THE OCCURRENCE, MOLECULAR IDENTIFICATION AND ANTIBIOTICS RESISTANCE PATTERN OF FAECAL BACTERIA IN PUBLIC AND PRIVATE WELLS IN PAIKO, NIGER STATE

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ABSTRACT

Well water has become a main source of water supply for both drinking and domestic use in many rural and urban areas in Nigeria due to inadequate provision of potable pipe-borne water. This study identified the occurrence of faecal bacteria and their antibiotic resistance pattern in different public and private wells of Paiko, Niger State. Sixty (60) water samples were collected and used for this study and it comprises of 32 private wells and 28 public wells. The water samples were inoculated on Eosin Methylene Blue (EMB) agar using pour plate methods for faecal bacteria isolation. The result of the molecular identification of the bacteria isolates and their percentage identity revealed the presence of *E. coli*, *Enterobacter aerogenes, Klebsiella pneumoniae* and *Citrobacter cronae*. The frequency of occurrence of the bacteria are *Klebsiella pneumoniae* 22 (33.8%), *Enterobacter aerogenes* 18 (27.7%), *E. coli* 14 (21.5%), and *Citrobacter cronae* 11 (16.9%). Antibiotic susceptibility test showed a high susceptibility of the bacteria to meropenem 65 (100%), ciprofloxacin 65 (100%), trimethoprim-sulfamethoxazole 64 (98.5%) and streptomycin 62(95.4%). While a significant level of resistance was observed with cephalexin 35 (53.8%) a class of cephalosporin antibiotics. The physicochemical parameters of this study were significantly different at the 5% level ($p>0.05$) from the required permissible limit of World Health Organization (WHO) and Standard Organization of Nigeria (SON) for potable water. Sensitization of the public on how to situate wells and its proper maintenance would reduce the risk of major water borne pathogens to public health.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

All living things from the simplest plants and microorganisms to complex living systems depend on water for survival (Onifade and Ilori, 2008). Water possesses unique chemical and physical characteristics, it occupies approximately 70% of the earth; making it earths most abundant compound (Obi and Okocha, 2007). One of the eight fundamental elements essential in primary healthcare globally is a safe and potable drinking water supply. Accessibility to potable drinking water improves health and productivity and these cumulates into the ability to earn a good living. The availability and accessibility of clean, fresh, potable water can contribute to sustainable development and the alleviation of poverty (Al-Bratty *et al.*, 2017). However, access to and supply of this necessary resource remains a major problem in many nations and communities around the world (World Health Organization (WHO), 2004).

In Niger state the sedimentary and basement complex rocks underlain have varying capacities for holding water year-round (Obaje, 2009; Olasehinde, 2010). In raining seasons, the state experiences heavy down pour which often lead to flooding in some communities, while once the dry season sets-in even rivers run dry, giving rise to difficulties in accessing water of suitable quality. Most medium-sized towns including Paiko have consistently encountered similar problem of poor water quality supply since in the 1980s. Less than 20% of the people living in Niger state currently have access to potable water. Morenikeji *et al*. (2000) claimed that access to potable water has been steadily declining in Niger State since the 1980s. Rural residents are now obliged to use well water for drinking and household use due to constrain and inadequate supply of pipe-borne water. In certain instances, hand-dung wells are situated near unsensitized locations like garbage dumps, drainages, pit latrines, or soak-away systems.

Some of these wells are frequently open, making them vulnerable to pollution of different kinds and degrees (Ikem *et al*., 2002).

Waterborne pathogens are categorized into three main groups: bacteria, viruses, and protozoans (Desouky *et al.*, 2003). Bacteria are majorly responsible for most waterborne diseases such as typhoid, paratyphoid fever, dysentery, diarrhoea, cholera. Many bacteria pathogens most of which are of enteric origin have been reported in water. These bacteria include *E. coli, Shigella* sp., *V. cholerae, C. jejuni, S. typhi, Aeromonas* spp., enterotoxigenic *Bacteroides fragilis* and enteropathogenic *E. coli* (Fricker, 2003).

Assessment of drinking water quality has majorly been carried out using culture techniques (Gantzer *et al.*, 1998). However, the method does not allow the detection of some specific water borne pathogens and it is also time consuming. Recent advances in molecular technology techniques for the detection of pathogens in drinking water showed significant promise in improving the safety of water supplies by precisely detecting and identifying these pathogens (Martin *et al*., 2008). Molecular techniques have several advantages over standard culture techniques for specific detection and quantification of waterborne pathogens, as they are highly sensitive, rapid, specific and can be readily automated and standardized (Tekpor *et al.*, 2017). Due to its high sensitivity, specificity, simplicity and rapidness compared to culture techniques, the Polymerase chain reaction is the most commonly adopted molecular tool (WHO, 2006).

Realizing that well water in Paiko is widely used for domestic activities as well as drinking by the people of the community. This study was designed to ascertain the potability and qualities of water from such wells and the antibiotics resistance pattern of faecal bacteria detected from the well water.

1.2 Statement of the Research Problem

In many countries, including Nigeria, access to clean drinking water has been a major problem (Vincent *et al*., 2010). Most rural regions in Nigeria, including the study area Paiko, lack access to potable drinking water (Aweda *et al*., 2020). Only a small percentage of people are able to afford and rely on treated and purified water, especially for drinking. Therefore, the primary source of both drinking water and domestic water used by the local population in Nigeria is ground water (well water) (Abaje *et al*., 2009).

Nigeria faces major disease epidemics as 63 million people lack inadequate access to safe and potable drinking water (Ogundipe *et al*., 2017). The resurgence of cholera is a devastating epidermic facing the country where more than 30,000 cases of cholera and 800 deaths were been reported in the year 2021, according to the Nigeria Centre for Disease Control (NCDC) (Food Safety News, 2021). The NCDC also reported the outbreak was aggravated by poor access to clean water, poor sanitation, open defecation and hygiene issues.

Annually, water-borne diarrheal diseases result to 502,000 diarrheal deaths due to ingestion of unsafe drinking water (WHO, 2018) and an estimated 150,000 children in Nigeria lost their lives each year due to diarrheal-associated illness (Bademosi, 2018). Treatment of these patients is additionally compromised by antimicrobial resistance bacterial (National (Nigeria) Action Plan for AMR, 2017). Therefore, there is a pressing need to contain these problems by constantly evaluating source of domestic and drinking water sources such as wells for the presence of faecal bacteria and to also examine the occurrence of antibiotic resistance pattern of the faecal bacteria.

1.3 Aim and Objectives of the Study

The aim of this study was to determine the occurrence, molecular identity and antibiotics resistance pattern of faecal bacteria in public and private wells in Paiko, Niger State.

The objectives of the study were to:

i. isolate and identify faecal bacteria from well water

ii. determine the occurrences of faecal bacteria in public and private wells

iii. evaluate the antibiotic resistance pattern of the bacterial isolates

iv. determine the physicochemical properties of the well water.

1.4 Justification for the Study

Previous studies on well water also known as ground water carried out in Paiko by Aweda *et al*. (2020) isolated and identify microorganism based only on cultural and morphological characteristics. Hence this study explores the use of molecular technique as a confirmatory method for the identification of faecal bacteria. A variety of molecular techniques have been employed to study the microbial composition of the human gastrointestinal tract. Results from these studies have shown that many microorganisms detected by molecular techniques cannot be cultured using conventional methods (Joao, 2010). Molecular techniques such as PCR and sequence analysis provide further information by giving specific identity and phylogenetic characteristic of strains identified (Girones *et al.*, 2010).

This study further evaluates the antibiotic resistance pattern of the bacterial isolates which was ignored by previous studies carried out in Paiko from the well water source. The development of antibiotic resistance among pathogenic bacteria is a global public health issue and that includes Nigeria, a developing country. The major risk factor for an increase in bacteria resistance is the high and indiscriminate use of antibiotics. The emergence of bacteria resistance to antibiotics is a proof of the potential post antibiotics era which could constitute a menace to present and future medical advances (Osuntokun and Komolafe, 2019). Identification of faecal bacterial contamination in well water and the evaluation of the antibiotic

resistance pattern associated with the bacteria isolates in a community like Paiko can help detect waterborne pathogen and the risks associated it.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of Water

Water is a vital natural resource required for life to exist (Bates, 2000). Water of drinking quality is of utmost importance to human physiology as well as essential to man's existence (Idowu *et al*., 2011; Ezeh *et al*., 2020). Though the surface of the earth majorly made up of water, only a little fragment of it is usable, making this resource very limited (Roy, 2019).

Water is needed for almost all of man's activities and it is connected to all forms of life. It is required in the maintenance of metabolic activities and homeostasis in living cells as all cellular functions occur in fluid medium (Adzitey *et al*., 2015). The human body is made up of approximately sixty percent water by weight in adult males, fifty percent in females, and roughly about seventy percent in new born infants (Svagzdiene *et al*., 2010).

European Food Safety Authority (EFSA, 2010) estimated the human dietary requirement for water to be about 2 litres daily for an average adult, as regular intake of sufficient amounts is crucial in the maintaining good health. However, water safety and quality are important attribute of drinking water to ensure it meets standard for human consumption. Various contaminants can affect water quality causing negative effect on human and animal health. Suitability and quality of water for drinking and domestic use are usually determined by various elements such as the concentration of organic and inorganic substances, taste, odour and colour (Rahmanian *et al*., 2015). Water for human consumption is expected be free of pathogens and meet certain standards.

Humans and animals consume water from a range of sources (rain, stream, wells, and borehole) some of which can be contaminated by various pathogens and chemical toxins (Adzitey *et al*., 2015). Groundwater is an essential resource that exists almost everywhere under most

landscapes. It is an important source of fresh water for many parts of the world. In Africa, many cities, towns and villages depend almost entirely on it for their domestic, agricultural and industrial needs (Jidauna *et al*., 2013). As accessibility and availability of clean fresh water plays an essential role in economic growth and social welfare, it is also a fundamental element in health, food production, and poverty reduction (Pachepsky *et al*., 2011; Mpenyana-Monyatsi *et al*., 2012; Malhotra *et al*., 2015; Edessa *et al*., 2017). As such drinking water must not contain contaminants, such as microbial pathogens, toxic physical and chemical residues and unwanted organoleptic properties (Vidyasagar, 2007).

Globally, water scarcity, poor water quality, and inadequate sanitation have a severe impact on the livelihood options, food security, and educational prospects for underprivileged households (Sivaranjani *et al*., 2015; Pal *et al*., 2018; Ezeh *et al*., 2020). According to various studies millions of people globally use highly contaminated water sources (Liang *et al*., 2006). Scarcity of safe drinking water is a major problem affecting significant proportion of people in third world nations (Ayantobo *et al*., 2012). The increasing number of individuals with no access to quality drinking water is mostly as a result of the ever-increasing population predominantly in developing nations and the incapability or involuntariness of the government to offer adequate water supply facilities (United Nations Educational Scientific Cultural Organization (UNSDG), 2015).

In Africa, about 320 million people remain excluded from safe drinking water supplies (Bazié, 2014) and in Western Africa quality drinking water supply coverage is below 60 percent (Conseil des Ministres Africains de l'Eau (CMAE), 2008). To date, about 30% of the global population is affected by water scarcity and it is projected to rise beyond this if proper measures are not put in place to curtail it (United Nations Sustainable Development Goals, 2018). In Nigeria, the public water supply is poor, with water scarcity affecting millions of homes (Ezeh *et al*., 2020).

Globally, an estimated 1.1 billion people lack adequate and potable water supply and over 460 million people suffer from acute water shortages, and about 2.4 billion lacking adequate sanitary facilities (WHO, 2012). Scarcity of safe drinking water poses a great danger to the wellbeing of individuals exposing them to various water borne diseases (Pal *et al*., 2018). Millions of individuals especially children die every year from diseases associated with poor water quality, sanitation and hygiene (Okpasuo *et al*. 2019). Nearly 1,000 infants die daily as a result of water and sanitation-related diarrheal infections (United Nations Children's Fund (UNICEF), 2018).

Approximately 3.1 percent of global annual motility and 3.7 percent of annual morbidity are a result of unsafe water, lack of basic sanitation and hygiene. In Nigeria, the growing demand, coupled with constrained supply of potable pipe-borne water has pushed rural and some urban inhabitants to fall back to using various water sources such as well water for domestic uses, such wells in most situations are located in unsanitary locations. Some of these wells are usually open making them vulnerable to various kinds and degrees of contamination (Jidauna *et al*., 2013).

Geological conditions, industrial operations, and agricultural practices are potential sources of water contamination. These contaminants can also be divided into five categories: radionuclides, disinfectants, inorganics, and microbes. Compared to organic chemicals, inorganic chemicals make up a larger part of the pollutants in drinking water (Azrina *et al*., 2011). Groundwater quality is generally stable overtime as it is chiefly determined by the chemical composition of the rocks serving as aquifer. However, in most developing nations access to safe drinking water is fast becoming a mirage due to poor infrastructural facilities, inapt farm practices as well as indiscriminate dumping of wastes. The disposal and leakage of human and animal wastes matter into both surface and underground water sources has been reported as the major source of pathogens in water bodies (Marathe *et al*., 2017).

Water is considered a vehicle for the spread of infectious agents (Mpenyana-Monyatsi *et al*., 2012). It is well established that diseases are spread mainly through human and animal excreta contaminated water supplies. *Salmonella* sp., *Shigella* sp., pathogenic *E. coli, V. cholerae, Y. entercolitica, Campylobacter* sp., viruses including hepatitis A and hepatitis E, rotavirus, and different parasites are among the microbial pathogens that poses a substantial risk of disease in drinking water (Mpenyana-Monyatsi *et al*., 2012; Malhotra *et al*., 2015).

Water pollution is a global menace that poses great threat to human health. The WHO estimated that there are four billion cases of diarrhoea annually in addition to millions of illnesses associated with poor water quality. Globally more than 3 million people die of water-related diseases each year, including 1.2 million infants (Malhotra *et al*., 2015).

According to Davis (2010), water-related diseases mainly diarrhoea kill more than 25,000 people and about 5000 infants daily, most of which are preventable. In developing countries, diarrhoea and other water related diseases kill an estimated 1.8 million children per year (Johnson *et al*., 2011). Developing countries such as Nigeria are plagued with water-related diseases, accounting for about 10 percent of the disease burden in such countries (Mari *et al*., 2018).

As pollution, population, and environmental degradation increase, water sources are increasingly threatened by both chemical and microbiological contaminants. A wide range of pathogens can contaminate water, and routine monitoring of their presence is impractical. The examination of chemical parameters like pH, turbidity, conductivity, total suspended particles, total dissolved solids, total organic carbon, and heavy metals are few of the scientific methods that have been established to evaluate pollutants in water (Rahmanian *et al*., 2015). If the values of these parameters exceed the safe limits recommended by the WHO and other regulatory authorities then the water quality is poor (WHO, 2011). Monitoring indicator organisms like

coliforms, especially *E. coli*, which is a more precise sign of faecal contamination, is a key component of determining the microbiological quality of drinking water (Odonkor and Ampofo, 2013; Malhotra *et al*., 2015).

2.1.1 Use of well water in Nigeria

Treated pipe-borne water is limited to metropolitan areas in Nigeria, the supplied quantity is inadequate and the frequency epileptic. Supply is entirely lacking in certain areas within the metropolis. More than 52% of Nigerians lack access to clean water (Raphael *et al*., 2018). Poor socioeconomic development, a growing industrial base, shoddy planning, insufficient funding and sloppy implementation are seen to be the main causes of Nigerians poor access to clean and safe water (Oluwasanya, 2009).

Due to the poor water supply in the country, an increasing number of people in semi urban and urban areas depend on any easily accessible and cheaper alternative water sources such as wells and other surface water sources for drinking and domestic purposes (Idowu *et al*., 2011; Singh *et al*., 2015; Raphael *et al*. 2018). Such water sources are usually susceptible to external contamination (Moyo *et al*., 2004)

Wells are a low-tech, inexpensive way to address the problems of water supply in rural and urban areas (Seamus, 2000). Shimizu *et al*. (1980) showed that well water is contaminated by microbial pathogens depending on its location. Thus, wells situated in unhygienic areas could be contaminated according to their proximity to pollution sources. Water supplies are polluted by contaminants including microorganisms, heavy metals, nitrates and salts due to poor waste management, industrial discharges, and excessive utilization limited water resources (Adeyemi *et al*., 2007). National Demographic Health Survey (NDHS) survey result showed that in Nigeria, over 35 percent of the population use wells as their primary water source (NDHS, 2014).

Concerns over the quality of well water have received wide attention among researchers. Water from wells has been reported to be polluted through physical processes, geochemistry of the environment and anthropogenic activities, exposing users of such waters to various health hazards (Omofonmwan and Eseigbe, 2009).

2.2 Water Quality

Quality of drinking water signifies its suitability for consumption. The composition of water, which is impacted by both natural and human processes, determines its quality. In order to define water quality, various organizations, including the WHO, have established standards or safe limits of chemical and microbiological contaminants in drinking water. As such the quality of water determined on the basis of these standards and if values surpass these acceptable limits, then human health is put at risk (Akter *et al*. 2016).

Water Quality is the chemical, physical and biological features of water, usually in respect to its suitability for designated use. Water required for drinking, agricultural and industrial use each has varying defined chemical, physical and biological standards needed to fulfil the respective purpose (Roy, 2019).

Drinking water according to the WHO ought to be clear, colourless, odourless, tasteless, free of pathogens and any toxic chemicals (Pantelić *et al*., 2017), as such knowing the properties of water is important for determining water quality (Liu *et al*., 2018).

Ground water served as the primary source of potable water in many countries (Kumpel and Nelson, 2014). The quality of drinking water has decreased emphatically as a result of pollution from industrial and agricultural sector, and resource over-extraction (McGlynn, 2011). This is also worsened by the raising demand due to the ever-increasing population (Bešić *et al*., 2011), as such, maintaining adequate water quality is one of today's most pressing challenges in terms of the environment and public health (Kassenga, 2007).

Analysing water quality is crucial for public health. The ideal drinking water should be free of any faecal or harmful microorganisms. A sensitive way of determining the water quality is by detecting faecal indicator bacteria in water. The frequency of bacteria in a water sample is referred to as the bacteriological quality of such water, and it is an indicator of how safe the water is to consume (WHO, 2012).

2.2.1 Parameters of water quality

Various scientific procedures have been developed to evaluate drinking water quality. These procedures include analysis of the physicochemical parameters which form part of the drinking water quality indicators. These parameters include: pH, chloride, phosphorus, and colour, turbidity, alkalinity, electrical, conductivity, nitrate, manganese total hardness and heavy metals. These parameters affect water quality, when their values are above the safe standard limits recommended by the WHO and other regulatory bodies (WHO, 2011).

According to Spellman (2017), there are three water quality parameters and they include:

- Physical
- Chemical and
- Biological parameters.

These parameters are monitored to ensure that quality of water meets regulatory standards and is safe for human use (Rahmanian *et al*., 2015).

2.2.1.1 Physical parameters

Monitoring the physical aspect of water quality is essential to determine if the water is polluted or not. Physical quality of water can be determined by:

a. Turbidity

Turbidity is the cloudiness of water (Adesiji *et al*., 2014). It is the measure of the ability of light to pass through water. High turbidity is usually due to suspended matter including silt, clay and other organic materials in water (Alley, 2007; Omer, 2019). Turbidity in drinking water is esthetically unacceptable as it makes the water look unappetizing. It causes an increase in the cost of water treatment (Davis, 2010), the particulates provide hiding places for pathogens shielding them from disinfection process and suspended particles also provides adsorption media for heavy metals and hazardous organic (Edzwald, 2011).

b. Temperature

Temperature influences some aspects of water quality including viscosity, solubility, odour, and chemical reactions (APHA, 2005). As such sedimentation, chlorination processes and biological oxygen demand (BOD) are dependent on temperature (Davis, 2010). Also, temperature impacts the biosorption process of dissolved heavy metals (Abbas *et al*., 2014).

c. Colour

Decayed organic matter such as vegetation and inorganic matters affects the colour of water, which is detested for aesthetic reasons, rather than health reasons. The colour of water sample is measured by comparing it with standard colour solutions or coloured glass disks. Colour is graded from a scale of 0 (clear) to 70 colour units. Pure water is colourless, which is equivalent to 0 colour units (APHA, 2005).

d. Taste and odour

Presence of foreign matter such as organic materials, inorganic compounds, or dissolved gasses in water can cause a change in taste and odour (Omer, 2019). These materials can be from natural, domestic, or agricultural sources.

e. Solids

Solids occur in water either in solution or in suspension. Glass fibre filters are used to identify the types of solids in water (Tchobanoglous *et al*., 2003). The suspended solids are usually retained on top of the filter while dissolved solids passes through the filter with the water sample. The filtered water sample is then evaporated and the solids residue is known as the total dissolved solids (TDS) (Omer, 2019).

f. Electrical conductivity (EC)

This is a measure of a solution's capacity to conduct electrical current (Tchobanoglous *et al*., 2003). Because ions in solution carry electrical current, conductivity rises as ion concentrations rise (Omer, 2019).

2.2.1.2 Chemical parameters

a. pH

pH is the negative logarithm of the hydrogen ion concentration (Spellman, 2017), a dimensionless number indicating the strength of acidity or basicity of a solution (Hammer, 2011). It is an important parameter of water quality. pH ranges from 0-14, with 7 being neutral. Values below 7 shows acidity, whereas values above 7 indicates basic solutions. Pure water at 25˚C is usually neutral, with a pH around 7.0 (Omer, 2019). Safe pH ranges for drinking water and domestic use are from 6.5 to 8.5 for (WHO, 2011).

b. Dissolved oxygen (DO)

Dissolved oxygen is an important test of water pollution; as such it is an important water quality parameter for streams, rivers and lakes (Omer, 2019). Higher concentration of dissolved oxygen signifies better water quality (Tchobanoglous *et al*., 2003). Dissolved oxygen has no direct effect on public health; however, water with little or no oxygen tastes unpalatable.

c. Biochemical oxygen demand (BOD)

Microorganisms metabolize organic material, consuming oxygen. They breakdown these organic matters into simpler compounds and utilize the released energy for growth and reproduction. During this process, the oxygen consumed is the dissolved oxygen in the water. This need for oxygen is called the biochemical oxygen demand; as such the higher the organic material available in the water, the higher the BOD required by microorganisms (Tchobanoglous *et al*., 2003).

d. Chemical oxygen demand (COD)

The COD is a measure of all organics that is the biodegradable and the non-biodegradable matter (Omer, 2019). COD value of a sample tends to be always higher than its BOD value.

e. Toxic inorganic substances

Various inorganic toxic substances in trace amounts can be detected in water; posing a threat to human health. Some toxic substances are from natural sources however they mostly result from industrial activities and improper management of hazardous waste (Omer, 2019). Toxic inorganic substances are split into two categories:

- Metallic compounds: Includes toxic heavy metals such as selenium, barium, lead, silver, mercury, arsenic, chromium and cadmium (Järup, 2003). Each of which causes wide range of effects that differ from one metal to the other (Campanella *et al*., 2016).
- Non-metallic compounds: This includes nitrates (NO_3-) and cyanides (CN-), Cyanide causes oxygen deprivation by binding the haemoglobin sites, preventing RBCs from conveying oxygen (Davis, 2010). Resulting in a blue skin colour syndrome called cyanosis. It also causes chronic effects on the central nervous system and thyroid.

f. Toxic organic substances

Over 100 compounds found in water have been reported as toxic organic compounds (Davis, 2010). They are naturally found in water and are usually man-made pollutants. They include insecticides, pesticides, solvents, detergents, and disinfectants. They can be measured using gas chromatography (GC), high-performance liquid chromatography (HPLC) and mass spectrophotometry.

2.2.1.3 Biological parameters

Microorganisms are ubiquitous in nature, widely disseminated in all environments (Wiesmann *et al*., 2007). In raw water and also in drinking water there are always specific amounts of microorganisms, most of which are not harmful; however, some of them are pathogenic causing serious health issues under specific conditions.

a. Bacteria

Bacteria are single-celled organisms, they occur in three basic cell forms: bacillus, coccus, and spiral. They reproduce rapidly under favourable conditions; this rapid growth allows visible colonies of bacteria on suitable nutrient medium making it possible to detect and enumerate bacteria in water. Numerous waterborne diseases including typhoid and paratyphoid fever, leptospirosis, tularaemia, shigellosis, and cholera are caused by various species of pathogenic bacteria, such as *Salmonella* sp., *Shigella* sp., pathogenic *Escherichia coli*, *Vibrio cholerae*, *Yersinia enterocolitica* and *Leptospira* sp.

b. Algae

Algae are autotrophic microscopic plants, containing photosynthetic pigments, such as chlorophyll (Wiesmann *et al*., 2007). They are primarily nuisance organisms in the water supply as they affect the taste and odour of water (Alley, 2007). Although certain species have serious environmental and public health impact (Wiesmann *et al*., 2007).

c. Viruses

Viruses are the smallest biological structures known to contain all genetic information necessary for their own reproduction. Infectious hepatitis and poliomyelitis are caused by waterborne viral pathogens (Chakraborty *et al*., 2020). Water treatment and disinfection process typically renders the majority of waterborne viruses inactive (Shannon *et al*., 2010).

d. Protozoa

Protozoa are microscopic single-celled organisms that consume organic substances, bacteria, and algae as food (Pando, 2018). Aquatic protozoa, also known as zooplankton, float freely in the water. They develop cysts that are challenging to destroy during disinfection process.

2.3 Concept of Indicator Organisms

Several species of pathogens are capable of contaminating water as such it is technically difficult, time consuming, impractical and expensive to analyse each species at regular intervals. Therefore, the use of indicator organisms was pioneered to allow a more cost effective, rapid, more frequent and less sophisticated routine analysis of water samples.

Indicator organisms suggest the occurrence of pathogens, when the indicator organism is present in a particular sample (Odonkor and Ampofo, 2013). These organisms are usually used in assessing faecal contamination level in water, instead of identifying all pathogens of concern because the presence of an indicator organism in water indicates probable contamination with other pathogens (Pandey and Soupir 2013; Pandey *et al*., 2014).

The attributes of indicator organisms are:

- their concomitant presence with the pathogens;
- they should be easy to detect and quantify;
- their test methods should be widely available and cost-effective (Odonkor and Ampofo, 2013; Alhamlan *et al*., 2015).

E. coli (most common), coliforms, thermotolerant or faecal coliforms, heterotrophic bacteria, faecal streptococci, and sulphite-reducing anaerobes are among the species regularly utilized as indicators in bacteriological assessments of water quality (Odonkor and Ampofo, 2013).

2.4 *Escherichia coli*

Largest numbers of *Escherichia coli* are found in the intestinal flora of human and animals. *E. coli* is usually analysed separately from coliform bacteria because they tend to appear exclusively with faecal contamination. As such it used as a direct indicator organism for faecal contaminations (OECD/WHO, 2003). The WHO recommended that no detectable amounts of *E. coli* should be found in 100 mL of drinking water (WHO, 2011). Most strains of *E. coli* are harmless, but some strains are pathogenic, due to *E. coli* detection been quite simple, cheap, and can survive longer than other bacteria in water, it is stated as the best indicator for faecal contamination (Jessoe, 2013). As such testing for the presences of *E. coli* is considered extremely in revealing recent faecal contamination, unsanitary processing, and presence of other enteric bacterial pathogens (Odonkor and Ampofo, 2013).

2.5 Water Quality Analysis

2.5.1 Bacteriological assessment of water quality

Monitoring the presence of indicator organisms and pathogens allows for the evaluation of the bacterial quality of water as well as the efficacy of treatment methods. *E. coli* isolation or count, total coliforms count, faecal coliform count, *Pseudomonas* sp. count, and *enterococci* sp. count are the most frequently used bacteriological analyses (Svagzdiene *et al*., 2010). Rompre *et al*. (2002) described a number of methods for enumerating coliforms in water. These include the traditional multiple tube fermentation, membrane filtering methods, and heterotrophic plate count [HPC] tests (Varga, 2011). The ATP test, which analyzes the active microorganisms in water quickly through the detection of adenosine triphosphate (ATP), is another bacteriological test used to evaluate the quality of drinking water

2.6 Water Treatment

Water treatment is required due to the existence of harmful undesirable physical, chemical, and microbiological constituents in water (Edberg, 2005). Microbiological assessment of drinking water mainly covers detection of coliforms and total bacterial count. Presence of coliforms in water is a prominent indicator of possible faecal contamination (Akbar *et al*., 2013; Pantelić *et al*., 2017).

Major microbiological contaminants of water are pathogenic bacteria, such as pathogenic *E. coli* strains, *Salmonella*, *Shigella* and *Vibrio cholerae*; parasites, such as *Cryptosporidium parvum, Giardia lamblia* and pathogenic viruses (Odonkor and Ampofo, 2013). When present at considerable viable levels in drinking water these organisms potentially cause diseases. As such, necessary treatments are required to ensure removal or inactivation of such organisms and other undesirable contaminants (Senior and Dege, 2005).

A number of water treatment methods for the removal or inactivation of pathogens are available. The most common include: Filtration, Reverse osmosis, Distillation, Ozonation, Chlorination and UV radiation (Senior and Dege, 2005). With each treatment method possessing its unique strength and limitation for target group of microorganisms (Edberg, 2005).

Filtration is the most commonly used microbiological treatment method for drinking water. The method uses filters, screens, and granular material or membranes to hold particulate material, including microorganisms. The sizes of particles that accumulate in the filter are usually between 0.001 and 100μm diameter (Senior and Dege, 2005). This method is particularly effective against protozoan parasites (Edberg, 2005).

In addition to changing the mineral composition of water, reverse osmosis also eliminates germs. It is a membrane process that is conceptually similar to filtration, but it uses pumps to

supply the necessary pressure and flow velocity over the membranes instead of filtration (Senior and Dege, 2005). However, this strategy makes it difficult to maintain the membranes properly, which might result in bacterial build-up that creates entry points for contamination (Edberg, 2005).

In distillation, water is boiled and resulting hot vapours are cooled, condensed and collected (APHA, 2012). The treatment normally results in the production of sterile, pathogen-free water if carried out properly. However, inadequate equipment maintenance can result in re-contamination of the water passing through the pipes after leaving the still (Edberg, 2005).

Water can also be treated using ozonation, which utilizes ozone, a potent disinfectant with high energy and short half-life. Due to its powerful oxidizing capacity, which kills bacteria by damaging their cell membranes, this treatment is frequently employed in the bottle water industry. It also oxidizes unwanted minerals, particularly dissolved manganese and iron. The usual effectiveness of ozone treatment against bacteria and viruses does not extend to parasites (Edberg, 2005).

Chlorination is another widely used water treatment method carried out using chemical oxidant chlorine, an economical water treatment chemical. As in other oxidation treatments, the process destroys microbial cells (Senior and Dege, 2005). Trihalomethanes (THMs) are undesirable chemical by-products that result from chlorination treatment after reacting with organic pollutants in water (Rosenfeldt *et al*., 2009). Since excessive chlorine residuals are also harmful to human health, these pollutants are neutralized by applying subsequent activated carbon adsorption (Senior and Dege, 2005). Municipal tap water systems are mostly treated with chlorine either in aqueous hypochlorite or chlorine dioxide gas forms or in combination (Carter *et al*., 2000).

Ultraviolet (UV) radiations are also widely used for water treatment. UV radiation achieves microbicidal activity via the effect of radiation energy at around a 260nm wavelength, causing the destruction of nucleic acid bases resulting in cell death (Edberg, 2005). The absence of chemical by-products after the process is one of the main advantages of using UV treatments.

Whilst each treatment method possesses unique advantages, their anti-microbial activity limitations should be acknowledged. Hence, combined use of these treatments can compensate for each method's limitation and improve the quality of the final water product (Wang *et al*., 2013).

2.7 Water Pollution

Contaminants in drinking water affect water quality and consequently human health. Geological conditions, sewage slots, industrial, agricultural activities, and treatment plants are potential sources of contamination. These contaminants are categorized as microorganisms, inorganics, organics, radionuclides, and disinfectants with the inorganic chemicals holding greater portion as contaminants in drinking water (Azrina *et al*., 2011)

Water pollution is a global problem and poses a serious threat to human life. According to the WHO, more than 3 billion people lack access to clean water for their daily requirements (WHO, 2018). Water sources are easily polluted especially in third world countries where proper environmental sanitation is a challenge (Gyau-Boakye and Dapaah-Siakwan, 2000). Increased usage of metal-based fertilizer in these countries has resulted in continued rise in concentration of metal pollutants in fresh water reservoir due to water run-off (Adefemi and Awokunmi, 2010).

With increasing levels of contamination, the risk of contracting waterborne illnesses rises. Water contaminated with infectious organisms, the majority of which are due to faecal contamination, is the main means of transmission of waterborne infections. As these disease-

causing organisms move through water sources and spread through individuals handling food and water, they are more common in places with inadequate sanitation (Alhamlan *et al*., 2015). These contagious infections primarily come from non-point sources like agriculture, wildlife, and urban runoff as well as point sources like sewage discharge. Point discharges are easily managed by treating the source; however, non-point sources are of serious threat due to their wide dissemination (Alhamlan *et al*., 2015).

The quality of groundwater can deteriorate due to inadequate source protection and poor resource management. Microbial and chemical contaminants have been detected in groundwater (Pritchard *et al*., 2008) and numerous sources of contamination have been pointed out. This includes land disposal of sewage effluents, sludge and solid waste, septic tank effluents, urban runoff and agricultural, mining and industrial practices (Allamin, 2015).

The chemical characteristics of natural waters are a reflection of the soils and rocks with which the water has been in contact (Pantelić *et al*., 2017). Such organic and inorganic compounds may contaminate water (Hartman *et al*., 2015). Organic compounds are derived from living organism as well as industrial sources while chemical contaminants that pollute groundwater, resulting in a long-term influence on public health, microbiological pollution has an immediate effect on a large number of people. Chemical pollutants such as hydrocarbons, metals, cyanide, arsenic, various synthetic chemicals, and soluble forms of nitrogen and phosphorus can all cause distinct types of poisoning (Pritchard *et al*., 2008).

2.8 Waterborne Diseases

Waterborne diseases are directly transmitted by drinking water contaminated with microbial pathogens. Such diseases include cholera, dysentery, typhoid fever, hepatitis A and E, giardiasis, cryptosporidiosis and amoebiasis (Okpasuo *et al*., 2019; Ahmed and Kafayos, 2020). Contaminated water plays an important role in diseases transmission (Katsi *et al*., 2007).

Individuals from third world countries are faced with higher risk of water-borne diseases than those in developed countries (Nazemi *et al*., 2016). Poor environmental sanitation and hygiene plays an important role in the dissemination of waterborne diseases. Ahmed and Kafayos (2020) pointed out that one of the fastest ways of contracting waterborne diseases is through drinking contaminated water. Clasen and Bastable (2003) also reported that contaminated, unclean or tainted water are major causes of morbidity and mortality throughout the world while Ismail and Elnaeem (2017) opined that one of the main causes of death and illness in developing worlds is waterborne disease associated with poor sanitary condition.

In sub-Saharan Africa the most common waterborne diseases are cholera, typhoid, infectious hepatitis, giardiasis, amoebiasis and dracunculiasis, with cholera been the most devastating (Ahmed and Kafayos, 2020). Waterborne diseases are not an exclusive problem affecting human health; they also tend to be a huge social and socioeconomic problem (Ubosi, 2018). Waterborne diseases affect millions of people in Africa (Fenwick, 2006). The WHO estimated that about 3.4 million people, mostly children, die from water-related diseases annually (WHO, 2014). According to the UNICEF (2014) assessment, 4000 children die daily as a result of contaminated water.

Polluted surface waters can contain various pathogenic organisms mostly of faecal origin, from point sources such as municipal wastewater treatment plants (Chigor *et al*., 2010; Odjadjare and Okoh 2010; Nwabor *et al.*, 2016) and drainage from areas where livestock are handled (Förstner and Wittmann, 2012), or from non-point sources such as domestic and wild animal defecation, malfunctioning sewage and septic systems, storm water drainage and urban runoff (Chigor *et al*., 2012).

The most vulnerable population group to waterborne diseases include children below the age of five, elderly individuals, individuals suffering from malnutrition, pregnant woman, immune-

compromised individuals, individuals suffering from chemical dependencies and individuals predisposed to other illnesses. The most common, and ironically the most dangerous, waterborne disease in developing countries is diarrhoea (WHO, 2011; UNICEF, 2018). In developing countries diarrhoea is responsible for approximately 2.5 million deaths annually, mostly affecting children below the age of five (Obi *et al*., 2004).

Most waterborne diseases are caused by enteric pathogens or parasites that are transmitted via the faecal oral route. There spread depends on several factors including: survival of these pathogens in the water, infectious dose of the pathogen, the microbiological and physicochemical quality of the water, presence or absence of water treatment and lastly the season of the year (Theron and Cloete, 2002).

Some natural factors can affect pathogen concentrations in water. They include geographical location, climate, and land use. High temperature and amount of precipitation have been shown to have a correlation with the number of faecal coliform and *enterococci* contamination (Staley *et al*., 2012). Precipitation is a good facilitator for the transfer of contaminants. However, land use impact has a greater influence than climate variations (St Laurent and Mazumder, 2014).

The survival of microorganisms in water is dependent on the availability of nutrient and water temperature. Infectious dose of most waterborne bacterial pathogens is between 10^7 - 10^8 cells, though some enteric pathogens are capable of causing infection at lower doses (Leclerc *et al*., 2002). Viruses are able to survive extended periods in the water and their infectious dose can be as low as 1 to 10 infectious particles. For parasites the infectious dose usually depends on host susceptibility and strain virulence (Masago *et al*., 2002). The infectious dose of *Giardia* might be as low as 10 oocysts and the presence of 30 oocysts of *Cryptosporidium* might be enough to cause infection (Hrudey *et al*., 2003).

Salmonella

Salmonella is a rod-shaped, Gram-negative, facultative anaerobic, non-spore forming and predominantly motile bacteria belonging to the Enterobacteriaceae family (Eng *et al*., 2015). They are often distinguished by the O, H, and Vi antigens. There are over 2600 serotypes of *Salmonella* in the genus that have been identified; the majority of them may adapt to a range of animal hosts, including humans (Ruby *et al*., 2012; Andino and Hanning, 2015; Eng *et al*., 2015; Whiley *et al*., 2017; Liu *et al*., 2018). Most *Salmonella* infections in humans are caused by the *Salmonella* enterica subsp. enterica, which accounts for more than half of the known serotypes (Eng *et al*., 2015).

Worldwide *Salmonella* infection remains a major public health problem, contributing to health and economic burden of both developed and developing countries (Eng *et al*., 2015). *Salmonella* species are a major contributor to intestinal illness around the world and are also the cause of serious systemic illnesses like typhoid and paratyphoid fevers (Levantesi *et al*., 2012). The most frequent manifestation of *Salmonella* infection are gastroenteritis, bacteraemia, and enteric fever. *Salmonella* has been identified as the most frequently isolated food and waterborne pathogen worldwide, causing an estimated 93.8 million food and water borne illnesses and 155,000 fatalities per year. There are no substantial animal reservoirs for the *Salmonella* species that cause typhoid fever and other enteric fevers, thus they are primarily transferred via faecal oral route. Human carriers who are asymptomatic aid in the disease's propagation (Eng *et al*., 2015).

Escherichia coli

Escherichia coli are Gram negative, rod-shaped, facultative anaerobes, they are considered a normal bowel flora of different species of mammals and birds (Gill *et al*., 2006). *Escherichia coli* is the most common specie of the genus *Escherichia* and an important member of the family

Enterobacteriaceae. They are one of the most predominant bacteria in human and animal intestinal tract and are among the foremost bacterial species to colonize the gastrointestinal tract, where they establish themselves as commensals and remain resident throughout the life (Wassenaar, 2016). Commensal strains of *E. coli* seldom cause diseases unless in immuno-compromised hosts. However, various highly adapted strains of *E. coli* have developed specific virulence factor, which confers enhanced capabilities to adapt to new niches, enabling them cause wide range of infections in the gastrointestinal system (Ndlovu *et al*., 2015).

Pathogenic *E. coli* are majorly categorized into five groups: Enterotoxigenic, Enterohemorrhagic, Enteroinvasive, Enteropathogenic and the newly recognized group called Enteroadherentaggregative *E. coli* (Omolajaiye *et al*., 2020).

As a member of the faecal coliform group *Escherichia coli* have been utilized as the primary indicator of faecal contamination in fresh and marine water as its presence indicates the potential presence of other pathogenic bacteria and it also suggests the extent as well as the nature of the contaminants (Khan and Gupta, 2020).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This research was carried out in Paiko town at the Paikoro local government area of Niger State, situated in the South-Eastern region of Niger State (Figure 1). The administrative headquarter of Paikoro is situated in Paiko town. It is bounded by latitude 9° 17' 10″ N and longitudes 6° 35' 1″ E Due to its proximity to important towns like Minna, Suleja, and the capital of the country, Abuja, it is a highly inhabited community that is undergoing very rapid development. The distance between Paiko and Minna is 23km and the distance between Paiko and Abuja is 142km by road. The mean annual rainfall is between 1200mm-1300mm spanning from April to October with maximum rain recorded in August (Aweda *et al.*, 2020).

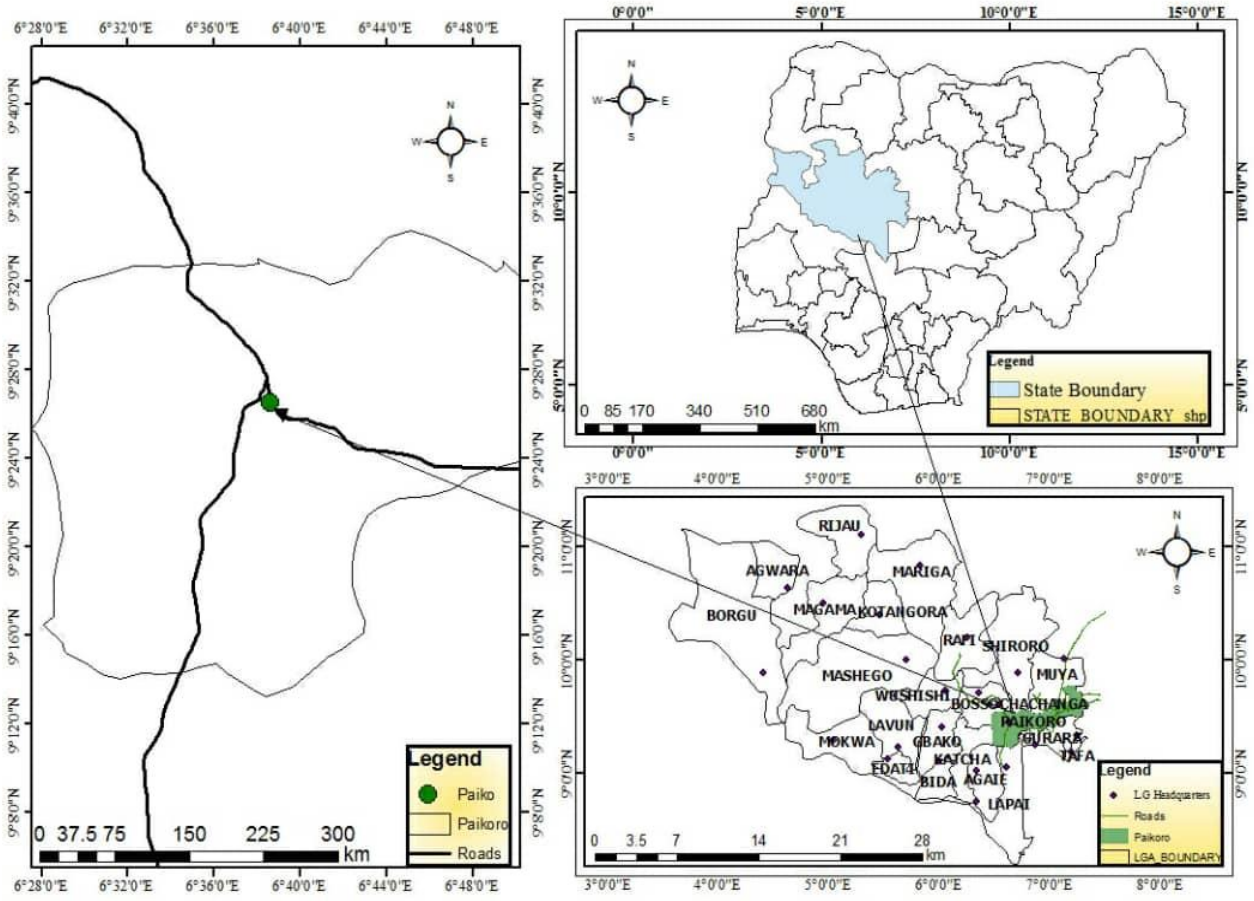

Figure 1: Map of Study Area in Niger State

Source: Geography Department, FUT Minna.

3.2. Sampling and Sample Size

Sixty (60) water samples comprising of 32 private wells and 28 public wells were strategically and randomly selected from 5 different regions in Paiko town. The places are Gbadan, Anguwan Liman, Millionaire's Quarters, Lugodna and P&P. The number of samples size of private and public wells differ because there were more wells situated in household which are meant for private use than those constructed outside for the general use of the public.

3.3. Sample Collection and Processing

The water samples from the wells were collected using 200 mL bottles that were clean and sanitized. The sampling bottle was secured with a suitable-sized stone, opened, and lowered into the well until fully submerged in water without touching the well's sidewalls, hitting the bottom, or upsetting any sediment. The bottle was filled and then removed by rewinding the rope (Chiranjay *et al.*, 2012). All the collected water samples were properly labeled and immediately transported to the Microbiology Laboratory of FUT Minna in an ice pack (Nwankwo *et al.*, 2020). They were then stored in the refrigerator at 4℃ for 18 hours prior to analysis to avoid microbial action affecting their concentration (Bala, 2006; Kihupi *et al*., 2016). All the samples were collected at the peak of the dry season between February and April.

3.4 Microbiological Analysis of the Water Sample

Ten-fold serial dilution was aseptically carried out by transferring 1 mL of the homogenized suspension into a sterile test tube containing 9 mL of sterile, distilled water and subsequent dilutions were made from the above-mentioned dilution (Bala *et al.*, 2016). Using a sterile pipette, 0.1 mL aliquot of the dilution factor of 10^{-4} was aseptically transferred into petri dishes. Using pour plate technique, prepared molten MacConkey agar (about 45°C) was dispensed into replicate plates containing the inoculum, gently swirled and allowed to solidify. The cultured plates were incubated for 24 hours at 37°C. After the incubation period, the faecal coliform growth count was estimated. Distinct colonies on the MacConkey agar plate were aseptically sub-cultured onto freshly prepared solidified selective and differential media, Eosin Methylene

blue (EMB) and incubated at 37°C for 24 hours to obtain a pure bacteria isolate. These pure bacterial isolates were subjected to biochemical tests for further characterization of the presumptive isolates and subsequently, molecular testing for the identification (Rodger *et al*., 2017).

3. 5 Identification of Bacterial Isolates

The faecal coliform bacteria isolated were identified based on colony morphology, Gram staining and various biochemical tests including citrate utilization test, indole test, oxidase test, Voges Proskauer (VP) and methyl red test (Hemraj *et al*., 2013). The procedures are highlighted in Appendix I

3.6 Molecular Identification of Isolates

3.6.1 DNA extraction

Genomic DNA extraction was carried out using Spin Column Bacteria DNA Preparation Kit. Following the manufacturer's instructions, Bacteria cells were harvested from 500 µl aliquot of 10,000g for 1 minute. The residual pellet was re-suspended in 300/µL of suspension Buffer and 2µl of Lysozyme Solution. The mixture was homogenized 30°C for 1 hour. Re-suspended cells were recovered by centrifugation at 10,000g for 1min and lysed by adding 300/µL of Lysis Buffer after which 2/µL RNase A and 8/µL proteinase K solution were added; followed by incubation at 60°C for 10minutes. The tube was cooled on ice for 5minutes. Binding buffer (300µL) was then added to the mixture and vortexed briefly; the mixture was cooled on ice for 5minutes and thereafter centrifuged at 10,000g for 5 minutes. The supernatant was transferred directly into the spin column and centrifuged at 10,000g for 1minutes to trap the DNA. The trapped DNA was washed twice with washing buffer after which it was eluted with 50µl elution buffer into a clean eppendorf tube (Jena Bioscience, 2021).

3.6.2 Polymerase chain reaction (PCR) 16s rRNA amplification

Each PCR reaction mixture consisted of 12.5µl mastermix (2x JENA Ruby hot start mastermix), 1µl 27F 5'AGA GTT TGA TCM TGG CTC AG3' and TAC GGY TAC CTT GTT ACG ACT T 3', 1µl DNA template and 9.5µl reaction volume of 25 µl. PCR amplification was carried out in an Applied Biosystem 2720 Thermocycler. The mixture was subjected to an initial denaturation at 94°C for 3min; followed by 35 cycles of 94°C for 45s, 55°C for 60s and 72°C for 60 seconds and a final termination at 72°C for 10mins and chilled at 4°C. The integrity of the amplified about 1.5 Mb gene fragment was checked on a 1.5% Agarose gel to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel (Rodger *et al*., 2017).

3.6.3 Gel electrophoresis

Agarose gel (1.5%) already prepared was used to confirm positive amplification. Ten microliters of each PCR product were loaded into the wells with the 100bp DNA ladder loaded. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100 bp molecular weight ladder that was ran alongside experimental samples in the gel (Odeyemi *et al*., 2018).

3.6.4 Sequencing

The amplified products were purified and sequenced by Sanger sequencing method using Applied Biosystem (AB1 3500XL) Genetic Analyzer and was done by Inqaba Biotec, Pretoria, South Africa. The resulting sequences were compared in the Basic Local Alignment Search Tool (BLAST) Gene Bank (Inqaba Biotechnology Industry, 2021)

3.7. Antibiotic Susceptibility Profile

Antibiotic susceptibility test was carried out using Kirby Bauer disk diffusion method on Mueller Hinton Agar. Suspension of the test organism was prepared in normal saline to the turbidity of 0.5 McFarland standard and streaked onto Nutrient Agar. Antimicrobial disc of Ampicillin (AMP)10µg; Cephalexin (CEP)10µg; Meropenem (MEM)10µg; Streptomycin (S) 30µg; Sulfamethoxazole-trimethoprim (SXT) 30µg; Ciprofloxacin (CPX) 10µg; Augmentin (AUG) 25µg; and Erythromycin (E) 10µg were applied aseptically on the surface of replicate inoculated plates. The plates were allowed to solidify for a while at room temperature and then incubated at 37°C for about 18-24 hours. The diameter of the zone of inhibition around the antibiotic discs was measured and interpreted in accordance with the breakpoints and criteria recommended by the Clinical Laboratory Standards Institute(CLSI) to determine which is susceptible, intermediate or resistance (CLSI, 2018).

3.8 Physicochemical Properties

All the water samples collected were subjected to physicochemical analysis using Rodger *et al*. (2017) standard method for the examination of the water and wastewater (SMEWW). The procedures are highlighted in Appendix II

3.9 Data Analysis

The data obtained were presented as means ± standard deviation and analysed using statistical analysis software (SAS) Version 9.4. Comparisons between different groups was carried out using one way Analysis of Variance (ANOVA) and their means were separated using Duncan's Multiple Range Test (DMRT) (Yalta, 2008).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Distribution of coliform bacteria from well water samples

Out of the 60 water samples analysed, 36 (60.0%) were positive for bacterial growth while 24 (40.0%) samples had no growth. Both the private and public wells had 18 positive coliform growths and each representing 30.0% respectively. The distribution of the coliform from the well water samples is shown in Table 4.1.

4.1.2 Faecal coliform count

The mean faecal coliform count of Public and Private well water samples from various locations in the study area is shown in Table 4.2. The result shows that private wells of Lugodna area had the highest level of contamination (12.21±4.214cfu/ml) followed by public wells of P&P area (5.71±1.656cfu/ml) and the lowest contaminated wells was in Gbadan area.

4.1.3 Biochemical characterization test

Presumptive characterization of the isolates was carried out based on colony morphology and various biochemical tests. The tests carried out presumed the presence of *Klebsiella* species, *Enterobacter* species, *E. coli*, *Salmonella* species *and Proteus* species as shown in Table 4.3.

Table 4.1: Distribution of Coliform Bacteria from Well Water Samples

Source	NSS	NPS	PP (%)	NNS	PN (%)
Private well	32	18	30.0	14	23.3
Public well	28	18	30.0	10	16.7
Total	60	36	60.0	24	40.0

Key: NSS: Number of Sample Screened; NPS: Number of Positive Samples; PP: Percentage Positive; NNS: Number of Negative Samples; PN: Percentage Negative.

Table 4.2: Mean Faecal Coliform Count of Private and Public Well Water Samples from various Locations

Sample Area	Type of Well Water	Mean Total Colony Count ($x10^4$ cfu/mL)
Gbadan Area	Public Wells	0.14±0.143 [a]
	Private Wells	0.71±0.354 [a]
Angwan Liman Area	Public Wells	2.07±0.934 [b]
	Private Wells	0.93±0.508 [a]
Millionaire's Quarters Area	Public Wells	1.64±0.843 [b]
	Private Wells	0.43±0.228 [a]
Lugodna Area	Public Wells	3.64±1.768 [c]
	Private Wells	12.21±4.214 [e]
P&P Area	Public Wells	8.71±2.736 [d]
	Private Wells	5.71±1.656 [cd]

Values are in ± mean S.E.

(*S.E = Standard error of Mean*)

Values within groups bearing the same superscript are not significantly different at the 5% level ($p<0.05$).

Table 4:3 Biochemical Characterization of the Bacteria Isolated from the Public and Private Wells

Organisms	Gram Reaction	Shape	Indole	MR	Motility	VP	Citrate	Urease
Klebsiella	-	Rod	-	-	-	+	+	+
Enterobacter	-	Rod	-	-	+	+	+	-
Escherichia coli	-	Rod	+	+	+	-	-	-
Salmonella	-	Rod	-	+	+	-	-	-
Proteus	-	Rod	-	+	+	-	+	+

+ Positive: - Negative

4.1.4 Molecular identification of faecal coliform bacteria

Identification of six isolates was carried out using the 16s rRNA sequence analysis. The gel electrophoresis of selected isolates is shown in Plate 1. The sequences obtained were queried in GenBank of the NCBI and the bacterial isolates were identified and confirmed to be *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Escherichia* coli, *Klebsiella aerogenes* and *Citrobacter cronae* strain as shown in Table 4.4 and Plate 1.

4.1.5 Frequency of occurrence of bacteria isolated from public and private well water samples

Klebsiella pneumoniae 22 (33.8%) was the most frequent bacteria isolated followed by *Enterobacter aerogenes* 18 (27.7%), *Escherichia coli* 14 (21.5%) and the least occurring was *Citrobacter cronae* 11 (16.9%). The frequency of occurrence of the bacterial isolated is shown in Table 4.5.

4.1.6 Antibiotic susceptibility profile of isolated *Citrobacter cronae*

The antibiotic susceptibility profile of *Citrobacter cronae* is shown in Table 4.6. *C. cronae* were highly susceptible 11(100%) to meropenem, ciprofloxacin, trimethoprim-sulfamethoxazole, erythromycin and streptomycin. While low level of resistance was observed in cephalexin and ampicillin 2(18.2%).

4.1.7 Antibiotic susceptibility profile of isolated *Enterobacter aerogenes*

Enterobacter aerogenes were highly susceptible 18(100%) to meropenem, ciprofloxacin, trimethoprim-sulfamethoxazole and streptomycin. While significant level of resistance was observed to only cephalexin 8 (44.4%). The antibiotic susceptibility profile of *E. aerogenes* is shown in Table 4.7.

Table 4.4: Basic Local Alignment Search Tool (BLAST) Result of the Selected Isolate

Isolate code	Scientific Name	Max Score	E Value	% Ident	Accession
PRW28	*Enterobacter aerogenes*	1610	0.0	99.45%	NR_102493.2
PRW31	*Escherichia coli*	2115	0.0	98.11%	NR_114042.1
PRW32	*Klebsiella pneumoniae*	1700	0.0	98.87%	NR_117683.1
PUW16	*Enterobacter aerogenes*	1697	0.0	99.26%	NR_102493.2
PUW22	*Citrobacter cronae*	1543	0.0	97.78%	NR_170426.1
PUW26	*Citrobacter cronae*	1669	0.0	99.15%	NR_170426.1

PRW= Private Well; PUW=Public Well

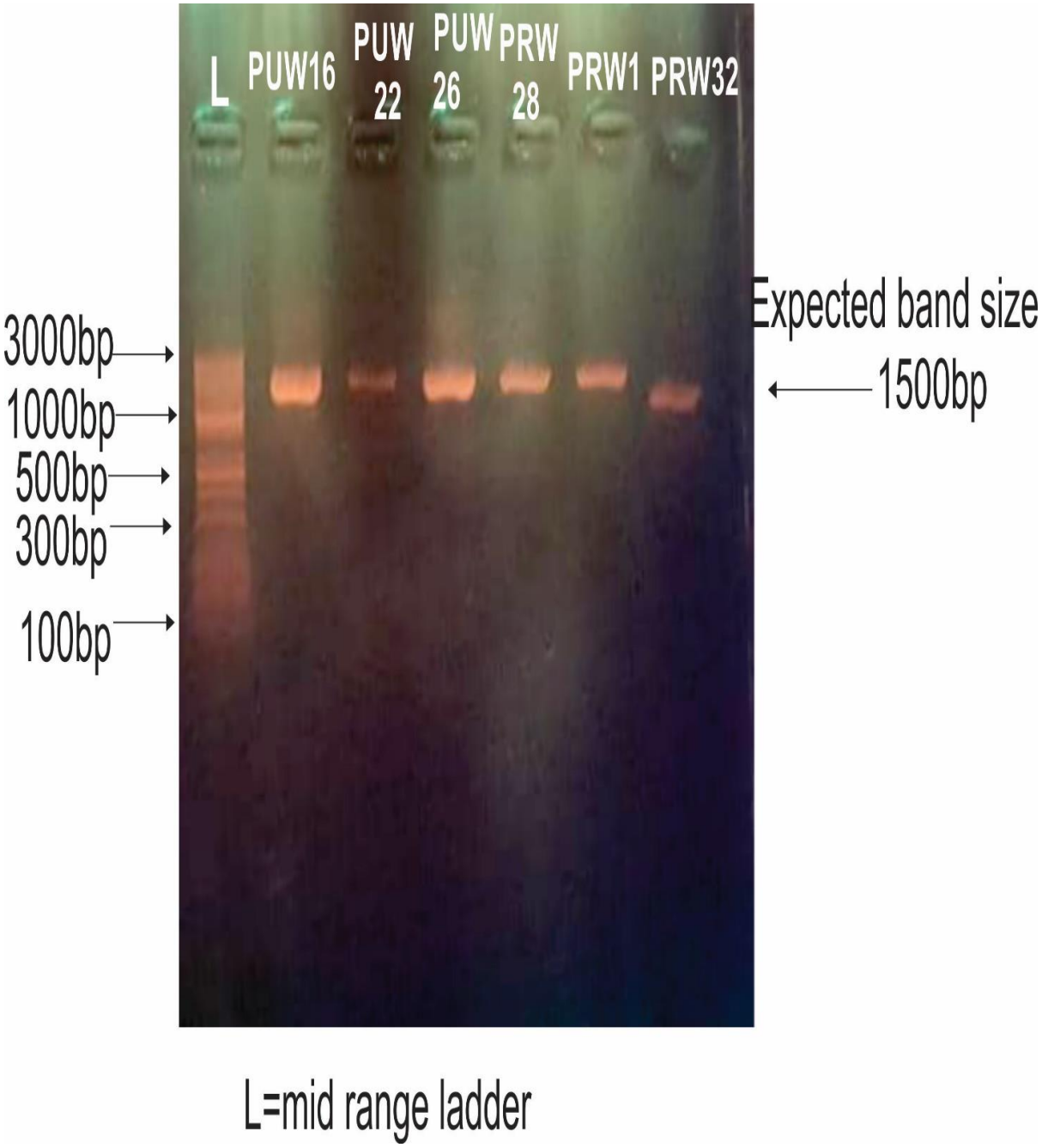

Plate 1: The gel electrophoresis of the bacteria PCR amplicons. The first lane had the DNA ladder followed by the samples tested: PUW 16, PUW 22, PUW 26, PRW 28, PRW 1, PRW 32

Table 4.5: Frequency of Occurrence of Bacteria Isolated from Public and Private Well Water Samples

Isolates	**Frequency**	**%**
Klebsiella pneumoniae	22	33.84
Enterobacter aerogenes	18	27.70
Escherichia coli	14	21.54
Citrobacter cronae	11	16.92
Total	**65**	**100**

Table 4.6: Antibiotic Susceptibility Profile of *Citrobacter cronae*

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
MEM	11 (100.0)	0 (0.0)	0 (0.0)
CPX	11 (100.0)	0 (0.0)	0 (0.0)
SXT	11 (100.0)	0 (0.0)	0 (0.0)
E	11 (100.0)	0 (0.0)	0 (0.0)
S	11 (100.0)	0 (0.0)	0 (0.0)
CEP	7 (63.6)	2 (18.2)	2 (18.2)
AMP	9 (81.8)	0 (0.0)	2 (18.2)
AU	9 (81.8)	2 (18.2)	0 (0.0)

MEM: Meropenem; CPX: Ciprofloxacin; AMP: Ampicillin; CEP: Cephalexin; SXT: Trimethoprim-sulfametoxazole; E: Erythromycin; AU: Augmentin; S: Streptomycin.

Table 4.7: Antibiotic Susceptibility Profile of *Enterobacter aerogenes*

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
MEM	18 (100.0)	0 (0.0)	0 (0.0)
CPX	18 (100.0)	0 (0.0)	0 (0.0)
SXT	18 (100.0)	0 (0.0)	0 (0.0)
E	14 (77.8)	1 (5.6)	3 (16.7)
S	18 (100.0)	0 (0.0)	0 (0.0)
CEP	5 (27.8)	5 (27.8)	8 (44.4)
AMP	10 (55.6)	5 (27.8)	3 (16.7)
AU	9 (50.0)	5(27.8)	4 (22.2)

MEM: Meropenem; CPX: Ciprofloxacin; AMP: Ampicillin; CEP: Cephalexin; SXT: Trimethoprim-sulfametoxazole; E: Erythromycin; AU: Augmentin; S: Streptomycin.

4.1.8 Antibiotic susceptibility profile of isolated *E. coli*

The isolated *E. coli* were highly susceptible to meropenem, ciprofloxacin, trimethoprim-sulfamethoxazole 14 (100%) and streptomycin 13 (92.9%). Moderate level of resistance was observed to cephalexin 5 (35.7%). The antibiotic susceptibility profile of *E. coli* is shown in Table 4.8.

4.1.9 Antibiotic susceptibility profile of isolated *Klebsiella pneumoniae*

The antibiotic susceptibility profile of *Klebsiella pneumonia*e is shown in Table 4.9. Isolated *K. pneumoniae* were highly susceptible to meropenem, ciprofloxacin 22 (100%), trimethoprim-sulfamethoxazole 21 (95.5%) and streptomycin 20 (90.9%). While a significant level of resistance was observed in cephalexin 10 (45.5%).

4.1.10 Antibiotic susceptibility profile of all the bacterial isolates

The isolates obtained from the well water samples were highly susceptible to meropenem 65 (100%), ciprofloxacin 65 (100%), trimethoprim-sulfamethoxazole 64 (98.5%) and streptomycin 59 (90.8%). While significant level of resistance was observed in only cephalexin 25 (38.5%). The antibiotic susceptibility profile of the bacterial isolates is shown in Table 4.10.

4.1.11 Physicochemical properties of public and private well water samples

The results of the physicochemical properties of public and private well water samples from Paiko local government are presented in Table 4.11.

Table 4.8: Antibiotic Susceptibility Profile of *E. coli.*

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
MEM	14 (100.0)	0 (0.0)	0 (0.0)
CPX	14 (100.0)	0 (0.0)	0 (0.0)
SXT	14 (100.0)	0 (0.0)	0 (0.0)
E	8 (57.1)	6(42.9)	0 (0.0)
S	13 (92.9)	1 (7.1)	0 (0.0)
CEP	4 (28.6)	5 (35.7)	5 (35.7)
AMP	9 (64.3)	3 (21.4)	2 (14.3)
AU	9 (64.3)	5(35.7)	0 (0.0)

MEM: Meropenem; CPX: Ciprofloxacin; AMP: Ampicillin; CEP: Cephalexin; SXT: Trimetoprim-sulfametoxazole; E: Erythromycin; AU: Augmentin; S: Streptomycin.

Table 4.9: Antibiotic Susceptibility Profile of *Klebsiella pneumoniae*

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
MEM	22 (100.0)	0 (0.0)	0 (0.0)
CPX	22 (100.0)	0 (0.0)	0 (0.0)
SXT	21 (95.5)	0 (0.0)	1 (4.5)
E	17 (72.3)	2 (9.1)	3 (13.6)
S	20 (90.9)	1 (4.5)	1 (4.5)
CEP	6 (27.2)	6 (26.1)	10 (45.5)
AMP	15 (68.1)	4 (18.2)	3 (13.6)
AU	13 (59.1)	4 (18.1)	5 (22.7)

MEM: Meropenem; CPX: Ciprofloxacin; AMP: Ampicillin; CEP: Cephalexin; SXT: Trimethoprim-sulfametoxazole; E: Erythromycin; AU: Augmentin; S: Streptomycin.

Table 4.10: Antimicrobial Susceptibility Profile of all the Bacterial Isolates

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
MEM	65 (100.0)	0 (0.0)	0 (0.0)
CPX	65 (100.0)	0 (0.0)	0 (0.0)
SXT	64 (98.5)	0 (0.0)	1 (1.5)
E	50 (76.9)	9 (13.8)	6 (9.2)
S	59 (90.8)	3 (4.6)	1 (1.5)
CEP	22 (33.8)	18 (27.7)	25 (38.5)
AMP	43 (66.2)	12 (18.5)	10 (15.4)
AU	40 (61.5)	16 (24.6)	9 (13.8)

MEM: Meropenem; CPX: Ciprofloxacin; AMP: Ampicillin; CEP: Cephalexin; SXT: Trimethoprim-sulfamethoxazole; E: Erythromycin; AU: Augmentin; S: Streptomycin.

Table 4.11: Physicochemical Properties of Private and Public Well Water Samples from Paiko Local Government Area

Study Location Area	Type of Well Water	Ph	Turbidity (NTU)	Electrical Conductivity (µs/cm).	TDS (mg/L)	Temp (˚C)	TSS (mg/L)	BOD (mg/L)	COD (mg/L)
Gbadan Area	Public Wells	7.14±0.025 ^{ab}	9.50±4.50 ^{a}	504.00±111.0 ^{ab}	328.0±72.0 ^{abc}	27.70±1.0 ^{b}	4.00±2.0 ^{a}	0.40±0.0^{abc}	100.50±3.50 ^{a}
	Private Well	7.46±0.26 ^{b}	0.00±0.0 ^{a}	605.00±33.0 ^{ab}	393.4±21.6^{abc}	27.70±1.0 ^{b}	2.00±2.0 ^{a}	0.20±0.00 ^{a}	0.00±0.00 ^{a}
Angwan Liman Area	Public Wells	7.34±.065^{b}	13.0±13.0 ^{a}	275.51±274.5 ^{a}	461.5±205.5^{bc}	27.60±1.10^{b}	7.50±5.50^{a}	0.60±0.00 ^{ab}	102.00±2.0^{f}
	Private Well	7.12±0.68 ^{ab}	23.0±14.0 ^{a}	294.5±293.50 ^{a}	540.0±158.0 ^{c}	27.60±1.10 ^{b}	44.0±18.0^{a}	0.20±0.0 ^{a}	0.0±0.0 ^{a}
Millionaire's quarters Area	Public Well	6.92±0.12 ^{ab}	2.63±2.63 ^{a}	326.00±116.0 ^{a}	212.0±75.0^{abc}	28.75±0.05 ^{b}	4.07±0.07 ^{a}	1.40±1.0 ^{ab}	5.50±0.50 ^{b}
	Private Well	6.95±0.045 ^{ab}	11.0±11.0 ^{a}	270.50±18.5^{a}	176.00±12.0^{ab}	28.75±0.05^{b}	11.5±9.50 ^{a}	1.25±0.05 ^{ab}	90.00±2.0 ^{e}
Lugodna Area	Public Well	6.48±0.19 ^{a}	18.0±15.0 ^{a}	574.00±122.0^{ab}	373.0±79.0^{abc}	28.75±0.50^{b}	24.0±17.0 ^{a}	0.70±0.30 ^{ab}	40.00±5.00^{c}
	Private Well	6.32±0.26^{a}	50.0±50.0 ^{b}	475.50±143.5^{a}	309.0±93.0 ^{abc}	28.75±0.50^{b}	2.50±2.50^{a}	1.20±0.00^{ab}	70.00±3.0 ^{d}
P&P Area	Public Well	6.80±0.07 ^{ab}	132.0±132.0^{c}	477.50±74.5 ^{a}	310.5±48.5 ^{abc}	28.70±0.10^{b}	145.5±140.5^{a}	1.55±0.45 ^{b}	0.00±0.00 ^{a}
	Private Well	6.96±0.08 ^{ab}	45.0±35.0^{b}	315.00±149.0 ^{a}	69.10±38.9 ^{a}	28.70±0.10^{b}	1.50±0.50 ^{a}	1.55±0.45 ^{b}	38.00±3.0 ^{c}

WHO STANDARDS FOR WATER	6.5 – 8.5	5.00	1000	500.0	25.00	>250.0	<5.00	5.0±0.00

KEYS: TDS: Total Dissolved solids; TEMP: Temperature; TSS: Total Suspended solids; BOD: Biological oxygen demand; COD: Chemical Oxygen Demand.

Values are in ± mean S.E. (*S.E = Standard error of Mean*)

Values within Groups bearing the same superscript are not significantly different at the 5% level ($p<0.05$).

4.2 Discussion

4.2.1 Distribution of positive coliform bacteria from well water samples

This study investigates the occurrence of faecal bacteria and antibiotics resistance patterns in public and private wells in Paiko. From the study, it shows that there was an overall high distribution of Positive coliform bacteria growth 36(60.0%) out of the 60 water samples collected and analyzed from the study area. This exceeded the values recommended by (WHO, 2011) international standard, which states that no sample should contain faecal coliform or *E. coli* in drinking water source This result confirms the report of Aweda *et al*. (2020) who observed the same from the research study carried out in Paiko. The reason for these may be attributed to the sample collection which was done during the peak of dry season March – April and also because faecal coliform bacteria are thermotolerant bacteria which are able to grow and thrive in high temperature. Omotoso *et al*. (2018) reported that in dry season the volume of water from wells are reduced thereby increasing the concentration of microorganism. Edokpayi *et al*. (2018) confirms the same reports and further stated of *E. coli* was predominant during dry season. This contradict Nnane *et al.* (2011) report stating that greater incidence of pathogen load was more likely to occur during rainy season when there was more incidence of rainfall.

4.2.2 Faecal coliform count

Total coliform count from the study area shows Gbadan area having a lower coliform count (0.14±0.143 and 0.71±0.354/cfu) compare to Lugodna area which had the highest coliform (3.64±1.768 and 12.21±4.214/cfu) count followed by P&P (8.71±2.736 and 5.71±1.656/cfu) in both the public and private wells. The water sample of Gbadan, Lugodna and P&P are significantly different at the 5% level (p>0.05). The result may be attributed to the clustering together of the building in the areas which is a characteristic of a slum with improper waste disposal and open defecation. Stream filled with debris and defecation was observed in the area

and there could have been an inflow of these stream water into the ground water thereby causing contamination. Some of the wells from the study area were observed to be left uncovered or had covers with cracks and improperly kept fetchers which could have also led to the contamination of the well water. Moyo *et al*. (2004) made similar observation of unprotected well water sources been susceptible to external contamination from surface runoff, windblown debris, human and animal faecal pollution and unsanitary collection methods in Zakat district, Zimbabwe.

Due to limitations in space, crowding and poor drainage network, pit latrines were observed to be extensively used in some of the study area. The seepage from these underground pits into nearby wells might have led to higher distribution of bacteria in the private wells. This observation agrees with a report by Aromolaran and Amodu (2021) which attributed the close proximity of hand dug wells in residential area to pit latrines and soak away, a major repository for pathogenic microorganism which is a source of contaminations in well water.

4.2.3 Molecular identification of faecal coliform bacteria

The sequencing result of the six bacterial isolates carried out using PCR revealed over 90 percent identity after blasting at the gene bank. The coliform bacteria identified were *Klebsiella pneumoniae, Enterobacter aerogenes, Escherichia coli* and *Citrobacter cronae* as shown in Table 4.4. The isolation and identification of *Klebsiella pneumoniae* strain from the wells of the study area is a likely indication of faecal contamination in the wells. This is similar to a report by Aromolaran and Amodu (2021) who molecularly identified *K. pneumoniae* from the water samples collected from private hand-dug wells in various communities within Ondo town, Southwest Nigeria and Bello *et al.* (2017) who also identified *K. pneumoniae* in sachet water in Ondo town. A study carried out by Ribeiro *et al*. (2017) reported the presence of *Citrobacter portucalensis* another *Citrobacter* species from the well water samples in Cantanhede City, Center of Portugal.

4.2.4 Frequency of occurrence of bacteria isolated from well water samples

The frequency of the bacteria isolated and identified from the study area are *Klebsiella* sp. (33.8%), *Enterobacter* sp. (27.7%), *E. coli* (21.5%) and *Citrobacter* sp. (16.9%). Data obtained from this study had similar bacteria but different frequencies of occurrence from those carried out in ground water source in Argentina by Liliana *et al*. (2008), where *E. coli* (37.5%) had the highest frequency followed by *Klebsiella* (23,5%), *Enterobacter* (17.3%) *and Citrobacter* (12.3%). Laniyan *et al*. (2016) also reported high occurrence of E. coli (37.7%) followed by *Proteus* (14.6%) and the least been *Klebsiella* sp. from hand dug wells in some areas of Ibadan, Oyo state. The difference in the rate of occurrences from this study could be attributed to the difference in environment, locations and life style of the people in the respective regions. However, this study is in agreement with Emmanuel *et al*. (2015) findings which reported a higher prevalence of 100% *Klebsiella pneumoniae* in all the well water samples collected from the study areas in Zaria. Chiranjay *et al*. (2012) also recorded a significant prevalence of *Klebsiella* sp. in well water samples of Karnataka, India.

The presence of these coliform bacteria in the wells could be a result of defective wells and these defects could come in the form of a missing cover or defective well cover which was observed in most of the wells in the study area. It may also be as a result of contaminant seepage through cracks or holes inside and outside the well casing, which allows water that has not been filtered through the soil to enter the well which is common in wells made of concrete, clay tile, or brick as reported by New York State Department of Health (NYSDH, 2017)

Klebsiella species are thermotolerant coliforms found in the feces of healthy humans and animals, and are easily detected in sewage-polluted water. Their presence signifies possible faecal contamination and likely presence of other pathogens (Emmanuel *et al*., 2015). *Klebsiella* are well-established opportunistic pathogens and are not normally considered as

causative agents of intestinal disease. Nevertheless, these organisms have been occasionally reported as potential pathogens in acute gastroenteritis (Guglielmettia and Bartoloni, 2003)

The presence of *E. coli* is considered a definitive indicator of faecal contamination in drinking water. The natural habitat of these organisms is the intestinal tract of humans and higher animals. This indicator is used to assess the potential public health risk of drinking water, a key element of most drinking water quality guidelines (WHO, 2004).

Citrobacter spp. occurs in the intestine of humans and animals and shed in the feces of both human and animals. It can also be found in varied environments such as water, soil, and sewage as reported by Oberhettinger *et al.* (2020) who reported *Citrobacter* as opportunistic pathogens causing wound infections, abscesses, severe forms of meningitis, endocarditis or bloodstream infections.

Enterobacter are ubiquitous in nature; their presence in the intestinal tract of animals result in their wide distribution in soil, water, and sewage. It has been found to live in various wastes, hygienic chemicals, and soil. They are also found in plants. In humans, multiple *Enterobacter* species are known to act as opportunistic pathogens (Rogers, 2020). *Enterobacter aerogenes* can cause gastrointestinal infections, urinary tract infections (UTIs), skin and soft tissue infections, respiratory infections, and adult meningitis

The presence of these coliform bacteria in the wells of Paiko indicates that the water is not fit for drinking and the risk of contracting water-borne disease is high. Positive faecal coliform especially *E. coli* are considered indication of faecal pollution in wells as reported by New York State Department of Health (NYSDH, 2017).

4.2.5 Antibiotic susceptibility profile of all bacterial isolates

All the bacterial isolates identified from this study were highly susceptible to meropenem 65 (100%), ciprofloxacin 65(100%), trimethoprim-sulfamethoxazole 64(98.5%) and streptomycin

62(95.4%). While a high level of resistance to cephalexin 35(53%) a first-generation cephalosporin was recorded as shown in Table 4.10. Rizzo *et al*. (2019) reported a similar resistance of *Enterobacter* sp, *E. coli*, *Klebsiella* sp, *and Citrobacter* sp to cephalosporin in healthcare associated infections in California, US.

In this study also, a significant level of *Klebsiella pneumoniae* resistance to cephalexin antibiotics 11(47.8%) was recorded. This correlates with a similar study by Ghartimagar *et al*. (2020) who reported that *klebsiella pneumoniae* from ground water source of Kathmandu Valley, Nepal was highly resistant to Cefazolin which is also a first-generation cephalosporin of the same class with cephalexin. Aromolaran and Amodu (2021) recorded similar resistance of *klebsiella pneumoniae* also to cefuroxime, augmentin, and ampicillin antibiotics from some well water samples of Ondo town.

The high level of cefalexin resistance in this study area may be associated with misuse and over use of antibiotics among human and also indiscriminate use of antibiotics in the treatment of animals. This could result into the antibiotics been shed in the faeces of both man and animal thus, directly or indirectly contaminating drink water source (WHO 2020).

4.2.6. Physicochemical properties of the well water samples.

Physicochemical properties of the water sample from the study area as shown in Table 4.13 indicates the pH to be within permissible limits of 6.5-6.8 by World Health Organization (WHO). Turbidity of Gbadan private well and Millionaire's quarters public well 0.00±0.0 and 2.63±2.63 were within the acceptable range of WHO while the remaining public and private wells were significantly higher at (p>0.05) than the recommended WHO limit value of 5.0 NTU. High turbidity may indicate presence of disease-causing pathogenic bacteria which causes symptoms such as, cramps, nausea, diarrhoea and headaches as reported by Maitera *et al*. (2010). Similar study by Alexander *et al*. (2019) stated that turbidity is been caused by the

presence of suspended insoluble materials such as clay and silt particles, discharges industrial wastes or sewage, or the presence of large numbers of microorganisms mainly occurring in surface water which makes them unfit for use. Excessive turbidity protects microorganisms from effects of disinfectants and stimulates the growth of bacteria in water has reported by Alexander (2008).

Electrical conductivity parameters were all significantly lower at (p>0.05) 270.50±18.5 - 605.00±33.0µs/cm than the set standard of Nigeria Agency for Food and Drug Administrative Control (NAFDAC) and WHO which is 1000µs/cm. Alexander *et al*. (2019) reported a similar study carried out in Michika and Environs, Adamawa State. Electrical conductivity is a quantitative measure of the ability of water to pass electric current. Total dissolve salt parameters were all observed to be significantly lower at (p>0.05) 69.10±38.9 – 461.5±205.5mg/L than the WHO set standard of 500mg/L, except for private well of Angwan Liman with 540.0±158.0mg/L which was a bit higher at (p>0.05) than the permissible standard which is an indication that they are unsuitable for drinking.

The temperature of all the water samples were a bit above 25°C, the acceptable standard range by Standard Organization of Nigeria (SON, 2007). It has been suggested by WHO (2006) that solar radiation, clear atmosphere and low water level increases the temperature of water body. Total suspended solid (TSS) parameters of the samples were lower than the permissible standard of (WHO) meaning the water samples were devoid of suspended solid. Biochemical oxygen demand (BOD_5) of all the samples were within the acceptable standard by WHO. The Chemical oxygen demand (COD) parameters of private well samples of Gbadan, Angwan Liman as well as public well samples of Millionaire's quarters and P&P were all within the permissible range of WHO, while the other wells sample were significantly above the permissible limits. High level of Chemical oxygen demand may be due to the presence of chemicals that could be organic or inorganically caused by the inflow of domestic, livestock

and industrial waste that contains elevated levels of organic pollutants as reported by Jannat (2019).

CHAPTER FIVE

5.0 CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION TO KNOWLEDGE

5.1 Conclusion

The results of this study revealed the presence of *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Escherichia coli* and *Citrobacter cronae* in the well water sample taken from Paiko, Niger State.

There was a significant occurrence of these faecal bacteria in both the public and private wells with each having a positive percentage distribution of 30.0% respectively. This therefore, makes the well water unsafe for human consumption and a potential health risk to the consumers.

The bacteria isolated showed more resistance to cephalexin but were susceptible to meropenem, ciprofloxacin, trimethoprim-sulfamethoxazole and streptomycin which indicate that they are not multiple drug resistance bacteria.

The physicochemical properties of the water samples from some of the wells in this study area were significantly different at the 5% probability level than the permissible accepted limit of WHO and SON which indicates that the water from these wells were unfit for drinking.

5.2 Recommendations

The following recommendations were made from the findings of this study:

i. Disinfection such as boiling, chlorination, using ultraviolet rays or ozonation is recommended before using the water from these wells for drinking and domestic purposes.
ii. The National Environmental Standards and Regulation Enforcement Agency, The Nigerian Industrial Standards Organization and other regulatory bodies responsible for

well construction and water quality must take necessary steps to ensure proper citing, construction, and maintenance of wells to minimize contamination.

iii. Periodic assessment of well water quality should be done routinely to eliminate or reduce the health risks on individuals and communities as a whole.

iv. Public enlightenment should be constantly carried out on the health risk implication of poor maintenance of well in homes and communities.

v. There is need for the government to provide potable water to the public to avoid outbreak of cholera like it's been witnessed in recent times.

vi. Further comparative studies of public and private wells involving more water samples and isolates during rainy and dry season is required to better understand the occurrence of faecal coliform bacteria in wells and to also track the source of the faecal contamination using microbial source tracking technique (MST).

5.3 Contributions of Research to Knowledge

This research contributed to the body of knowledge by the creation of awareness that the majority of well water in Paiko are not suitable for drinking and when consumed, could pose health challenges to the populace.

It also showed the importance of using molecular method for the evaluation of drinking water sources for the detection of possible pathogenic bacteria.

Awareness of the possibility of waterborne disease-causing pathogens resistance to cephalosporin antibiotics in Paiko town was also indicated in the research.

REFERENCES

Abaje, I. B., Ati, O. F. & Ishaya, S. (2009). Nature of portable water supply and demand in Jema's local Government area Kaduna State, Nigeria. *Research Journal of Environment and Earth Science*, 1(1), 16-21.

Abbas, S. H., Ismail, I. M., Mostafa, T. M., & Sulaymon, A. H. (2014). Biosorption of heavy metals: a review. *Journal of Chemical Sciences and Technology*, 3(4), 74-102.

Adefemi, S. O., & Awokunmi, E. E. (2010). Determination of physico-chemical parameters and heavy metals in water samples from Itaogbolu area of Ondo-State, Nigeria. *African Journal of Environmental Science and Technology*, *4*(3).

Adesiji, A. R., Adeoye, P. A., & Gbadebo, A. O. (2014). Investigating the effects of sewage on the groundwater quality in Minna, Niger State, Nigeria. *Ethiopian Journal of Environmental Studies and Management*, *7*(1), 82-89.

Adeyemi, O., Oloyede, O. B., Oladiji, A. T., (2007). Physicochemical and microbial characteristics of leachate-contaminated groundwater. *Asian Journal of Biochemistry*, 2 (5), 343–348.

Adzitey, F., Nafisah, S., & Haruna, A. (2015). Antibiotic susceptibility of *Escherichia coli* isolated from some drinking water sources in Tamale Metropolis of Ghana.

Ahmed, A. A., & Kafayos, Y. (2020). Prevalence of Waterborne Diseases in Bade, Nguru and Machina Local Government Areas of Yobe State-Nigeria. *International Journal of Tropical Disease & Health*, 35-46.

Akbar, A., Sitara, U., Khan, S. A., Muhammad, N., Khan, M. I., Khan, Y. H., & Kakar, S. U. R. (2013). Drinking water quality and risk of waterborne diseases in the rural mountainous area of Azad Kashmir Pakistan. *International Journal of Biosciences*, *3*(12), 245-251.

Akter, T., Jhohura, F. T., Akter, F., Chowdhury, T. R., Mistry, S. K., Dey, D., & Rahman, M. (2016). Water Quality Index for measuring drinking water quality in rural Bangladesh: a cross-sectional study. *Journal of Health, Population and Nutrition*, *35*(1), 1-12.

Al-Bratty, M., Arbab, I. A., Alhazmi, H. A., Attafi, I. M., Javed, S. A. & Al-Rajab, A. J. (2017). ICP-MS determination of trace metals in drinking water sources in Jazan area. Saudi Arabia Current World Environment, 12 (1), 6–16.

Alexander, P. (2008) Evaluation of ground water quality of Mubi town in Adamawa State Nigeria. *African Journal of Biotechnology*. 7(11), 1712-1715.

Alexander, P., Bwatanglang, I. B. & Daniel, J. (2019). Assessment of Physicochemical and Bacteriological Parameters of Borehole and Hand Dug Well Water in Michika and Environs, Adamawa State, Nigeria. *Microbiology Research Journal International*. 28(6), 1-11.

Alhamlan, F. S., Al-Qahtani, A. A., & Al-Ahdal, M. N. A. (2015). Recommended advanced techniques for waterborne pathogen detection in developing countries. *The Journal of Infection in Developing Countries*, *9*(02), 128-135.

Allamin, I. A. (2015). Physicochemical and bacteriological analysis of well water in Kaduna Metropolis, Kaduna State. *Open Access Library Journal*, *2*(06), 1.

Alley, E. R. (2007). *Water quality control handbook*. McGraw-Hill Education.

Andino, A., & Hanning, I. (2015). Salmonella enterica: survival, colonization, and virulence differences among serovars. *The Scientific World Journal*, *2015*.

American Public Health Association (APHA). (2005). Standard Methods for the Examination of Water and Wastewater, 21st ed. American Public Health Association, Washington, D.C

American Public Health Association (APHA). (2012). Standard Methods for the Examination of Water and Wastewater Faecal Coliform (Thermotolerant Coliform) Procedure (pp. 9-74). Washington, DC: American Public Health Association, American Water Works Association, Water Environment Federation.

Aromolaran, O. & Amodu, O. A. (2021). Antibiotic susceptibility pattern of Klebsiella pneumoniae and Pseudomonas aeruginosa isolated from some drinking wells in Ondo town southwest Nigeria. *Journal of Applied Science and Environment Management*. 25 (1), 59-63. doi: https://dx.doi.org/10.4314/jasem.v25i1.8

Aweda, A. K., Amadi, A. N., Kyari, R., Samuel, A. & Samson, B. A. (2020). Investigation of Groundwater Quality from Selected Wells in Paiko, North central Nigeria. *Journal of Geography and Geology*, 12 (1), doi:10.5539/jgg.v12n1p1.

Ayantobo, O. O., Oluwasanya, G. O., Idowu, O. A., & Eruola, O. A. (2012). Water quality evaluation of hand-dug wells in Ibadan, Oyo State, Nigeria. *Special Publication of the Nigerian Association of Hydrological Sciences*, 231-239.

Azrina, A., Khoo, H. E., Idris, M. A., Amin, I., & Razman, M. R. (2011). Major inorganic elements in tap water samples in Peninsular Malaysia. *Malaysian Journal of Nutrition*, *17*(2), 271-276.

Bademosi, A. (2018). Why outbreak of waterborne diseases persists in North Central States. Available online at: https://www.tribuneonlineng.com/164805/. Accessed on the 24th April, 2021.

Bala J. D., (2006). Occurrence of Faecal Coliforms in Water Sources in Jimeta-Yola. Nigeria. *Journal of Environmental Sciences*, 10 (2), 64-69.

Bala, J. D., Kuta, F. A., Adabara, N. U., Abioye, O. P., Adelere, I. A., Abdulsalam, R.,Adel, A.S. Al-Gheethi, Kaizar, H. & Mamman, L. H (2016). Bacteriological and Physicochemical Assessment of Packaged water sold in Minna, Niger state, Nigeria. *International Journal of Applied Biological Research*. 7(2), 43–50.

Bates, A. J. (2000). Water as consumed and its impact on the consumer—do we understand the variables? *Food and chemical toxicology*, *38*, S29-S36.

Bazié, J. B. (2014). Accès à l'eau: l'Afrique entre abondance et pénurie. *Après-demain*, (3), 28-29.

Bello, O. O., Oni, M. O., Bello, T. K. & Amoo, O. T. (2017). Occurrence of antibiotic resistant bacteria in sachet and bottled water brands in Ondo City, Nigeria. *International Journal of Advanced Research and Publications,* 1(6), 58-68.

Bešić, A., Obradović, Z., Pašalić, A., & Žilić, A. (2011). Microbiological composition of untreated water during different weather conditions. *Journal of health sciences, 1*(2), 68-74.

Campanella, B., Onor, M., D'Ulivo, A., Giannecchini, R., D'Orazio, M. & Petrini, R. (2016). Human exposure to thallium through tap water: A study from Valdicastello Carducci and Pietrasanta (northern Tuscany, Italy). *Science of the Total Environment*, 548:33-42

Carter, J. T., Rice, E. W., Buchberger, S. G., & Lee, Y. (2000). Relationships between levels of heterotrophic bacteria and water quality parameters in a drinking water distribution system. *Water Research, 34*(5), 1495-1502.

Chakraborty, M., Vyas, S., Bhalerao, U., Gangopadhayya, A., Srivastava, M., Barman, M. K., & Bhukya, P. (2020) Occurrence and fate of viruses in water. In *Legacy, Pathogenic and Emerging Contaminants in the Environment* (pp. 147-180). CRC Press.

Chigor, V. N., Umoh, V. J., Okuofu, C. A., Ameh, J. B., Igbinosa, E. O., & Okoh, A. I. (2012). Water quality assessment: surface water sources used for drinking and irrigation in Zaria, Nigeria in a public health hazard. *Environmental Monitoring and Assessment, 184*(5), 3389-3400.

Chigor, V. N., Umoh, V. J., Smith, S. I., Igbinosa, E. O., & Okoh, A. I. (2010). Multidrug resistance and plasmid patterns of Escherichia coli O157 and other E. coli isolated from diarrhoeal stools and surface waters from some selected sources in Zaria, Nigeria. *International Journal of Environmental Research and Public Health, 7*(10), 3831-3841.

Chiranjay, M., Shashidhar, V., Vanadana, K. E., Shamanth, A. S. & Afrin, S. (2012). Microbial quality of well water from rural and urban households in Karnataka, India: A cross-sectional study. *Journal of Infection and Public Health*, 1(3), 221-268.

Clasen, T. F., & Bastable, A. (2003). Faecal contamination of drinking water during collection and household storage: the need to extend protection to the point of use. *Journal of water and health, 1*(3), 109-115.

Clinical and Laboratory Standards Institute (CLSI). (2018). Performance standards for antimicrobial disk susceptibility tests; Approved standard. Clinical and Laboratory Standards Institute, Wayne, PA, (12), M02-A13.

Conseil des Ministres Africains de l'Eau (CMAE). (2008). Un Aperçu de la situation de l'eau potable et de l'assainissement en Afrique (An Overview of the Situation of Drinking Water and Sanitation in Africa). Conseil des Ministres Africains de l'Eau, African Ministers' Council on Water, Sharm El Sheikh, Egypt.

Davis, M. L. (2010). *Water and Wastewater Engineering: Design Principles and Practice.* McGraw-Hill Education.

Desouky, A. H., Kheiralla, Z. H., Zaki, S., Rushdy, A. A. & Abd-El- Raheim, W. (2003). Multiplex-PCR and PCR-RFLP assays to monitor water quality against pathogenic bacteria. *Journal of Environmental Monitoring,* 5, 86.

Edberg, S. (2005). Microbiology of treated bottled water. In D. Senior & N. Dege (Eds.), *Technology of Bottled Water* (2nd ed.). Oxford: Blackwell Publishing Ltd, (2) 4-6.

Edessa, N., Geritu, N., & Mulugeta, K. (2017). Microbiological assessment of drinking water with reference to diarrheagenic bacterial pathogens in Shashemane Rural District, Ethiopia. *African journal of microbiology research*, *11*(6), 254-263.

Edokpayi, J. N., Odiyo, J. O., Popoola, E. O. & Msagati, T. A. M. (2018). Evaluation of microbiological and physicochemical parameters of alternative source of drinking water: a case study of Nzhelele River, South Africa. *The Open Microbiology Journal* 12, 18–27

Edzwald, J. (2011). *Water quality & treatment: a handbook on drinking water*. McGraw-Hill Education.

European Food Safety Authority (EPSA). (2010). Scientific opinion on Dietary Reference Values for water. *European Food Safety Authority (EFSA) Journal*, *8*(3), 1459.

Emmanuel, A. A., Fatima, J. G. & Giwa, A. (2015). Microbiological assessment of well waters in Samaru, Zaria, Kaduna, State, Nigeria. *Annals of African Medicine*, 14, 32-38, doi:10.4103/1596-3519.148732

Eng, S. K., Pusparajah, P., Ab Mutalib, N. S., Ser, H. L., Chan, K. G., & Lee, L. H. (2015). Salmonella: a review on pathogenesis, epidemiology and antibiotic resistance. *Frontiers in Life Science*, *8*(3), 284-293.

Ezeh, C. C., Udourioh, G. A., Echoke, J. C., & Matthews-Amune, O. C. (2020). Well Water Quality Assessment: A Case of Gaba and Zuma Communities, Bwari Area Council, Abuja, Nigeria. *Journal of Applied Sciences and Environmental Management*, *24*(6), 967-974.

Fenwick, A. (2006). Waterborne infectious diseases—could they be consigned to history? *Science*, *313*(5790), 1077-1081.

Food Safety News (2021). Breaking News for Everyone. News Desk. https://www.foodsafetynews.com/2021/08/cholera-outbreak-kills-800-in-nigeria/ Accessed on the 10th September, 2021.

Förstner, U., & Wittmann, G. T. (2012). *Metal pollution in the aquatic environment*. Springer Science & Business Media.

Fricker, C. R. (2003). The presence of bacteria in water after regrowth. *Applied Environmental Microbiology*, 43, 49–60.

Gantzer, C., Maul, A., Audic, J. M. & Chwartzbrod, L. (1998). Detection of infectious enteroviruses, enterovirus genomes, somatic coliphages, and Bacteroides fragilis phages in treated wastewater. *Applied Environmental Microbiology*, 64 (11), 4307–4312.

Ghartimagar, S., Khatri, P., Neupane, S., Joshi, D. R., & Joshi, T. P. (2020). Evaluation of Ground Water Quality of Kathmandu Valley and Antibiotic Susceptibility test against Klebsiella pneumoniae. *Tribhuvan University Journal of Microbiology*, 7, 83–90.

Gill, S. R., Pop, M., DeBoy, R. T, Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., Gordon, J. I., Relman, D. A., Fraser-Liggett, C. M. & Nelson, K. E. (2006). Metagenomic analysis of the human distal gut microbiome. *Science*, 312(5778), 1355–9.

Girones, R., Ferrus, M. A., Alonso, J. L., Rodriguez-Manzano, J., Calgua, B., de Abreu Corre^a, A. & Bofill-Mas, S. (2010). Molecular detection of pathogens in water–the pros and cons of molecular techniques. *Water Research*, 44(15), 4325-4339.

Guglielmetti, P. & Bartoloni, A. (2003). Escherichia coli. Food Poisoning by Species other than *Escherichia coli*. *Encyclopedia of Food Science and Nutrition*. 2, 2166-2169. Doi:https://doi.org/10.1016/B0-12-227055-X/00415-6

Gyau-Boakye, P., & Dapaah-Siakwan, S. (2000). Groundwater as source of rural water supply in Ghana. *Journal of Applied Science and Technology*, *5*(1), 77-86.

Hammer, M. J (2011). Water and Wastewater Technology. 7th ed. Upper Saddle River: Pearson education.

Hartman, L. J., Coyne, S. R., & Norwood, D. A. (2015) Development of a novel internal positive control for Taqman based assays. *Molecular Cell Probes*, 19, 51–59.

Hemraj, V., Diksha, S., & Avneet, G. (2013). "A review on commonly used biochemical test for bacteria," *Innovare Journal of Life Sciences*, 1(1), 1-7

Hrudey, S. E., Payment, P., Huck, P. M., Gillham, R. W., & Hrudey, E. J. (2003). A fatal waterborne disease epidemic in Walkerton, Ontario: comparison with other waterborne outbreaks in the developed world. *Water Science and Technology*, *47*(3), 7-14.

Idowu, A. O., Oluremi, B. B., & Odubawo, K. M. (2011). Bacteriological analysis of well water samples in Sagamu. *African Journal of Clinical and Experimental Microbiology*, *12*(2).

Ikem, A., Osibanjo, O., Sridhar, M. K. C., & Sobande, A. (2002). Evaluation of groundwater quality characteristics near two waste sites in Ibadan and Lagos, Nigeria. *Water, Air, and Soil Pollution*, *140*(1), 307-333.

Inqaba Biotechnology Industry (Pty) Ltd. (2021). Africa's Genomics Company. Sanger Sequencing. https://inqababiotec.co.za/services/sanger-sequencing/

Ismail, M. I. H. & Elnaeem, S. H. E. (2017). Epidemiology of water borne diseases and the knowledge, attitudes and practices of basic school children towards them in El-Obeid, North Kordofan State, Sudan. *European Journal of Pharmaceutical and Medical Research*. 4, 233-237.

Jannat, N., Mottalib, M. A. & Alam, M. N. (2019). Assessment of Physicochemical Properties of Surface Water of Mokeshbeel, Gazipur, Bangladesh. *Journal of Environmental Science Current Research, 2, 014.*

Järup, L. (2003). Hazards of heavy metal contamination. *British medical bulletin*, *68*(1), 167-182.

Jena Bioscience. (2021). Molecular Biology, DNA Clean up, PCR Purification Kit - Column Kit. Spin-column based DNA cleanup from PCR samples. Jena Bioscience, Jena, Germany. Retrieved from https://www.jenabioscience.com/molecular-biology/dna-cleanup/pp-201-pcr-purification-kit-column-kit

Jessoe, K. (2013). Improved source, improved quality? Demand for drinking water quality in rural India. *Journal of Environmental Economics and Management*, *66*(3), 460-475.

Jidauna, G. G., Dabi, D. D., Saidu, B., Abaje, B., & Ndabula, C. (2013). Assessment of well water quality in selected location in Jos, Plateau State, Nigeria. *International Journal of Marine, Atmospheric & Earth Sciences*, *1*(1), 38-46.

Joao, P. S. C (2010). Water Microbiology, Bacterial Pathogen and Water. *International Journal of Research and Public Health*. 7(10), 3657-3703 doi: 10.3390/ijerph7103657

Johnson, G. C., Zimmerman, T. M., Lindsey, B. D., & Gross, E. L. (2011). Factors affecting groundwater quality in the Valley and Ridge aquifers, eastern United States, 1993-2002: U.S. *Geological Survey Scientific Investigations Report*, 5115, 70.

Kassenga, G. R. (2007). The health-related microbiological quality of bottled drinking water sold in Dar es Salaam, Tanzania. *Journal of water and health*, *5*(1), 179-185.

Katsi, L., Siwadi, J., Guzha, E., Makoni, F. S., & Smits, S. (2007). Assessment of factors which affect multiple uses of water sources at household level in rural Zimbabwe–A case study of Marondera, Murehwa and Uzumba Maramba Pfungwe districts. *Physics and Chemistry of the Earth, Parts A/B/C*, *32*(15-18), 1157-1166. doi: http://dx.doi.org/10.1016/j.pce.2007.07.010.

Khan, F. M., & Gupta, R. (2020). *Escherichia coli* (*E. coli*) as an Indicator of Faecal Contamination in Groundwater: A Review. In *International Conference on Sustainable Development of Water and Environment* (pp. 225-235). Springer, Cham.

Kihupi, C. S. M., Yohana, L., Saria, J. A., & Malebo, H. M. (2016). Faecal contamination of drinking-water in Tanzania's commercial capital, Dar Es Salaam: implication on health of the consumers. *SM Journal of Public Health and Epidemiology*, 2(1), 1025-9.

Kumpel, E., & Nelson, K. L. (2014). Mechanisms affecting water quality in an intermittent piped water supply. *Environmental science & technology*, *48*(5), 2766-2775.

Laniyan, T. A., Olatunji, A. S., Bayewu, O. O. Mosuro, G. O., Odukoya, M. O., Kenjinu, S. & Odunuga, S. T. (2016). Physicochemical assessment and bacteriological studies of hand-dug wells of major markets in southwest Nigeria. *Arabian Journal of Geoscience*, 9, 261-269. https://www.infona.pl/resource/bwmeta1.element.springer-doi-10_1007-S12517-015-2105-Z

Leclerc, H., Schwartzbrod, L., & Dei-Cas, E. (2002). Microbial agents associated with waterborne diseases. *Critical Reviews in Microbiology*, *28*(4), 371-409.

Levantesi, C., Bonadonna, L., Briancesco, R., Grohmann, E., Toze, S., & Tandoi, V. (2012). Salmonella in surface and drinking water: occurrence and water-mediated transmission. *Food Research International*, *45*(2), 587-602.

Liang, J. L., Dziuban, E. J., Craun, G. F., Hill, V., Moore, M. R., Gelting, R. J., . . . Roy, S. L. (2006). Surveillance for waterborne disease and outbreaks associated with drinking water and water not intended for drinking - United States, 2003-2004. *Morbidity and Mortality Weekly Report*, *55*(SS12), 31-65.

Liliana S. L., Jose M. A., & Luis L. M (2008). Occurrence of antimicrobial-resistant Enterobacteriaceae in water from different sources in a subtropical region of Argentina. *Interdisciplinary Journal of Applied Science*. 2(2), 28-32.

Liu, H., Whitehouse, C. A., & Li, B. (2018). Presence and persistence of Salmonella in water: the impact on microbial quality of water and food safety. *Frontiers in Public Health*, *6*, 159.

Maitera, O. N., Ogugbuaja, V.O., Barminas, J. T. (2010). An assessment of the organic pollution indicator of river Benue in Adamawa state, Nigeria. *Journal of Environmental Chemistry and Ecotoxicology*, 2(10), 110-116.

Malhotra, S., Sidhu, S. K., & Devi, P. (2015). Assessment of bacteriological quality of drinking water from various sources in Amritsar district of northern India. *The Journal of Infection in Developing Countries*, *9*(08), 844-848.

Marathe, N. P., Pal, C., Gaikwad, S. S., Jonsson, V., Kristiansson, E., & Larsson, D. J. (2017). Untreated urban waste contaminates Indian river sediments with resistance genes to last resort antibiotics. *Water research*, *124*, 388-397.

Mari, L., Casagrandi, R., Rinaldo, A., & Gatto, M. (2018). Epidemicity thresholds for water-borne and water-related diseases. *Journal of theoretical biology*, *447*, 126-138.

Martin, R., De Lorenzo, V., & Cohewell, R. R. (2008). Molecular assessment of bacterial pathogens, a contribution to drinking water safety. *Current Opinion in Biotechnology*. 19(3), 274–280. Doi 10.1016/j.copbio.2008.04.004

Masago, Y., Katayama, H., Hashimoto, A., Hirata, T., & Ohgaki, S. (2002). Assessment of risk of infection due to Cryptosporidium parvum in drinking water. *Water science and technology*, *46*(11-12), 319-324.

McGlynn, C. D. (2011). An island environment: Saltwater intrusion, groundwater management and water privatization in Cebu (Doctoral dissertation, Rutgers, The State University of New Jersey-New Brunswick).

Morenikeji, W., Sanusi, Y. A. & Jinadu, A.M. (2000). The Role of Private Voluntary Organizations in Community and Settlement Development in Niger State, A Research Report Submitted to the Centre for Research and Documentation, Kano.

Moyo, S., Wright, J., Ndamba, J. and Gundry, S. (2004). Realising the maximum health benefits from water quality improvements in the home: a case from Zaka district, Zimbabwe. *Physics and Chemistry of the Earth*. 29: 1295-1299.

Mpenyana-Monyatsi L., Onyango, M. S., Momba, M. N. B (2012). Groundwater quality in South African Rural Community: a Possible Threat to Public Health. *Polish Journal Environmental Studies*, 21(5), 1349-1358.

National (Nigeria) Action Plan for Antimicrobial Resistance (AMR) 2017-2022 (2017). Federal Ministries of Agriculture, Environment and Health, Nigeria. Available online at: https://ncdc.gov.ng/themes/common/docs/protocols/77_1511368219.pdf (Accessed Date: 28th March, 2020).

Nazemi, K., Salari, S., & Eskandani, M. A. (2016). Prevalence of *Salmonella* spp. in water sources of sistan: a descriptive cross-sectional study. *Journal of Experimental Biology*

and Agricultural Sciences, 4, 748-755. DOI: http://dx.doi.org/10.18006/2016.4(VIS).748.755

Nigeria Demographic and Health Survey (NDHS). (2014). National Population Commission (NPC) [Nigeria] and ICF International Nigeria Demographic and Health Survey 2013. Abuja, Nigeria, and Rockville, Maryland, USA: NPC and ICF International.

Ndlovu, T., Le Roux, M., Khan, W. & Khan, S. (2015). Co-Detection of Virulent *Escherichia coli* Genes in Surface Water Sources. *Public Library of Science One* 10(2), e0116808, doi: 10.1371/journal.pone.0116808

New York State Department of Health, Center for Environmental Health (NYSDH). (2017). https://www.health.ny.gov/environmental/water/drinking/coliform_bacteria.htm. (Accessed Date: 1st December, 2021)

Nnane, D. E., Ebdon, J. E. & Taylor, H. D. (2011). Integrated analysis of water quality parameters for cost-effective faecal pollution management in river catchments. *Journal of Water Research*, 45(6), 2235- 2246.

Nwabor, F. O., Nnamonu, E. I., Martins, P. E., & Ani, O. C. (2016). Water and water borne diseases: a review. *International Journal of Tropical Diseases & Health*, 12(4), 1-14

Nwankwo, C. E. I., Barika, P. N., Akani, N. P., & Amadi, O. N. (2020). Physicochemical and mycological examination of groundwater in Rumuosi community, Rivers State, Nigeria. *Research Journal Microbiology*, 15, 82-89.

Obaje, N. G (2009*). Geology and Mineral Resources of Nigeria. Lecture Notes in Earth Sciences*. Published by Springer Dordreccht Heidelberg, London. New York.

Oberhettinger, P. Schüle, L., Marschal, M., Bezdan, D., Stephan, O., Dörfel, D., Vogel, W., John, W. R., Willmann, M., & Peter, S.1 (2020). Description of *Citrobacter cronae* sp. nov., isolated from human rectal swabs and stool sample. *International Journal of Systematic and Evolutionary Microbiology*, 70, 2998–3003. Doi:10.1099/ijsem.0.00410

Obi, C. L., Bessong, P. O., Momba, M. N. B., Potgieter, N., Samie, A., & Igumbor, E. O. (2004). Profiles of antibiotic susceptibilities of bacterial isolates and physico-chemical quality of water supply in rural Venda communities, South Africa. *Water SA*, *30*(4), 515-519.

Obi, C. N. & Okocha, C. O. (2007). Microbiology and physicochemical analysis of selected borehole waters. *Journal of Engineering and Applied Science*, 25(7), 920-929.

Odeyemi A. T., Ayantola K. J. & Peter S. (2018). Molecular Characterization of Bacterial Isolates and Physicochemical Assessment of Well Water Samples from Hostels at Osekita, Iworoko-Ekiti, Ekiti State. *American Journal of Microbiological Research*, 6(1), 22-32.

Odjadjare, E. E., & Okoh, A. I. (2010). Physicochemical quality of an urban municipal wastewater effluent and its impact on the receiving environment. *Environmental monitoring and assessment*, *170*(1), 383-394.

Odonkor, S. T., & Ampofo, J. K. (2013). *Escherichia coli* as an indicator of bacteriological quality of water: an overview. *Microbiology research*, *4*(1), 5-11, doi:10.4081/mr.2013.e2

Organisation for Economic Co-operation and Development (OECD/WHO). (2003). Assessing Microbial Safety of Drinking Water. Improving Approaches and Methods. Organisation for Economic Co-operation and Development (OECD). World Health Organization (WHO). Paris, France.

Ogundipe, S., Obinna, C & Olawale, S (2017). Nigeria faces disease epidemics as 63m lack access to safe water. Vanguard News Nigeria. https://www.vanguardngr.com/2017/06/nigeria-faces-disease-epidemics-63m-lack-access-safe-water/ (Accessed on 12th November, 2021).

Okpasuo, O. J., Okafor, F. C., Aguzie, I., Ikele, C., & Anunobi, J. (2019). Spatiotemporal trend of waterborne disease in Enugu Urban, Nigeria: A retrospective study. *International Journal of tropical disease & Health*, 1-13.

Olasehinde, P. I. (2010). The Groundwater of Nigeria: A Solution to Sustainable National Water Needs. Inaugural Lecture Series 17, Federal University of Technology, Minna. September, 2010.

Oluwasanya, G. (2009), Better Safe than Sorry: Towards Appropriate Water Safety Plans for Urban Self Supply Systems in developing countries, Phd. Thesis, Cranfield University. 459 pp.

Omer, N. H. (2019). Water quality parameters. *Water quality-science, assessments and policy*, 18.

Omofonmwan, S.I. and Eseigbe, J.O.(2009) "Effects of Solid Wastes on the Quality of Underground Water in Benin Metropolis, Nigeria". *Journal of Humanities and Ecology* Vol. 26(2) 99-105.

Omolajaiye, S. A., Afolabi, K. O. & Iweriebor, B. C. (2020). Pathotyping and Antibiotic Resistance Profiling of Escherichia coli Isolates from Children with Acute Diarrhea in Amatole District Municipality of Eastern Cape, South Africa. *Biomedical Research International*, Article ID 4250165, https://doi.org/10.1155/2020/4250165

Omotoso, A. J., Dada, E. O., & Oyedeji, O. C. (2018). Physicochemical and Microbiological Assessment of Selected Hand-Dug Wells for Water Quality in Ilesa Metropolis, Southwest Nigeria. *Notulae Scientia Biologicae*, 10(1):52-59. doi: 10.15835/nsb10110058

Onifade, A. K. & Ilori, R. M. (2008). Microbiological analysis of sachet water vended in Ondo State, Nigeria. *Environmental Research Journal*., 2, 107-110.

Osuntokun and Komolafe (2019). Antibiotics Resistance Pattern of Bacteria Isolated from faecal Pellets of Supella longipalpa (cockroach) found in Akungba Akoko, Ondo State. Nigeria. *Journal of Analytical Techniques and Research*. 1(1), 010-024. doi: 10.26502/jatri.003

Pachepsky, Y., Shelton, D. R., McLain, J. E., Patel, J., & Mandrell, R. E. (2011). Irrigation waters as a source of pathogenic microorganisms in produce: a review. *Advances in agronomy*, *113*, 75-141.

Pal, M., Ayele, Y., Hadush, M., Panigrahi, S., & Jadhav, V. J. (2018). Public health hazards due to unsafe drinking water. *Air Water Borne Disease*, *7*(1000138), 2.

Pandey, P. K., & Soupir, M. L. (2013). Assessing the impacts of E. coli laden streambed sediment on E. coli loads over a range of flows and sediment characteristics. *JAWRA Journal of the American Water Resources Association*, *49*(6), 1261-1269.

Pandey, P. K., Kass, P. H., Soupir, M. L., Biswas, S., & Singh, V. P. (2014). Contamination of water resources by pathogenic bacteria. *Applied Microbiology and Biotechnology Express*, *4*(1), 1-16.

Pando, Y. (2018). Microorganisms and Biological Behavior of Living Organisms. *Microreviews in Cell and Molecular Biology*, 4(2).

Pantelić, N. Đ., Dramićanin, A. M., Milovanović, D. B., Popović-Đorđević, J., & Kostić, A. Ž. (2017). Evaluation of the quality of drinking water in Rasina district, Serbia: physicochemical and bacteriological viewpoint. *Romanian Journal of Physics*, *62*(9-10).

Pritchard, M., Mkandawire, T., & O'neill, J. G. (2008). Assessment of groundwater quality in shallow wells within the southern districts of Malawi. *Physics and Chemistry of the Earth, Parts A/B/C*, *33*(8-13), 812-823.

Rahmanian, N., Ali, S. H. B., Homayoonfard, M., Ali, N. J., Rehan, M., Sadef, Y., & Nizami, A. S. (2015). Analysis of physiochemical parameters to evaluate the drinking water quality in the State of Perak, Malaysia. *Journal of Chemistry*, *2015*. http://dx.doi.org/10.1155/2015/716125

Raphael, O., John, O. O., Sandra, U. I. & Sunday, A. C. (2018). Assessment of borehole water quality consumed in Otukpo and its environs. *International Journal of Ecological Science and Environmental Engineering*, 5 (3), 71–78.

Ribeiro, T. G., Gonçalves, B. R., da Silva, M. S., Novais, A., Machado, E., Carrico, J. A. & Peixe, L. (2017). *Citrobacter portucalensis* sp. nov., isolated from an aquatic sample. *International Journal of Systematic and Evolutionary Microbiology*, 67, 3513–3517. doi:10.1099/0.002154

Rizzo, K., Horwich-Scholefield, S., & Epson, E. (2019). Carbapenem and Cephalosporin Resistance among Enterobacteriaceae in Healthcare-Associated Infections, California, USA. *Emerging Infectious Diseases*, 25(7), 1389-1393.

Rodger, B. B., Eugene W. R., & Steven, P. (2017). Standard Methods for the Examination of Water and Wastewater analysis, SMEWW, 23rd Edition, American Public Health Association Washington D.C. https://doi.org/10.2105/SMWW:2882.002 Accessed on the 30th Dec,2021.

Rogers,K.(2020).EnterobacterEncyclopediaBritannica,https://www.britannica.com/science/Enterobacter. Accessed 2nd December, 2021.

Rompre, A., Servais, P., Baudart, J., de-Roubin, M. R., & Laurent, P. (2002). Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. *Journal of microbiological methods*, 49(1), 31-54. doi:10.1016/s0167-7012(01)00351-7

Rosenfeldt, E. J., Baeza, C., & Knappe, D. R. (2009). Effect of free chlorine application on microbial quality of drinking water in chloraminated distribution systems. *Journal-American Water Works Association*, *101*(10), 60-70.

Roy, R. (2019). An introduction to water quality analysis. *ESSENCE International Journal Environmental Rehabilitation. Conserv. IX (1),* 94-100.

Ruby, T., McLaughlin, L., Gopinath, S., & Monack, D. (2012). *Salmonella* long-term relationship with its host. *FEMS microbiology reviews*, *36*(3), 600-615.

Seamus C. (2000), Hand dug shallow wells, principles of Hand dug wells. *Series manuals on drinking water supply.* Vol. 5, pp 29-33.

Senior, D. A. G & Dege, N. (2005) Technology of Bottled Water. Blackwell, Oxford. ISBN 1-4051-2038-X

Shannon, M. A., Bohn, P. W., Elimelech, M., Georgiadis, J. G., Marinas, B. J., & Mayes, A. M. (2010). Science and technology for water purification in the coming decades. Nanoscience and technology: *a collection of reviews from nature Journals*, 337-346.

Shimizu, T., Agatomo H., & Kigotake T. (1980). Bacteria contamination of drinking water from wells in Miyazaki, Miyazaki University, Japan, Pp 21-28.

Singh, S., Raju, N. J., & Ramakrishna, C. (2015). Evaluation of groundwater quality and its suitability for domestic and irrigation use in parts of the Chandauli-Varanasi region, Uttar Pradesh, India. *Journal of Water Res. Protection*, *7*(7): 572-587.

Sivaranjani, S., Rakshit, A., & Singh, S. (2015). Water Quality Assessment with Water Quality Indices. *International Journal of Biological Science. 2*(2): 85-94.

Spellman, F. R. (2017). *The drinking water handbook*. Third Edition. United State. CRC Press eBook.

St Laurent, J., & Mazumder, A. (2014). Influence of seasonal and inter-annual hydro-meteorological variability on surface water faecal coliform concentration under varying land-use composition. *Water Research*, *48*, 170-178.

Staley, C., Gordon, K. V., Schoen, M. E., & Harwood, V. J. (2012). Performance of two quantitative PCR methods for microbial source tracking of human sewage and implications for microbial risk assessment in recreational waters. *Applied and environmental microbiology*, *78*(20), 7317-7326.

Standard Organization of Nigeria (SON) (2007). Nigerian Standard for Drinking Water Quality. Abuja, Nigeria: SON Publishing Company, 554, 5-22.

Svagzdiene, R., Lau, R., & Page, R. A. (2010). Microbiological quality of bottled water brands sold in retail outlets in New Zealand. *Water Science and Technology*, 10, 689-699.

Tchobanoglous, G., Burton, F. L., & Stensel, H. D. (2003). Wastewater engineering treatment and reuse (No. 628.3 T252s). Boston, US: McGraw-Hill Higher Education.

Tekpor, M., Akrong, M., Asmah, M. H., Banu, R. A. & Ansa, E. D. O. (2017). Bacteriological quality of drinking water in the Atebubu-Amantin district of the Brong-Ahafo region of Ghana. Applied Water Science, 7, 2571–2576.

Theron, J., & Cloete, T. E. (2002). Emerging waterborne infections: contributing factors, agents, and detection tools. *Critical Reviews in Microbiology*, *28*(1), 1-26.

Ubosi, N. I. (2018). Prevalence of diarrhoea among infants of child welfare clinics at two teaching hospitals in Lagos, Nigeria. *Journal of Applied Sciences and Environmental Management*, *22*(10), 1707-1711.

United National Educational Scientific and Cultural Organization (UNESCO) (2015). The United World Water Development Report: *Water for a Sustainable World*, Paris, UNESCO. Unsustainable growth, (1), 10-13.

United Nation Children's Fund (UNICEF) (2014) World Water Day 2025: 4,000 children die each day from a lack of safe water., http://www.unicef.org/wash/index_25637.htm

United Nation Children's Fund (UNICEF) (2018) Report on Water, Sanitation and Hygiene. Retrieved from www.unicef.org on September 13, 2021

United Nations Sustainable Development Goals (UNSDG) (2018) Report Retrieved from www.un.org on September 15, 2021

Varga, L. (2011). Bacteriological quality of bottled natural mineral waters commercialized in Hungary. *Food Control*, *22*(3-4), 591-595. doi: 10.1016/j.foodcont.2010.10.009

Vidyasagar, D. (2007). Global minute: water and health–walking for water and waterwars. *Journal of perinatology*, *27*(1).

Vincent, N. C., Veronica, J. U., Stella, I. S., Etinosa, O. I. & Anthony, I. O. (2010). Multidrug resistance and plasmid patterns of *Escherichia coli* 0157 and other *E. coli* isolated from diarrheal stools and surface waters from some selected sources in Zaria, Nigeria. *International of Journal Environment Research and Public Health*, 7, 3831-3841.

Wang, H., Pryor, M. A., Edwards, M. A., Falkinham III, J. O., & Pruden, A. (2013). Effect of GAC pre-treatment and disinfectant on microbial community structure and opportunistic pathogen occurrence. *Water Research*, *47*(15), 5760-5772.

Wassenaar, T. M. (2016). *Escherichia coli*. *European Journal of Microbiology & Immunology*, 6(3), 147-161.

Whiley, H., Gardner, M. G., & Ross, K. (2017). A review of *Salmonella* and squamates (lizards, snakes and amphibians): implications for public health. *Pathogens*, *6*(3), 38.

World Health Organization (2004). Guidelines for Drinking Water Quality, Vol. 1, 3rd edition World Health Organization, Geneva. Available from: http://www.who.int/water_sanitation_health/dwq/gdwq3_contents.pdf. Accessed on the 28th November, 2021.

World Health Organization World Health Organization (WHO). (2006). WHO guidelines for drinking water quality, 3rd (current) edition, including the first addendum. Volume 1. Recommendations. World Health Organization, Geneva

World Health Organization (WHO). (2011). *Guidelines for drinking water quality*, 4th edition. World Health Organization: Geneva.

World Health Organization (WHO). (2012). UN-Water global annual assessment of sanitation and drinking-water (GLAAS) 2012 report: the challenge of extending and sustaining services.

World Health Organization (WHO). (2014) *Progress on Drinking Water and Sanitation*: 2014 Update. World Health Organization, Geneva

World Health Organization (WHO). (2018). Nigeria: Cholera Outbreak. Available online at: https://reliefweb.int/disaster/ep-2017-000077-nga. Accessed on the 16th August, 2021.

World Health Organization (WHO). (2020). Antibiotics Resistance. Available from: https://www.who.int/news- room/fact-sheets/detail/antibiotic-resistance. Accessed on the 30th November, 2021.

Wiesmann, U., Choi, I. S., & Dombrowski, E. M. (2007). Fundamentals of biological wastewater treatment. John Wiley & Sons.

Yalta, A. T. (2008). The accuracy of statistical distributions in Microsoft® Excel 2007. *Computational Statistics & Data Analysis*, 52(10), 4579-4586.

APPENDICES

APPENDIX A

BIOCHEMICAL TEST PROCEDURES

A. Gram staining

A thin smear of the pure 24 hours old culture is prepared on clean grease-free slide; it is allowed to dry and fixed by passing over gentle flame. The smear is then stained by adding 2 drops of crystal violet (Primary stain) solution for 60 seconds and then rinsed with water. The smear is again flooded with Lugol's iodine (Mordant) for 30 seconds and rinsed with water before decolourizing with 70 % alcohol. The smear is then counter stained with Safranin for 60 seconds before rinsing with water, and then allowed to air dry. The smear is mounted on a microscope and observed under oil immersion objective lens (x100). Gram negative cells appear pink or red while gram positive organisms appear purple.

B. Citrate utilization test

Appropriate amount of Simon citrate agar was prepared according to manufacturer's standard (24 g – 1000 mL), dispensed into test tubes (5 mL each) and autoclaved at121°C and 15 psi for 15 minutes. The autoclaved test tubes are allowed to cool and gel in a slanted position. The tubes are then inoculated with the test organism and incubated at 37°C for 24-48 hour. Observe for colour change from green to blue indicating a positive result and a negative result will show no change in colour.

C. Indole test

Appropriate amount of peptone broth is prepared according to manufacturer's standard, dispensed into test tubes (About 4 ml each) and sterilized. Fresh culture of the test organisms (18-24 hrs) is aseptically inoculated into the broth and incubated for 24 hours at *37°C.* After incubation about 0.5 ml of kovac's reagent is added to the broth culture

and observed for the presence or absence of coloured ring at the top of the broth. A positive indole test is indicated by the formation of a pink to red colour ("cherry-red ring") in the reagent layer on top of the medium within seconds of adding the reagent. If a culture is indole negative, the reagent layer will remain yellow or be slightly cloudy.

D. Methyl red test

Appropriate amount of Nutrient broth is prepared according to manufacturer's standard, dispensed into test tubes (About 4 ml each) and sterilized. Fresh culture of the test organisms (18-24 hours) is aseptically inoculated into the broth and incubated for 24 hours at *37°C* change After incubation about 5 drops of the methylred solution is added to the broth culture and observed for colour. Positive results are indicated by a bright red colour and no change is observed for negative results.

E. Oxidase test

A piece of filter paper is placed on a clean petri dish and 3 drops of oxidase reagent is dropped on it. The test organism is smeared on it. An oxidase positive organism will produce a blue-purple colour in 10 seconds, while an oxidase negative organism will produce no colouration.

F. Voges - proskauer test

Appropriate amount of Nutrient broth is prepared according to manufacturer's standard, dispensed into test tubes (About 4 ml each) and sterilized. Fresh culture of the test organisms (18-24 hours) is aseptically inoculated into the broth and incubated *37°C* for 24 hours at. *After incubation about* One millilitre of 40 % KOH and 3 ml of 5 % alpha-naphthol is added and shakened well. Colour formation will be observed. Organism positive for Voges Proskauer gives a pink colour within 2-5 minutes.

APPENDIX B

DETERMINATION OF PHYSICOCHEMICAL PROPERTIES

A. pH

The pH of the water samples was analysed using the Wagtech pH meter. After standardizing the pH meter with a standard solution, 10mL of each of the water samples was poured into a sterile beaker and the anode of the pH meter was dipped into it and readings was obtained when it is stable. The value obtained determines the acidity or alkalinity of the water.

B. Turbidity

Turbidity was measured using DR 2000 (HACH) spectrometer. Freshly prepared distilled water was filled into 25ml sample cell and another containing sample water to be analysed. The spectrophotometer was programmed to the number of turbidities which was 750 and 450mm wavelength. The distilled water was inserted first into the cell holder. The knob was set at zero to standardize the instrument after which the sample was inserted into the cell holder and the “Read” button was pressed and results was displayed in Formazine Turbidity Unit (FTU=NTU).

C. Electrical Conductivity

Conductivity of water indicates concentration of electrolytes. 100mL of distilled water and 100mL of water sample was poured into two separate beakers. The conductivity meter was switched on and its sensor rod was dipped into the beaker containing distilled water to standardize it. It was then further dipped into a second beaker containing water sample and readings was taken and reported in microsiemens/cm.

D. Total Suspended Solid (TSS)

This parameter was determined by pouring 100mL of the sample through a pre-weighted filter (glass fibre) of specific pore size. The filter was again weighed after undergoing drying process which removed all the remnant water in the filter. The weight gain of the filter, is a dry weight

measure of the particulates present in the water samples. Results were reported in milligrams of solids per litre of water (mg/L). TSS was calculated using Equation 3.1

$$TSS\ (mg/L) = \frac{A-B}{\mathrm{V}} \qquad (3.1)$$

Where: A = mass of filter + dried residue (mg)

B = mass of filter (pre-weight) (mg)

V = volume of sample filtered (L)

E. Temperature

Temperature of water samples was determined by a handheld thermometer. A thermometer was dipped in the water samples and allow to stay for 3 minutes after which the reading on the calibration was read and recorded.

F. Total Dissolved Solid (TDS)

A clean and dry evaporating dish was weighed. 100ml of sample was filtered through a filter paper and the filtrate was taken in an evaporating dish. The sample was evaporated on hot water bath. When the whole water was evaporated, the weight of the evaporating dish and the weight of the evaporating dish after cooling in a desiccator was noted and differences was calculated using Equation 3.2.

$$TDS\ (g/L) = \frac{A-B}{\mathrm{V}} X\ 100 \qquad (3.2)$$

Where: A= final weight of evaporating dish (g), B = initial weight of evaporating dish (g), and V= volume of sample taken (ml).

G. Biological Oxygen Demand (BOD_5)

Biochemical Oxygen Demand is the amount of oxygen consumed by microorganisms while they decompose organic matter under aerobic condition at specified temperature. The BOD5 was determined using azide modification of Winkler's method. BOD5 bottle was prepared and incubated at 200C for 5 days in the dark. After five days, the incubated bottle was poured with

mixing 2 mL of orthophosphoric acid. This was shaken gently and titrated with sodium thiosulphate to the end point where there was colour change. The titre value represents dissolve oxygen on day five. Biochemical Oxygen Demand was then calculated as the difference between dissolve oxygen on day one and that on day five.

H. Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is the oxygen requirement of a sample for oxidation of organic and inorganic matter. 50 ml of the water sample was taken in a reflux flask, and 10 mL of potassium dichromate solution with 1 g mercuric sulphate was thoroughly mixed. 10 mL of concentrated sulphuric acid containing silver sulphate was added through the open-end of the condenser carefully and mixed by swirling motion. The reflux apparatus was operated for around 1 hour and allowed to cool. The flask was removed, and its content was diluted to 150 mL with distilled water. To the resulting solution, three drops of the ferroin indicator were added. This sample was titrated with standard ferrous ammonium sulphate to an end point where blue-green colour just changed to reddish-brown. Chemical oxygen demand (COD) of the blank sample was then calculated.

APPENDIX C

Clinical and Laboratory Standard Institute (CLSI) Interpretation Chart for Antibiotics

Antibiotics	Code	Disk content	Susceptible(S)	Intermediate(I)	Resistant (R)
Imipenem	IMP	10µg	≥ 23	20–22	≤ 19
Meropenm	MEM	10µg	≥ 23	20–22	≤ 19
Ciprofloxacin	CPX	10µg	≥21	16–20	≤15
Gentamicin	CN	10µg	≥15	13–14	≤ 12
Trimethoprim sulfamethoxazole	SXT	25µg	≥16	11–15	≤10
Colistin	CT	10µg	≥14	12-13	≤11
Fosfomycin	FOS	50µg	≥ 16	13–15	≤ 12
Amoxicillin-clavulanic acid	AMC	30µg	≥ 18	14–17	≤ 13
Ampicillin	PN	10 µg	≥17	14-16	≤13
Cefpodoxime	CPD	10µg	≥21	18-20	≤17
Nalidixic acid	NA	30µg	≥19	14-18	≤13
Chloramphenicol	CH	30µg	≥18	13-17	≤12
Cefixime	CFM	5µg	≥19	16-18	≤15

Source: Clinical Laboratoray Standard Institutes (CLSI, 2018)

APPENDIX D

DIAMETER OF ANTIBIOTIC ZONES OF INHIBITION IN MILLIMETRES

S/N	SAMPLE CODE	MEM	CPX	SXT	E	S	CEP	PN	AU
1	PRW-3 (1)	35	37	33	17	33	14	-	-
2	PRW-3 (2)	27	34	30	-	24	-	11	-
3	PRW-4 (1)	25	30	27	27	26	18	20	12
4	PRW-4 (2)	25	31	16	-	26	15	13	12
5	PRW-7 (1)	27	30	30	19	25	16	15	16
6	PRW-7 (2)	28	31	33	14	25	16	15	15
7	PRW-11 (1)	27	40	17	33	-	23	19	27
8	PRW-11 (2)	27	35	30	17	22	18	20	20
9	PRW-12 (1)	25	31	29	17	24	22	20	22
10	PRW-12 (2)	25	35	31	30	24	20	21	30
11	PRW-15 (1)	26	30	32	12	20	14	14	17
12	PRW-17 (1)	26	35	30	28	27	25	23	22
13	PRW-18 (1)	30	38	35	27	26	20	13	15
14	PRW-18 (2)	23	33	27	31	17	18	-	14
15	PRW-20 (1)	29	39	35	25	34	27	24	32
16	PRW-20 (2)	28	37	30	22	29	25	29	20
17	PRW-20 (3)	27	35	34	30	32	29	22	24
18	PRW-20 (4)	26	44	22	20	31	27	23	25
19	PRW-22 (1)	29	40	27	26	30	26	25	21
20	PRW-22 (2)	25	27	26	14	23	12	15	17
21	PRW-22 (3)	30	27	10	13	12	-	11	-

22	PRW-22 (4)	32	35	26	12	26	27	15	14
23	PRW-23 (1)	30	32	31	15	27	12	17	11
24	PRW-23 (2)	30	36	28	27	26	34	30	25
25	PRW-25 (1)	33	40	27	17	27	25	22	17
26	PRW-25 (2)	31	36	32	30	27	17	23	20
27	PRW-27 (1)	27	37	30	15	28	28	15	12
28	PRW-27 (2)	35	36	32	33	30	23	22	13
29	PRW-28 (1)	26	30	27	13	26	-	-	-
30	PRW-29 (1)	26	36	28	20	30	25	26	22
31	PRW-29 (2)	26	37	27	24	26	22	19	19
32	PRW-30 (1)	28	35	29	28	27	26	20	22
33	PRW-30 (2)	30	35	29	30	27	32	23	34
34	PRW-31 (1)	29	37	30	20	30	26	22	20
35	PRW-31 (2)	28	32	26	20	22	19	19	21
36	PRW-32 (1)	27	31	27	24	20	25	21	20
37	PRW-32 (2)	27	33	33	22	25	20	22	14
38	PUW-2 (1)	28	31	27	30	24	-	18	17
39	PUW-6 (1)	29	31	23	28	27	25	19	16
40	PUW-6 (2)	30	38	31	29	29	24	23	21
41	PUW-7 (1)	27	30	28	25	21	24	16	18
42	PUW-7 (2)	28	35	27	19	24	22	18	17
43	PUW-8 (1)	30	32	34	36	25	23	17	20
44	PUW-9 (1)	35	32	29	16	29	26	12	25
45	PUW-11 (1)	29	31	27	15	22	15	16	20

46	PUW-12 (1)	30	40	35	30	30	27	17	29
47	PUW-13 (1)	29	31	25	17	25	15	15	17
48	PUW-13 (2)	30	30	32	20	23	17	12	18
49	PUW-13 (3)	32	30	35	27	20	20	20	-
50	PUW-16 (1)	29	31	30	24	27	22	17	20
51	PUW-16 (2)	30	34	27	20	23	23	20	19
52	PUW-17 (1)	28	31	26	18	20	22	17	22
53	PUW-18 (1)	30	42	24	21	27	21	19	20
54	PUW-19 (1)	31	27	25	27	19	21	15	19
55	PUW-19 (2)	34	35	31	27	20	22	20	20
56	PUW-20 (1)	30	39	27	29	27	24	17	14
57	PUW-20 (2)	29	35	30	21	21	24	19	21
58	PUW-21 (1)	31	35	27	27	21	23	20	21
59	PUW-22 (1)	30	34	25	22	25	20	15	-
60	PUW-22 (2)	29	30	25	27	25	25	21	22
61	PUW-23 (1)	40	35	20	-	-	-	-	-
62	PUW-25 (1)	31	35	25	23	27	15	20	15
63	PUW-26 (1)	28	37	31	27	24	20	20	18
64	PUW-26 (2)	29	30	27	23	24	27	19	17
65	PUW-27 (1)	30	29	30	25	21	24	24	20

APPENDIX D

ANTIBIOTIC SUSCEPTIBILITY PROFILE

S/N	SAMPLE CODE	MEM	CPX	SXT	E	S	CEP	PN	AU
1	PRW-3 (1)	S	S	S	I	S	R	R	R
2	PRW-3 (2)	S	S	S	R	S	R	R	R
3	PRW-4 (1)	S	S	S	S	S	R	S	R
4	PRW-4 (2)	S	S	S	R	S	R	R	R
5	PRW-7 (1)	S	S	S	S	S	R	I	I
6	PRW-7 (2)	S	S	S	R	S	R	I	I
7	PRW-11 (1)	S	S	S	S	R	I	S	S
8	PRW-11 (2)	S	S	S	I	S	R	S	S
9	PRW-12 (1)	S	S	S	I	S	R	S	S
10	PRW-12 (2)	S	S	S	S	S	R	S	S
11	PRW-15 (1)	S	S	S	R	S	R	I	I
12	PRW-17 (1)	S	S	S	S	S	I	S	S
13	PRW-18 (1)	S	S	S	S	S	R	R	I
14	PRW-18 (2)	S	S	S	S	S	R	R	I
15	PRW-20 (1)	S	S	S	S	S	S	S	S
16	PRW-20 (2)	S	S	S	S	S	I	S	S
17	PRW-20 (3)	S	S	S	S	S	S	S	S
18	PRW-20 (4)	S	S	S	S	S	S	S	S
19	PRW-22 (1)	S	S	S	S	S	S	S	S

20	PRW-22 (2)	S	S	S	I	S	R	I	I
21	PRW-22 (3)	S	S	R	R	I	R	R	R
22	PRW-22 (4)	S	S	S	R	S	S	I	I
23	PRW-23 (1)	S	S	S	I	S	R	S	R
24	PRW-23 (2)	S	S	S	S	S	S	S	S
25	PRW-25 (1)	S	S	S	I	S	I	S	I
26	PRW-25 (2)	S	S	S	S	S	R	S	S
27	PRW-27 (1)	S	S	S	I	S	S	I	R
28	PRW-27 (2)	S	S	S	S	S	I	S	R
29	PRW-28 (1)	S	S	S	R	S	R	R	R
30	PRW-29 (1)	S	S	S	S	S	I	S	S
31	PRW-29 (2)	S	S	S	S	S	R	S	S
32	PRW-30 (1)	S	S	S	S	S	S	S	S
33	PRW-30 (2)	S	S	S	S	S	S	S	S
34	PRW-31 (1)	S	S	S	S	S	S	S	S
35	PRW-31 (2)	S	S	S	S	S	R	S	S
36	PRW-32 (1)	S	S	S	S	S	I	S	S
37	PRW-32 (2)	S	S	S	S	S	R	S	I
38	PUW-2 (1)	S	S	S	S	S	R	S	I
39	PUW-6 (1)	S	S	S	S	S	I	S	I
40	PUW-6 (2)	S	S	S	S	S	I	S	S
41	PUW-7 (1)	S	S	S	S	S	I	I	S

42	PUW-7 (2)	S	S	S	S	S	R	S	I
43	PUW-8 (1)	S	S	S	S	S	I	S	S
44	PUW-9 (1)	S	S	S	I	S	S	R	S
45	PUW-11 (1)	S	S	S	I	S	R	I	S
46	PUW-12 (1)	S	S	S	S	S	S	I	S
47	PUW-13 (1)	S	S	S	I	S	R	I	I
48	PUW-13 (2)	S	S	S	S	S	R	R	S
49	PUW-13 (3)	S	S	S	S	S	R	S	R
50	PUW-16 (1)	S	S	S	S	S	R	I	S
51	PUW-16 (2)	S	S	S	S	S	I	S	S
52	PUW-17 (1)	S	S	S	S	S	R	S	S
53	PUW-18 (1)	S	S	S	S	S	R	S	S
54	PUW-19 (1)	S	S	S	S	S	R	I	S
55	PUW-19 (2)	S	S	S	S	S	R	S	S
56	PUW-20 (1)	S	S	S	S	S	I	S	I
57	PUW-20 (2)	S	S	S	S	S	I	S	S
58	PUW-21 (1)	S	S	S	S	S	I	S	S
59	PUW-22 (1)	S	S	S	S	S	R	I	R
60	PUW-22 (2)	S	S	S	S	S	I	S	S
61	PUW-23 (1)	S	S	S	R	R	R	R	R
62	PUW-25 (1)	S	S	S	S	S	R	S	I
63	PUW-26 (1)	S	S	S	S	S	R	S	S

64	PUW-26 (2)	S	S	S	S	S	S	S	I
65	PUW-27 (1)	S	S	S	S	S	I	S	S