PREVALENCE AND VIRULENCE FACTORS OF PATHOGENIC BACTERIA FROM OGBESE RIVER IN SOME COMMUNITIES OF OVIA NORTH EAST LOCAL GOVERNMENT AREA OF EDO STATE.

BY

Benedicta Iyobosa IDAHOSA

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UNIVERSITY OF BENIN

BENIN CITY

FEBRUARY 2020

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A THESIS WRITTEN IN THE DEPARTMENT OF MICROBIOLOGY AND SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY (M.Phil) IN ENVIRONMENTAL AND PUBLIC HEALTH MICROBIOLOGY, UNIVERSITY OF BENIN

FEBRUARY, 2020

CERTIFICATION OF THESIS

We the undersigned attest and declare that the thesis of **Mrs**. **Benedicta Iyobosa IDAHOSA** titled Prevalence and Virulence Factors of Pathogenic Bacteria from Ogbese River in some Communities of Ovia North East Local Government Area of Edo State has successfully passed the anti-plagiarism and do not violate any copyright regulations.

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APPROVAL

This is to certify that this work has been accepted in partial fulfillment of the requirements for the award of Master of Philosophy (M.Phil) in Environmental and Public Health Microbiology, University of Benin, Benin City.

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DEDICATION

This thesis is dedicated to Almighty God for his grace and wisdom and to all who support girl-child Education.

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ABSTRACT

All living things need water and rivers constitute one of the most important sources of water for domestic, industrial and agricultural activities. This study was carried out to evaluate the physicochemical properties and prevalence of pathogenic bacteria in Ogbese River, Ovia North East Local Government Area, Edo State.

Water samples (twenty-four) were collected from 12 stations at Ogbese Town, and another 12 stations at Olumoye towns along Ogbese river course during the months of June to December, 2018 and February 2019. Bacteriological analysis involving membrane filtration and multiple tube fermentation techniques were employed to isolate bacterial pathogens. Extraction of genomic DNA, amplification, sequencing and blasting were used to identify bacterial isolates. Antibiogram and curing of bacterial isolates were performed using Kirby-Bauer disk diffusion method. Hemolysin production, serum resistance, Sereny test, Ileal loop assay and suckling mouse tests were carried out on the bacterial isolates. The physicochemical parameters and heavy metals analyses were evaluated using standard methods for water quality. Data were analysed using analysis of variance and unpaired Student's *t* test.

The highest bacterial count was obtained in station 5 at Ogbese River ($104.2 \pm 32.0 \times 10^3$ cfu/ml) while the least count was obtained at station 2 during the dry season ($42.4 \pm 0.25 \times 10^3$ cfu/ml). The most probable number (MPN) was least in station 5 in Ogbese town during the dry season (1 MPN/100 ml) and highest in station 1 during wet season (740 MPN/100 ml). The bacterial isolates from Ogbese River were *Salmonella enterica, Escherichia coli, Staphylococcus aureus, Vibrio cholerae, Shigella flexneri, Enterobacter cloacae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa. Enterobacter cloacae* (67 %) and *Escherichia coli* (67 %) were the most dominant or prevalent bacterial isolates in Ogbese town during the wet season, while *Pseudomonas aeruginosa* (50 %) and *Escherichia coli* (50 %) were the most dominant in Olumoye community during the wet season. Most of the bacterial isolates

were toxigenic with *Escherichia coli* (83.33 %) being the bacterium having the highest frequency of enterotoxin production. All isolated bacteria were found to be invasive but *Shigella* and *Klebsiella* were found to have a 100 % frequency of invasiveness. *Shigella flexneri* (100.00 %), *Vibrio cholerae* (83.33 %) and *Salmonella enterica* (55.55 %) had the highest frequency of serum resistance amongst the bacterial isolates evaluated in the study. A few of the isolates had higher percentage prevalence relative to their hemolytic behaviour. *S. aureus* (25.00 %) and *Klebsiella pneumoniae* (25.00 %) were the isolates with the least prevalence of hemolytic behaviour. All the isolates with the exception of two were found to contain bacterial plasmids for resistance to antibiotics and were of public health importance.
The pH of the water samples in Ogbese town was 6.85±0.04 for wet while it was 6.65±0.08 for dry season. The turbidity of water samples from Ogbese River was 9.83±1.32NTU (dry season) and 32.00±2.16 NTU (wet season) in Ogbese town while it was found to be 8.83±1.01 NTU (dry season) and 30.83±5.92 NTU (wet season) in Olumoye town. Transition metals like lead, zinc and iron were detected only during wet season across the towns assessed. These make the water polluted and unfit for consumption. Proper disinfection techniques and general enlightenment should be adopted to make the water potable for human consumption.

CHAPTER ONE

INTRODUCTION

In human existence water forms part of life and this points out one of the reasons people settle densely in places they are sure of adequate water supply. All living things need water because it supports life, and rivers constitute one of the most important sources of water for many activities which include: industrial, agricultural and domestic uses. These have to do with drinking, bathing, fishing, transportation, washing, generating electricity, and irrigation, in refineries, cooling of equipment and in recreational activities (Florke and Alcamo, 2013). The Government has been the major supplier of potable water for its populace in Nigeria but with the increasing population and subsequent expansion in settlement, it has become difficult to satisfy the water needs of its citizens. This has resulted in individuals and communities depending on other sources of water such as rivers, streams, boreholes and wells. Although rivers aid and sustain culture, economic needs and advancement of any nation, however, the activities in these rivers make its water team with potentially pathogenic microorganisms; hence, it has been recorded as a potential carrier of pathogenic organisms, virulent enough to cause human morbidity and mortality (Fawell and Nieuwenhuijsen, 2003).

About 70 million Nigerians have no potable water and 335,000 children die annually from water-borne diseases, (UNICEF, 2014). In developing countries of the world, four-fifth of all illnesses are caused by water borne diseases with diarrhea leading to dehydration and the major cause of childhood death (WHO, 2018). Faecal coliforms, opportunistic pathogens and heterotrophic forms have all been isolated from raw surface water and bacteria isolates identified include *Klebsiella oxytoca, Escherichia coli, Enterobacter cloacae, Salmonella enterica, Vibrio cholerae and Shigella* spp. (Mulamattathils*et al*., 2014; Gupte*et al*., 2015). Some of these have been implicated in gastrointestinal disease and epidemic outbreaks (Sharma *et al*., 2017).

In Africa, 3.4 million people mostly children die each year and four thousand children each day through water related illness (UNICEF, 2014). River Thames in London was reported to be the cause of a large outbreak of gastrointestinal illness, which involved one thousand one hundred (1100) swimmers, with diarrhea, vomiting, abdominal cramps and nausea that lasted for nine days (Hall *et al*., 2017). In another report, the raw surface water of Bassaseachic waterfalls and its main rivers used as drinking water by the inhabitants in Mexico was reported to be the cause of a seasonal cholera problem and diarrhea (Delgado-Gardea, *et al*., 2016). In another report, agricultural produce such as vegetables, fruits and cereals contaminated with pathogenic *Escherichia coli* and *Salmonella* serovars from surface water used by the producer for washing were implicated in an epidemic outbreak in Southern Georgia, USA (Antaki*et al*., 2016). Globally, about 80% of all diseases and death in developing countries are water-related as a result of polluted water (Ayeni*et al*., 2011, Aderibigbe*et al*., 2008). The numbers of waterborne disease outbreaks that have been reported in Nigeria demonstrate that transmission of pathogens by drinking water remains a significant cause of illness (Nwidu*et al*., 2008).

The pathogenicity of an organism is determined by its virulence factors. Virulence factors of water-borne pathogens include membrane associated virulence factors that aid the bacterium in adhesion and invasion of the host cells, enterotoxins and cytotoxins such as hemolysins and leucocidin as well as invasiveness (Sharma *et al*., 2017).

River Ogbese runs through several communities including Ogbese, Olumoye, Agekpanon, Ayede and others in Edo and Ondo States. Inhabitants of these rural communities depend on the river for many activities, as rural communities, such as farming especially in dry season, fishing, cassava processing, washing, drinking, bathing and transporting of their farm produce. Most of the farming are done close to the river banks especially maize, okra and varieties of other vegetables which supply Edo and neighboring states during dry seasons

when such vegetables are scarce. Consequently, agricultural, human and animal excrement pollute the river water and may render it unfit for consumption according to WHO/UNICEF, (2014); and Orjiekwe (2013).Data on bacteriological quality of this river is scarce and where available, it is not comprehensive. For example, Orjiekwe*et al.* (2013) only identified coliforms as contaminants of Ogbese River but did not identify the individual bacterial species. Akinbile and Omoniyi (2018) reported on the water quality index of the river, while Ololade and Ajayi(2009) reported that some major rivers along Ondo (Ogbese inclusive) contains some pathogenic bacteria. However, bacteriological studies on available water studies are necessary for developing disease preventive and control strategies.

1.1Aim and Objectives

The aim of this study was toassess the bacteriological quality of Ogbese River in Ogbese and Olumoyetowns/communities in Ovia North East Local Government Area of Edo State, with a view to ascertaining the suitability and potability of water quality.

The specific objectives of this study were to:

1. determine the bacteria load in water samples from Ogbese River, specifically at Ogbese and Olumoye.
2. identify potential pathogenic bacteria in the water samples.
3. determine the antibiotic susceptibility profiles of the bacterial pathogens.
4. ascertain the genetic source of antibiotic resistance.
5. ascertain potential virulence factors such as hemolysis, enterotoxin production and invasiveness in some of the isolates.
6. determine the physicochemical parameters of the water quality

CHAPTER TWO

LITERATURE REVIEW

Water is very essential to the survival of all organisms and the human body is composed of approximately 70% water by mass (Enger and Smith, 2004). Safe drinking water is a human birthright as much a birthright as clean air. An adequate supply of safe and portable water assist in preventing the spread of gastrointestinal diseases, supports domestic and personal hygiene and improves the standard of living (Kangpe*et al*. 2014). However, much of the world's population does not have access to safe drinking water. This is because dead vegetation, metal leachates from solid waste dumps; leaching of rocks, sewage, industrial wastes and agricultural chemicals return eventually to the river by run offs (Ademola, 2008). As population increases, the need of water for domestic, transport, power, agricultural and industrial purposes also increase (Kangpe*et al*., 2014). Apart from expansion as a result of increase in population, other reasons such as crises has led to the development of settlement towns in Nigeria. These private water sources are not subjected to any quality standard before usage, thereby resulting in the consumption of contaminated water (Kangpe*et al*., 2014). According to WHO (2012), about 2 million people die annually due to diarrhea diseases, most of them are children less than 5 years of age.

Contaminants such as bacteria, viruses, heavy metals, nitrates and salt have polluted water supplies as a result of inadequate treatment and disposal of waste from humans and livestock, industrial discharges, and over-use of limited water resources (Wright *et al*., 2004).

2.1 Domestic Sources of Water

Domestic water supply is the water used for every household or domestic purposes such as consumption, bathing and food preparation (WHO, 2007). Sources of domestic water supply are categorized into improved, which are protected from outside contamination and

unimproved, not adequately protected from outside contamination (WHO, 2011). The unimproved include rivers and streams, which are the main crux of this research work. Water being used for different purposes depends on its chemical, physical and biological characteristics (Umegbolu and Offor, 2017). Improvements in source water quality generally depend on expensive, long-term, centralized projects, such as construction of water treatment plants, and water distribution systems (Mintz*et al*., 1995), hence safe drinking water for all remains an indefinable and expensive goal. In 1990, more than 1 billion people depended on rivers, streams, or other unsafe surface sources for drinking water (WHO, 1996). In urban and rural areas in Nigeria, water sources vary from natural sources like rivers, ponds, streams, rainwater, and human made sources like well, boreholes, and pipe borne water (Niyi and Felix, 2007). In many developing countries, even municipal piped well water is unsafe, because of inadequately maintained pipes, low pressure, intermittent delivery, lack of chlorination (Mintz*et al*., 1995). In many cities and rural communities of Nigeria, central chlorination of the municipal water system is undoubtedly insufficient to maintain adequate free chlorine residuals at distribution sites, and this could be a primary source of water-borne infections and diseases. The limited access to water supplies by a significant proportion of the Nigerian rural population has been blamed on institutional and socioeconomic factors (Ezenwaji*et al*., 2016).

According to Toyobo and Tanimowo (2011), the prevailing water inadequacies is attributed to poverty in many rural communities of Nigeria, poor sustainability of water infrastructure, inadequate technology, paucity of funds and lack of political charisma in detecting the needs of the of the people. Parts of the Northern territories like Yobe, Borno, Adamawa Zamfara and Jigawa states are noted for water shortages as they have aggravated refugee and food crises, forcing some residents to migrate southwards (Goni, 2006; Abaje*et al*., 2009).

2.2 Microbial Contamination of potable water sources in Nigeria

Quality water supply is one of the requirements for human existence. The quality of water is examined to assess its hygienic level as well as its suitability for general use (Ohanu*et al*., 2012). Due to environmental conditions and activities of human, clean, pure and safe water which exist in nature gets contaminated (Eze and Madumere, 2012). The high microbial population from surface water could have stemmed from soil pollution which is leached into the surface water via runoff. The microbial load could increase in potable water, if the containers used in collection and storage are contaminated (Izah andIneyougha, 2015). This could manifest in the water quality.

Pathogenic microbes pose a greater health threat to domestic water users than most chemical contaminants, for a number of reasons which according to TCEQ (2007) include the fact that pathogens can cause disease after a single exposure, while most chemical contaminants may require months or even years of exposure before causing a negative health effect. Pathogens also do not affect the taste, smell, or appearance of the water; on the other hand, chemical contaminants make the water taste, smell, or look different, especially if the chemicals are present at levels that would pose a short-term risk. Again, pathogen levels may rise very quickly, while chemical levels tend to remain constant. Consequently, it is relatively easy (though somewhat costly) to periodically test for chemical contaminants, while it is both difficult and costly to continuously test for most pathogens. More so, a disease caused by a pathogen can usually be passed from person to person, while the health effects caused by chemicals affect only those that actually consume the contaminated water. Lastly, waterborne illnesses caused by pathogens can be a serious health risk for the elderly, infants, chemotherapy patients, and other individuals with a delicate or compromised immune system.

Contaminated water by microorganisms has been linked to infectious and parasitic diseases. The microbial populations are typically highest in surface water and rain water, followed by ground water and least in sachet and bottled water. Aji*et al.,* (2015)surveyed the sachet water in Jere and Maiduguri, Borno state, Nigeria and reported that over 80% of the sachet water used for drinking purposes contains coliforms, hence making such water unfit for human consumption. However, Akpoborie and Ehwarimo (2012) reported absence of coliform in bottled and sachet water sold in Warri, Delta state, Nigeria. Hence, the microbiological quality of the water produced commercially depends on the level of sanitary practices employed by the producers. The microbial density which is the total heterotrophic bacteria count; total coliform, fecal coliform often exceeds the recommended limits in most water samples consumed without proper treatment. The World Health Organization/Food and Agricultural Organization recommended a maximum permissible limit of 1.0 x 10^2 CFU/ml for potable water (Oludare and Sikiru, 2012; Sunday *et al*., 2014). Standard Organisation of Nigeria (SON) set the maximum permissible level of total coliform as 10 CFU/ml and the values for thermotolerant coliform or *E. coli,* Faecal *Streptococcus* and *Clostridium perfringens* spore as 0 CFU/100ml.

Storage duration usually influence the microbial counts of potable water (Eze*et al*., 2013), materials used in covering water during storage (Dami*et al*., 2012), and season (Olorode*et al*., 2015). Eze*et al*. (2013) reported that microbes such as *Staphylococcus aureus*, *Escherichia* coli*, Proteus, Pseudomonas, Enterobacter, Salmonella, Klebsiella, Bacillus* spcies were the dominant microbial diversity found in the different potable water sources in Nigeria. To a lesser extent, *Alcaligenes faecalis, Aeromonas, Micrococcus, Citrobacter, Streptococcus, Vibrio, Shigella, Enterococcus, Flavobacterium,* and *Chromobacterium* species etc. Information of fungal diversity in potable water sources is few in literature. This could be attributed to the non-use of fungal species as indicator organisms in water quality. As such,

authors focus more on total heterotrophic bacteria counts and total enteric counts such as coliforms, *Salmonella*-*Shigella* counts and *Vibro* counts. Also, uncommon bacteria such as *Clostridium perfringens, Phaerotilus, Erwinia* and *Yersinia* species were only reported in Foma River, Ita-Nmo, Ilorin, Nigeria (Agbabiaka and Oyeyiola, 2012).

According to Adewoye and Adewoye (2013), bacteriological quality of potable water is vital and as such monitoring and surveillance must be given utmost consideration due to the fact that disease outbreak have been associated with poorly treated or contaminated water. However, Agbabiaka and Oyeyiola (2012) showed the fungal diversity of Foma River, to include *Curvularia, Penicillium, Rhizopus, Fusarium, Mucor, Cladosporium, Saccharomyces, Mortierella*and *Asprgillus*Species. The microbial diversity and density in the potable water sources is a reflection of the contamination level. For the packaged water, the high microbial load could be due to the method and hygienic conditions of the producer and their environment. Egbe*et al.* (2013) reported that contamination of rain water occurs due lack of a protective covering thus, enhancing external source. Bello *et al.* (2013) reported that a plethora of factors can enhance the microbial contamination of potable water sources. Amongst the several factors listed, include poor sanitary conditions of water collection and storage containers/tanks, unhygienic techniques in dispensing water from household storage containers (including faecally contaminated hands and dippers), lack of protection against contamination introduced by vectors and insufficient cleaning of vessels to prevent biofilm formation and accumulation of sediments and pathogens. Hence, the presence of these bacteria is an indication that the water sources are not potable for human consumption.

The occurrence of pathogens in water is a sign that such waters may result in the transmission of waterborne diseases (Bello *et al*., 2013; Egbe*et al*., 2013). The occurrence of coliform in the potable water sources could be due to the presence of human and animal’s excreta in water (Aboh*et al*., 2015), which could provide appropriate nutrients required for growth and

proliferation. Generally, *E. coli* and *Enterobacter aerogenes* in potable water indicates presence of recent faecal matters (Egbe*et al*., 2013). Isikwue and Chikezie (2014) reported that feacal coliform in water is influenced by presence of wastewater and septic system effluent, animal waste, sediment load, temperature and nutrients levels. International standards for water quality aimed at preventing pathogenic microbes in potable water, which is because pathogens that contaminate water could transmit infectious diseases (Bukar*et al*., 2015).

2.4 Waterborne Diseases

Waterborne diseases are caused by pathogenic microorganisms, which are directly transmitted when contaminated fresh water is consumed. They are very rampant especially in sub-Saharan Africa due to lack of access to clean water and poor sanitation (Olajuyigbe, 2012). In Africa, it has been estimated that every child has five episodes of diarrhoea per year and that 800,000 children die each year from diarrhoea and dehydration. According to Wittenberg (1998), infective diarrhoea is predominantly a disease of poverty, overcrowding and environmental contamination. Waterborne outbreaks of enteric disease have occurred either when public drinking water supplies were not adequately treated after contamination with surface water or when surface waters contaminated with enteric pathogens have been used for recreational purpose (Johnson *et al*., 2003). Water borne illness is caused by various bacteria, virus, protozoa and pathogenic microorganisms and usually occurs as a result of poorly treated drinking water and wastewater or a natural disaster, like flooding and environmental pollutants (Adeyinka *et al*. 2014). Waterborne disease include cholera, typhoid fever, dysentery, diarrhoea, hepatitis and guinea worm.

In Nigeria, about 90 million people are without access to safe drinking water and 130,000 children under the age of 5 die annually from preventable waterborne diseases (Adeyinka *et*

al., 2014). Some states in Nigeria are predominantly rural states with over 65% of the population living in rural areas. The greatest problem facing responsible government and non-governmental organizations is how to improve the quality of life of the rural population (Adeyinka *et al*., 2014).Like other developing countries, the issue of access to potable water is very important in Nigeria, where 48% (about 67 million Nigerians) depend on surface water for domestic use, 57% (79 million) use hand dug wells, 20% (27.8 million) harvest rain, 14% (19.5 million) have access to pipe borne water, and 14% have access to borehole water sources (FGN, 2007).

Nigeria is one of the countries in the world that has unsafe water supplies due to the un-coordinated efforts of various federal, state and local agencies. Given the low quality of drinking water in Nigeria, most Nigerians will usually contract a waterborne illness. A total of 5600 cholera cases and 340 cholera deaths were reported between December 1995 and May 1996 (attack rate=86.3 per 100,000 population) in the state of Kano (Hutin, *et al*. 2003). The Guardian Newspaper on Tuesday October 26th, (2010) reported that Cholera has killed more than 1,500 persons and infected nearly 40,000 people in Nigeria in its worst outbreak for nearly two decades. The lethal waterborne disease spread to Nigeria's West African neighbours Cameroon, Chad, Niger and Benin. In the same news report, it was estimated that 1,555 people have died from cholera in Nigeria since January of the same year and 38,173 cases have been reported according to data released by the United Nations and World Health Organisation. A research by Oguntoke*et al*. (2009) on the association of water-borne diseases, morbidity pattern and water quality in parts of Ibadan City, Nigeria, reported that there was a significant difference in the occurrence of waterborne disease amongst residential areas ($P<0.05$). Typhoid fever had the highest occurrence (39.3%) followed by bacillary dysentery and cholera. More so, about 45% of water-borne diseases were reported in the months of July to September. Faecal coliform contamination of water samples ranged between 0.1×10^4 and

1.8 x 10^4 CFU/ml. Furthermore, 18% of rainwater and 23.6% of well water samples were positive to *Vibriocholerae*, *Salmonellatyphi* and *Shigelladysenteriae*. Adeyinka *et al*. (2014) reported that an estimated 23,441 cases of cholera were reported in Nigeria in year 2002 alone. The number was reduced by almost 50% (11,993 cases) in 2003. Report on Cholera in 2008 increased to 17,854 cases. There was a staggering 104,154 cases of typhoid cases reported in 2002 alone. The number of cases drastically reduced to 39, 377 cases two years later. The reduction that occur for both disease condition could partly be due to the awareness and consciousness about the danger of the disease and possible means of spread. Drancunliasis and hepatitis are also waterborne disease with 2,588 and 9451 cases reported in 2002 respectively but as at 2007, the cases had been greatly reduced to 1 in 5,239 case(s) respectively. Generally, the number of incidences of waterborne illness in some part of Nigeria is high compared to other major causes of illness (Adeyinka *et al*., 2014). Contamination of drinking water with pathogens have also been reported in several towns across Nigeria (Biu*et al*., 2009; Adekunle*et al*., 2007; Ibrahim *et al.,* 2000). Waterborne outbreaks of enteric disease have occurred either when public drinking water supplies were not adequately treated after contamination with surface water or when surface waters contaminated with enteric pathogens have been used for recreational purpose (Johnson *et al*., 2003). A retrospective study by Raji and Ibrahim (2011) on the prevalence of water borne disease in North West Nigeria opined that the safety of drinking water to the people in this part of the country has been of major concern because of frequently reported incidences of waterborne infections. It was reported that there were high incidences of waterborne infections namely typhoid, cholera, dysentery, diarrhoea and gastroenteritis in the three towns, and that these are more frequent in children below the age of 5 years. Gender distribution of diarrhoea and dysentery cases in 2004 and 2005 showed on average that more female than male residents contracted the infections. The number of cases of waterborne infections in the

three towns increased from 10.03% in 2004 to 14.14% in 2005. Diarrhoea, constituting 6.23% in 2004 and 10.04% in 2005 was the most commonly reported cases of waterborne infection in the three towns. Shuni recorded the highest (8.95%) incidences of diarrhoea infection in 2004, followed by Tambuwal (6.23%) and Sokoto had the least (4.81%) while in 2005 Sokoto had the highest (11.99%) followed by Tambuwal (10.23%) and Shuni had the least (7.55%).

Although, the symptoms of gastrointestinal disorders (nausea, diarrhea, vomiting and abdominal pain) are usually mild and generally last a few days to a week and only a small percentage of those affected will visit a health facility, there is untold impact on human productivity (Owolabi*et al*., 2014).

2.4 Pathogenic Determinants of Waterborne Disease Agents

2.4.1 *Vibrio cholerae*

Cholera caused by toxigenic *Vibrio cholerae* is a major public health problem in developing countries, where outbreaksoccur in a regular and seasonal pattern and are associated with poverty and poor sanitation (Leclerc *et al*., 2002). Amongst the 193 currently recognized O serogroups*of V. cholerae*, only serogroups O1 and O139 have been associated with epidemics of cholera. The other serogroups usually referred to as non-O1, non O-139, can cause sporadic diarrhea (Chakraborty*et al*., 2000). This sharp distinction between serogroups is related to virulence associated genes. The strains belonging to serogroups O1 and O139 (more than 95%) produce cholera toxin (CT) and colonization factor known as toxin co-regulated pilus (TCP) that is co-ordinately regulated with CT production (Leclerc *et al*., 2002). In contrast, the strains of non-O1 non-O-139 (more than 95%) serogroups lack these two virulence genes. From 1817 to 1994, seven distinct pandemics of cholera occurred; all these were caused by *V. cholerae* O1 (Basu*et al*., 2000). During the first six pandemics, cholera remained principally confined to South and Southeast Asia, whereas the seventh

pandemic reached West and East Africa and South America (Leclerc *et al*., 2002). In late 1992, *V. cholerae* belonging to a non-O1 serogroup (now referred as O139 Bengal) caused explosive epidemics of cholera through India, Bangladesh, and neighbouring countries (Basu*et al*., 2000). *V. cholerae* is now recognized as an autochtonous member of the microflora in many aquatic environments such as in riverine and estuarine areas. The close association of V. cholerae with surface water suggests the importance of water ecology and population interacting with the water and food also plays an essential role, although in many instances water is the source of contamination of foods (Albert *et al*., 1977). Many ecological aspects remain unknown to explain seasonal appearance epidemic *V. cholerae* strains and outbreaks of cholera. Take for example: the state of an aquatic reservoir of *V. cholerae* O1 or O139 being capable not only to survive in water but also to form a complete component of the ecosystem (Franco *et al*., 1997). It has been postulated that under stress conditions the vibrios can be converted to a viable but nonculturable form (VNC) that can be reverted back to live infectious bacteria (Jesudason*et al*., 2000). More so, the major pathogenic gene in toxinogenic *V. cholerae* are clustered in several chromosomic regions (CTX genetic element and TCP pathogenicity island) that are capable of being propagated horizontally to non-O1 and non-O139 strains by lysogenic conversion (Faruque*et al*., 1998). Later on, Chakraborty *et al*. (2000). Demonstrated the occurrence and expression of critical virulence genes in environmental strains of *V. cholerae* that appear to constitute an environmental reservoir of virulence genes. These data on the ecology of *V. cholerae* appear to be of great significance.

2.4.2 *Escherichia coli*

They are a Gram-negative bacteria, a member of the enterobacteriaceae, a normal flora of the gut and ubiquitous in the environment (Willey *et al*., 2008). It has been reported to cause a plethora of infections with varying virulence characteristics. The pathogenicity of some of the strains of *E. coli* has been attributed in part to the production of toxin cytotoxic for Vero cells

(Leclerc *et al*., 2002). The toxin was appropriately named Verotoxin (VT), and the group of *E. coli* that produce VT became known as the Verotoxinproducing*E. coli* (VTEC) (Charts, 2000). Subsequently, other verotoxin-producing *E. coli* strains have been associated with hemorrhagic colitis and hemolytic ureic syndrome. *E. coli* O157:H7, however, is recognized as the most common cause of VTEC-related human illness. VTEC, including *E. coli* O157:H7, are strongly associated with cattle, and they can clearly pass through stomachs of ruminants. The transmission of VTEC O157 is often foodborne, particularly from contaminated ground beef or raw milk, or person-to-person, and contact with farmed animals has also resulted in human infection. The waterborne route of VTEC O157 infection was first clearly demonstrated by an unusually large outbreak by unchlorinated municipal water in the Missouri Community (Swerdlow*et al*., 1992). Since then, waterborne VTEC O157 has been described in sporadic cases and in outbreaks of illness. Chalmers *et al*. (2000) have analyzed published outbreaks of VTEC O157 associated with recreational waters, private and municipal supplies. Despite the potential for large contamination of environment with VTEC O157, however, waterborne infection is relatively rare because VTEC O157 is as susceptible to chlorination as bacterial indicators (Chalmers *et al*., 2000). Epidemiological investigations have elucidated the mechanisms by which *E. coli* O157:H7, *Shigella* spp., and campylobacters have become a source of concern recently. The most striking feature is the low inoculum of organisms that may trigger disease. As few as 10 to 100 organisms of the most virulent *S. dysenteriae* type 1 are sufficient to cause clinical dysentery, while the other species may require a 10 to 100 times more elevated dose (Dupont*et al*., 1989). The dose required to trigger campylobacteriosis is also low, probably no more than a few hundred bacteria (Black *et al*., 1988). *E. coli* O157:H7, like *Shigella* and *C. jejuni*, appears to have a low infectious dose, approximately some hundred organisms or less (Griffin *et al*., 1991).

2.4.3*Pseudomonas aeruginosa*

P. aeruginosa is a bacterium, ubiquitous in the environment, inhabiting soil, fresh waters and plants (Leclerc *et al*., 2002). The bacterium has been recovered/isolated from several vegetables, like cucumbers, tomatoes, radishes, lettuces and onions at rates capable of reaching 103/g (Stiles, 1981). Its presence is constant and abundant in waste waters and consequently in surface waters that receive polluted effluents (Leclerc *et al*., 2002). Its growth in water is not directly linked to the organic matter content, because it can develop abundantly in the purest of waters. *P. aeruginosa* is a species of considerable versatility and a significant pathogen that can cause infection in a variety of plants, insects, and warm-blooded animals. Its extreme resistance to antibiotics explains why this ubiquitous bacterium has adapted to colonize the skin and mucous membranes of patients (Willey *et al.,* 20058). As some *P. aeruginosa* strains are capable of producing enterotoxins, the enteropathogenicity of this species was sometimes surmised (Leclerc *et al*., 2002). Since 1894, several publications have recognized this bacterium as an enteric pathogen and causative agent of diarrhoea in infants and children (Williams and Cameron, 1897; Hunter and Ensign, 1974; Florman and Schifrin, 1950). Enteric disease associated with septicaemia was described earlier by Dold (1918) as "Shangai fever", a prolonged febrile illness that affected both children and adults. As expounded by Hardalo and Edberg (1977) the colonization of children by *P. aeruginosa* quickly ceases once the environmental sanitary conditions are corrected. Community acquired *P. aeruginosa* gastrointestinal disease with sepsis rarely occurs in healthy infants, that is, those who lack identified underlying immunological or haematological problems (Lepow, 1994). On the other hand, *P. aeruginosa* can be a colonizer of the gastrointestinal tract in immunocompromised and hospitalized adults and children. In the past, Hoadley (1977) considered the presence of *P. aeruginosa* in drinking water as a public health risk. However, *P. aeruginosa* is predominantly an environmental organism and fresh surface water is an

ideal reservoir (Leclerc *et al*., 2002). As a consequence of contemporary lifestyles, *P. aeruginosa* reaches relatively high numbers in food and on moist surfaces. Daily, substantial numbers of the species are ingested with our food, particularly with raw vegetables, while our body surfaces also are in continuous contact with the organism.

2.4.4 ***Salmonella***

Salmonella are typical members of the family Enterobacteriaceae, facultative anaerobes, Gram-negative bacilli, flagellated, non-spore forming. They are able to grow on a wide range of relatively simple media. They can be distinguished from other members of the family by their biochemical characteristics and antigenic structure. Their normal habitat is the animal intestines. There are well over 2000 different antigenic types of *Salmonella.* They were originally classified as separate species, but it is now generally accepted that they represent serotypes of a single species, *Salmonella enterica*. Various subspecies are recognized, but most of the serotypes that infect mammals are found in a subspecies also designated enterica. Therefore, the full correct designation is, for example *S. enteric* sub species *enterica* serotype Enteritidis. This is abbreviated to *S.* serotype *Enteritidis* or simply *S. Enteritidis* (Maciorowski*et al*., 2004). In poultry, the serotypes include *S. gallinarum, S. pollurum,* and *S. arizona*.

Non typhoid *Salmonella* spp. are estimated to be the third most common cause of human food borne illness in the United States, causing approximately 1.3 million cases annually (Tauxe, 2002). Being ubiquitous in nature, the bacterium can quickly be spread vertically and horizontally throughout poultry flock or livestock herd. Humans can be exposed to the bacteria by consuming improperly prepared eggs, meat or milk from infected animals or from foods contaminated by the faeces of infected animals (Crump *et al.,* 2002). Human outbreaks of *Salmonella enterica* serovars Hadar, Heidelberg, Virchow, and Agona in cattle and

chickens have been traced to contaminated bone, meat and fish meal (Crump *et al.,* 2002). The serovars causing human food borne illness have evolved over time, with outbreaks of *S. enterica* ser. Agona in the 1970s, *S. enterica* ser. *Enteritidis* in the 1980s, and *S. Enteric Typhimurium* DT104 in the 1990s (Crump *et al.,* 2002; Tauxe, 2002). *S. enterica* ser. Agona became a major public health risk in the United States in the 1970s and became the eighth most common isolate in cases of human salmonellosis by 1972 (Crump *et al.,* 2002). Epidemiology investigations revealed that people became ill after consuming contaminated poultry. The ultimate source of the epidemic was traced back to a poultry raising facility in Mississippi that had used feed derived from fish meal imported from Peru. It has been estimated that *S. enterica* ser. Agona has cause over 1 million cases of human illness since its introduction into the American food chain. Foodborne *Salmonella* spp. contamination in feeds detection methodologies and control measures has been extensively reviewed elsewhere and will not be discussed here. The physiology of *Salmonella* spp. lends itself quite well to the contamination and survival on a wide range of feeds and feed ingredients. Some feeds, such as bone, meat, and fish meal, have been associated with high rates, in some surveys ranging from 0.17 to 0.43 %, of *Salmonella* contamination (Maciorowski*et al.,* 2004). *Salmonella* spp. are organisms that have developed diverse mechanisms to survive outside of their preferred niche, the intestine, as reviewed by Foster and Spector (1995). The pathogen may utilize a starvation stress response (SSR) to resist low concentrations of available carbon, nitrogen, and phosphorus (Harder and Dijkhuizen, 1983). *Salmonella* spp. may utilize a stationary-phase acid tolerance response (ATR) in order to survive transient low pH levels, genes for catalase (*katE*and *katG*) to tolerate peroxide, 10 different proteins for superoxide radicals and mechanisms involving glutamate, K+ ions, proline, or trehalose to maintain osmotic pressures (Foster and Spector,1995). *Salmonella* spp. have found to be particularly resistant to dehydration. Survival of the bacterium in meat and bone meal, dry milk and

poultry feed has been shown to be inversely related to moisture content, with greater survivability seen at a water activity of 0.43 and 0.52 than at 0.75 (Juven*et al.,*1984). These adaptations have allowed *Salmonella* spp. to be potentially resistant to environmental stress, to survive in multiple environments and, ultimately, to infect human hosts.

Other pathogens that are capable of causing waterborne include viruses, protozoans and algae. Viruses are intracellular parasites that can only replicate inside host cells. They are capable of causing diseases of man and beast. Viruses have been found to cause gastrointestinal disorder popularly known as stomach flu, which is a function of ingesting contaminated food and water (Goodgame, 1999). For the protozoans, a notable species of the *Cryptosporidium* has been implicated to cause disease of the intestinal tract (zoonosis), which can be contracted via contaminated food and water (Wright, 2012). For algae, the cyanobacteria also known as the blue green algae has been reported to cause health hazards and amongst which, is the gastrointestinal illness (Figgart*et al*., 2017).

2.5 Antibiotics and Antibiotic Resistance

Antibiotics were originally defined as substances produced by one micro-organism which inhibits growth of other micro-organisms. The advent of synthetic antibiotics has however resulted in a modification of this definition.

Therefore antibiotic now refers to a substance produced by a micro-organism or to a similar substance (produced wholly or partly by chemical synthesis), which in low concentrations inhibits the growth of other micro-organisms. There are four major sources of antibiotics namely:-

i. Micro-organisms for example bacitracin and streptomycin
ii. Synthetic like chloramphenicol
iii. Semi synthetic like the penicillins and the cephalosporins.
iv. Plant products (Willey *et al*., 2008).

Mechanisms of antibiotics

Antibiotics affect microorganisms in several ways with variation from one antibiotic to the other (Dubey and Maheshwari, 2013). They can be grouped as those that:-

i. Inhibit cell wall synthesis (ampicillin, cephalosporin, β-lactam, Vancomycin, Bacitracin)
ii. Inhibit nucleic acid function (Nitroimidazole, Nitrofurans, Quinolones, Rifampicin) or intermediate metabolism (Sulphonamides, trimethoprin)
iii. Damage cell membrane hence interfere with its function (Polymyxin, polygene)
iv. Inhibit protein synthesis (Aminoglycosides, Fenicols, Lincosamides, Macrolides, Streptogramins, Pleuromutilins, Tetracyclines)
v. Inhibit respiration, that is antagonism of metabolic pathways

2.6 Antibacterial resistance

Resistance is the ability of a micro-organism to withstand the effects of antibiotics. Antibiotic resistance may evolve via natural selection acting upon random mutation.

It can also be engineered by applying an evolutionary stress factor on a population of bacteria. Once a gene is generated, bacteria can then transfer the genetic information in a horizontal fashion (between individuals of same species) by plasmid exchange. If a bacterium carries several resistance genes, it is described as multi resistant or informally, a superbug. The antibiotic action against the pathogen can be seen as an environmental pressure to the pathogenic bacteria such that those which have a mutation allowing them to survive will live to reproduce. They will then pass these traits to their offspring resulting in a fully resistant colony (Wikipedia-The Free Encyclopedia). Resistance to antibiotics may be either intrinsic or acquired. Intrinsic resistance is when the organism lacks the target site for the agent or has other features that always render it resistant to the antibiotic. Acquired resistance is applicable to those organisms that were previously susceptible to the antibiotic in question.

This later form of resistance causes great concern because of its potential for reducing the range of previously useful antibiotics available. Resistance may also be either phenotypic or genotypic. There are two types of phenotypic resistance, the L-form bacteria which lack a cell wall but survive in an isosmolar environment despite continued exposure to antibiotics (Inglis et al., 2003). The other type is called persisters because they represent a small fraction of the initial bacterial population that persists after the start of antimicrobial therapy. Genotypic resistance is determined by resistance genes carried in the chromosome or plasmids and there is a possibility of genes being a re-assorted leading to a unique recombinant. Recombination ensures more efficient adaptation to a rapidly changing antibiotic environment. Sequences of genes that have the ability to recombine with both the chromosome and plasmids are known as transposons. The accumulation of resistance factors on a transposon may result in chromosomally coded antibiotic resistance being transferred first to a plasmid and then to other species (Inglis et al 2003).

It is possible for resistance to be acquired in a week's time. For example, in enteric *E. coli* strains, they become resistant in a week's time after chicken being infected with this *E. coli*. This is quite alarming in terms of the efficiency of chemotherapy. Resistance to antibiotics typically occurs by one or more of the following mechanisms:-

i. Inactivation of the drug (enzyme-mediated resistance)
ii. Alteration of the target site.
iii. Reduced cellular uptake (altered transport)
iv. Increased efflux

In summary, bacteria utilize four main resistant strategies, these are;

1. Modification of their permeability either by becoming impermeable to antibiotics or by actively excreting the antibiotic accumulated in the cell.

2. Modification of the antibiotic by producing enzymes capable of modifying and directly inactivating antibiotics.
3. Modification of target; - Bacteria modify the structure of the antibiotic`s target molecule which is usually an essential metabolite of the bacterium or express an alternative target molecule not inhibited by the drug thus escaping the antibiotic`s toxic effect.
4. Over production of the target such that even if some are destroyed by the effect of the antibiotic, there will still be enough left to keep the bacteria living (EMEA public., 1999).

2.7 Mechanisms for inter-bacterial transfer of resistance

Three mechanisms have been identified for inter- and intra-transfer of genetic material, including resistance genes. These mechanisms are:

i. Transduction- This is common in Gram positive bacteria. Transmission of genetic material from one bacterium to another is by bacteriophages.

ii. Transformation- This involves direct transfer of free DNA originating for example from lysed bacteria.

iii. Conjugation- This is common in Gram- negative bacteria. The latter is the most important mechanism of inter- and intra-bacterial transfer of resistance. A plasmid or other genetic material is transferred from the donor bacterium to the recipient via cytoplasmic bridge (pilus). Conjugation may occur between bacteria of the same species, within species of the same genera or between species of different.

2.8 Factors that influence antibiotic resistance of bacteria

Pharmacokinetic characteristics of different classes of antibiotics may favour the development of resistance as well as dose regime (like insufficient dose, too short duration of treatment or long term use), active concentration or route of excretion of the drug.

i. The long term use of sub-MIC (sub-therapeutic doses) is regarded as one of the major factors responsible for development of resistance. This exerts a potent selective pressure for the emergence of resistant clones that already pre-existed in the bacterial population. The progressive emergence of insensitive bacteria and of acquired resistance in human clinical settings and the veterinary fields reflects the "tuning of these micro-organisms to antibiotic polluted" ecosystems.

The amount of antibiotics used is also a selective force.

2.9 Antibiotic Resistance of Bacteria in the Environment

Jury *et al.* (2011) defined antibiotics as a group of compounds with antimicrobial activity, which are either synthetic, semi-synthetic or naturally occurring. They find wide application in medicine (veterinary and human medicine) for the treatment and prevention of a number of infections and diseases as well as they have been used as growth promoters in animal intensive industries (Jury *et al*., 2011). The phenomenon of multi-resistance or simply the resistance to several antibiotics of two or more different classes by microbes is a major concern in medicine. Infections and death due to multi-resistant superbugs are on the increase with each passing day according to Levy (1992). Resistance to antibiotic can be established in the digestive tract of both humans and animals in accordance to Launay*et al*. (2004). The spread of resistant strain within the gastrointestinal tract (GIT) is made possible by the phenomenon of co-occurrence of bacteria in high population density as well as sub-lethal antibiotics doses. It is often assumed as well as known that effluent emanating from pharmaceutical manufacturing plants and waste water treatment facilities receiving wastewater from sewage, hospitals and veterinary clinic are some of the notable sources through which antibiotics are released into water bodies (Karthikeyan and Meyer, 2006). This

could be attributed to the cause of multiresistant bacterial pathogens in the environment. Towards the end of the 20th century, several antibiotics classes, were discovered in sewage as well as its treatment plants (STPs) (Jury *et al*., 2011). These include ß-lactams, trimethoprim, sulphonamides fluoroquinolones, macrolides, and tetracycline (Karthikeyan and Meyer, 2006). Many of these antibiotics find their way into water bodies unchanged as they are not completely metabolized during therapeutic use. Various researchers have reported that wastewater harbours bacteriophage carrying various genes for antibiotic resistance, which is an easy route for spreading resistance to antibiotics amongst bacterial communities (Jury *et al*., 2011). Trickling filters, activated sludge tanks and others STPs are examples of biological reactors, which creates conditions for bacteria to encourage their activity as well as proliferation (Jury *et al*., 2011). They decrease viable population of faecal bacteria such as enterococci and coliforms (Jury *et al*., 2011) depending on the efficiency of the plant (Martins de Costa and friends, 2006; Jury *et al*., 2011). However, relatively high number of bacteria still remains the effluents, up to 10^3cfu/ml$^{-1.}$ Enterococci have been reported (Jury *et al*., 2011). Some of these bacteria harbour genes for resistance even in the wastewater (Jury *et al*., 2011). Reinthaler*et al*. (2005), on the phenomenon of bacterial resistance and multi-resistance reported that 40% of the strains of *E. coli* recovered from or isolated from sewage were found to be resistant to more than one antibiotics. While another staggering sum of about 9.8% were shown to be multi-resistant in that they were resistant to more than three classes of antibiotics. Szczepanowski and colleagues, (2005); Jury and friends, (2011) posited that various plasmids which have been found to harbour different genes for resistance to about ten (10) antibiotics, have been recovered or isolated from treatment plants for sewage. Aside from the health implications, the dual presence of resistant bacteria as well as the antibiotics in the environment creates a huge concern for potential ecological impact according to Jury *et al*. (2011).

2.10 Management of Diseases and Resistance to Antibacterial Drugs

For more than 60 years, antibacterial drugs a have been regarded as the panacea to cure infections, whether or not their use is appropriate, and whether the infection was acquired in the community or in the hospital setting (WHO, 2018). Already in his Nobel Prize speech in 1945, Alexander Fleming, who discovered penicillin, warned that bacteria could become resistant to these remarkable drugs. Indeed, the development of each new antibacterial drug has been followed by the detection of resistance to it. The development of resistance is a normal evolutionary process for microorganisms, but it is accelerated by the selective pressure exerted by widespread use of antibacterial drugs (Walsh, 2000). Resistant strains are able to propagate and spread where there is non-compliance with infection prevention and control measures.

Use of antibacterial drugs has become widespread over several decades (although equitable access to antibacterial drugs is far from being available worldwide), and these drugs have been extensively misused in both humans and food-producing animals in ways that favour the selection and spread of resistant bacteria (WHO, 2018). Consequently, antibacterial drugs have become less effective or even ineffective, resulting in an accelerating global health security emergency that is rapidly outpacing available treatment options. Until the 1970s, many new antibacterial drugs were developed to which most common pathogens were initially fully susceptible, but the last completely new classes of antibacterial drugs were discovered during the 1980s (Silver, 2011). It is essential to preserve the efficacy of existing drugs through measures to minimize the development and spread of resistance to them, while efforts to develop new treatment options proceed.

2.11 Health and Economic Burden due to Antibacterial Resistance

For several decades antimicrobial resistance (AMR) has been a growing threat to the effective treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. AMR results in reduced efficacy of antibacterial, antiparasitic, antiviral and antifungal drugs, making the treatment of patients difficult, costly, or even impossible (WHO, 2018). The impact on particularly vulnerable patients is most obvious, resulting in prolonged illness and increased mortality. The magnitude of the problem worldwide and the impact of antimicrobial resistance (AMR) on human health, and on costs for the health-care sector and the wider societal impact, are still largely unknown. Some estimates of the economic effects of AMR have been attempted, and the findings are disturbing. For example, the yearly cost to the US health system alone has been estimated at US $21 to $34 billion dollars, accompanied by more than 8 million additional days in hospital (WHO, 2018). Because AMR has effects far beyond the health sector, it was projected, nearly 10 years ago, to cause a fall in real gross domestic product (GDP) of 0.4% to 1.6%, which translates into many billions of today's dollars globally (WHO, 2018). Patients with infections caused by bacteria resistant to a specific antibacterial drug generally have an increased risk of worse clinical outcomes and death, and consume more healthcare resources, than patients infected with the same bacteria not demonstrating the resistance pattern in question. Available data are insufficient to estimate the wider societal impact and economic implications when effective treatment for an infection is completely lost as a result of resistance to all available drugs.

2.12 Regulatory Standards and Guidelines for Potable Water

Safe drinking-water is suitable for all usual domestic purposes, including personal hygiene (WHO, 2006). Safe drinking water, as defined by the Guidelines, does not represent any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages. The Guidelines describe reasonable minimum requirements of

safe practice to protect the health of consumers and derive numerical “guideline values” for constituents of water or indicators of water quality.

The WHO standard for heterotrophic bacteria in potable water supplies states that the total heterotrophic bacterial count should not be more than 100 CFU/ml (WHO, 1996). The WHO standards for total and fecal coliforms are 1 to 10/100 ml and 0/100 ml, respectively (USEPA, 1976; WHO, 1982, 2003).Nonetheless, water must be free from pathogenic organisms and organisms indicating fecal contamination, such as fecal coliform bacteria like *Escherichia coli*, enterococci bacteria and coliphage viruses. Water must not contain more than 10 total coliforms per 100 ml of samples collected and at least 90% of samples must be free from total coliform bacteria. Thus, the drinking water is considered contaminated when *E. coli* is detected in the water sample, when greater than 10 total coliforms are detected in two or more water samples within a 30-day period. Bacterial guidelines for drinking water are critical because they regulate water that directly affects human health. It is thus imperative to ensure safe and bacteria-free drinking water (WHO, 2006). Drinking water becomes contaminated when faeces containing pathogens are deposited or flushed into the water. If treatment is insufficient, or if the water distribution system is inadequate, drinking water may contain sufficient numbers of pathogens to cause illness (O’Connor, 2002). In general, every country has its own set of guidelines for drinking water. However, most of these guidelines are similar for different countries, and the same indicator microorganisms are used. WHO (1999, 2006) recommends that *E. coli* or thermotolerant coliform and total coliform bacteria should not be detected in any 100 ml sample of water intended for drinking.

2.13 Physicochemical Parameters for Safe Drinking Water

2.13.1 Total Dissolved Solids (TDS)

This parameter arises from the dissolved substances from organic compounds as well as decomposition of inorganic substances such as nitrate and carbonate. The recommended limits for total dissolved solids stipulated by Nigerian Industrial Standard (NIS) (2007) and WHO (2008) are in the limits of 500 and 1500 respectively. Values above the recommended standard for drinking water pose a threat to health

2.13.2 Electrical Conductivity (EC)

Electrical conductivity (EC) in natural waters is the normalized measure of the water's ability to conduct electric current. This is mostly influenced by dissolved salts such as sodium chloride and potassium chloride. The recommended limits are values within 1000µS/cm set by Nigerian Industrial Standard (NIS) (2007) and WHO (2008)

2.13.3 Total Alkalinity

The alkalinity in water is mainly due to the presence of bicarbonates. It is a measure of the capacity of the water to neutralize acids and it reflects its inherent resistance to changes in pH. No specific value is available for WHO water quality standard for this parameter. However, the limit stipulated by NIS (2007) is in the values of 100mg/L.

2.13.4 Calcium

Calcium is an element that is found naturally and in abundance in the earth crust. It is an important and abundant element in the human body and an adequate intake is essential for normal growth and health. Ca^{+} is the most important element causing hardness in water. The threshold limits of 45mg/L and 67.3mg/L are given by Nigerian Industrial Standard (NIS) (2007) and WHO (2008). When there is hardness in water, it would not form lather with soap

as such, washing clothes with the water won't be interesting. It could also consume more soap since lather formation or foaminess will be hampered.

2.13.5 Total Iron

Iron is the fourth most abundant element, by weight, in the earth's crust. Iron in groundwater is normally present in the soluble ferrous (Fe^{2+}) form. It is easily oxidized to the insoluble ferric (Fe^{3+}) state upon exposure to air. The amount of Fe in water varies depending on the geology of the area and other chemical constituents of the water. Underground water normally contained Fe^{2+} due to lack of enough oxygen in the aquifer. Iron is also known to promote the growth of iron bacteria in water and also makes the water distasteful. Apart from its unpleasant taste, iron forms rust in water and it can cause clogs and stains pipes. The acceptable limits of 0.30 mg/L each are recommended by Nigerian Industrial Standard (NIS) (2007) and WHO (2008) as the maximum permissible limit and does not affect the taste of water.

2.13.6 Copper

Copper and its compounds are widely distributed in nature, and copper is found frequently in surface water and in some groundwater. Copper is an essential element in human metabolism, and it is well-known that deficiency results in a variety of clinical disorders, including nutritional anaemia in infants. Although the intake of large doses of copper has resulted in adverse health effects.

2.13.7 Fluoride

Fluoride content in drinking water is of interest because it its concentration in optimum dose is beneficial and its concentration in excess of the optimum dosage adversely affect the health of consumers. High fluoride concentration in the ground water and surface water in many parts of the world is a cause of great concern. The main source of fluoride in ground water is

fluoride-bearing rocks such as Fluorspar, Fluorite, Cryolite, Fluorapatite and Hydroxylapatite (Meenakshi*et. al*., 2004). It has been established that drinking water should have a fluoride concentration from of 1-1.5 mg/L as recommended by Nigerian Industrial Standard (NIS) (2007) and WHO (2008). At this concentration, fluoride is beneficial as it prevents tooth caries especially among children. However, the consumption of water that contains fluoride in excess of 1.5 mg/l can cause dental and skeletal fluorosis.

2.13.8 Nitrate and Nitrite

Elevated nitrate levels in drinking water are often caused by groundwater contamination from animal waste, excessive use of fertilizers, or seepage of human sewage from private septic systems. Nitrite is of particular health concern in the body because it causes the hemoglobin in the blood to change to methemoglobin. Methemoglobin reduces the amount of oxygen that can be carried in the blood. This results in cells throughout the body being deprived of sufficient oxygen to function properly. This condition is called methemoglobinemia (ATSDR, 2000). Nitrite can react with secondary amines in human stomach to form the highly carcinogenic nitroso compounds (Gray, 2008). The recommended limits of 50mg/L were given by Nigerian Industrial Standard (NIS) (2007) and WHO (2006).

2.13.9 Manganese

Manganese occur naturally in many surface water and groundwater sources and in soils that may erode into these waters. However, human activities are also responsible for much of the manganese contamination in water in some areas (WHO, 2011). The Nigerian Industrial Standard (NIS) (2007) and WHO (2006) recommends a threshold limits of 0.5mg/L and 1-1.5mg/L Mn levels respectively in drinking water.

2.13.10 Sulphate

Sulphate minerals are widely distributed in nature, and the sulphate anion ($SO4^{2-}$) is a common constituent of unpolluted water. Sulphates may be leached from most sedimentary rocks, with appreciable contributions from such sulphate deposits as gypsum. The recommended limits for sulphate as stipulated by NSDWQ and WHO is in the value of 200mg/L.

2.13.11 Chloride

Chlorides are widely distributed in nature, usually in the form of sodium, potassium, and calcium salts (NaCl, KCl, and $CaCl_2$), although many minerals contain small amounts of chloride as an impurity. Chlorides in water are more of a taste than a health concern, although high concentrations may be harmful to people with heart or kidney problems (Weiner, 2000). The recommended limits of 250mg/L and 200mg/L are set by (NIS) (2007) and WHO (2006).

2.13.12 Turbidity

Turbidity measures the degree to which water losses its transparency due to the presence of suspended particles. Turbidity in water arises from the presence of very finely divided solids (which are not filterable by routine methods). The existence of turbidity in water affects its acceptability to consumers and it will also affect markedly its utility in certain industries (EPA, 2001). It represents an important aspect of water quality. The presence of microorganisms is often associated with increased turbidity; hence low turbidity reduces the potential for transmission of infectious diseases (DWAF, 1996). Turbidity is deemed as the cloudiness of a liquid as a result of particulate matter being suspended within it. Its importance is highlighted by the fact that suspended solids interfere with effective chlorination/disinfection and helps to shield bacteria (Asano, 2007). Additionally, suspended solids also serve as a place of attachment for bacteria (Hurst, 1996). The general WHO

standard set for drinking water is a turbidity <0.1 NTU. A turbidity >5 NTU is considered unhealthy.

2.13.13 pH

The Nigerian Industrial Standard (NIS) (2007) and WHO (2008) Guidelines for drinking water quality states that the pH ranges of drinking water fall between 6.5 and 8.0.

2.13.14 Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)

BOD and COD are used to measure the amount of oxygen used and equate it to the amount of organic matter within the water sample. BOD measures the amount of oxygen used by microorganisms to oxidize organic matter present within the water sample (Nielsen, 2003). Water with BOD levels <4mg/L are deemed as clean, while those >10 mg/L are considered polluted and unsafe as delineated by Nigerian Industrial Standard (NIS) (2007) and WHO (2008). COD is used to measure the oxygen equivalent of organic matter of a sample through the use of a chemical oxidant. COD values ought to be less than 10 mg/L at the end of treatment of water as delineated by (NIS) (2007) and WHO (2006).

2.13.15 Total Hardness

No specific value was mentioned by WHO as the threshold limit for Total Hardness in water. However, the limit set by Nigerian Industrial Standard (NIS) (2007) and must not exceed 150mg/L.

2.13.16 Temperature

Though not defined Nigerian Industrial Standard (NIS) (2007) and World Health Organization (WHO), the temperature value of safe drinking water should appeal to the consumer.

2.13.17 Colour

The colour of drinking water reflects the presence of suspended matter. Therefore, the more suspended matter in water the greater is the colour. In exceptional circumstances however, color may arise naturally from the presence of colloidal Iron/Manganese in water. The recommended limits set for colour by both The Nigerian Industrial Standard (NIS) (2007) and WHO (2008) is 15pt.co. unit.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sampling Location

Ogbese River is in Ovia North East L.G.A of Edo State (Figure 1)which was captured at Ogbese and Olumoye towns. The area lies within Latitude E6^0 SE8^0 and longitude N4^0 NE6^0. Many towns and villages also depend on Ogbese River. The population of Ovia North East L.G.A as at 2016 was 159,500 persons. The river has its source from Adede-Ekiti in Ekiti State and flows through Ondo State to Ogbese in Edo State, 10km East of Akure. Olumoye is about 12km from Ogbese town. River Owan flows into River Ogbese before Olumoye town whose occupations are mostly farming and fishing. River Ogbese flows down-stream through many towns (including Olumoye) and villages with adjoining streams and rivers to form Ovia River (Ministry of lands survey and housing, Benin City, 2017).

3.2 Sample Collection

Samples of raw surface water were collected from Ogbese River at Ogbese and Olumoye towns during the months of June to December, 2018 and February 2019. Six (6) sampling points of 50 meters apart were randomly evaluated at Ogbese (site A) and six (6) sampling points in Olumoye (site B). Sampling was carried out aseptically using sterile 500 ml flasks by dipping the flasks into the surface water before opening the lid, covered immediately after obtaining water samples, and transported on ice to laboratory for analysis (Delgado-Gardea*et al.,* 2016).

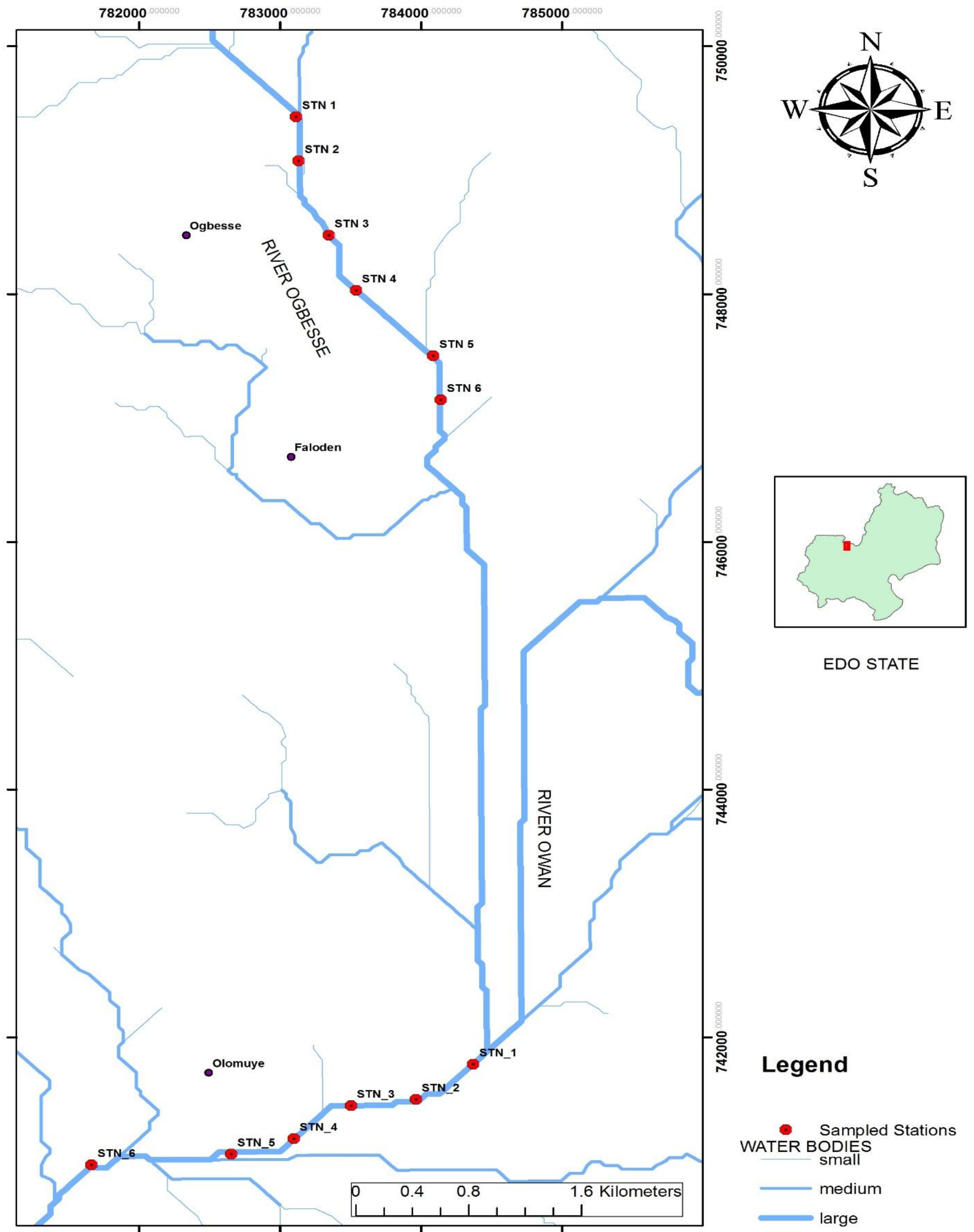

Fig. 3.1. Map of Ogbese River in Ovia North East Local Government Area and the sampling sites

3.3 Bacteriological Analysis of Water Samples

3.3.1 Isolation of Bacteria

One hundred milliliter volume of each water sample was filtered through 0.45mm pore-sized filter (cellulose nitrate membranes). The membranes were aseptically placed on plates of nutrient agar, for total heterotrophic plate counts and MacConkey Agar to enumerate total coliform counts, *Salmonella Shigella* Agar (SSA), Bile Esculin Agar (BAA), Mannitol Salt Agar (MSA), Thiosulfate Citrate Bile Salt Sucrose (TCBS) Agar, and Eosin methylene blue agar were used for bacteriological analysis (Bridson, 2006). The cultured plates were incubated for 24-48 hours at 37^0C. Distinct colonies were purified by subculture using streak plate method on sterile agar plates and further incubated at 37^0C for 24hrs. Stock cultures on nutrient agar slants were prepared from the purified cultures and kept at 4^0C for further characterization (Mulamattathil*et al.,* 2014).

3.3.2 Most Probable Number (MPN) to determine total feacal coliform

The modified method of Sood*et al.* (2015) was employed for this assay. The total and faecal coliform counts were determined using the multiple-tube fermentation technique as well as the Most Probable Number (MPN) of organisms present per 100 ml of samples. Briefly, 50 ml, 10 ml and 1 ml double and single strength MacConkey broth were inoculated with 10 ml, 1 ml, and 0.1 ml in triplicate with aliquots of the seasons/site samples. The broth, which contains an inverted Durhan tube, was incubated at 37^0C for 24 h. One milliliter (1 ml) of gas-positive samples were inoculated into eosin methylene blue agar and MacConkey agar for fecal and total coliform confirmation. The inoculated plates were incubated at 37^0C for 24 h while the negative samples were further incubated at the same temperature for an additional 48 h as confirmatory test for the presence of total and fecal coliforms. The water

samples, which exceed the WHO standard (0 MPN/100 ml), were analysed using standard microbiological techniques for identification of pathogen.

3.4 Identification of Bacterial Isolates and Molecular Assay

Standard bacteriological procedures involving colonial characteristics such as size, elevation, pigments, margin and shape were used. In addition, the morphological characteristics of the isolates were determined by Gram's staining technique. Biochemical characterization, which include catalase, oxidase, coagulase production, citrate utilization, indole production and sugar fermentation were also evaluated (Cheesbrough, 2006; Holt *et al*., 2002). The biochemical test for the isolates were based on the cultural and morphological characteristics on selective media.

3.4.1 Extraction of DNA from Isolates

DNA was extracted from the pure cultures of all isolated bacteria in the study. An aliquot of 1.5 ml culture in a broth was aseptically dispensed into Eppendorf tube before centrifugation for 5 min. at 10,000 xg. The supernatant was discarded and 200 µl of autoclaved deionized water (sterile distilled water (SDW)) was added to the collected pellet and vortexed. The cap of the Eppendorf tube was pieced with a needle and the tube was boiled in a heating block at 100 °C for 10 min. After boiling, the Eppendorf tube was kept in ice for 10 min and then centrifuged at the 10.000 rpm for 10 min. Briefly, 100 µl supernatant containing the bacterial chromosomal DNA was then collected and used for PCR amplification and further studies (Islam *et al*., 2016).

3.4.2 Identification of bacteria by sequencing of the 16s rRNA

PCR amplification was performed using Applied Biosystemverti thermal cycler (Model FTC41H2D, UK). The primers for PCR amplification were obtained from Sigma-Aldrich. Universal Primer 27 forward **5^{1}** AGAGTTTCCTGGCTCAG **3^{1}** reverse

5'ACGGCTACCTTGTTACGATT **3'**. A 20µl reaction mixture which contains 2µl of 10X assay buffer, 0.5µl of $MgCl_2$, 1µl each of forward and reverse primer (5pmol), 1µl dNTP mix of 2.5 mM, 0.5µl of Taq polymerase, 13.5µl of high performance liquid chromatography (HPLC) grade water and 1µl of template DNA was used for the PCR analysis. The following amplification for 16s rRNA was used which is initial denaturation for 4 min at 95ºC followed by 38 cycles of denaturation, annealing and extension (94ºC for 1 min, 59.9ºC for 2 min and 72ºC for 2 min) with a final extension for 20 min at 72ºC followed by hold for infinity at 4ºC. A 2.5% agarose gel was used to detect the presence of the PCR products via electrophoresis and also to analyze the size of amplified product of PCR. DNA ladders or markers of 100bp provided by Puregene was used (Basavaraj*et al.,* 2014).

3.4.3 Blasting of Amplified PCR Sequences and Deposition of Sequences in Gene bank

Following sequencing of the amplified bacteria DNA, blasting was carried out on the sequences using the national centre for biotechnology information (NCBI) blast website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Bacterium with the highest homology chosen and the sequences were deposited in the GenBank(https://submit.ncbi.nlm.nih.gov/subs/genbank/)to obtain ascension numbers for the isolated bacteria.

3.5. Standardization of Bacterial Culture

Standardized bacteria culture were prepared using the method delineated by Bauer *et al.* (1996) where 0.5 ml of $Bacl_2$ was added to 99.5 ml of 1% H_2SO_4. The corresponding turbidity (referred as 0.5 McFarland standard) was used as control for the turbidity of bacterial suspension prepared via the addition of bacteria colony in a sterile diluent. More bacterial cells were added to the diluent until the turbidity becomes identical to the 0.5 McFarland standard.

3.5.1 Antibiotic Susceptibility Testing

The disc diffusion method was used for susceptibility testing as described by the Clinical and Laboratory Standards Institute (2017). The isolates from the slants were first cultured in nutrient broth. The turbid broth was then diluted to be equivalent to a 0.5 McFarland standard (1.5 x 10^8cfu/ml). Swab sticks were used to spread the turbid inocula on the Muller Hinton agar plates. The antibiotics used were pefloxacin (Pef) 10µg, gentamycin (GEN) 30µg, ampiclox (APX) 30µg, cefuroxime (zinnacef (Z)) 20µg, amoxicillin (AM) 30µg, ceftriaxone (rocephin (R) 30µg, ciprofloxacin (CPX) 10µg, streptomycin (S) 30µg, septrin (SXT) 30µg erythromycin (Ery) 10µg and Carbapenem. The antibiotic discs were carefully and firmly placed on the inoculated Muller Hinton agar plates using a sterile pair of forceps. The plates were inverted and incubated for 37 °C for 24 h. The diameter of the zone of inhibition were measured in millimeters (mm) using a meter rule. The results were taken as resistant, intermediate or sensitive, according to the standards stipulated by Clinical and Laboratory Standards Institute (2017).

3.5.2 Multiple Antibiotic Resistance (MAR) Index

The MAR index is a good tool for health risk assessment, which identifies if the isolates are from a region of high or low antibiotic use. A MAR index of 0.2 and above indicates a 'high-risk' source of contamination (Davis and Brown, 2016). The multiple antibiotic resistance MAR index was determined for each isolate using the methods delineated by Chitanand *et al.,* (2010). In this method, the percentage of antibiotic resistance of a particular species of bacteria with a definite sample number (*say n =5)* is calculated and used as the numerator while the sum of the percentage of the entire antibiotics used in the study is taken as the denominator. The formula above is similar to the conventional method for determining the MAR index of a bacterium which other studies have calculated MAR index as ($^{a}/_{b}$) where a = Number of antibiotics an isolate is resistant to and; b = Total number of antibiotics used in

the study (Krumpernam, 1983). MAR index higher than 0.2 identifies organisms that originate from high-risk sources of contamination, where antibiotics are often used (Davis and Brown, 2016).

3.5.3Plasmid Curing

Furthermore, the isolates that were multi-resistant during antibiotic sensitivity experiment were subjected to plasmid curing. The acridine orange required for plasmid curing, was prepared directly before use by dissolving acridine orange 7.5 mg in 100 ml in sterile distilled water. Following the dissolution of 7.5 mg of acridine orange in 100 ml (75 µg/ml), several other concentrations (30, 50, and 70 µg/ml) were prepared. One milliliter (1ml) of the different concentrations of acridine orange were added to 5 ml of tryptic soy broth. After which, the test bacteria isolates standardized to 1.5 x 10^8cfu/ml (100 µl suspension) was inoculated into nutrient broth containing different concentrations of acridine orange and incubated for 48 hours at 35°C. Similarly, control cultures were prepared without acridine orange in nutrient broth. After incubation, serial dilutions were prepared from each of the acridine orange-culture suspensions and then inoculated onto Mueller Hinton agar and screened again for antibiotic susceptibility using standardized disc diffusion method (CLSI, 2017).

3.5.4 Hemolysin Production

Hemolysin production was detected using the method described by Drews*et al*. (2005). This involves the inoculation of the organism isolated from water samples onto a 5% sheep blood agar plates. This was incubated aerobically for 24 h at 37 °C. The presence of a clear zone around the colonies were taken as positive for hemolysin production (Egbe and Enabulele, 2014).

3.5.5 Determination of Serum Resistance

The serum resistance assay was performed by the method described by Egbe and Enabulele, (2014). Normal serum from apparently healthy individuals were pooled and the serum was inactivated at 56 ^{o}C for 30 minutes in an incubator. The test organisms that were grown in nutrient broth overnight at 37 ^{o}C were standardized to 10^6 cells per ml in 5mls fresh nutrient broth and incubated at 37 ^{o}C for 2 h. This broth cultures was centrifuged at 1500g for 5 minutes and the sediment was re-suspended in 5ml sterile phosphate buffered saline. Equal volumes (1ml each) of pooled normal human serum was added to 1mls of bacterial- broth complex and incubated in a water bath at 37 ^{o}C. Viable count was performed on the culture after incubating at 0 hour and 3 hours intervals using the pour plate method. If after the incubation the viable count dropped to less than 1% of the initial value (comparing counts at 0 and 3 hours), the isolates was regarded as sensitive to the normal human serum. If more than 90% of the cells survived after 3 hours, the isolates is said to be resistant to the pooled normal human serum (Egbe and Enabulele, 2014).

3.6 Sereny Test for Invasiveness

This test was carried out to evaluate the ability of pathogenic bacteria obtained in the study to invade and proliferate in the epithelial cells. A pure culture of freshly prepared bacteria grown overnight on agar plates at 37 ^{o}C are harvested and suspended in a freshly prepared broth standardized to give approximately 1.5 x 10^8cells per ml. A matured healthy mouse, albino strains of 16-21 weeks were used per isolate and were infected with 1.5 x 10^8 viable cells per eye of a known Gram-negative enteropathogenic isolates. The right eye of the mouse receives the bacteria inoculum while the left eye receives sterile saline as a control according to the method delineated by Murayama *et al*. (1986). The macroscopic changes observed in the mice eyes include conjunctivitis, redness and swelling of the palpebral,

hyperemic bulber conjunctiva, bulbopalpebral adhesion and epidermal hyperemia from the eyes to the nose as a function inflammation.

3.6.1 Enterotoxin assay

This was carried out according to the method of Everests*et al*. (1999) using known Gram-negative enteropathogenic isolates on rabbit ileal loops assay. A healthy rabbit weighing less than 2 kg was anesthetized, the peritoneal cavity was opened with sterile surgical blade and the bowel was carefully washed with pre-warmed sterile water. The length of the bowel making the loops is cut at both ends and both surfaces was claimed. The ileal loops are ligatedated into five-centimeter (5 cm) segments and 2 cm intervals. Required number of loops were constructed by tying with ligatures. The ends of the isolated intestine were closed with sutures. Then 0.5ml aliquot of the different suspensions of the test organisms were introduced into each of the ligated segments of the ileal. Controls were inoculated with only sterile water. The ileal loop was then placed in a clean bowl for 18 h. Positive loops show intense inflammatory response and fluid accumulation in segments that contain enterotoxigenic organisms, the fluid was measured using syringe while taking note of the viscosity, texture and colour. Accepted or minimal results of 0.25 ml and above is considered as positive Everests*et al*. (1999).

3.7 Determination of Physico-Chemical Parameters

3.7.1 pH and Temperature

The hydrogen ion concentration (pH) of each sample and temperature were measured using a HACH digital pH/temperature meter. The electrode probe was inserted into a glass beaker containing about 20 ml of the sample and the result was read from the screen and recorded. The pH meter will be calibrated before and after each readings using freshly prepared pH buffers (7.00), (4.00) and (9.00).

3.7.2 Alkalinity

Total alkalinity was determined by titrimetric method using standardized trioxocarbonate (IV) acid, phenolphthalein and methyl orange indicator. The development of a pink color indicates the presence of carbonate. Then 2 drops of 0.1% methyl orange indicator was added and titrated with standard 0.25N H_2CO_3. A colourless reaction gave the endpoint (APHA, 1993).

3.7.3Total Suspended Solids

This was determined by filtering a measured volume (50 ml) of the sample via a standard glass fibre filter. The filtrate (filtered liquid) was thereafter added to a pre-weighed ceramic evaporating dish, which was placed in a drying oven at a temperature of 103 ^{0}C. it was thereafter cooled and weighed. The total suspended solids in mg/ml was calculated using the formula below:

$$mg\ of\ suspended \frac{solids}{L} = \frac{(A - B)x\ 100}{mL\ of\ sample}$$

Where: A = weight of filter paper plus dried residue (mg)

B= weight of filter paper (mg)

3.7.4 Electrical conductivity

The electrical conductivity of each water sample was determined using a portable conductivity meter. A 50 ml of the sample was collected with a beaker, the plastic electrode probe was inserted into the sample, and the result in microsiemens (µs/cm^{-1}) read from the screen. The meter was calibrated using distilled water after each measurement (APHA, 1993).

3.7.5 Turbidity

The turbidity of the respective water samples were be determined using spectrophotometric method. A five (05) ml of the sample was dispensed into a curvette and placed in the light chamber and the absorbance was measured at a specific wavelength (450 nm) using distilled water as blank. The turbidity values were recorded in nepholometric turbidity unit (NTU) (APHA, 1993).

3.7.6 Phosphate

Ten (10) ml of the water sample was dispensed onto a clean curvette. About 4 ml of phosphate reagent containing ammonium molybdate, antimony potassium tartate and ascorbic acid was also added to the curvette containing the water sample. A one (01) ml of 95 % ethanol and 1 ml of concentrated H_2SO_4 was added. It was shaken, and left for 5 min to allow for color development. The absorbance was determined at a specific wavelength (560 nm) using a spectrophotometer (Radojevic and Bashkin, 1999).

3.7.7 Nitrate

Ten (10) ml of the sample was placed in a test tube, followed by the addition of 2 ml NaCl solution, this mixture was swirled and 10 ml of H_2SO_4 solution was also added. The resultant solution was also swirled and allowed to stand. A sample blank was also prepared. To the first test tube containing the mixture of the sample, NaCl and H_2SO_4, 0.5 ml of brucine – sulphanilic acid reagent was added and the test tube was swirled and left to stand for 20 minutes. The test tubes were allowed to develop color and the absorbance reading of the solution was taken using a spectrophotometer at a specified wavelength (450 nm) (Ademoroti, 1996).

3.7.8 Sulphate

Ten (10) ml of the water sample was decanted onto a clean curvette. A one (01) ml of 95 % isopropyl alcohol, 0.5 ml of glycerol and 5 ml of conditioning reagent, which consist of NaCl, BaCl and Citric acid, were added to the curvette containing the sample. The solution was left to stand for 5 min to allow colour development, after which the absorbance was read at a specific wavelength (540 nm) using a spectrophotometer (APHA, 1993).

3.7.9 Dissolved Oxygen (DO)

The dissolved oxygen content is the amount of available oxygen present in the water (Venkatesharajuik*et al*., 2010). The dissolved oxygen value depends on a number of physical, chemical, biological and microbiological processes (Abida and Harikrishna, 2008). A 250 ml DO bottles were filled to the brim with samples, taking care to minimize contact with air. A 100 ml of the sample solution was measured to which 2 drops of starch indicator was added. The resulting dark blue solution was titrated against a colourless 0.0125M Thiosulphate solution (Ademoroti, 1996).

3.7.10 Biological Oxygen Demand (BOD)

Biological oxygen demand is a measure of the oxygen in the water that is required by the aerobic organisms (Abida and Harikrishna, 2008). The water sample was aerated using an air pump. A measured dilution of the water sample was done and seeding of the water sample was also conducted. Determination of the Dissolved Oxygen (DO_1) using Wrinkler's method on a suitable portion of the seeded water was carried out. An incubation bottle was filled to the brim with the remainder of the diluted water sample. The bottle was screw capped and incubated in the dark for 5 days at 20°C. On the 5th day, the DO value was determined. The BOD value was the result of the difference of the respective DO values divided by the percentage dilution (Ademoroti, 1996).

3.7.11 Chemical Oxygen Demand (COD)

The chemical oxygen demand is a measure of the oxygen equivalent of organic matter in a sample that is susceptible to oxidation by a strong oxidizing agent (Radojevic and Bashkin, 1999). The COD values for the water samples were determined using the colorimetric procedure as described by Ademoroti (1996). A measured volume of the sample was added to 5 ml of high range COD reagent (HACH). This mixture was placed in a COD reactor for about 1 h. and upon cooling; the absorbance of the mixture was read at a specified wavelength using a HACH DR 2010 Spectrophotometer.

3.8Data Analysis

The obtained data in this research were exposed to version 21.0 of SPSS statistical package. Unpaired students *t*-test was used to show significant difference between isolates from microbial counts between towns. The *p*-value less than (<) 0.05 were considered statistically significant.

CHAPTER FOUR

RESULTS

The total heterotrophic and coliform count of water samples obtained from Ogbese River in Ogbese and Olumoye towns is shown in Table 4.1. It was revealed that bacteria count was higher during the wet seasonthan the dry season in both towns assessed in the study. The highest bacterial count was obtained in station 5 at Ogbese River (104.2±32.0 x 10^3cfu/ml) for wet while the least count was obtained at station 2 during the dry season (42.4±0.25 x10^3cfu/ml). Similar results were obtained in Olumoye community during wet and dry season.

The most probable number (MPN/100 ml) of bacteria from water samples in Ogbese River at Ogbese and Olumoye town is shown in Table 4.2. The MPN of water samples was highest in wet season compared to dry season in all the six water collection stations. MPN was least in station 5 in Ogbese town during the dry season (1 MPN/100 ml) and highest in station 1 in during wet season (740 MPN/100 ml). For water samples obtained from Olumoye town, the MPN was highest (500 MPN/100 ml) in station 2 during wet season and lowest in station 4 during dry season (1 MPN/100 ml).

The typical enteric pathogens isolated were *Enterobacter cloacae* (67 %) and *Escherichia coli* (67 %) were the most dominant or prevalent bacterial isolates in Ogbese town during the wet season, while *Pseudomonas aeruginosa* (50 %) and *Escherichia coli* (50 %) the most dominant in Olumoye community during the wet season (Table 4.3).

The frequency of enterotoxin producing bacterial isolates from Ogbese River is shown in Table 4.4. Most of the bacterial isolates were found to produce bacteria toxins (toxigenic) with *Escherichia coli* (83.33 %) being the bacterium having the highest frequency of enterotoxin production. Isolates of *Salmonella enterica* (66.66 %) and *Vibrio cholerae* (66.66 %) were also found to have high frequency of toxigenic strains. The bacterium with the least frequency of enterotoxin production was *Staphylococcus aureus* (25.00 %).

Table 4.1. Total heterotrophic and coliform count of water samples obtained from Ogbese River in Ogbese and Olumoye towns

Towns/stations	Heterotrophic count (x 10^3cfu/ml)		Coliform count (x 10^3cfu/ml)	
	Wet period	Dry period	Wet period	Dry period
Ogbese 1	85.8±11.5	59.8±1.17	0.06±0.02	0.02±0.15
Ogbese 2	75.2±9.8	42.4±0.25	0.10±0.02	0.09±0.22
Ogbese 3	80.0±0.00	65.0±1.26	0.22±0.45	0.15±0.15
Ogbese 4	85.4±21.2	66.5±0.89	0.31±0.58	0.26±0.65
Ogbese 5	104.2±32.0	68.9±0.98	0.43±0.28	0.32±0.52
Ogbese 6	95.8±62.5	72.3±0.29	0.25±0.28	0.16±0.02
Olumoye 1	108.30±0.12	97.9±2.23	0.55±0.05	0.37±0.42
Olumoye 2	89.40±2.14	77.7±0.75	0.35±0.05	0.25±0.52
Olumoye 3	56.8±0.20	45.2±1.21	0.29±0.00	0.21±0.12
Olumoye 4	74.20±4.8	56.5±0.19	0.30±0.00	0.26±0.17
Olumoye 5	94.5±2.00	76.6±0.12	0.42±0.15	0.40±0.00
Olumoye 6	79.9±0.12	65.2±0.89	0.42±0.52	0.44±0.23
WHO standard	0.1±0.00	0.1±0.00	0.00±0.00	0.00±0.00

Legend: values are mean ± standard error of mean

Table 4.2. Most probable number (MPN/100 ml) of bacteria from water samples in Ogbese River

Sample Location/station	Ogbese		Olumoye	
	Dry season	Wet season	Dry season	Wet season
1	38	740	10	120
2	20	220	55	500
3	5	100	20	250
4	3	50	1	110
5	1	100	10	90
6	10	250	30	100

Table 4.3. Prevalence (%) of Gram-negative enteric bacteria in Ogbese River water samples

Bacterial isolates	Ogbese (*n*=12)		Olumoye (*n*= 12)	
	Wet season	Dry season	Wet season	Dry season
Salmonella enterica	3(50)	2(33)	3(50)	1(17)
Shigella flexneri	2(33)	0(0)	3(50)	1(17)
Enterobacter cloacae	4(67)	2(33)	3(50)	0(0)
Escherichia coli	4(67)	1(17)	3(50)	1(17)
Vibrio cholerae	3(50)	0(0)	3(50)	0(0)
Klebsiella pneumoniae	3(50)	1(17)	2(33)	1(17)
Pseudomonas aeruginosa	3(50)	1(17)	3(50)	2(33)

Legend: *n* = number of water samples, 6 samples per season for both communities

Table 4.4 Frequency of enterotoxin producing bacterial isolates from Ogbese River

Isolates	Number of isolates	Positive (%)
Escherichia coli	6	5 (83.33)
Shigella flexneri	6	2 (33.33)
Salmonella enterica	9	6 (66.67)
Staphylococcus aureus	4	1 (25.00)
Vibrio cholerae	9	4 (66.67)

Legend: n = number of isolates

The frequency of invasive enteric bacterial isolates from Ogbese River is presented in Table 4.5. All isolated bacteria were found to be invasive but *Shigella* and *Klebsiella* were found to have a 100 % frequency of invasiveness.

The serum resistance of isolated bacteria from Ogbese River is shown in Table 4.6. *Shigella flexneri* (100.00 %), *Vibrio cholerae* (83.33 %) and *Salmonella enterica* (55.55 %) had the highest frequency of serum resistance amongst the bacterial isolates evaluated in the study. *S, aureus* (20.00 %) and *P. aeruginosa* (40.00) were the isolates with the least frequency of serum resistance.

The prevalence of hemolytic bacterial isolates from Ogbese River is shown in Table 8. Majority of the bacterial isolates were found to be hemolytic (alpha and beta hemolysis). But a few of the isolates had higher percentage prevalence relative to their hemolytic behaviour. *S. aureus* (25.00 %) and *Klebsiella pneumoniae* (25.00 %) were the isolates with the least prevalence of hemolytic behaviour. Others include *Shigella flexneri* (66.67 %) and *P. aeruginosa* (60.00 %).

The antibacterial susceptibility (%) of bacterial isolates from Ogbese River is shown in Table 4.8. Majority of the bacterial isolates were susceptible to ciprofloxacin, streptomycin and perfloxacin with varying degree of susceptibility. All bacterial isolates but *Vibrio* (17) were resistant to cefuroxime and augmentin while gentamicin, ciprofloxacin streptomycin and septrin appear to be the antibiotics with the most efficacy in the study.

Antibiotics resistance profile from Ogbese River before and after curing of plasmids is presented in Table 4.9. All the isolates with the exception of 2 were found to contain bacterial plasmids for resistance to antibiotics. The two isolates, which were not found to contain plasmids, include *Vibriocholerae* and *Klebsiella pneumoniae*. Other bacterial isolates were

found to become sensitive or susceptible to the antibiotics they were initially resistant to before the curing process.

Table 4.5. Frequency of invasive enteric bacterial isolates from Ogbese River

Isolates	Number of isolates	Morphology	Positive (%)
Escherichia coli	6	produced inflammation of the right eye	4 (66.67)
Shigella flexneri	6	Invaded the epithelium with conjunctivitis of the right eye (redness of the eye)	6 (100.00)
Salmonella enterica	9	Produced inflammation reaction and ulceration of the epithelium of the right eye	5 (83.33)
Vibrio cholerae	6	produced inflammation of the right eye	4 (66.67)
P. aeruginosa	5	produced inflammation of the right eye	4 (80.00)
Klebsiella pneumoniae	4	produced inflammation of the right eye	4 (100.00)
Control	3	Left eye inoculated with 0.1 ml normal saline no inflammation and ulceration	

Table 4.6. Serum resistance of isolated bacterial from Ogbese River

Isolates	No. tested	Positive (%)
Escherichia coli	06	3 (50.00)
Shigella flexneri	06	6 (100.00)
Salmonella enterica	09	5 (55.55)
Staphylococcus aureus	04	1 (25.00)
Vibrio cholerae	06	5 (83.33)
P. aeruginosa	05	2 (40.00)
Klebsiella pneumoniae	04	2 (50.00)

Table 4.7. Prevalence of hemolytic bacterial isolates from Ogbese River

Isolates	No. tested	Positive (%)
Escherichia coli	06	2 (33.33)
Shigella flexneri	06	4 (66.67)
Salmonella enterica	09	3 (33.33)
Staphylococcus aureus	04	1 (25.00)
Vibrio cholerae	06	3 (50.00)
P. aeruginosa	05	3 (60.00)
Klebsiella pneumoniae	04	1 (25.00)

Table 4.8: Antibacterial susceptibility (%) of bacterial isolates from Ogbese River

Isolates	No. of isolates	CIP	STR	SXT	ERY	PEF	CN	APX	Z	AMX	R	MEM
E. coli	4	4(100)	4(100)	4(100)	2(50)	3(75)	4(100)	1(25)	0(0)	0(0)	3 (75)	4(100)
S. flexneri	5	5(100)	5(100)	5(100)	2 (50)	5(100)	5(100)	0(0)	0(0)	0(0)	0(0)	4(80)
S. enterica	6	6(100)	6(100)	3(50)	3(50)	6(100)	6(100)	3(50)	0(0)	0(0)	3(50)	4(57)
S. aureus	4	4(100)	2 (50)	3 (75)	1 (25)	2(50)	2(50)	0 (0)	0 (0)	0 (0)	0 (0)	-
V. cholerae	6	6(100)	6(100)	6(100)	3(50)	6(100)	6(100)	4(67)	1(17)	1(17)	2(33)	4(67)
P. aeruginosa	5	4(80)	4(80)	3(60)	2 (50)	5(100)	5(100)	0(0)	0(0)	0(0)	0(0)	5(100)
Klebsiella pneumoniae	4	2(50)	4(100)	3(75)	2(50)	3(75)	4(100)	1(25)	0(0)	0(0)	3 (75)	4(100)

Legend: CIP = ciprofloxacin, STR= streptomycin, SXT= septrin, ERY= erythromycin, PEF= perfloxacin, CN= gentamicin, APX = ampicillin, Z= cefuroxime, AMX = augmentin, R = ceftriaxone, MEM = meropenem

Table 4.9. Plasmid-mediated antibiotics resistance in isolates from Ogbese River

Isolates	Before curing	After curing	Plasmid borne resistance (markers)
E. coli	ERY, PEF, APX, Z, AMX	PEF, Z	ERY, APX, AMX
E. coli	ERYAPX, Z, AMX	-	-
E. coli	APX, Z, AMX	Z	APX, AMX
E. coli	AMX, R,	-	-
S. flexneri	APX, Z, AMX, R, ERY, MEM	MEM	APX, Z, AMX, R, ERY,
S. flexneri	APX, Z, AMX, R, ERY,	-	-
S. flexneri	APX, Z, AMX, R	-	-
S. flexneri	APX, Z, AMX, R	-	-
S. flexneri	APX, Z, AMX, R	-	-
S. enterica	AMX, R, SXT, ERY, APX, R	-	-
S. enterica	AMX, R, SXT, ERY, APX, R	-	-
S. enterica	AMX, R, SXT, ERY, APX, R, MEM	SXT, MEM	AMX, R, ERY, APX, R,
S. enterica	AMX, R, MEM,	-	-
S. enterica	AMX, R,	-	-
S. enterica	AMX, R,	-	-
S. aureus	APX, Z, AMX, R, ERY, PEF	-	-
S. aureus	APX, Z, AMX, R, ERY, PEF	-	-
S. aureus	APX, Z, AMX, R, ERY, STR, CN	APX, Z, AMX, R, ERY, STR, CN	-
S. aureus	APX, Z, AMX, R, SXT, STR, CN	-	-
V. cholerae	AMX, Z, ERY, APX, R,	-	-
V. cholerae	AMX, Z, ERY, APX, R,	-	-
V. cholerae	AMX, Z, ERY, R,	-	-
V. cholerae	AMX, Z, R, MEM,	-	-
V. cholerae	AMX, Z, MEM,	-	-
V. cholerae	-	-	-
P. aeruginosa	APX, Z, AMX, R, CIP, ERY,	-	-
P. aeruginosa	APX, Z, AMX, R, STR, ERY,	Z, ERY,	APX, Z, AMX, R, STR, ERY
P. aeruginosa	APX, Z, AMX, R, SXT, ERY,	Z, AMX	APX, R, SXT, ERY
P. aeruginosa	APX, Z, AMX, R, SXT	-	-
P. aeruginosa	APX, Z, AMX, R	-	-
Klebsiella	APX, AMX, Z, R, PEF, CIP,	-	-
Klebsiella	APX, AMX, Z, ERY, CIP,	APX, Z, AMX, ERY, Z, CIP	-
Klebsiella	APX, AMX, Z, ERY,	-	-
Klebsiella	AMX, Z, SXT,	-	-

Legend: CIP = ciprofloxacin, STR= streptomycin, SXT= septrin, ERY= erythromycin, PEF= perfloxacin, CN= gentamicin, APX = ampicillin, Z= cefuroxime, AMX = augmentin, R = ceftriaxone, MEM = meropenem

The multiple antibiotic resistance index is shown in figure 2 (appendix). All isolated bacterial were found to be greater than the permissible limit of 0.20, which connotes that they are of public health importance. *S. aureus* and *P. aeruginosa*, had the highest MAR index.

The physicochemical properties of River Ogbese River in Ogbese and Olumoye in wet and dry seasons is shown in Table 10. The pH of the water samples in Ogbese town was 6.85±0.04 for wet and while 6.65±0.08 was obtained for dry season. The pH of water samples in Olumoye town was 6.18±0.14 (wet season) and 6.11±0.21 (for dry season). There was no significant difference ($p>0.05$) between the pH of water samples obtained from Ogbese town. The turbidity of water samples from Ogbese River was 9.83±1.32NTU (dry season) and 32.00±2.16 NTU (wet season) in Ogbese town while it was found to be 8.83±1.01 NTU (dry season) and 30.83±5.92 NTU (wet season) in Olumoye town. Statistically speaking, there was a significant difference between the turbidity of Ogbese river at both towns of collection during the wet and dry season ($p<0.05$). The BOD of Ogbese River water samples were found to be in the range of 0.93±0.13 mg/ml (dry season) to 1.03±0.25 mg/ml (wet season) at Ogbese town while it was recorded to be between 1.10±0.16 mg/ml (dry season) and 2.32±0.21 mg/ml (wet season) in Olumoye town. There was a statistical difference ($p<0.05$) between the river water samples obtained in Ogbese town and Olumoye town. More so, there was no significant difference in the BOD of water samples obtained for wet and dry season in Ogbese and Olumoye town.

Table 4.10. Physicochemical properties of River Ogbese River in Ogbese and Olumoye in wet and dry seasons

Physicochemical parameter	Ogbese town		Olumoye town		WHO standard
	Wet season	Dry season	Wet season	Dry season	
pH	6.85±0.04	6.65±0.08	6.18±0.14	6.11±0.21	6.50-8.50
Colour (pt.co.)	94.67±6.75	35.00±6.14	79.00±12.73	27.17±3.05	-
Turbidity (NTU)	32.00±2.16	9.83±1.32	30.83±5.92	8.83±1.01	5
Conductivity (μS/cm)	101.67±1.67	65.00±7.19	68.33±8.72	48.33±7.92	1000
Suspended solids (mg/ml)	6.50±1.93	4.80±1.22	14.17±2.41	6.00±0.37	<10
Chloride (mg/ml)	18.82±2.35	20.02±1.18	22.35±5.59	16.47±2.35	200-600
Nitrate (mg/ml)	6.50±1.93	4.83±1.22	14.17±2.41	6.00±0.37	40-50
Phosphate (mg/ml)	0.48±0.02	0.12±0.01	0.43±0.05	0.12±0.02	5
Sulphate (mg/ml)	7.50±0.50	2.83±0.31	10.50±0.81	5.67±1.28	200
Dissolved oxygen (mg/ml)	5.18±0.26	2.63±0.08	6.38±0.09	2.26±0.19	1-5
BOD (mg/ml)	1.03±0.25	0.93±0.13	2.32±0.21	1.10±0.16	10
Calcium	18.04±0.58	13.76±1.57	10.56±1.90	9.89±1.89	75
Magnesium	5.99±0.53	5.51±1.70	5.19±0.13	4.62±0.85	
Lead	0.19±0.00	BDL	0.14±0.00	BDL	0.001
Zinc	0.41±0.05	BDL	0.26±0.03	BDL	5
Iron	0.32±0.02	BDL	0.22±0.05	BDL	0.3

Legend: BDL= below detectable limit

CHAPTER FIVE

DISCUSSION

Water is a fundamental resource, integral to all environmental and social processes. Access to adequate safe drinking water is of prime importance to many governmental and international organizations since it is undoubtedly the core component of primary health care and a basic component of human development as well as a precondition for man's success to deal with hunger, poverty and death (SOPAC/WHO, 2005). The total heterotrophic bacterial count from Ogbese River revealed that bacterial count was higher during the wet season in all of the stations analyzed (from 5.68±0.02 x 10^4 cfu/ml in station 3 from Olumoye to 11.58±6.25 x 10^4 cfu/ml in station 6 from Ogbese community). The bacterial and coliform count obtained was however higher than the reported permissible limit by world health organization (2008) of 1 x 10^2 cfu/ml. There was however, no significant difference in the bacteriological quality (heterotrophic and coliform counts) of water in Ogbese River in Ogbese and Olumoye communities ($p>0.05$; *t*- test). The result obtained in this study is in consonance with the results of Potgieter*et al*. (2006) whose report indicated that faecal coliform was present in the river water sources (River Limpopo, South Africa) and they were in the range of 100 to 1,000 MPN/100 ml. These values were similar to the ones obtained from stations 1 and 2 in Ogbese and Olumoye communities where coliform count was found to be 740 and 500 MPN/100 respectively. Coutros (2004) suggested that based on estimation of total coliform, which is a nonspecific bacterial indicator of water quality, all unimproved and semi-improved water sources, which have exceeded the stipulated limit by recognized bodies, were to be considered as not potable. This of course should include water sources from rivers, wells, as well as stored harvested rainwater. One of the main reasons for bacterial contamination of river water sources as described by WHO (2007) is as a result of direct discharge of untreated sewage into rivers and stream, and inefficient management of water distribution system. The

contaminated water therefore has critical impact on all biotic components of the ecosystem and this could affect its use for other purposes. Water can also get contaminated by bacteria from air, sewage, organic waste, dead plants and animal (Ajayi and Adejumo, 2011). The inhabitants of Ogbese and Olumoye communities use the river for several domestic as well as commercial activities. Their lack of enlightenment and possible negligence on sanitation could contribute to the high bacterial burden on the River. The following bacterial isolates were obtained from Ogbese River in the study. They include *Salmonella enterica, Shigella flexneri, Enterobacter cloacae, Escherichia coli*, *Vibrio cholerae*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. These isolates were similar to the ones reported by Aliyu*et al.* (2016) who evaluated the bacteriological qualities of river Lavun, Bida, Niger State. Some of these isolates were found to have pathogenic potentials as indicated by their abilities to produce toxins (Table 4.4), invasiveness (Table 4.5) resistance to serum (Table 4.6) and production hemolysin (Table 4.7). Alotaibi (2009) opined that the primary source of human pathogens in water is mainly from human and animal waste. The Presence of pathogenic species could cause various diseases to man and other animals. The results obtained in this study, with respect to the isolation of pathogenic bacteria from river water source is similar to the results of Orjiekwe*et al.* (2013) who reported that Ogbese River water falls short of being potable on account of its high presence of coliforms 2.5×10^5cfu/ml. The bacteria count of Ogbese river in this study ($42.4 \pm 0.25 \times 10^3$cfu/ml to $104.2 \pm 32.0 \times 10^3$cfu/ml) and 0.02 ± 0.15 for dry to 0.55 ± 0.05 coliform count for wetwas also consistent with the results of Akinbile and Omoniyi (2018) who reported that the coliform count of Ogbese river for wet (1.79×10^4 cfu/l) and dry (9.97×10^3 cfu/l) seasons exceeded the maximum limits stipulated by WHO (2006) who gave values of 1×10^0, 1×10^1 and 1×10^1 cfu/l respectively.More to the fact that the water quality exceeded standards stipulated by WHO (2006), it was found to contain *Salmonella*, *Shigella, Enterobacter, E. coli* and *Staphylococcus*. Similar results were also

obtained when the bacteriological quality of Ikpoba River, Edo State was analysed by Ekhaise and Anyasi (2005) as they reported that *Enterobacter cloacae, Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* amongst other bacterial isolates were found in the River samples. In addition, Ololade and Ajayi (2009) reported that major rivers, which are situated along highways in Ondo State were found to contain potential pathogenic bacteria such as *E. coli, Staphylococcus aureus, Klebsiella* and *Pseudomonas.* Amongst the rivers analysed were Ose, Owena, Oluwa and Ogbese. The isolated bacterial species in this study are notable threat to public health safety especially in immunocompromised individuals as their effect could range from mild to life threatening infections depending on the immune state of the consumer. The results for physicochemical parameters tested for all water samples obtained in Ogbese River revealed that the pH, Temperature, turbidity, alkalinity amongst other parameters were within acceptable limit for portable water standard stipulated by WHO (2006)and also similar to reports by Ekhaise and Anyasi (2005); Ololade and Ajayi (2009), Aliyu*et al*. (2016), as well as Akinbile and Omoniyi (2018) who analysed Ikpoba river, Oluwa, Ogbese, Osa and Owena rivers, River Lavun and Ogbese River respectively. All water samples were close to neutrality on the pH scale. They however were lower than the benchmark given by WHO (2006) and NSDWQ (2007).

The presence of chlorides in natural waters such as Ogbese River could result from the leaching of chloride-containing rocks and soils with which the water comes in contact. The values obtained in the samples analyzed are within the limits set by WHO (2006).The values for chloride content obtained in this study across wet and dry seasons were within the aforementioned range of values.Sulphate ions (SO_4^{-2}) occur naturally in most water supplies and River water. Sulphates are formed due to the decomposition of various sulphur containing substances present in water bodies such as drugs and animal waste. The values obtained from all locations in this study are low comparable to the WHO (2006) permissible

limits. Nitrates indicates the presence of fully oxidized organic matter present in water. Although nitrates levels that affect infants do not pose a direct threat to older children and adults, they do indicate the possible presences of other more serious residential or agricultural contaminants such as bacteria or pesticides (Robert, 2006). The Phosphate content (0.12±0.01 – 0.48±0.02 mg/L) of the water samples obtained from Ogbese River were within acceptable limit (5 mg/L) for drinking water quality and this result is in consonance with the report of Ogunlana*et al*. (2010).

The pH (6.65±0.08 – 6.85±0.04) of the water samples analyzed were in consonance with the report of Shittu*et al*. (2008). Extreme pH in water is not usually healthy for consumers especially when the acidity is high. Low pH in water tend to be corrosive to some certain metals, asbestos and pipelines (UNICEF, 2008). WHO reported that health effect are more pronounced in pH extremes and Drinking water with pH above 11 can cause skin, eye and mucous membrane irritation. On the opposite end of the scale, pH values below 4 can also cause irritation due to the corrosive effect of the low pH levels (Engwa*et al*., 2015). WHO (2008) warn that extreme pH level can worsen existing skin conditions. The turbidity of the water samples depend on the solid matter present in suspended state, which is a measure of the light emitting properties of the water. This parameter is used however to indicate the quality of waste discharge in the water samples with respect to colloidal materials (Ezeribe*et al*., 2012) and as such, the water samples from Ogbese River (8.83±1.01 – 32.00±2.16 NTU) was found to be higher than WHO stipulated limit of 5 NTU. Water hardness is a property that is defined by the quantity of calcium and magnesium ions present in water. Hard water does not usually form enough lather with soap compared to soft water with little of theses ions present in it. The result obtained in this study revealed that calcium ion in the water samples were within acceptable or tolerable limits stipulated by WHO (2006) thus, the water is good enough to use for washing and other domestic purposes.

Heavy metal refers to any metallic chemical element that have a relatively high density and it is toxic or poisonous at low concentrations (Tchounwou*et al*. 2012). Heavy metals tend to bioaccumulate over time in living organisms without being excreted by any means and thus they pose a great danger in the food chain. Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lIower energy levels, and damage of the lungs, kidney, liver and other vital organs of the body (Tchounwou *et al*. 2012). Long-term exposure may result in slowly progressing physical, muscular and neurologic processes that mimic multiple sclerosis, Parkinson’s disease, Alzheimer’s disease and muscular dystrophy. Repeated long-term contact with heavy metals and their compounds have been implicated to cause cancer (Rubio *et al*., 2006). The heavy metal contents of Ogbese River samples analyzed in this study (Table 4.10) for zinc, iron and lead respectively gave interesting results. Zinc was found to be in trace amount (0.26±0.03 to 0.41±0.05 ppm) within the permissible acceptable limit (5 ppm) of WHO (2006) for drinking water quality. This result is in agreement with literatures on well water samples analyzed by Kangpe*et al*. (2014), Ogunlana*et al*. (2010) and Bisi-Johnson *et al.* (2014). However, Iron and lead were found to be above stipulated limit delineated by WHO (2006). This could pose possible risk to health (as described above) on prolong consumption of such water samples.

CONCLUSION

The detection of bacterial indicators of contamination and some pathogenic species in the river water samples is a course for concern as consumption could pose serious health problems, such as gastroenteritis, also known as infections diarrhea. It is an inflammation of the gastrointestinal tract; the stomach and small intestine. Symptoms may include diarrhea, vomiting, abdominal pain, fever, lack of energy and dehydration. It therefore implies that the water samples from Ogbese River is unsafe for consumption because of the high bacterial load which exceeded the limits stipulated by WHO. Proper disinfection techniques such as general enlightenment should be adopted to reduce microbial load before consumption of such water. It can also be inferred that most of the physicochemical parameters are within acceptable limits delineated by WHO and NSDWQ, however, the microbiological quality is the most important aspect of drinking water in relation to waterborne diseases.

CONTRIBUTION TO KNOWLEDGE

The following are the contribution to knowledge:

1. Occurrence of potentially pathogenic bacteria present in Ogbese River was documented
2. The bacterial and coliform count of Ogbese River was above the standard stipulated by world health organisation for potable water quality and as such, there is need for public awareness.
3. Ogbese River water samples were found to be contaminated with transition metals such as lead.

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APPENDIX I

BACTERIA IDENTIFICATION TABLE

Table 11. Cultural, morphological and biochemical characteristics of bacterial isolates from Ogbese River in Ogbese and Olumoye towns

Cultural								
Elevation	Convex	Low convex	Low convex	Convex	Convex	Low convex	Convex	Low convex
Margin	Smooth	Smooth	Entire	Smooth	Entire	Entire	Entire	Smooth
Colour on NA	Pale milky	Cream	Green	Cream	Mucoid milky	Cream	Swarming	Cream
Morphological								
Gram stain	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
Cell type	Rod	Rod	Long rods	Cocci	Short rods	Rod	Rod	Coma shaped
Arrangement	Single	Single	Chains	tetrads	Single	Single	Single	Single
Spore staining	ND	ND	ND	ND	ND	ND	ND	ND
Biochemical								
Catalase	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Coagulase	ND	ND	ND	Positive		ND	Negative	Negative
Lac. Ferm.	Negative	Negative	Negative	Positive	Positive	Positive	Negative	Negative
Maltose	Negative	Positive	Negative	Positive	Positive	Positive	Negative	Negative
Sucrose	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Positive
Glucose	Negative	Positive	Negative	Positive	Positive	Positive	Negative	Positive
Mannitol	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive
Indole	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Positive
Citrate	Negative	Positive	Positive	Negative	Negative	Positive	Negative	Positive
Urease	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative
Oxidase	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Positive
Gr. Diff. Agar	MCC, SSA	DCA, SSA	PCA	MSA	MCC, EMB	MCC, EMB	MCC	TCBS
Identity	*Shigella*	*Salmonella*	*Pseudomonas*	*Staphylococcus*	*E. coli*	*Enterobacter*	*Proteus*	*Vibrio*

Legend: MCC= MacConkey agar, PCA= *Pseudomonas* EMB= Eosin methylene blue agar, DCA = Deoxycholate citrate agar, TCBS = thiosulphate citrate bile salt sucrose agar, ND =Not determine, Gr. Diff. Agar = Growth on differential agar

The molecular characterization of the bacterial isolates from Ogbese River is presented in Table X. The bacterial isolates from Ogbese River include *Salmonella enterica, Escherichia coli, Staphylococcus aureus, Vibrio cholerae, Shigella flexneri, Enterobacter cloacae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

Table 12. Molecular characterization of bacterial isolates from Ogbese River

Sample code	Closest Similarity	Homology (%)	Accession number
ST 1OG	*Salmonella enterica*	99.96	MN317312
ST 2OL	*Escherichia coli*	100.00	MN317310
ST 4OL	*Staphylococcus aureus*	100.00	MN317311
ST 6OG	*Vibrio cholerae*	99.00	MN317313
ST 3OG	*Shigella flexneri*	99.25	MN317315
ST 4OL	*Enterobacter cloacae*	100.00	MN317308
ST 1OL	*Klebsiella pneumoniae*	100.00	MN317309
ST6 OG	*Pseudomonas aeruginosa*	100.00	MN326102

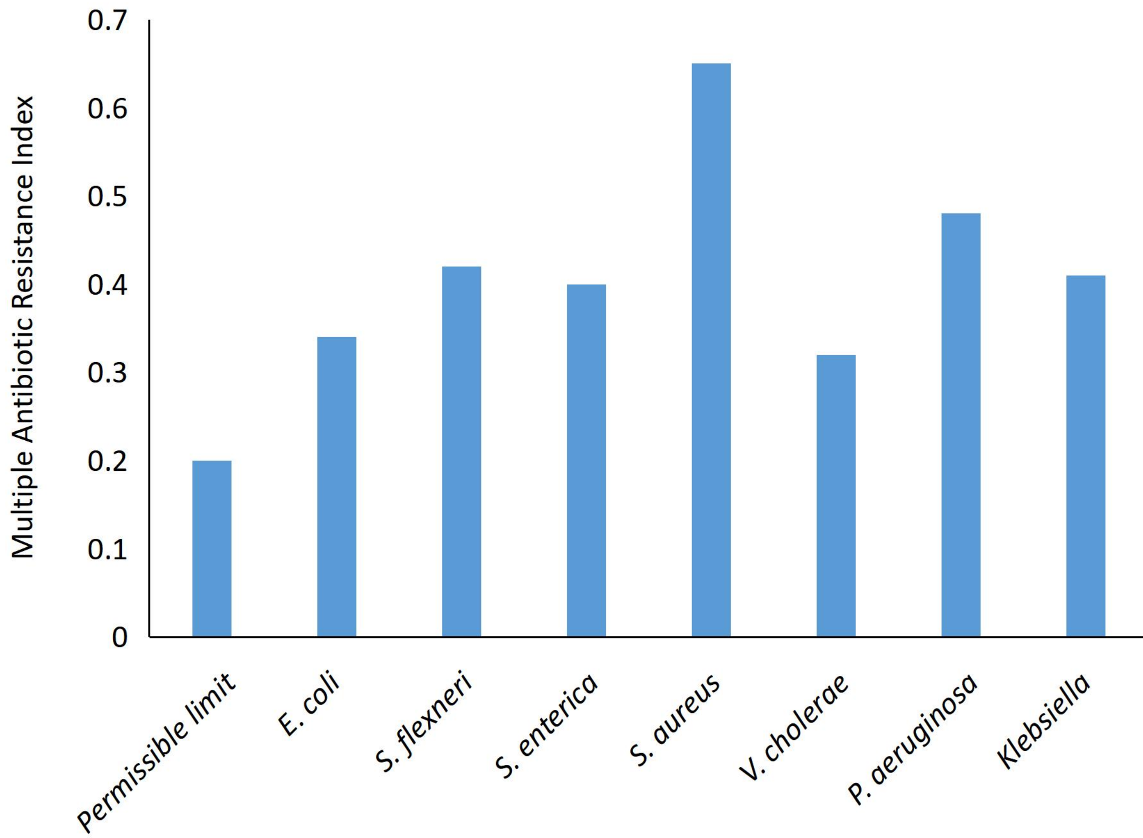

Figure 2. Multiple antibiotic resistance index of bacterial isolates from Ogbese River

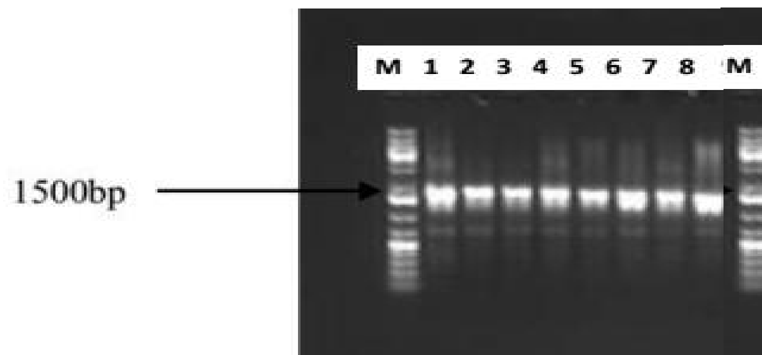

Plate 1. Gel electrophoresis of amplified bacterial DNA

Lane	Sample
Lane M	DNA ladder
Lane 1	ST 1OG
Lane 2	ST 2OL
Lane 3	ST 4OL
Lane 4	ST 6OG
Lane 5	ST 3OG
Lane 6	ST 4OL
Lane 7	ST 1OL
Lane 8	ST6 OG

>seq1 Enterobacter cloacae

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TAGTAACCCG

>seq2 Klebsiella pneumoniae

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>seq3 Escherichia coli

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>seq4 Staphylococcus aureus

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>seq5 Salmonella enterica

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>seq6 Vibrio cholerae

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>seq7 shigella

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