BACTERIOLOGICAL ANALYSIS OF SOME BOREHOLE WATER FOR THE INCIDENCE OF *ESCHERICHIA COLI* AND *SALMONELLA* SPP IN AUCHI METROPOLIS

BY

AGHAHOWA AISOSA	**AST/2382019720**
AIRUEDOMWINYA NANCY ADESUWA	**AST/2382070657**

DECEMBER, 2022

BACTERIOLOGICAL ANALYSIS OF SOME BOREHOLE WATER FOR THE INCIDENCE OF *ESCHERICHIA COLI* AND *SALMONELLA* SPP IN AUCHI METROPOLIS

BY

AGHAHOWA AISOSA	**AST/2382019720**
AIRUEDOMWINYA NANCY ADESUWA	**AST/2382070657**

BEING A PROJECT WORK SUBMITTED IN THE DEPARTMENT OF BIOLOGICAL SCIENCE LABORATORY TECHNOLOGY, SCHOOL OF APPLIED SCIENCE AND TECHNOLOGY AUCHI POLYTECHNIC, AUCHI, EDO STATE

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF HIGHER NATIONAL DIPLOMA (HND) IN BIOLOGICAL SCIENCE LABORATORY TECHNOLOGY, MICROBIOLOGY OPTION

DECEMBER, 2022

CERTIFICATION

We certify that this project work carried out by AGHAHOWA AISOSA (Matriculation Number AST/2382019720) and AIRUEDOMWINYA NANCY ADESUWA (Matriculation Number AST/2382070657) of the Department of Biological Science Laboratory, Auchi Polytechnic, Auchi. In partial fulfilment of the requirements for the award of Higher National Diploma (HND) in Microbiology Option.

________________________ ________________

MR. OZEKHOME M. CYRIL **Date**
(PROJECT SUPERVISOR)

________________________ ________________

MR. ANTHONY OHIMAI **Date**
(HEAD OF DEPARTMENT)

________________________ ____________________

MR. CHARLES OLUWASEUN ADETUNJI (PHD) **DATE**
EXTERNAL SUPERVISOR

DEDICATION

We dedicate this project to the Glory of God Almighty for the knowledge, vision, understanding and his profound mercies towards our lives and the times we spent in Auchi Polytechnic, Auchi.

ACKNOWLEDGEMENTS

Our most sincere gratitude go to God Almighty who has brought us this far in our academic career. We give Him all the thanks for the wisdom and knowledge in putting these scripts together and also for the successful completion of this project work.

Our hearty gratitude also goes to our wonderful project supervisor **Mr. Ozekhome M. Cyril** for his unending devotion, time and attention towards us, that greatly helped us in accomplishing this great work.

Our profound gratitude goes to our parents **Mr. and Mrs. Aghahowa and Mr. and Mrs. Airuedomwinya** and also to our brothers and sisters for their unconditional love and financial support towards the successful completion of this project work. May God continue to bless you all.

Our gratitude also goes to all our friends and well-wishers for their support, both financially, morally and for their advice during our trying times in the course of this project work and we pray God meet you all at your various points of need, AMEN.

TABLE OF CONTENTS

ABSTRACT

Borehole water represent an important source of water supply for population in Auchi metropolis. Although its consumption is being accentuated by limited access to potable water, the quality of water from borehole is unknown by the consumers. This study aimed at assessing the bacteriological analysis of some borehole water for the incidence of *Escherichia coli* and *Salmonella* spp. A total of six (6) borehole were chosen as sampling site for the analysis obtained result from total bacteria count of the water sample ranges from 9.5×10^5 to 1.5×10^7. The result showed that *Escherichia coli* and *Salmonella* species where not found in the borehole water samples. The absence of these contaminant maybe because the sites where these boreholes were far from latrine, drainage and domestic waste dump. There is a need water source and the importance of clean and healthy surrounding near water source and to implement household water treatment to improve the water quality and reduce water borne disease.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The portability of drinking water across the globe has increasingly continued to be in doubt, due to the problem of microbial contamination, high saline content and external human activity stress, thus making the water quality questionable and no longer guaranteed as safe anymore for human consumption. Nonetheless, with the increasing demand for water, irrespective of its portability; borehole has become a common source of drinking water in many countries across the globe, even in the country of this present study. Poor quality of drinking water has contributed to the increase in morbidity, due to water borne diseases especially in children and immuno-compromised individuals thereby increasing the burden of diseases in the region. Water borne disease is a huge concern and a major public health problem, because it affects the health of an individual in most cases when it is ingested into the body via faecal-oral route therefore; the need for the evaluation of microbial contamination of borehole water remains paramount and thoughtful towards good health and safety of all (Azuonwu *et al.*, 2020).

Borehole water is an increasingly important resource all over the world. It is the subsurface water that occurs beneath the water table in soils and geological formation that are usually saturated. It supports drinking water supplies, livestock needs, irrigation, industrial and many commercial activities. Borehole water is generally less susceptible to contamination and pollution when compared to surface water bodies. Borehole water pollution occurs widely from diverse sources such as water disposal facilities, industrial pollution, agricultural practices, atmosphere fallout, clearing of vegetation, over abstraction of borehole water and excavation below the water table. It not only affects water quality but also threatens human health, economic development and social prosperity. Microbiological health risks are associated with many aspects of water use, including drinking water in developing countries, irrigation, reuse of treated wastewater and recreational water use. It has been reported that drinking water supplies have a long history of association with a wide spectrum of microbial infections. Some microorganisms are native or adapted to saturated sediment and rock and are present in significant numbers in most water supply aquifers and even deep geoclinal formations. Biofilm formation sometimes encourages the growth of bacteria in wells and boreholewater. Events which occur between and within bacteria and plankton populations also affect water quality (Onuorah *et al.*, 2019).

Safe drinking water is still a challenging public health issue in the midst of lots of borehole water in-use in our various communities; thus, these further justify the trend that the integrity of such a source of drinking water might not be guaranteed in the end. Access to safe drinking water is a human right; and it is one of the basic requirements for good health. This has resulted to more level of awareness campaigns and Public Health echo regarding quality of borehole water.

An indicator known as “use of an improved source”, this indicator is used to monitor access to safe drinking water globally? Nevertheless, this indicator does not really measure the quality of water. Furthermore, water sources are either from the periphery called surface water or underneath referred to as borehole water. Globally, borehole water is the largest and most important source of potable water (Azuonwu *et al.,* 2020).

Although water is essential for life, it also remains an important source of disease transmission and a major cause of mortality in developing countries because of limitations in access and quality. Drinking water can become contaminated with foreign matter such as pathogenicbacteria (*Salmonella typhi, Shigella dysentariao, Escherichia coli, Klebsiella pneumonia* etc.), chemical substances (fertilizer, pesticides, metals etc.) and industrial effluents or other wastes, which deteriorates its quality; rendering it unfit for its intended use. Drinking water fact sheets, contaminated water can transmit diseases such diarrhea, cholera, dysentery, typhoid, and polio. Also, contaminated drinking-water is estimated to cause 502, 000 diarrheal deaths each year. This makes it expedient to ascertain the safety of drinking water by determining the extent to which it conforms to the standards set by regulatory bodies (Okoro *et al.,* 2017).

In Nigeria, access to safe drinking water and hygienic sanitation facilities still pose serious challenge to its rapidly growing population of more than 174 million people. Therefore, the project worked will focus on the bacteriological analysis of some borehole water for the incidence of *Escherichia coli* and salmonella sp.

1.2 STATEMENT OF PROBLEM

The short falls in the distribution of treated pipe borne hole water leads people to resort or alternative source of water which maybe unfit for human consumption. Borehole water can be polluted through leakage/improper plumbing, run off, agricultural activities, improper solid waste disposal, animal droppings near borehole water and especially shallow well.

The borehole water can be contaminated by pathogens which known to cause several disease conditions like water borne disease resulting in food intoxication and identification.

1.3 AIM/PURPOE OF THE STUDY

The study is to determine the bacteriological analysis of borehole water for the incidence of *Escherichia coli* and *Salmonella* spp in Auchi metropolis.

1.4 SIGNIFICANCE OF STUDY

Borehole water can help reduce the demand placed on the municipal supply by agricultural projects and related farming activities, as well as construction projects. Borehole water can be used for irrigation, maintaining parks and gardens, and even for watering livestock.

Although there are some bacteria in all borehole waters, and in general they carry out beneficial processes, some bacteria or other microorganisms (e.g., protozoa, viruses) may cause disease in humans. Naturally some microorganisms have learned to live on or in the human body. Many of these microorganisms do no harm, and are even beneficial because they compete with other microorganisms that might cause disease if they could become established in or on our bodies. A few microorganisms (called pathogens) can cause disease in humans. Some of these disease-causing microorganism are closely associated with humans and other warm-blooded animals. These pathogens are transmitted from one organism to another by direct contact, or by contamination of food or water. Many of the pathogens which cause gastrointestinal disease are in this category. Several human gastrointestinal pathogens produce toxins which act on the small intestine, causing secretion of fluid which results in diarrhea. Cells of the pathogen are shed in the feces, and if these cells contaminate food or water which is then consumed by another person, the disease spreads. Other pathogens are "opportunists", they may not be closely associated with humans or other mammals and they rarely cause disease in healthy adults. Instead, these may be common bacteria or fungi which exist in soil or water, but may cause disease in persons already weakened by a pre-existing disease.

1.5 SCOPE OF THE STUDY

The study is limited to the bacteriological analysis of borehole water for the incidence of *Escherichia coli* and *Salmonella* sp in Auchi metropolis.

1.6 DEFINITION OF TERMS

Some terms expressions or technical work used in the process of the research write up is clearly defined and their meaning was well explained. Some of these terms include;

Microorganism or Microbe: It is an organism of microscopic size, which may exist in its single-celled form or as a colony of cells.

Water: Water is an inorganic, transparent, tasteless, odorless, and nearly colorless chemical substance, which is the main constituent of Earth's hydrosphere and the fluids of all known living organisms.

Borehole Water: It is a source of water for wells, boreholes and springs. A borehole is a hydraulic structure which when properly designed and constructed, permits the economic withdrawal of water from an aquifer. It is a narrow well drilled with machine.

Escherichia coli, also known as *E. coli,* is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms.

Salmonella sp, is a genus of rod-shaped Gram-negative bacteria of the family Enterobacteriaceae. The two species of Salmonella are *Salmonella enterica* and *Salmonella bongori*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 CONCEPT OF BOREHOLE WATER

Water is essential for human existence, and its importance for individual health as well as the well-being of a nation cannot be overemphasized. Fresh water represents the main sources of safe water for household use, sustainable development, and human survival. Borehole water quality can be assessed by analyzing its total quality, which is physicochemical and microbiological quality. Physicochemical quality refers to the physical nature such as total dissolved solids, colour, temperature, hardness, hydrogen ion concentrations (pH), conductivity, alkalinity, and taste, and concentration of dissolved substances such as organic and inorganic chemicals including metals. Microbiological quality refers to the absence of disease-causing microorganisms in water. Microorganisms play a major role in water quality and the bacteria that are concerned with water borne diseases are *Salmonella sp.*, *Shigella sp.*, *E. coli* and *Vibrio cholera.* Access to quality and portable water devoid of pollutants and pathogenic organisms is a major challenge in the rural areas. Most people depend on borehole and well water for consumption and domestic purposes (Fakeye *et al.*, 2018).

In Nigeria, borehole water represents the major source of potable water. Due to the acute shortage of water supplies, the last decade has witnessed a rapid increase in sinking of boreholes. Moreover, available underground water sources especially in developing countries were becoming polluted due to; the increasing growth in human populations, industrialization, indiscriminate refuge dumpsites, and climatic changes. Literatures have shown that water was prompt to contamination regardless of the sources. Contaminants such as; bacteria, fungi, protozoans, viruses, heavy metals, nitrates and salts have polluted water supplies, as a result of inadequate treatment and poor disposal of wastes from humans and livestock, industrial discharges; and over-use of limited water resources (Bashir *et al.,* 2018).

Collected and stored borehole water microbial contamination is caused not only by the collection and use of faecally contaminated water that was not safe, to begin with, but also by the contamination of water (that was microbiologically safe initially), during storage. Unhygienic and imperfectly protected (poorly covered or open) water collection and containers for storage, unhealthy means of dispensing water from storage containers, including faecally contaminated dippers, hands, tools, lack of protection against vectors (flies, cockroaches, rodents, etc.) and inadequate cleaning of storage container to prevent biofilm formation and accumulation of sediments and pathogens, all are factors contributing to this problem.

2.2 HISTORY OF BOREHOLE WATER

According to Wikipedia "Borehole drilling has a long history. By at least the Han Dynasty (202 BC – 220 AD), the Chinese used deep borehole drilling for mining and other projects. The British sinologist and historian Michael Loewe states that borehole sites could reach as deep as 600 m (2000 ft). K.S. Tom describes the drilling process: “The Chinese method of deep drilling was accomplished by a team of men jumping on and off a beam to impact the drilling bit while the boring tool was rotated by buffalo and oxen.” This was the same method used for extracting petroleum in California during the 1860s (i.e. “Kicking Her Down”).

A Western Han Dynasty bronze foundry discovered in Xinglong, Hebei had nearby mining shafts which reached depths of 100 m (328 ft) with spacious mining areas; the shafts and rooms were complete with a timber frame, ladders and iron tools. By the first century BC, Chinese craftsmen cast iron drill bits and drillers were able to drill boreholes up to 4800 feet (1500 m) deep. By the eleventh century AD, the Chinese were able to drill boreholes up to 3000 feet in depth. Drilling for boreholes was time consuming and long. As the depth of the holes varied, the drilling of a single well could last nearly one full decade. It wasn’t up until the 19th century that Europe and the West would catch up and rival ancient Chinese borehole drilling technology”

Luckily, scientists and historians are not so keen on accepting “alien technology” as the answer to how they seemingly used advanced machinery so long ago. Research has shown that they created tools with copper “drill bits” that were used to core out boreholes. To allow the soft copper to get through hard materials, such as granite, they simply used water and an abrasive material, like sand. There are also signs that they may have known about certain chemical reactions that can help with the process.

2.3 TYPES OF BOREHOLE WATER

There are in three major categories of boreholes: dug wells, driven wells and drilled wells, which are commonly known as boreholes.

Dug wells, are holes in the ground dug by shovel or backhoe. Historically, a dug well was excavated below the groundwater table until incoming water exceeded the digger's bailing rate.

Driven well, is a small diameter well, assembled by joining lengths of steel pipe, 1¼ inches or 2 inches in diameter, with threaded couplings.

Drilled well, consists of a hole bored (a borehole) into the ground, with the upper part or the entire depth of the well-being lined with casing.

2.4 GENERAL IMPORTACE OF BOREHOLE WATER

Having access to a borehole or borehole water is an affordable option and is arguably the cheapest way to deliver water to homes and for crops. If there is a good flow of groundwater, once a borehole has been dug, it can be used for all water needs.

With a borehole water system an individual is independent from the mains water supply, thus not affected by future increases in the water price.

Borehole water is suitable for domestic uses such as toilet flushing, car and clothes washing as well as watering of grass, gardens and even crops. Also, a significant factor to consider is that the immediate environment where the borehole is dug can impact the water with contaminants. If there is a high level of contamination in the area near the borehole water, it is likely that the water will be affected (Onajite *et al.*, 2018).

Borehole water cannot be use for every form of consumption without purification treatment such as cooking and drinking. This cannot be done straight from the source due to the possibility of contaminant. As a result the water needs to be tested and water purification system installed which will increase the cost.

2.5 QUALITY STANDARD OF BOREHOLE WATER AND HEALTH

Water plays essential roles in supporting human life. It also has if contaminated great potential for transmitting a wide variety of diseases and illness. In the developed world, water related diseases are rare, due essentially to the presence of efficient water supply and waste water disposal systems. However, in the developing world perhaps a lot of people are without safe water supply and adequate sanitation (Okoro *et al.*, 2017).

In the developed world water related disease are rare, due essentially to the presence of efficient water supply and waste water disposal systems.

In rural areas, boreholes are often badly maintained or non-protected and water that they get is sometimes contaminated. The pollution of groundwater is generally increased by several human factors such as defecation in nature, presence of pit latrines, waste water, agricultural activities, farms and discharges of chemicals, in industrial sites, close to water points. These factors could have considerable impacts on the quality of boreholes water. Studies showed that bacterial contamination of groundwater would be due to lack of sanitation system, poor habits in management of wastes and the presence of latrines close to water sources (Djaouda *et al.*, 2018).

The effect of water pollution by chemical include cancer, arthritis, skin reaction, eruption, health disease, central nervous system problem, skin rashes, kidney problem and bronchitis. The principal microbial water borne disease are typhoid and paratyphoid fevers (Salmonellosis), cholera, bacillary, infectious hepatitis, dracontiasis and schistomiasis, others are food poisoning, amoebic dysentery, giardiasis, gastro enteritis, hepatitis A and poliomyelitis (Okoro *et al.*, 2017).

The incidents of water borne disease and epidemics nationwide arising from drinking water of doubtful quality have become of great concern. The primary purpose of the guideline for drinking water quality is the protection of public health. Water quality standard is a measure, principle or rule established by authority set to protect the water resource for uses such as drinking water supply, recreational uses and aesthetics, agriculture (irrigation and livestock watering), protection of aquatic life and industrial water supplies.

In order to maintain water quality, guidelines for drinking water was set up by the World Health Organization. A guideline value represents the level (a concentration or number) of a constituent that ensures aesthetically pleasing water and does not result in any significant risk to the health of the consumer (Djaouda *et al.,* 2018).

2.6 BOREHOLE WATER ANALYSIS

The method for the bacteriological analysis of water samples are many, which includes the multiple tube technique (MTT) and the membrane fitters techniques (MFT) are commonly used to determine the presence of coliforms and *E coli*. Water to be used at home must therefore be treated to exclude the pathogenic organism so that such water will be fit for human consumption.

In this wise, water collected form well, stream etc. must be steam, boiled and filtered before usage and major analysis of drinking water is to ensure that water does not transmit organism causing human disable to human health.

2.7 NATURAL ACTIVITIES OF BACTERIA IN BOREHOLE WATER

Over a century of research on naturally occurring bacteria and their activities allows us to interpret some of the roles of bacteria in borehole- water environments. We know that bacteria are found everywhere in our environment. They are common in air, soil, water and in the habitats of our daily lives. Bacteria are commonly present in soil at numbers of about 10^8-10^9 cells per gram. Bacterial slime (biofilms) on rocks in streams and rivers may contain 10^9 bacteria per square centimeter. Pristine lake waters contain many thousands of naturally-occurring bacteria per liter. These naturally occurring bacteria maintain the fertility of soil, they transform minerals and nutrients in water and sediments, and degrade leaf litter and other plant materials producing materials useful to other organisms. In addition, naturally occurring bacteria carry out activities useful to humans by degrading wastes in our landfills and compost piles, and cleansing water of the pollutants we add. We purposefully use some bacteria to make food (cheese, beer, sauerkraut), we put bacteria to work in sewage treatment plants, and we use them in biotechnology to produce chemicals. Therefore, microbiologists have learned a great deal about the types and activities of naturally-occurring bacteria. Based on principles learned from other environments, we would expect bacteria in borehole water to be able to:

1. Transform organic carbon to carbon dioxide (CO_2)
2. Use up oxygen when sufficient carbon is available for growth
3. Transform nitrogen between oxidized (e.g., nitrate - NO_3) and reduced (e.g., ammonium - NH_4 or nitrogen gas - N_2) forms
4. Transform iron between oxidized [Fe(III)] and reduced [Fe(II)] forms
5. Transform sulfur between oxidized (e.g., sulfate - SO_4) and reduced (e.g., sulfide - H_2S) forms
6. Produce methane
7. Degrade pesticides, fuels and other organic contaminants
8. Affect the distribution and solubility of some metals (e.g., arsenic, uranium, etc.)

Indeed, there is evidence for each of these activities in borehole water. The review articles cited above are good resources for more information and case examples. In addition, a general review of the role of bacteria in natural and augmented bioremediation of fuels and solvents in borehole water.

Most of the activities of bacteria in borehole water are the direct result of the astounding metabolic versatility of bacteria. Although humans and other vertebrate and invertebrate animals are primarily dependent on respiration using oxygen, some bacteria may respire using NO_3, SO_4, oxidized (ferric) iron [Fe(III)] or a variety of metals (such as arsenic or uranium) as the oxidant. In addition, in the absence of oxygen, bacteria may carry out processes such as methane production or fermentation. Finally, bacteria may be capable of growth on some organic compounds which are toxic to other organisms. The combination of these unique metabolic capabilities suggests that bacteria play important roles in pristine and contaminated borehole water environments. Nevertheless, bacteria are limited, as are all living things, by extremes of pH and temperature, by lack of nutrients to support growth, and by toxicity of some compounds. In addition, bacteria are subject to predation by larger microorganisms, such as protozoa. Each of these environmental features must be assessed when interpreting the role of bacteria in a particular borehole water process.

2.8 FACTORS THAT LEADS TO MICROBIAL CONTAMINATION OF BOREHOLE WATER

Borehole water has traditionally been considered to be the water source least susceptible to contamination by indicator bacteria or human pathogens. This is certainly true of borehole water from deep, confined aquifers. The microbiological quality of source waters for drinking water supply, the sources of contamination to borehole water environments, and the instances of waterborne disease outbreaks attributed to untreated or poorly-treated borehole water which contained pathogens. If fecal indicator bacteria or pathogens commonly associated with humans are present in borehole water in measureable numbers, there is most likely a nearby connection with a contaminated surface environment, such as a seepage from a waste lagoon or a contaminated surface water, or a subsurface source of contamination such as a septic tank, a broken or leaking sewer line, or an old or improperly designed landfill.

It is important to recognize that in spite of what we do know about bacteria and other microorganisms, we still know relatively little about their types, activities and habitats. For example, the ability of certain bacteria to grow by carrying out the reduction of Fe(III), arsenic or uranium was first demonstrated conclusively in the early 1990's. Likewise, the discovery of new pathogens, the association of common bacteria or protozoa with specific diseases, occurs on a relatively frequent basis. Bacteria, viruses and protozoa are difficult to study, and most microbiologists believe that we have identified fewer than 10% of the types of bacteria actually present in nature. We also have only a very rudimentary understanding of what types of activities bacteria carry out in nature, and the environmental factors which influence their activities and survival. We have even less information on bacteria in borehole water, since this field of study is so recent. It is likely that we will learn much more about the prevalence, activities, and significance of microorganisms in borehole water in the coming decade.

2.9 MICROBIAL CONTAMINATION OF BOREHOLE WATER AND ITS EFFECTS ON HEALTH

Microorganisms play an important role in water quality and the microorganisms that are concerned with water borne diseases are *Salmonella* species, *Shigella* species, *Escherichia coli* and *Vibrio cholera*. The presence of faecal coliforms of *Escherichia coli* and those listed earlier are indicators of contaminated water. The fecal indicator bacteria (*Escherichia coli*, fecal coliforms, fecal streptococci) are typically used to measure the sanitary quality of water for recreational, industrial, agricultural and water supply purposes. The fecal indicator bacteria are natural inhabitants of the gastrointestinal tracts of humans and other warm-blooded animals. These bacteria in general cause no harm. They are released into the environment with feces, and are then exposed to a variety of environmental conditions that eventually cause their death. In general, it is believed that the fecal indicator cannot grow in natural environments, since they are adapted to live in the gastrointestinal tract. Studies have shown that fecal indicator bacteria survive from a few hours up to several days in surface water, but may survive for days or months in lake sediments, where they may be protected from sunlight and predators. In borehole water, temperature, competition with bacteria found naturally in the water, predation by protozoa and other small organisms, and entrapment in pore spaces may all contribute to their demise. We assume that pathogens similar to the fecal indicator bacteria die at the same rate as fecal indicator bacteria. Therefore, if we find relatively high numbers of fecal indicator bacteria in an environment, we assume that there is an increased likelihood of pathogens being present as well. Unfortunately, some pathogenic bacteria, viruses and protozoans may have special survival mechanisms, such as cyst formation in *Cryptosporidium*, or attachment of viruses to particles, so that waters free of fecal indicator bacteria may still harbor these microorganisms. This is even true of water which has undergone treatment for drinking water purposes (Adeyemi *et al.*, 2020).

Although there are some bacteria in all borehole waters, and in general they carry out beneficial processes, some bacteria or other microorganisms (e.g., protozoa, viruses) may cause disease in humans. Naturally some microorganisms have learned to live on or in the human body. Many of these microorganisms do no harm, and are even beneficial because they compete with other microorganisms that might cause disease if they could become established in or on our bodies. A few microorganisms (called pathogens) can cause disease in humans. Some of these disease-causing microorganism are closely associated with humans and other warm-blooded animals. These pathogens are transmitted from one organism to another by direct contact, or by contamination of food or water. Many of the pathogens which cause gastrointestinal disease are in this category. Several human gastrointestinal pathogens produce toxins which act on the small intestine, causing secretion of fluid which results in diarrhea. Cells of the pathogen are shed in the feces, and if these cells contaminate food or water which is then consumed by another person, the disease spreads. Other pathogens are "opportunists": they may not be closely associated with humans or other mammals and they rarely cause disease in healthy adults. Instead, these may be common bacteria or fungi which exist in soil or water, but may cause disease in persons already weakened by a pre-existing disease.

There is no clear way to associate risk of disease with the bacteriological quality of borehole water and measured by the presence of fecal indicator bacteria. First, there is no direct association between the presence of fecal indicator bacteria and the presence of specific pathogens. Second, individuals are not equally susceptible to pathogens. Whether or not a pathogen is successful in causing disease is related to the health of the exposed individual and the state of his or her immune system, as well as to the number of pathogen cells required to make the person ill. Some pathogens can cause disease when only a few cells are present. In other cases, many cells are required to make a person ill. Children, elderly persons and persons with pre-existing illnesses are more susceptible to many pathogens than are healthy young or middle-aged adults. Third, it would be difficult to monitor for every possible pathogen. Each type of pathogen requires a specific test and many of these tests are time-consuming or expensive. Monitoring for each type of known pathogen would be prohibitively expensive. Finally, new pathogens are still being discovered. It was only about 5 years ago that a specific bacterium was identified as a cause of stomach ulcers in humans.

In addition, "old" bacteria are acquiring new "tricks" in that they are becoming resistant to antibiotics and are re-emerging as serious pathogens.

2.10 MICROBIAL CONTAMINATION OF BOREHOLE WATER

Escherchia coli

This organism is a rod-shaped facultative anaerobe, belonging to the genus Escherichia that mainly indicate feacal content contamination, most strains of this Gram-negative organism are harmless or cause relatively brief diarrhea but virulent strains, such as *E. coli* O157:H7 can cause severe symptoms including bloody diarrhea and vomiting.

Salmonella typhi

Salmonella typhi is the causative agent of typhoid fever, a serious disease condition with an annual global burden of approximately 16 million cases, leadinsg to 600,000 fatalities, *S. typhi* typically live in animal and human intestines and Humans become infected by the consumption of contaminated water or food. *S. typhi* was found in drinking water analyzed by 30% of the studies under review.

2.11 ECONOMIC IMPORTANCE OF BOREHOLE WATER

Access to safe water sources remains scarce in sub-Saharan African countries. We estimate the economic value of safe water from newly constructed boreholes in Nigeria. Water boreholes can help reduce the demand placed on the municipal supply by agricultural projects and related farming activities, as well as construction projects.

CHAPTER THREE

3.0 MATHERIALS AND METHODS

3.1 MATERIAL USED

The materials that were used for this project practical include the following; Beaker, conical flasks, pipettes, petri- dishes, nutrient agar, eosin methylene blue agar, salmonella-shigella agar, blender, weighing scale, colony counter, cotton wool, incubator, autoclave, detergent, Dettol, inoculating wire loop, lactophenol cotton blue, wooden clip (slide holder), masking tape, crystal violet stain, oil immersion, gloves, safranin stain, lugol’s iodine, acetone, wash bottle, droppers, work bench, water, spatula, gloves autoclave, bunsen burner, aluminum foil paper, distilled water, test tubes, test racks, cover-slips and sterile bottle water.

3.2 METHODS

3.2.1 Source and Collection of Samples

A total of six (6) samples of borehole water were collected from different locations in Etsako West Local Government Area of Edo State and were used for this project practical. The samples were immediately taken to the laboratory in sterile and airtight polythene bags for analysis and they were labelled A, B, C, D, E and F respectively.

3.2.2 Processing of Samples

The homogernate of each of the samples were aseptically collected with the aid of sterile pipette and kept for use as inoculum.

Table 3.1: Samples of Borehole Water

SAMPLE	SOURCES
A	Borehole water collected from cash momodu street iyekhei girls road
B	Borehole water collected from polytechnic hostel
C	Borehole water collected from first borehole sabo
D	Borehole water collected from water tanker, igbirra camp
E	Borehole water collected from GT plaza
F	Borehole water collected from opposite Uchi market

3.3 MICROBIOLOGICAL ANALYSIS

3.3.1 Sterilization of Apparatus

All glassware, apparatus, and laboratory workbench/table were sterilized before use. The glassware and apparatus were sterilized with the aid of the autoclave operated at 121^0C for 15 minutes, the laboratory work bench/table was sterilized with the aid of cotton wool soaked with methylated spirit.

3.3.2 Media Preparation

The media used for the analysis were; Nutrient Agar (NA), Salmonella Shigella Agar (SSA) and Eosin Methylene Blue Agar (EMBA). All the media were prepared according to the manufacturer's instructions and sterilized with the aid of an autoclave operation at 120^0C - 121^0C for 15 minutes using the autoclave.

3.3.3 Serial Dilution of Samples

1ml aliquot of each of the inoculation borehole water (obtained from table 3.2.2 above) were used for serial dilution of each of the samples. 9ml of sterile distilled water were aseptically dispensed into 6 sterile test tubes. Each of the 1ml aliquot of inoculum were separately transferred and diluted in the test tubes with the aid of sterile pipettes. The six folds serial dilution was used with test tubes labelled (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}).

3.3.4 Inoculation of Media

The method used for this inoculation is the pour plating technique. The petri dishes were labelled accordingly and inoculation of the serially diluted sample into the sterile petri dishes was carried out aseptically before pouring warm, freshly prepared and sterilized media agar into the petri dish and swirled gently to mix properly with the innocula before it solidifies.

3.3.5 Incubation of Culture Plates

All the inoculated culture plates were transferred to the incubator for incubation at 37^0C for 24 hours before enumeration and identification of isolates in order to get the total viable count.

3.3.6 Enumeration of Colonies

Colonies that developed after 24 hours of incubation were enumerated as colony-forming unit for all the samples of the borehole water. Colonies were enumerated with the aid of digital colony counter and only culture plate with colonies of between 30-300 were used for enumeration.

3.3.7 Identification of Microorganisms

The bacterial isolates that was obtained after enumeration was thereafter identified based on their cultural, morphological and biochemical characteristics.

The bacterial species were *Escherichia coli* and *Salmonella* spp which were not identified because it was not present in any of the samples of the borehole water.

GRAM STAINING

A thin smear of the bacteria was made on a clean grease free microscopic slide, air-dried and heat fixed by passing it through the Bunsen flame. The slide was placed on a staining rack and flooded with crystal violet for one minute and was rinsed with sterile distilled water, the slide was then flooded with lugols iodine solution for one minute and was rinsed with sterile distilled water, the slide was decolorized with ethanol for thirty seconds (30 secs) and rinsed with clean sterile distilled water. Grams iodine was added (mordant) for 60 seconds. The smear was gently rinsed with tap water. Alcohol (70% ethanol) was applied to decolorize it for 30 seconds. It was then rinsed with clean sterile distilled water again and allowed to dry. Lastly, the slide was filled with safranin for the next 1 minute and rinsed with sterile distilled water. The slide was drained and allowed to air dry, before viewing under the lens microscope using immersion oil, objective lens (x100). The gram-positive bacteria appeared purple while gram-negative bacteria colony appears red or pinkisk.

3.3.8 Biochemical Test (Bacteria)

INDOLE TEST

It is use to detect if bacteria possess enzymes trytophanase which degrades amino acid tryptophan to indole, pyruvic acid and ammonia.

Procedure: The test organism was inoculated into peptone water both in test tube and incubated at 37^0C for 48-96 hours. 0.5ml of Kovac's reagent is then added and shaken gently. A red colour indicate positive while a yellow colour indicate negative test result of bacteria.

METHYL RED TEST

It is use to determine the ability of bacteria to produce and maintain stable acid end products from glucose fermentation. The test detects the production of acid

Procedure: The test organism was inoculated in glucose phosphate broth in a test tube and incubated at 37^0C for 2 to 5 days about 5 drops of 0.4% solution of alcoholic methyl red solution was added and mixed thoroughly and the result was read immediately. A positive test gives bright red colour and yellow colour indicating negative test. Both organisms (*Staphylococcus aureus* and *Salmonella* spp tested positive to methyl red.

UREASE TEST

The test detects the ability of organisms to produce urease enzyme.

Procedure: The test organism is inoculated on the agar and incubated at 37^0C, observation was after 4 hours and overnight incubation, development of purple coloration indicates the production of urease i.e. positive test. *Staphylococcus aureus* appeared positive in urease test while *Salmonella* spp was negative.

MOTILITY TEST

It is aimed at identifying motile bacteria, motility can sometimes be referred to as the way an organism grow on solid media and it is determined by the presences or absence of flagella. The test is performed to distinguish the motile organism from the non-motile one.

Procedure: tubes containing the motility were inoculated by making a fine stab with a loopful of the culture to a depth of 1-2cm and it was inoculated at 37^0C for 24 to 48hours. Motility is observed by spreading of the organism outwards from the stab area.

CATALASE

The test is used to determine the ability of the organism to produce enzyme catalase that breaks down hydrogen peroxide to water and oxygen. It is also use to differentiate those bacteria that produce enzyme catalase.

Procedure: A small bacteria colony was taken from sample by using a sterile wire loop and a drop of catalase reagent (hydrogen perioxide) was added to the slide using a sterile pipette. The presence of catalases observed by bubbling indicated a positive result which gave oxygen production within 10 seconds while negative result test result of bacteria.

COAGULASE

It is an enzyme-like protein and cause plasma to dot by converting fibrinogen to fibrin. The test is performed on gram positive, catalase positive species to identify the coagulase positive *Staphylococcus aureus*. Coagulase is a virulence factor for *Staphylococcus aureus*.

Procedure: A drop of physiological saline was placed on a sterile test tube using a sterile pipette. With the sterile urine loop, a portion of the isolated colony is placed on each test-tube human plasma is added to the suspension and mix gently looking clumpy of the organism within 10 seconds. The presence of particles indicates positive result while the absence of particles indicate negative result

OXIDASE TEST

This test was carried out to identify bacteria species. This test is used to determined if an organism possess the cytochrome oxidase enzyme. The test is use as an aid for the differentiation of bacteria species.

Procedure: A piece of filter paper was placed in a clean sterile petri dish and 2 to 3 drops of fresh or nascent oxidase reagent was added. A colony of test organism was collected using a sterile wire loop and smeared on the filter paper and observation of blue purple colour within few seconds shows positive test and absence of color gives negative results.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

The results obtained for the Total Variable aerobic plate count in colony forming unit per 1ml of each of the borehole water. The samples are presented in Table 4.1 below.

Table 4.1: Total Viable aerobic plate count (cfu/ml) of samples of Borehole Water

Samples	Total Viable aerobic counts (cfu/ml)
A	1.3×10^{7}
B	9.3×10^{5}
C	1.1×10^{7}
D	1.2×10^{7}
E	7.5×10^{6}
F	1.5×10^{7}

The result obtained from the identified microorganisms in each of the samples analyzed is as presented on the table below;

Table 4.2: Incidence of *Escherichia coli* and *Salmonella* spp in Borehole Water

Samples	***Escherichia coli***	***Salmonella* spp**
A	-	-
B	-	-
C	-	-
D	-	-

Key: + = Present, - = Absent

4.2 DISCUSSION

The result from table 4.1 total viable aerobic plate count (cfu/ml) of samples of borehole water samples shows that samples F (1.5×10^7), A (1.3×10^7), D (1.2×10^7), C (1.1×10^7), E (7.5×10^6) has the highest of bacteria compared to sample B (9.3×10^5) which has the lowest growth of bacteria.

The results obtained from table 4.2 identified organisms and their presence in the sample analyzed shows that *Salmonella* spp and *Escherichia coli* were not found in any of the samples.

The reason may be that there was no faecal contamination and linkage in the borehole site which linked to septic tank pollution.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The study aimed at assessing the bacteriological quality of borehole water intended for human consumption in Auchi metropolis. The water from the boreholes assessed in Auchi were of pure quality in terms of the bacteriological parameters. The detection of total bacteria and coliform in significant numbers indicated the site where these boreholes were sited are far from latrine and drainages. In conclusion these result shows that majority of the borehole water in Auchi metropolis are safe for drinking.

5.2 Recommendation

This investigation suggested that not all borehole water is fit for human consumption. Hence, tests have to be done in order to determine if they are potable. The sites of borehole are very important as clean and hygienic environment promote safety of water.

The geologist drilling borehole have to be educated on the importance of ensuring the populance needed to be educated on the importance of maintaining clean and hygienic environment around the borehole to ensure the safety of water from the boreholes.

We recommend that all borehole in Auchi metropolis and other borehole in different location in Auchi Edo State should be well treated before consumption so as to prevent the spread of water borne diseases and also to ensure that the health of every individual in Auchi community is well protected.

REFERENCES

Adeyemi, O. A., Efunwole, T. K and Olanbiwoninu, A. A. (2020). Comparative bacteriological analysis of stored borehole water sources in Oyo town, Oyo State, Nigeria. *African Journal of Microbiology Research*. 14(1): 32-41.

Azuonwu, O., Ihua, N and Ohwondah, G. (2020). Bacteriological Risk Assessment of Borehole Sources of Drinking Water in Some Part of Port Harcourt Metropolis of Niger Delta, Nigeria. *Biomed Journal of Science & Technological Research*. 24(4): 18477- 18487.

Bashir, I., Adam, A. S., Yahaya, H.S., Makeri, D., Ntulume, I., Aliero, A. A and Afolabi, R. O. (2018). Assessment of bacteriological quality of borehole water in Wamakko Local Government, Sokoto State, Nigeria. *Novel Research in Microbiology Journal*. 2(6): 175-184

Djaouda, M., Lecké, A., Maïné, M. K., Wadoubé, Z., Liang, S., NOLA, M and Njiné, T. (2018). Assessment of bacteriological quality of groundwater from boreholes in Maroua (Far North Cameroon). *International Journal Biology Chemistry Science*. 12(2): 958-974.

Fakeye, O. D., Usiholo, I. F. Anyaele, O. O. and Daodu, A. (2018). Physico-chemical and Bacteriological assessment of Borehole Water in some selected rural communities of Edo State. *SAU Science Technology Journal*. 3(1): 58-69.

Okoro, N., Omeje, E. O and Osadebe, P. O. (2017). Comparative Analysis of Three Borehole Water Sources in Nsukka Urban Area, Enugu State, Nigeria. *Resources and Environment*. 7(4): 110-114.

Onajite, C. I., Onome, E. O., Oluwatoyin, F. I and Alexander, O. E. (2018). Comparative Microbial Analysis of Borehole Water and other Sources of Water in Benin Metropolis, Edo State. *Journal of Environmental Science and Public Health*. 2(4): 232-242.

Onuorah, S., Igwemadu, N and Odibo, F. (2019). Bacteriological Quality Assessment of Borehole Water in Ogbaru Communities, Anambra State, Nigeria. *Universal Journal of Clinical Medicine*. 7(1): 1-10.