BACTERIOLOGICAL ANALYSIS OF WATER TANK IN HALLS OF RESIDENCE

BY

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BEING A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCE LABORATORY TECHNOLOGY, SCHOOL OF APPLIED SCIENCE AND TECHNOLOGY, AUCHI POLYTECHNIC, AUCHI

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CERTIFICATION

This is to certify that this research work was carried out by **IMADE ODION RACHEAL (AST/238200221), IMATITIKUA ORIAKHI SOPHIA (AST/2382040991) and IMOROA BLESSING (AST/2382040927)** in the Department of Biological Science Laboratory Technology, School of Applied Science and Technology, Microbiology Option. We therefore certify that this research work is adequate both in scope and even quality in partial fulfilment of the requirements for the award of Higher National Diploma (HND).

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DEDICATION

This project work is dedicated to Almighty God in whom I live and have my being for his infinite kindness and love which was manifested all through the duration of this research work, to him be honour and glory now and forever and to my wonderful parents for their unrelenting efforts into actualizing this feat.

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TABLE OF CONTENTS

CHAPTER FOUR

CHAPTER FIVE

ABSTRACT

Drinking water free of pathogenic organisms is fundamental to breaking one of the principal transmission routes of infectious disease. The Bacteriological Analysis of Water Tank in Halls of Residence was carried out in the Microbiological laboratory of Auchi Polytechnic, Auchi, Edo State, Nigeria. Four water samples; Water from microbiology laboratory (A), Water from Engineering Laboratory (B), Water from Hostel C (C), Water from Hostel F (D) were specially collected in a clean sample container and transferred to the laboratory for further Bacteriological analysis. The samples were investigated and analyzed using standard analyticalmethods. This study revealed the bacterial load of the sample with values ranging from $5.9x10^5$cfu/ml - $7.9x10^5$cfu/ml. The morphological and biochemical characterization of the isolates encountered was determined. The bacterial isolates from this study are *Staphylococcus aureus, Vibrio cholera, Escherichia coli, klebsiella pneumoniae* and *Salmonella* spp. In this study, E. coli was most frequently observed (28.5%). It is concluded that water from these tanks is found to be unsatisfactory for the human consumption hence it is a health risk to use this water without purification. Therefore, Improving and expanding the existing water treatment and sanitation systems is more likely to provide safe and sustainable sources of water over the long term.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Water is one of the indispensable resources for the continued existence of all living things including man and adequate supply of fresh and clean drinking water is a basic need for all human beings (Edema *et al*., 2011). In nature, all water contains impurities; as water flows in streams, accumulates in lakes and filters through layers of soil and rock in the ground, it dissolves or absorbs substances it come in contact with, which may be harmful or harmless (Ogamba, 2004). One of the major critical problems in most developing countries today is the provision of an adequate and safe drinking water to its populace (Edema *et al*., 2011).

Water and water resources is very important for maintaining an adequate food supply and a productive environment for the all living organisms (Pimentel *et al*., 2004). Water is essential to life because it heavily influences public health and standard of living. Water is a very important nutritional requirement in order to sustain vital activities of human such as nutrition, respiration, circulation, excretion and reproduction. In addition water is also a life space as well as being one of the basic substances in the formation of life environment (Kılıç, 2020).

As human populations and economies grow, global freshwater demand has also increased rapidly. In addition to threatening the human food supply, water shortages severely reduce biodiversity in both aquatic and terrestrial ecosystems (Pimentel *et al*., 2004; Umeh *et al*., 2005). The negative effects of global population increase, climate change impacts, and lifestyle changes are exerting growing pressures upon our vital water resources leading to widespread water stress

in many countries. As a result, there is growing realization of the urgent need to conserve water (Pimentel *et al*., 2004).

Drinking water free of pathogenic organisms is fundamental to breaking one of the principal transmission routes of infectious disease. This has stimulated worldwide investment in the construction of water systems that are designed to meet stringent water quality standards (Trevett *et al*., 2004). Waterborne pathogens, including a variety of viral, bacterial, algal and protozoan agents, account for much of the estimated 4 billion cases and 2.5 million deaths from endemic diarrheal disease each year (Kosek *et al*., 2003; World Health Organization, 2017). Waterborne illnesses caused by bacteria found in contaminated household water tanks increases the risk of spreading waterborne diseases and may lead to many infectious outbreaks. World Health Organization (WHO) data on the burden of disease suggest that approximately 3.2% of deaths (1.8 million) and 4.2% of disability-adjusted-life years (61.9 million) worldwide are attributable to unsafe water, sanitation and hygiene (Khan and AlMadani, 2017).

Unsafe water is a global public health threat, placing persons at risk for a host of diarrheal and other disease as well as chemical intoxication (Hughes *et al.,* 2005; Nwabor *et al*., 2016). Unsanitary water particularly has devastating effects on young children in developing world. Each year, more than 2 million persons, mostly children less than 5 years of age, die of diarrheal disease (Kosek *et al.* 2003; WHO, 2022). Nearly 90% of diarrheal-related deaths have been attributed to unsafe or inadequate-water supplies and sanitation conditions affecting a large part of the world's population (Hughes *et al.,* 2005).

Water has the potential of transmitting a variety of enteric diseases such as cholera, typhoid fever, infectious hepatitis, amoebic and bacillary dysentery (Chemuliti *et al*., 2002). The main transmission routes are by consumption, contact or transfer that can be easily prevented by

the strict maintenance of good hygiene and sanitation as well as implementing strategies that will reduce or eliminate the presence of pathogenic microorganisms and filter the contaminated water to provide safe water for human usage and consumption (Nath and Bloomfield, 2016). According to WHO (2017) water, sanitation and hygiene contribute in preventing at least 9.1% of global disease burden and 6.3% of all deaths (Khan *et al*., 2017).

Various residences depend on borehole water stored in overhead tanks for their water supply.

1.2 Scope of the Study

The view of this research work is to analyze and estimate the numbers of bacteria present in water tanks in halls of residence in Auchi metropolis.

1.3 Purpose of the Study

The purpose of this study is to ascertain the bacterial loads of water from these tanks.

1.4 Objective of Study

The objectives of this study are:

- To attain the total bacterial count of the water samples.
- To determine the coliform counts (Most Probable Number) of the water samples.
- To determine the species of bacteria present in the water.

1.5 Significance of Study

This study is useful to residence users and every consumer of water in that, it will help them to understand the quality of water and to know if it is good or unfit for human consumption in other to prevent waterborne diseases.

1.6 Limitation of Study

Some of the limitations encountered in the course of this study include**:**

- ❖ High cost of materials like the Agars used in carrying out the analysis.
- ❖ Irregular power supply.
- ❖ Unavailability of vital equipment required for the analysis

CHAPTER TWO

2.0 LITERATURE REVIEW

The earth has an abundance of water but unfortunately, only about 0.3 % is usable by humans. The usable 0.3% is comprised of freshwater and lakes (0.009%), inland seas (0.008%), soil moisture (0.005%), atmosphere (0.001%), rivers (0.0001%), groundwater (0.279%), ocean (97.2%), glaciers and other ice (2.15%) (Bibi *et al*., 2016).

Water is essential for living things, both in the composition of their cells and in the environment in which they live. Organisms are made up of between 60 and 95 per cent water by weight, and even inert, dormant forms like spores and seeds have a significant water component. This dependence on water is a function of its unique properties, which in turn derive from its polar nature (Hogg, 2005).Water is an essential part of human nutrition either directly as drinking water or indirectly as constituent of food and served in various other applications of our daily life(Umeh *et al*., 2005).

To humans, water is indispensable. In the home, water is used for cooking, washing, bathing and other domestic uses. Water is the starting point of most industrial processes. The chemist says that water is a universal solvent following his findings that most chemicals are soluble in water (Ajobiewe *et al*., 2019). For the biologist, it is essential for the growth of organisms and for carrying out fermentation for the production of products useful to man (Ajobiewe *et al*., 2019).

The vast majority of people living in undeveloped countries depend heavily on surface waters as their primary sources of water and simultaneously, as their means of waste disposal. A majority of this population depends on unprotected/or contaminated water sources as a means of drinking water which can cause outbreaks of waterborne diseases (Jamielson *et al*., 2004). A

large percentage of the population in developing countries (majorly African countries) lack accessibility to potable water supply thus, they are compelled to use untreated water from other sources such as rivers, reservoir, springs, streams and groundwater for drinking and other domestic purposes (Jamielson *et al*., 2004).

The basic requirements for drinking water are that it should be free from pathogenic organisms, contain no compounds that have adverse effects on human health, be fairly clear, be non-saline, contain no compounds that cause an offensive taste or smell and cause no corrosion (Ajobiewe *et al*., 2019). Safe drinking water for human consumption should be free from pathogens such as bacteria, viruses and protozoan parasites, meet the standard guidelines for taste, odour, appearance and chemical concentrations, and must be available in adequate quantities for domestic purposes (Kirkwood, 2000). However, inadequate sanitation and persistent faecal contamination of water sources is responsible for a large percentage of people in both developed and developing countries which lack access to microbiologically safe drinking water and suffering from diarrhoeal diseases (WHO, 2002).

Waterborne illnesses can occur as a result of water supply contamination, especially from microbiological hazards(Fong *et al.,* 2007).Diarrhoeal diseases are responsible for approximately 2.5 million deaths annually in developing countries, affecting children younger than five years, especially children in areas devoid of access to potable water supply and sanitation (Obi *et al*., 2004). An examination of water quality is basically a determination of the organisms, minerals and organic compounds contained in the water.

To improve and enhance the microbiological quality and to reduce the potential health risk of water to these households, intervention strategies is needed that is easy to use, effective, affordable, functional and sustainable (Sobsey, 2002). Many different water collection and

storage systems have been developed and evaluated in the laboratory and under field conditions (Sobsey, 2002). In addition, a variety of physical and chemical treatment methods to improve the microbiological quality of water are available (Sobsey, 2002).Water treatment employs aeration, coagulation and flocculation, sedimentation, slow sand filtration and rapid filtration and disinfection. The principal methods of purifying water on a small scale are those used locally in areas where water harvesting is carried out include sedimentation, coagulation, boiling and filtration (Ajobiewe *et al*., 2019). Boiling is the most satisfactory way of destroying disease-producing organisms in water. It is equally effective whether the water is clear or cloudy, whether it is relatively pure or heavily contaminated with organic matter. Also, it destroys all forms of disease producing organisms usually encountered in water whether they are bacteria, viruses, spores, cysts or ova (Ajobiewe *et al*., 2019).

In Nigeria, treated pipe borne water is limited to urban areas and the quantity provided grossly inadequate and the frequency of supply epileptic. Such services may not even be available in certain areas within the metropolis. Due to this scenario, an increasing no of people in semi urban and urban areas in Nigeria depend on wells as their source of water supply (Idowu *et al*., 2011).

2.1 The Water Cycle

The Water cycle (known scientifically as the hydrologic cycle) refers to the continuous exchange of water within the hydrosphere, between the atmosphere, soil water, surface water, groundwater, and plants. Water can change state among liquid, vapour, and ice at various places in the water cycle (Anderson *et al*., 2012).

The Hydrologic cycle is the driving wheel of all the available water resources on the planet earth. Through this process, water is transformed from liquid to solid, solid to liquid,

liquid to vapour, vapour to liquid and vapour to solid states. The sun's radiation, acceleration due to gravity, ability of the water to flow and several other properties of water, make this transformation more effective and regular (Balasubramanian and Nagaraju, 2015).

The basic input to the world's water masses comes from precipitation. Precipitated rain (or) snow falls overland. Processes like infiltration and percolation moves the water down to the groundwater systems. Some amount of water flows towards the sea as runoff. The surface water collected in lakes, ponds, swamps, seas and oceans get evaporated into the atmosphere (Balasubramanian and Nagaraju, 2015).

The vegetation transpires the water collected from the soil moisture. Evaporated and transported water enters into the atmosphere as vapour. Collected water vapour gets condensed to form the clouds. Clouds move towards the land and starts precipitating again and these processes continue. This endless circulation of water is known as the Water (hydrologic) cycle (Balasubramanian and Nagaraju, 2015).

2.1.1 Components of the Water Cycle (Hydrologic Cycle)

The circulation of water masses seen in all spheres of the earth involves several causative factors and components. The major components of the hydrologic cycle are:

- Precipitation
- Evaporation
- Transpiration
- Evapotranspiration
- Surface Runoff
- Condensation
- Infiltration

- Groundwater base flow
- Sublimation
- Interception (Balasubramanian and Nagaraju, 2015).

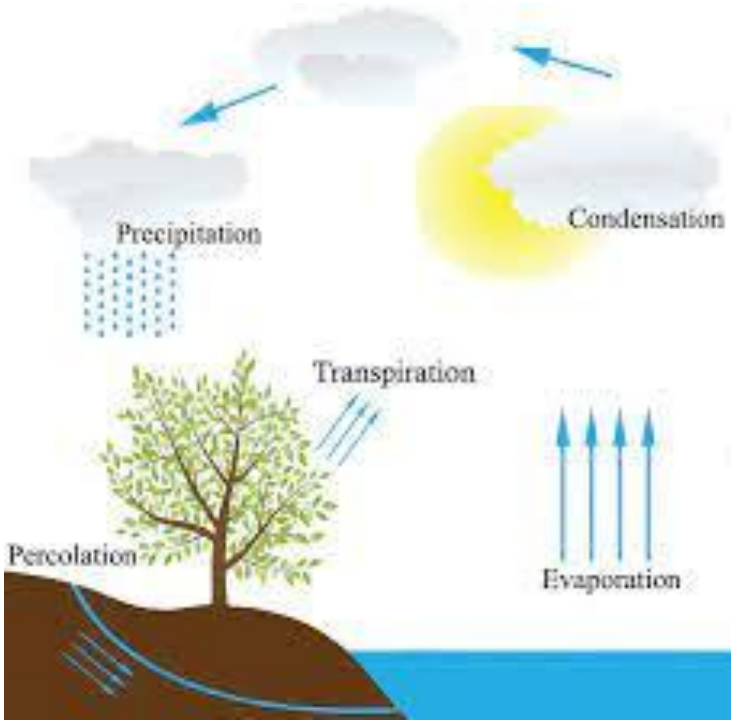

Fig.1.Aschematic view of the Water Cycle (hydrologic cycle process)

Source: (Sadollah *et al*., 2015)

2.2 Surface Water Sources

Surface water is any source of water that is open to the atmosphere and is subject to run off from the land. Hence, it is very likely to contain microorganisms that can cause sicknesses. In some areas, a substantial portion of the surface drinking water is derived from bank filtration that carries a diverse chemicals' and pathogens' load (Tufenkji *et al*., 2002) and requires purification. Surface water is accumulated on the ground or in a stream, river, lake, reservoir, or

ocean. The volume of water depends mostly on the amount of rainfall but also on the size of the watershed, the slope of the ground, the soil type and vegetation, and the land use (Katsanou and Karapanagioti, 2017).

2.3 Ground Water Sources

Groundwater is a major source of drinking water worldwide and is hosted in aquifers. Hydrological recharge of aquifers hugely varies geographically and strongly depends, among other factors, on climate, geology, soil type, vegetation, and land use (Scanlon *et al*., 2002). Groundwater is covered by soils and sediments and is considered to be less vulnerable than surface water. Its abstraction though requires drilling and pumping equipment that is not always available or sustainable especially in developing countries. Groundwater is more evenly distributed compared to surface water, though much of it is nonrenewable fossil water. Groundwater is a critical source of water that can cover the human needs, because it is part of the limited budget of freshwater (Katsanou and Karapanagioti, 2017).

2.4 Water Storage Tanks

A water tank is a container for storing water. Almost all water tanks were made from different types of elements as long as it has the ability to store. The means and make of these tanks evolved with time (Mang *et al.,* 2021).

Water storage tanks, also known as cisterns, are primarily used to store water for domestic and consumptive purposes in households or buildings. Cisterns are typically found in areas where a potable water source is not available in the community, the area yields low well water capacity, or the groundwater quality is poor. Cisterns can also be used to store water:

a) to supplement a low yielding private water well

b) as an emergency water supply

c) for seasonal/occasional use

2.5 Drinking Water Quality

Drinking water quality is a relative term that relates the composition of water with effects of natural processes and human activities. Deterioration of drinking water quality arises from introduction of chemical compounds into the water supply system through leaks and cross connection (Napacho and Manyele, 2010). Drinking water quality requirements have been focused on elimination of pathogenic organisms for over 100 years (Schroeder, 2003).

The quality of water is affected by an increase in anthropogenic activities and any pollution either physical or chemical causes changes to the quality of the receiving water body (Aremu *et al*., 2011). Chemical contaminants occur in drinking water throughout the world which could possibly threaten human health. In addition, most sources are found near gullies where open field defecation is common and flood-washed wastes affect the quality of water (Messeret, 2012).

2.6 Waterborne Diseases

Waterborne Diseases are those diseases that are transmitted through the direct drinking of water contaminated with pathogenic microorganisms. Contaminated drinking water when used in the preparation of food can be the source of food borne disease through consumption of the same microorganisms. Most waterborne diseases are characterized by diarrhea, which involves excessive stooling, often resulting to dehydration and possibly death (Nwabor *et al*., 2016). According to the World Health Organization, diarrhea accounts for an estimated 4.1% of the total daily global burden of disease and is responsible for the deaths of 1.8 million people every year (WHO, 2017).Waterborne or water related diseases encompass illnesses resulting from both direct and indirect exposure to water, whether by consumption or by skin exposure during

bathing or recreational water use. It includes disease due to water-associated pathogens and toxic substances (Satnwell, 2010). A broader definition includes illness related to water shortage or water contamination during adverse climate events, such as floods and droughts, and diseases related to vectors with part of their life cycle in water habitats (Satnwell, 2010).

2.6.1 Water-washed Diseases

Water washed or water scarce diseases are those diseases which thrive in conditions with freshwater scarcity and poor sanitation. Control of water-washed diseases depends more on the quantity of water than the quality. Examples of water washed diseases includes; Scabies, Typhus, Yaws, Relapsing fever, Trachoma, Conjunctivitis and Skin ulcers (Manetu and Karanja, 2021).

2.6.2 Impact of Waterborne Diseases

Waterborne diseases can have a significant impact on the economy, locally as well as internationally. People who are infected by a waterborne disease are usually confronted with related costs and often a huge financial burden. This is especially the case in less developed countries. The financial losses are mostly caused by e.g. costs for medical treatment and medication, costs for transport, special food, and by the loss of manpower. Many families must even sell their land to pay for treatment in a proper hospital. On average, a family spends about 10% of the monthly households income per person infected. Some of the roles are as follows:

- ✓ Droughts can cause increased concentrations of effluent pathogens, overwhelming water treatment plants and contaminating surface water. Older water treatment plants are particularly at risk.
- ✓ Changes in ocean and coastal ecosystems, including changes in pH, nutrient and contaminant runoff, salinity, and water security that can cause degradation of fresh water,

particularly in areas where much of the population uses untreated surface water for daily consumption and activities.

- ✓ Increased frequency of intense extreme weather events can cause flooding of water and sewage treatment facilities, increasing the risk of waterborne diseases.
- ✓ Indirectly, the lack of water can cause pressure on agricultural productivity, crop failure, malnutrition, starvation, population displacement, and resource conflict.

Changes can occur in the distribution and concentrations of chemical contaminants in coastal and ocean waters through the release of contaminants previously locked in polar ice sheets, or in runoff from coastal and watershed development.

2.7 Microbial Contamination of Water

The microbial contamination of water is often of faecal nature related to humans (water sewage treatment plants, combined sewage overflow (CSO), non-collective sewage systems), domesticated animals (manure spreading, pit stock overflow), or wildlife (Jung *et al*., 2014). The main origins of microbial contamination of natural aquatic resources are discharges of water treatment plants, decontamination stations, hospitals, industries considered as point sources, etc. Correlation between pathogens concentrations and urban activities is well documented (Marsalek and Rochfort, 2004; Selvakumar and Borst, 2006).

On the other hand, diffuse sources (slurry, manure, sludge application…) may also be considered. The abundance and importance of pathogens in water depend on factors such as the contamination level, pathogens' persistence in water bodies, biological reservoirs (including aquatic plants and sediments) and the ability of pathogens to be transported (Dechesne *et al*., 2006).

The land use management practices and the size of the watershed also influence the survival of microorganisms (Harmel *et al*., 2010). Streams flowing through areas partly or fully covered with pastures are more contaminated than those running through forests and cultivated areas (Jung *et al*., 2014).

2.8 Bacteriological Analysis of Water

Microbial contamination is by far the most serious public health risk associated with drinking-water supplies. It is impractical to analyze water for every individual pathogen, some of which can cause disease at very low doses. Instead, since most diarrhea-causing pathogens are faecal in origin, it is more practical to analyze water for indicator species that are also present in faecal matter. The use of indicator organisms in the bacteriological analysis of water has remained the mainstay of water bacteriology. For many years, total coliforms have been used as indicators in evaluating water quality for several water uses with respect to faecal contamination (Obasi *et al.*, 2021).

Not all coliforms are from faecal source. Hence, feacal coliforms and pathogenic forms such as *Escherichia coli* are now used largely as bacteriological indicators (Mahmud *et al*., 2019).

2.9 Bacteriological Quality of Drinking Water

The bacteriological quality of drinking water refers to the level of occurrence of microorganisms in the final product, and is an index determining whether the water is safe for human consumption (WHO, 2012). Also, the bacteriological quality of drinking water is defined by parameters which separate acceptability [safe] from unacceptability [unsafe] (Codex, 2001), based on the measurement of multiple indicator species of microorganisms present in a water

sample. If the bacteriological testing results are within the required standard limits, then the water is said to be of good bacteriological quality for human consumption (APHA *et al*., 2012).

2.10 Prevention and Control of Waterborne Diseases

According to Patel (2019), in affected individuals, antibacterial, anti-parasitic, or antiviral medications are used for treatment depending on the nature of the disease. However, common precautions to keep the surroundings hygienic can do wonders to stop the spread of these ailments (Patel, 2019). Additionally, maintaining personal hygiene also reduces the occurrence of water-borne diseases dramatically. One should make sure that their drinking water should be filtered and purified. Also, the water used for cooking at home should also be equally pure. Being mindful of the surroundings, avoiding street food (especially if the place of preparation is visibly dirty), and covering and storing the food safely at home are some basic tips for prevention of such diseases (Patel, 2019).

Governments of the countries with high incidence of water-borne diseases, often run health check-up and awareness campaigns. They educate and sensitize the communities about the risks and common precautions. Avoiding the water clogging (e.g., from rain) surrounding the houses is an important step to prevent water-borne diseases. Apart from the precautions at an individual level, several other approaches including mass recycling of water and carbon sequestration are employed to control the water-borne diseases (Patel, 2019). Protecting the natural water sources and lands is another important strategy to combat climate change. Effective irrigation techniques have been developed by agricultural scientists for optimal usage of water during farming. Creation of "green spaces" and the responsible use of the natural resources are at the core of sustenance (Patel, 2019).

CHAPTER THREE

3.0 MATERIALS AND METHODOLOGY

3.1. Materials

Four samples of water, nutrient agar, Macconkey Agar, Eosin Methylene Blue Agar, measuring cylinder, petri dishes, beaker, 10ml pipette, flat bottom flask, weighing balance, test tubes, test tube rack, autoclave, incubator, glass slide, microscope, cotton wool, distilled water, wire lop, crystal violet, lugos iodine, acetone alcohol, safranine, hydrogen peroxide, conical flasks, syringe, gram reagents and hand gloves.

3.2 Sample Collection

Four water samples; Water from microbiology laboratory (A), Water from Engineering Laboratory (B), Water from Hostel C (C), Water from Hostel F (D) were specially collected in a clean sample container and transferred to the microbiology laboratory of Auchi Polytechnic, Auchi for further Bacteriological analysis.

3.3 Sterilization of Materials

All glass wares were first washed with detergent and rinsed with distilled water, wrapped with aluminum foil after drying and sterilized by dry heat method in the oven at a temperature of 160^{o}c for 2 – 3 hours.

3.4 Disinfection of working area

The working area was disinfected thoroughly before and after use with ethanol (75%). Cotton was soaked in ethanol and used to clean the working bench, a Bunsen burner was put on and the flame was allowed to burn, this helped in sterilizing the air in the laboratory.

3.5 Culture Media

The media used in this study is Nutrient Agar, Macconkey Agar and Eosin Methylene blue Agar. The media were prepared according to the manufacturer's specification.

3.5.1 Preparation of Nutrient Agar

7g of Nutrient Agar powder was weighed using a weighing balance and dispensed into a beaker; 250mls of distilled water was measured using a measuring cylinder and dispensed into the beaker containing the agar powder; it was stirred to dissolve for 10mins. The mixture was transferred into a conical flask and the neck of the flask was corked with cotton wool wrapped in aluminum foil. It was autoclaved at a temperature of 121^{o}C and pressure of 15psi for 15 – 20 minutes. The sterilized agar was allowed to cook to about 45^{o}C and then aseptically poured into petri dishes and allowed to set.

3.5.2 Preparation of Macconkey Agar

11.79g of Macconkey agar powder was weighed using a weighing balance and dispensed into a beaker; 250mls of distilled water was measured using a measuring cylinder and dispensed into the beaker containing the agar powder; it was stirred to dissolve for 10mins. The mixture was transferred into a conical flask and the neck of the flask was corked with cotton wool wrapped in aluminum foil. It was autoclaved at a temperature of 121^{o}C and pressure of 15psi for 15 – 20 minutes. The sterilized agar was allowed to cook to about 45^{o}C and then aseptically poured into petri dishes and allowed to set.

3.5.3 Preparation of Eosin Methylene Blue Agar

9g of Eosin Methylene blue agar powder was weighed using a weighing balance and dispensed into a beaker; 250mls of distilled water was measured using a measuring cylinder and dispensed into the beaker containing the agar powder; it was stirred to dissolve for 10mins. The

mixture was transferred into a conical flask and the neck of the flask was corked with cotton wool wrapped in aluminum foil. It was autoclaved at a temperature of 121^{o}C and pressure of 15psi for 15 – 20 minutes. The sterilized agar was allowed to cook to about 45^{o}C and then aseptically poured into petri dishes and allowed to set.

3.6 Preparation of inoculums and inoculations

Serial dilution was used in carrying out the process in which 1ml from the sample water was dispensed into the first test tube containing 9mls of water which was labeled 10^{-1} from this 1ml was also taken and dispensed into the second test tube labeled 10^{-2}. The process was continued till the last test tube labeled 10-5.

Then pour plate method was used in introducing the serially diluted water sample into the various petri dishes before the Agars (Nutrient Agar, Macconkey Agar and Eosin Methylene Blue Agar) was introduced (only 0.5ml of the mixture from dilution 10^{-1} and 10^{-3}). The sample plates were incubated using the incubator for 24 hours for the growth of bacteria.

3.7 Characterization and identifications of isolates

The bacterial isolates from the different samples were grouped on the basis of colonial morphology. The criteria used were the size of the colony, color, surface, edge, slope and elevations.

3.8 Microscopic Examination of the Isolates

Smear of each of the different bacterial isolates were stained using Gram's method of staining to study the morphological appearance of the cell as well as their gram reactions (whether they are gram positive or gram negative) and cell arrangement. Gram positive cells

retained the purple colour of the initial dye while the gram negative cells retained the pinkish-red color of the counter stain (Oyeleke and Manga, 2008).

3.8.1 Gram Staining Techniques

A thin smear each of the pure 24hours old culture was prepared on clean grease-free slides, allowed to air dry then heat fixed by passing swiftly over a burning flame. The slides containing the smears were flooded with crystal violet solution for 60sec, rinsed with distilled water and blotted to remove water residue. The smears were again flooded with lugos Iodine which acts as a mordant for 60secs, rinsed with distilled water and blotted. They were then decolorized with acetone alcohol for a few seconds and rinsed after which they were counter stained with safranin for 30 – 60 secs, washed with distilled water and kept to air dry before they were observed under the oil immersion objectives of the microscope. Gram negative cells appeared pink or red while the gram positive organisms appeared purple.

3.9 Biochemical Test

Biochemical test were performed to confirm the identity of the isolated bacteria, 24 hours old cultures were used to perform the biochemical test.

3.9.1 Catalase test

The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small innoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles occurs.

A loopful of 24hours old culture was placed on a clean slide and drop of 3% hydrogen peroxide was placed on it. Gas seen as white bubbles indicated the presence of catalase enzymes which is a positive result.

3.9.2 Coagulase test

Coagulase is a protein enzyme produced by several microorganisms that enables the conversion of fibrinogen to fibrin. A drop of physiological saline is placed on each end of a slide, with a wire loop, a portion of the isolated colony was emulsified into each drops to make two thick suspensions and mixed. Then clotting is observed closely.

3.9.3 Oxidase Test

This test is used to determine if an organism possesses the cytochrome oxidase enzyme. A piece of small filter paper was soaked in 1% kovac oxidase reagent and allowed to dry, a sterile wireloop was used to pick a well isolated colony from a fresh (18-24hours culture) from a bacterial plate and rubbed on to treated filter paper and observed for color changes.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

The results for the bacteriological analysis of water are presented in the tables below.

Table 1:

Plate count isolated from different water samples.

SAMPLES	PLATE COUNT (cfu/ml)
A	$7.9x10^5$
B	$6.5x10^5$
C	$6.3x10^5$
D	$5.9x10^5$

Key: A= MICROBIOLOGY LAB; B= ENGINEERING LAB; C = HOSTEL C; D = HOSTEL F

Table 2:

Biochemical Test

Sample	Gram Stain	Coagulase Test	Catalase Test	Oxidase Test
A1	+	+	+	-
A2	-	-	+	-
B3	-	-	+	-
C3	-	-	+	-
D2	-	-	+	+
D3	-	-	+	-

KEY; += PRESENT; -= ABSENT

Table 3:
Morphological, cultural and Biochemical characteristics of Bacterial isolate from water sample

Sample	Cultural Characteristics	Morphological arrangement of cell	Probable organism
A1	Curved colonies, 0.8mm in size and creamy colour on NA	Rod like and distributed	*Staphylococcus aureus*
A2	Raised round colonies, 0.12mm in size and Pink colour on MAC	Short chains, singly in pairs and sometimes in clusters	*Escherichia coli*
B3	Convex colonies colonies, 0.9mm in size, colorless colonies on EMB	Short rod in singular arrangement	Salmonella spp.
C3	Raised round colonies, 0.5mm in size and pink on EMB	Short rod-shaped in single and cluster arrangement	*Klebsiella pneumoniae*
D2	Raised round colonies, 0.4mm in size and colorless but later changed to slightly Pink color on MAC	curved rods or comma-shaped in single arrangement	*Vibrio cholerae*
D3	Raised round colonies, 0.3mm in size and Metalic green sheen on EMB	Short chains, singly in pairs and sometimes in clusters	*Escherichia coli*

Key: NA= Nutrient agar; EMB= Eosin methylene blue; MAC = Macconkey agar

Table 4:

Occurrence of Bacteria isolated from the different water samples

SAMPLE/ISOLATES	A	B	C	D
Staphylococcus aureus	+	+	+	+
Vibrio cholerae	+	+	-	-
Escherichia coli	+	+	+	+
Salmonella	+	+	+	+
Klebsiella pneumoniae	+	-	+	-

KEY; += PRESENT; -= ABSENT

Table 5: Percentage of Occurrence of Isolate

Isolate	Percentage
Escherichia coli	28.5%
Staphylococcus aeureus	26%
Salmonella spp.	25%
Vibrio cholerae	10.5%
Klebsiella pneumoniae	10%

4.2 Discussion

This study revealed the bacterial load of the sample with values ranging from $5.9x10^5$cfu/ml - $7.9x10^5$cfu/ml (Table 1). The biochemical results of bacteria isolates were recorded in Table 2 above. The Cultural and morphological characteristics of bacteria isolates were recorded in Table 3 above. The bacterial isolates from this study are *Staphylococcus aureus, Vibrio cholera, Escherichia coli, klebsiella pneumoniae* and *Salmonella* spp. The result shows that all the water samples in the above four places, i.e. Microbiology laboratory (Sample A), Engineering Laboratory (Sample B), Hostel C (Sample C) and Hostel F (Sample D) were contaminated with high amount of bacterial population than acceptable limit. The occurrence of bacteria isolate from this student is recorded in Table 4 while the percentage of occurrence of isolate was recorded in Table 5.

The water examined in this study has clearly demarcated that it is loaded with indicator organisms which are indication of fecal pollution and also human interference (Jung *et al*., 2014). *Escherichia coli* was the predominant bacteria along with *Salmonella, Vibrio cholera, Klebsiella pneumonia* and *Staphylococcus* spp. isolated in this present study which correlated with the study of Paneerselvam, (2014) who isolated *Escherichia coli* and as the commonest organisms respectively.

The major diseases that could arise from bacteriological contamination of water include typhoid, diarrhea and cholera (Uzoigwe and Agwa, 2012). The high abundance of bacteria isolated from the water sample as seen in this study indicate the presence of high feacal contamination and health risk for the human consumption due to high pathogens presence in the water sample (Franciska *et al*., 2005). According to WHO recommendations, there should no fecal coliforms in 100 ml drinking water and the reason for the gross contamination of ground

waters by pathogens as observed in this study may be due to openness and shallowness of this ground water that allows easy entrance of particles from the surroundings. It may also be due to poor sanitary condition around the areas where such wells are located (Anyanwu and Okoli, 2012). Coliforms are the primary bacterial indicator for faecal pollution in water and they are most abundant bacteria in water responsible for waterborne diseases such as typhoid, dysentery, diarrhea and also been implicated in mortality across the world (WHO, 2011).

Surface water are particularly liable to pollution from animals and birds, and *Salmonella spp*. may be detected even when only a small number of indicator organisms are present, e.g. *Escherichia coli*. Additionally, some authors highlighted that the different rates of survival of Salmonella and *E. coli* in non-host environment suggest that *E. coli* may not be an appropriate indicator of *Salmonella spp*. contamination (Polo *et al*., 2005). *Salmonella spp.* is a recognized human pathogen and its waterborne transmission has been well-documented (Polo *et al*., 2005). Presence of *Salmonella spp.* in waterways indicates the spread of the agent in the environment, highlighting the importance of fecal contamination of the water environment in the spread of salmonellosis (Cabral, 2010). Nutrients which support the growth of bacteria find the way into drinking water after disinfectant has been applied to it through the seepage and when the environmental condition favour their growth. Some disinfectants are selective in killing particular organisms while others survive or develop resistant towards it. Ward *et al*. (2006) reported that disinfection itself can be selective for a variety of bacteria has been demonstrated by the results of work of several researchers.

In this study, E. coli was most frequently observed (28.5%) (Table 5). This result indicates that there is a high probability of exposure of household members to this bacterium through water tanks. A recent study in Oman revealed that the storage reservoirs at pumping

station and water distribution lines to residential houses complied with local drinking water standards, however, regrowth of several opportunistic pathogens occurred in the household water tanks.

The reason for high number of bacterial colonies might be due to inadequate maintenance of water reservoirs and the mixing of sewage into the reservoirs or directly into the rivers. These sources of bacterial contamination include leakage in pipes, cross contamination from waste waters (Roohul *et al*., 2012), surface runoff, pasture, and other land areas where animal wastes are deposited. Additional sources include seepage or discharge from septic tanks, sewage treatment facilities and natural soil /plant bacteria (EPA, 2003).

This study have therefore proposed that the bacteriological quality of water in a residence water storage tank might depend on the source from which it was drawn and also the handling of the water in the tank by the users.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Bacteriological quality of household water tanks in residences in Auchi for the presence of various types of bacteria in household water tanks was studied. The analysis showed the occurrence of *E. coli, Salmonella*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Vibrio cholerae* in the water tanks studied. It is concluded that water from these tanks is found to be unsatisfactory for the human consumption hence it is a health risk to use this water without purification.

5.2 Recommendation

The following recommendations are made:

- Improving and expanding the existing water treatment and sanitation systems is more likely to provide safe and sustainable sources of water over the long term.
- Strict hygienic measures should be applied to improve water quality and to avoid deleterious effects on public health, by using periodical monitoring programmes to detect sewage pollution running over local hydrological networks and valleys.

Reference

Ajobiewe, H. F., Ajobiewe, J. O., Mbagwu, T. T., Ale, T. and Taimako, G. A. (2019). Assessment of Bacteriological Quality of Borehole Water, Sachet Water and Well Water in Bingham University Community. American Journal of Medicine and Medical Sciences, 3(9), 96-103.

Anderson, R. G., Lo, M. H. and Famiglietti, J. S. (2012). Assessing surface water consumption using remotely-sensed groundwater, evapotranspiration, and precipitation. *Geophysical Research Letters*, *39*(16).

Anyanwu, C. U. and Okoli, E. N. (2012). Evaluation of the bacteriological and physicochemical quality of water supplies in Nsukka, Southeast, Nigeria. *African Journal of Biotechnology*, *11*(48), 10868-10873.

American Public Health Association (APHA) (2012). Standard methods for the examination of water and wastewater. 20th ed. American Public Health Association, American Water Works Association and Water Environment Federation. New York, NY.

Aremu, M. O., Olaofe, O., Ikokoh, P. P. and Yakubu, M. M. (2011). Physicochemical characteristics of stream, well and borehole water sources in Eggon, Nasarawa State, Nigeria. *J Chem Soc Niger*, *36*(1), 131-136.

Balasubramanian, A. and Nagaraju, D. (2015). The hydrologic cycle. *Mysore, UO, Ed*, 1.

Bibi, S., Khan, R. L., Nazir, R., Khan, P., Rehman, H. U., Shakir, S. K. and Jan, R. (2016). Heavy metals analysis in drinking water of Lakki Marwat District, KPK, Pakistan. *World applied sciences journal*, *34*(3), 15-19.

Cabral, J.P.S. (2010). Water microbiology. Bacterial pathogens and water. *Int. J. Environ. Res. Public Health.* 7:3657-3703.

Chemuliti, J. K., Gathua, P. B., Kyule, M. M. and Njeruh, F. M. (2002). Bacteriological qualities of indoor and out-door drinking water in kabera sub-location of Nairobi, Kenya. *East African medical journal*, *79*(5), 271-273.

Dechesne, M., Soyeux, E., Loret, J. F., Westrell, T., Stenström, T. A., Gornik, V. and Medema, G. J. (2006). Pathogens in source water. *Microbiological Risk Assessment: A Scientific basis for managing drinking water safety from source to tap*, 1-42.

Edema, M. O., Atayese, A. O. and Bankole, M. O. (2011). Pure water syndrome: bacteriological quality of sachet-packed drinking water sold in Nigeria. *African Journal of Food, Agriculture, Nutrition and Development*, *11*(1).

EPA (2009). *Source Water Protection Practices Bulletin. Managing Stormwater Runoff to Prevent Contamination of Drinking Water*. United States Environmental Protection Agency (EPA); Washington, DC, USA. Office of Water (4606).

Fong, T. T., Mansfield, L. S., Wilson, D. L., Schwab, D. J., Molloy, S. L. and Rose, J. B. (2007). Massive microbiological groundwater contamination associated with a waterborne outbreak in Lake Erie, South Bass Island, Ohio. *Environmental health perspectives*, *115*(6), 856-864.

Franciska, M. S., Marcel, D. and Rinald, L. H. (2005). Escherichia coli 0157: H7 in drinking water from private supplies. *Journal of Netherlands Water Resource*, *39*, 4485-4493.

Harmel, R. D., Karthikeyan, R., Gentry, T. and Srinivasan, R. (2010). Effects of agricultural management, land use, and watershed scale on E. coli concentrations in runoff and streamflow. *Transactions of the ASABE*, *53*(6), 1833-1841.

Hogg, N. (2005): Current meter data at station ACM3_WHOI_907 (CMDB code rcm01080). *PANGAEA*.

Hughes, J. M. and Koplan, J. P. (2005). Saving lives through global safe water. *Emerging Infectious Diseases*, *11*(10), 1636.

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

Jamieson, R., Gordon, R., Joy, D. and Lee, H. (2004). Assessing microbial pollution of rural surface waters: A review of current watershed scale modeling approaches. *Agricultural water management*, *70*(1), 1-17.

Jung, A. V., Le Cann, P., Roig, B., Thomas, O., Baurès, E. and Thomas, M. F. (2014). Microbial contamination detection in water resources: interest of current optical methods, trends and needs in the context of climate change. *International Journal of Environmental Research and Public Health*, *11*(4), 4292-4310.

Katsanou, K. and Karapanagioti, H. K. (2017). Surface water and groundwater sources for drinking water. *Applications of Advanced Oxidation Processes (AOPs) in Drinking Water Treatment*, 1-19.

Khan, M. A. and AlMadani, A. M. A. A. (2017). Assessment of microbial quality in household water tanks in Dubai, United Arab Emirates. *Environmental Engineering Research*, *22*(1), 55-60.

Kılıç, Z. (2020). The importance of water and conscious use of water. *International Journal of Hydrology*, *4*(5), 239-241.

Kosek, M., Bern, C. and Guerrant, R. L. (2003). The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bulletin of the world health organization*, *81*, 197-204.

Mahmud, Z. H., Islam, M. S., Imran, K. M., Hakim, S. A. I., Worth, M., Ahmed, A. and Ahmed, N. (2019). Occurrence of Escherichia coli and faecal coliforms in drinking water at source and household point-of-use in Rohingya camps, Bangladesh. *Gut pathogens*, *11*(1), 1-11.

Manetu, W.M. and Karanja, A.M. (2021) Waterborne Disease Risk Factors and Intervention Practices: A Review. *Open Access Library Journal*, 8, 1-11.

Manga, M., Ngobi, T. G., Okeny, L., Acheng, P., Namakula, H., Kyaterekera, E. and Kibwami, N. (2021). The effect of household storage tanks/vessels and user practices on the quality of water: a systematic review of literature. *Environmental Systems Research*, *10*(1), 1-26.

Marsalek, J. and Rochfort, Q. (2004). Urban wet-weather flows: sources of fecal contamination impacting on recreational waters and threatening drinking-water sources. *Journal of Toxicology and Environmental Health, Part A*, *67*(20-22), 1765-1777.

Messeret, B. (2012). Assessment of drinking water quality and determinants of household potable water consumption in Simada District, Ethiopia (Doctoral dissertation, Cornell University).

Napacho, Z. A. and Manyele, S. V. (2010). Quality assessment of drinking water in Temeke District (part II): Characterization of chemical parameters. *African journal of environmental science and technology*, *4*(11), 775-789.

Nath, K. J. and Bloomfield, S. M. J. (2016). Household water storage, handling and point-of-use treatment. Vol. 34. In *International Scientific Forum on Home Hygiene*.

Nwabor, O. F., Nnamonu, E. I., Martins, P. E. and Ani, O. C. (2016). Water and waterborne diseases: a review. *Int. J. Trop. Dis. Health*, *12*(4), 1-14.

Obasi, K., Amadi, C., Azuamah, Y., Ede, A., Braide, W., Amadi, A., Iro, O. and Okereke, E. (2021). Bacteriological Quality of Drinking Water among Residents of Umuahia, Southern Nigeria. *Int'l J. Research and Review.* 2349-9788.

Obi, C. L; Bessong, P. O; Momba, M. N; Potgieter, N; Samie, A. and Igumbar, E.O. (2004): Profiles of antibiotics susceptibility of bacterial isolate and physicochemical quality of water supply in rural Venda communities, South Africa. Water S. A., 30(4): 515-519.

Ogamba, A.S. (2004). Packaged drinking water, how safe? Professionalism IPAN News.

Oyeleke, S. B. and Manga, S. B. (2008). Essential of laboratory practical in microbiology to best publisher Minna, Nigeria. Pp. 36-75.

Panneerselvam, A., (2014). Isolation and identification of bacteria from lake water in and around ranipet area, vellore district. *International Journal of Pharmaceutical and Biological Archives*, *3*(4), 1008-1011.

Patel, H.H. (2019, February 27). Water-Borne Diseases. News-Medical. Retrieved on May 03, 2022 from https://www.news-medical.net/health/Water-Borne-Diseases.aspx.

Pimentel, D., Berger, B., Filiberto, D., Newton, M., Wolfe, B., Karabinakis, E. and Nandagopal, S. (2004). Water resources: agricultural and environmental issues. *BioScience*, *54*(10), 909-918.

Roohul-Amin, A. S.S., Anwar, Z. and Khattak, J.Z.K. (2012). Microbial analysis of drinking water and distribution system in new urban Peshawar. *Curr. Res. J. Biol. Sci.* 4(6): 731-737.

Sadollah, A., Eskandar, H., Bahreininejad, A. and Kim, J. H. (2015). Water cycle algorithm with evaporation rate for solving constrained and unconstrained optimization problems. *Applied Soft Computing*, *30*, 58-71.

Satnwell, S. R. (2010). Classification of Water related diseases in water and health. Encyclopedia of Life Support Systems (EOLSS).

Scanlon, B. R., Healy, R. W. and Cook, P. G. (2002). Choosing appropriate techniques for quantifying groundwater recharge. *Hydrogeology journal*, *10*(1), 18-39.

Selvakumar, A. and Borst, M. (2006). Variation of microorganism concentrations in urban stormwater runoff with land use and seasons. *Journal of Water and Health*, *4*(1), 109-124.

Sobsey, M. D. and J. S. E. Leland (2002). Antiprotozoan and Anthelmintic Agents. In: Disinfection, Sterilization, and Preservation. 5th edition. S. S. Block (ed.). New York, Lippincott Williams & Wilkins.

Trevett, A. F., Carter, R. C. and Tyrrel, S. F. (2004). Water quality deterioration: a study of household drinking water quality in rural Honduras. *International journal of environmental health research*, *14*(4), 273-283.

Tufenkji, N., Ryan, J. and Elimelech, M. (2002). The promise of bank filtration. *Environmental science & technology*. 36. 422A-428A.

Umeh, C. N., Okorie, O. I. and Emesiani, G. A. (2005). Towards the provision of safe drinking water: The bacteriological quality and safety of sachet water in Awka, Anambra State. In The book of abstract of the 29th annual conference & general meeting on microbes as agents of sustainable development, organized by Nigerian society for microbiology (NSM), University of Agriculture, Abeokuta (p. 22).

Uzoigwe, C.I. and Agwa, O.K. (2012). Microbiological quality of water collected from boreholes sited near refuse dumpsites in Port Harcourt, Nigeria. *Afr J Biotechnol*., 11(13): 3135- 3139.

Ward, M.H., de-Kok, T.M., Levallois, P., Brender, J., Gulis, G., VanDerslice, J. and Nolan, B.T. (2006). Response to dietary nitrate: where is the risk? *Environ. Health Perspect*., 114, pp. A459-A460.

World Health Organization. Global WASH Fast Facts [Internet]. WHO; c2010 [cited 5 May 2011]. Available from: http://www. cdc.gov/healthywater/global/wash_statistics.html.

World Health Organization (2017). Diarrhoeal disease. https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease.

World Health Organization (2022). Drinking Water. https://www.who.int/news-room/fact-sheets/detail/drinking-water.