamination level of 10mg/l. Cadmium (Cd) and Lead (Pb) contents of boreholes, wells, and brands of sachet waters were above the minimum permissible limits set by NAFDAC (0.003mg/l and 0.01mg/l respectively). However, Lead (Pb) was not detected in the bottled water brands sampled. Total coliforms and *Escherichia coli* were detected in sachet water brands (80%), bottled water brands (20%), borehole (100%) and well water (100%). Enterococci were recovered from sachet water brands (70%), borehole (100%) and well water (100%). There were no statistically significant differences ($P \leq 0.05$) between the total coliform counts of the sachet water brands and borehole

water in Zaria, therefore, the purity of sachet water as claimed by the manufacturers is doubtful. Bacteriological counts were higher during the wet season than dry season. Antibiogram of *Escherichia coli* O157:H7 (80%) and *Enterococcus* spp (37.6%) isolates showed multiple antibiotics resistance (MAR) with MAR indices of 0.3 and above. Polymerase chain reaction (PCR) amplified some housekeeping genes such as tuf gene of *Enterococcus* genus and antibiotics resistance genes such as glycopeptides Vancomycin and teicoplanin resistance gene VANR. *Escherichia coli* O157:H7 extended spectrum β-lactamase genes: bla-TEM and bla-CTX-M genes. Most of the sachet water brands fell below NAFDAC and WHO drinking water standards

6.3 Recommendations

1. The National Agency for Food and Drug Administration and Control (NAFDAC) should intensify efforts in the routine monitoring of activities in the packaged drinking water industry. Testing of market samples will be a good way of detecting if the water is actually pure as claimed by these producing companies.
2. It is advisable that Well water, shallow contaminated boreholes and municipal tap water should be avoided as sources of raw water for the production of packaged drinking. Ground waters such as boreholes when properly constructed and maintained provide a relatively safer source of raw water in terms of microbial load compared to unprotected water sources such as river, spring and well waters.
3. Appropriate treatment processes should be utilised for production of quality and safe packaged drinking waters while regulatory agencies should stipulate adequate sanitation and hygienic practices as a condition for routine recertification of packaged water producers in Nigeria.

4. Receptacles for drawing water from open wells should be kept clean and permanently attached to a windlass when not in use; Well lids must be kept dry and clean and should be constructed as a single unit. Wells should be sited at higher elevations so as not to serve as a sink during rainfall; Wells should be sited at least 30 m away from septic tanks, latrines and rubbish dumps; access to wells and boreholes by domestic and grazing animals should be restricted by fencing.
5. Legal framework should be put in place at the national level to put stringent laws to regulate the citing of wells and boreholes, as findings from this study corroborate observations of Erah *et al.* (2002) that indiscriminate sinking of boreholes and wells without proper geological surveys contributes to the presence of faecal coliform in underground water.
6. Enactment of appropriate legislations to regulate the handling and disposal of e-waste and lead accumulators by battery chargers in order to control pollution of the environment by lead wastes is likewise recommended.
7. The local mass media should disseminate information on the need for a more careful handling and disposal of materials that contain heavy metals such as lead especially lead paints.
8. Intensive education of the Nigerian population on correct treatment procedures of water for domestic use should be done on the electronic media especially TV and radio on continual basis.
9. It is recommended here that mothers should practice six months exclusive breastfeeding of babys to avoid babys from being exposed to the intake of contaminated water with high nitrate levels which could cause blue-baby syndrome (Methaemoglobinaemia).

10. It is also recommended here that chlorinating agents be provided by the government at heavily subsidized prices to trained personnel to assist in the elimination of pathogenic microorganisms in the untreated water supplies.
11. Most importantly, the government at all levels in Nigeria should also be admonished and take the issue of supply of adequately treated water to the public as an essential public service.

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APPENDICES

Appendix I: Biochemical Characterization and Identification of Presumptive Isolates of *Escherichia coli* O1517:H7 using the Microgene® GnA-ID system.

Isolate code	Lysine	ornithine	H2S	Glucose	mannitol	xylose	ONPG	Indole	Urease	V.P.	Citrate	TDA	Identification NO.	Identity
SMW24	+	+	-	+	+	+	+	+	-	-	-	-	6760	*E. coli*
WS103	+	-	-	+	+	+	+	+	-	-	-	-	4760	*E. coli*
SGW28	+	+	-	+	+	+	+	+	-	-	-	+	6761	*E. coli*
SG48	+	-	-	+	+	-	-	+	-	-	-	-	4620	*E. coli*
TW91	+	+	-	+	+	+	+	+	-	-	-	-	6760	*E. coli*
WT11	+	-	-	+	+	+	-	+	-	-	-	-	4720	*E. coli*
ZW4	+	-	-	+	+	+	+	+	-	-	-	-	4760	*E. coli*
ZC13	+	-	-	+	+	+	-	+	-	-	-	+	4721	*E. coli*
WS2	+	+	-	+	+	+	+	+	-	-	-	-	6760	*E. coli*
WS400	+	+	-	+	-	+	+	+	-	-	-	+	6561	*E. coli*

+=positive, -=negative

Appendix II: Biochemical Characterization and Identification of *Enterococcus* spp. Isolated from water samples in Zaria using the microgene Strep-ID system.

Isolate code	HIP	AHE	BHE	MEL	SOR	INU	LAC	ARA	RIB	ESC	VP	PHS	BGA	PYR	ARG	IDENTIFICATION OCTAL CODE	IDENTITY
H312	+	-	-	-	+	-	+	-	+	+	+	+	+	+	+	42577	*Enterococcus faecalis*
W212S	+	-	-	-	+	-	+	-	+	+	+	+	+	+	+	42577	*Enterococcus faecalis*
W203b	+	-	-	+	+	-	+	+	+	+	-	+	+	+	+	46757	*Enterococcus faecium*
W5	+	-	-	+	-	-	+	+	+	+	-	+	-	+	+	44753	*Enterococcus faecium*
W3m	+	-	-	+	+	-	+	+	+	+	+	-	+	+	+	46767	*Enterococcus faecium*
H211	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+	44777	*Enterococcus faecium*
W189	-	-	-	-	+	-	+	+	+	+	+	-	-	+	+	02763	*Enterococcus faecium*
H414	+	-	-	+	-	-	+	+	+	+	-	-	+	+	+	44747	*Enterococcus faecium*
C11	+	-	-	+	-	-	+	+	+	+	+	-	+	+	+	44767	*Enterococcus faecium*
W4b	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	47776	*Enterococcus gallinarum*
W301	+	-	-	+	-	+	+	+	+	+	+	-	+	-	+	45765	*Enterococcus gallinarum*
W5b07	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	47777	*Enterococcus gallinarum*
W1a205	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	47777	*Enterococcus gallinarum*
H5a10	+	-	+	+	-	-	+	+	+	+	+	-	+	+	+	54767	*Enterococcus durans*
W2k	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	07777	*Enterococcus mundtii*
H232	-	-	-	+	-	+	+	+	+	+	-	-	+	+	+	05747	*Enterococcus casseliflavis*

+=positive, -=negative



Appendix III: Microgen^{TM} GnA-ID Kit For the Identification of *Escherichia coli*



Appendix IV: Microgen^{TM} STREP-ID For the Identification of *Enterococcus* species**.**





Appendix V: Work in Progress for the Heavy Metal Analysis at the Mulstipurpose Laboratory of Ahmadu Bello University, Zaria, Nigeria.



Appendix VI: Site5- Children Drinking Well Water



Appendix VII: site2- Children Fetching Water from Hand-Operated Borehole



Appendix VIII: Site5- Filtration Units in a Plastic Bagged Sachet Drinking Water Production Factory