MICROBIOLOGICAL ANALYSIS OF BOREHOLE WATER IN UNIBEN HOSTELS

BY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY,

FACULTY OF LIFE SCIENCES,

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BENIN CITY.

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF

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CERTIFICATION

We certify that this project work was carried out by NWACHUKWU CHINEMEREM BETHEL in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc.) Degree in Microbiology.

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DR. O. N. IGIEHON **DATE**

(Project Supervisor)

_________________________ ____________________

Professor (Mrs). F. I. AKINNIBOSUN **DATE**

(Head of Department)

DEDICATION

This project is dedicated to the Almighty God, my helper and my source, my parents and also to my lovely parents Mr. and Mrs. Nwachukwu Innocent

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I wish to express my appreciation to God Almighty, who has been the source of my inspiration and has been faithful to me, comforted and guided me.

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ABSTRACT

Bacterial and fungal contaminations of borehole water in school hostels is a prevalent issue, particularly in regions where boreholes serve as primary water sources and hygiene practices may be challenging to maintain. This study was aimed at assessing the microbial contamination of water in student's residential halls in University of Benin, Benin City. The bacterial isolates were characterized and identified using morphological and biochemical methods. The percentage distribution and frequency of the isolates were evaluated using statistical method. From the result obtained in this study, the total heterotrophic bacterial counts ranged from 4.46 log_{10} cfu/ml to 4.86 log_{10} cfu/ml, Coliform count ranged from 2.84 to 3.13 log_{10} cfu/ml and fungal counts ranged from 4.40 to 4.85 log_{10} cfu/ml. Using the cultural, morphological and biochemical test, the isolates obtained in this study include, *Escherichia coli, Klebsiella sp, Staphylococcus sp, Bacillus sp, Citrobacter*, *Enterobacter* sp, *Bjerkandera sp, Aureobasidium sp, Scedosporium sp,* and *Mucor sp.* The contamination of borehole water in school hostels by various bacterial and fungal pathogens, such as *Escherichia coli, Klebsiella*, *Staphylococcus*, *Bacillus*, *Citrobacter*, *Enterobacter, Bjerkandera sp*, *Aureobasidium sp*, *Scedosporium sp*, and *Mucor sp*, presents serious public health risks. These microorganisms, arising from fecal contamination, soil infiltration, and environmental factors, can cause a range of infections, from gastrointestinal illnesses to skin, respiratory, and systemic diseases.

1.0 CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Water is the universal solvent capable of dissolving nearly all solutes, which is important to living and non-living things (Koda *et al*., 2017). Water is a resource that can be used for a wide range of purposes, including household, industrial, commercial, hydroelectric power, transportation, recreation, and more. River water is crucial to the growth of nations. Water is extraordinarily abundant on the planet as a whole, but fresh potable water is not always available at the right time or the right place for human or ecosystem use (Ababiaka and Sule, 2011). Water quality is defined as the set of physical, chemical and biological characters that must be satisfied in order to ensure that the water supplied is safe for the consumer.

Water pollution can cause adverse health effects for a representative number of people over predictable periods of time and is due to population growth, industrial development and urbanization (Awoyemi *et al*., 2014). In the recent past, expanding human population, industrialization, intensive agricultural practices and discharges of massive amount of wastewater into rivers have resulted in deterioration of water quality (Sale). The improper management of water systems may cause serious problems in availability and quality of water since water quality and human are closely related. For degradation of the quality of surface and groundwater, one of its origins is the direct discharge of contaminated water from domestic, industrial and agricultural sources into bodies of water (Falowo *et al*., 2017). Unfortunately, clean, pure and safe water only exists briefly in nature and is immediately polluted by prevailing environmental factors and human activities. Water from most sources is therefore unfit for immediate consumption without some sort of treatment. The industrial pollutants associated with organic matter, inorganic dissolved solids and other unwanted chemicals cause serious problems in the water quality (Mahaurpawar, 2015). Water related diseases continue to be one of the major health problems globally due to consumption of contaminated water (WHO, 2022).

Waterborne diseases constitute a serious public health problem in developing countries. In low- and middle-income nations, 58% of all diarrheal disease-related deaths are caused by inadequate access to safe water, poor hygiene, and unimproved sanitation conditions (WHO, 2014). Drinking water quality, high population densities, resulting from uncontrolled urbanization, coupled with poor hygiene and inadequate sanitation facilities play an important role in the emergence and transmission of waterborne diseases in urban environment (Sobsey, 2002; Negera *et al*., 2017).

However, boreholes are often badly maintained or non-protected and water that they get is sometimes contaminated (Amadou *et al*., 2014). 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 (Kirschnner *et al*., 2009). 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*., 2014; Dovonou *et al*., 2017). Microbiological pollution of water sources is often determined by counting faecal bacteria considered as bio-indicators such as coliforms and *E. coli* (Ashbolt *et al*., 2001; APHA, 2012). This is due to the fact their population is proportional to the amount of feces entering into environment (Páll *et al*., 2013). The enumeration of Heterotrophic Aerobic and Mesophilic Bacteria (HAMB) informs on the general microbiological quality of water. This bacterial group contains the following taxa*: Aeromonas, Enterococcus, Bacillus, Pseudomonas, Klebsiella, Flavobacterium, Citrobacter, Serratia,* *Acinetobacter, Proteus, Alcaligenes, Enterobacter* and *Moraxella* (Gerba, 2019). Generally, these bacteria are not pathogenic, but among them opportunistic pathogens which may have impacts on human health, especially in immunocompromised populations, children and old persons consuming soiled water, can be found (Bartram *et al*., 2013). The development of the microbial communities in surface water and groundwater would be related to meteorological factors, physico-chemical and biological characteristics of the biotope (Hounsounou *et al*., 2016).

Indiscriminate dumping of solid wastes, chemicals, sewage from homes and industries, and agricultural runoffs are some of the anthropogenic activities that compromise the quality of water sources (Yahaya *et al*., 2022a). One other source of environmental contaminants in water is dumpsites. Dumpsites are the most widely used methods of disposing of municipal solid wastes, industrial wastes, and hazardous wastes because they are effective and cheap (Ferronato and Torretta, 2019; Obiekezie *et al*., 2019). Dumpsites are open grounds where truckloads of waste are deposited, containing mainly industrial, medical, agricultural, electronic, and domestic waste (Ziraba *et al*., 2016). Properly managed dumpsites are hygienic, but poor management may cause organic waste degradation and, coupled with acidic rain, elicit leachate which can percolate into the ground and contaminate groundwater (Omorogieva and Andre-Obayanju, 2020; Ozbay *et al*., 2021).

Leachate from dumpsites contains hazardous substances like heavy metals, dissolved organic matter, inorganic macro components, microorganisms, and xenobiotic organic compounds (Daniel *et al*., 2021). Of all the environmental contaminants known to have the capability of marring the sanctity of groundwater, heavy metals are the most studied due to their toxicity, persistency, and nondegradability (Uddin *et al*., 2021). Some heavy metals, including cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), and arsenic (As), are toxic, even at low concentrations, making them a threat to both plant and animal survival (Olayiwola *et al*., 2017; Otomewo, 2022). On the other hand, some heavy metals such as cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn) are beneficial but they become harmful after certain doses (Sabejeje *et al*., 2014). Microorganisms are also a top environmental and health concern worldwide. Some of the microorganisms often detected in dumpsite leachate include *Escherichia coli, Enterobacter*, *Bacillus*, *Salmonella, Aspergillus niger, Aspergillus flavus, Rhizopus, Serratia marcescene, Klebsiella aerogenes, Staphylococcus aureus, Alcaligens* species, and *Proteus* species (Daniel *et al*., 2021). These pathogens have been linked to the outbreaks of typhoid, cholera, diarrhea, and skin rashes, among others (Daniel *et al*., 2021).

1.2 AIM AND OBJECTIVES

This project aimed to explore the microorganisms frequently associated with borehole water around hostels in University of Benin.

The specific objectives of this work:

1. Isolate and identify bacteria and fungi from borehole water in Uniben
2. Determine the total bacteria and fungi load
3. carryout antibiotic sensitivity testing on the bacterial isolates

2.0 CHAPTER TWO

LITERATURE REVIEW

2.1 BOREHOLE WATER

Water is one of the most essential natural resources needed by every living organism (Duru *et al*., 2017; Onyango *et al*., 2018). Whether it is used for drinking, bathing, food production or recreational purposes; portable and accessible water supplies are crucial for public health (Yusuf, 2007; WHO. 2018; WaterLife. 2018). Today the major challenges in developing countries include among others; the unprecedented human populations growth and climatic changes, which have culminated in pollution of available natural water resources (Olaoye and Olaniyan, 2012). According to the United nations medium population projection; over 2,8 billion people in 48 countries will be affected with water stress by 2025 (Hinrichsen *et al*., 1998; WHO. 2018). Against this background, global water security was adopted as one of the top most agenda of international organizations (Aboh *et al*., 2015).

According to Amenu, (2014), water was broadly grouped into two sources; surface and underground water. Surface water includes; rivers, streams, ponds and lakes, whereas, underground water includes wells and borehole waters among others. In Nigeria, borehole water represents the major source of potable water (Getso *et al*., 2018). Due to the acute shortage of water supplies, the last decade has witnessed a rapid increase in sinking of boreholes (Lateef *et al*., 2012). Moreover, Hati *et al*., (2011) reported earlier that the current 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. Clean water is priceless and has limited resources that man has begun to treasure only recently after decades of pollution with wastes (Oparaocha *et al*., 2010; Getso *et al*., 2018).

According to Chapman and Kimstach, (2014); Obioma *et al*., (2017) groundwater was the most important component of the hydrological cycle, and was an important source of potable water in Africa. Groundwater provides a reasonably constant water supply for domestic use; livestock and irrigation; in addition, it was not likely to dry up under natural conditions thereby buffering the effects of rainfall variability across seasons (Calow *et al*., 2010). According to Bolaji and Tse, (2019), in many arid and semi-arid areas of Africa; borehole water was a means of coping with water deficiencies especially in areas where rainfall was scarce; or highly seasonal and surface water was extremely limited.

Groundwater appeared as vulnerable as surface water; because superficial sources of pollution were being numerous (Deutsch, 2013). Literatures have shown that water was prompt to contamination regardless of the sources (Oludair and Aiyedun, 2016; Onyango *et al*., 2018). 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 (Onyango *et al*., 2018; WHO. 2018; WaterLife. 2018). Hamil and Bell, (1986) stated that pathogenic bacteria can survive long period underground and may have a life span of about 4 years. Reports from previous research works showed that the majority of borehole waters in Nigerian communities were microbiologically unsafe for drinking; which therefore placed the community at risk of waterborne diseases (Hati *et al*., 2011; Bello *et al*., 2013; Okoro *et al*., 2017). The most frequent bacteria associated with waterborne diseases were the enteric bacteria such as; Escherichia coli, Shigella spp. and Salmonella spp.; which according to WHO. (2018) have been associated with the estimated 80% of diseases affecting developing countries.

2.2 BOREHOLE WATER QUALITY

Globally safe drinking water is of concern because of its importance to humans and other life forms (Mukherjee, Sundberg, Sikdar and Schutt, 2022). It is now certain that poor water quality results in poor health and low productivity which invariably limits poverty alleviation and economic recovery in any region, as the cost implications of getting quality water is increasingly getting beyond the reach of an average Nigerian. There is water, water everywhere but no water to drink is a well known adage. People are not only dying for water, they are also dying of it (Duru, 2019; Yuri, Adriano and Lutiane, 2022).

High incidence of communicable diseases that reduce vitality and economic productivity are often linked to low access to safe drinking water and sanitation. Disease from unsafe water and lack of basic sanitation kill more people every year than all forms of violence, including war (World Health Organization (WHO), 2004). Children are the most vulnerable group affected as a result of their bodies not being matured and strong enough to resist attack due to diarrhea, dysentery and other water related ailments. The dangers of neglect to this important issue is causing death of over 530 children every day due to water borne diseases in Nigeria (Ochekpe, 2012). This is like three airplane loads of children crashing everyday in Nigeria. Further, a startling statistics by WHO (2012) and United Nations International Children Education Fund (UNICEF) (2012) indicate that about 1.4 million children die every year from diarrhea caused by unclean water and poor sanitation and about 4000 children die in a day which is equivalent to one child every 20 seconds. This is as a result of the inability of the masses to foot the cost of quality clean water.

This is a human tragedy attributable to an entirely preventable public health crisis that calls for global effort in terms of quality and quantity as well as making the cost of assessing clean water within the reach of everyone. All these facts apart, it is known that water has measurable constituents such as physico-chemical and bacteriological properties to varying degrees. The quantities and the levels of toxicity in the growing numbers of boreholes in Nigeria do not seem to be sufficiently known. This raises the issue of the quality of water from boreholes and their wholesomeness for human consumption. According to United Nations (2022) poor water quality constrains improvement in standard of living, food security and attainment of the Sustainable Development Goal (SDGs). In different parts of Nigeria, the situation is quite pathetic.

Effort by governments in the past decades to improve the water situation has not yielded the expected results. Government has neither spent time nor invested enough to alleviate this pathetic situation. The quality of most water sources are affected by frequent use of pit latrines in rural communities of Nigeria and some urban centers; without any consideration for factors such as soil type, topography and geology. Indiscriminate dumping of waste on land does not help matters either. Borehole systems are the fastest growing sources of potable water now in Nigeria (Ume and Chukwuemeka, 2019), and in other climes (Makonjo and Calford, 2022). According to Forester, (2000) over 400 million rural people in Sub – Saharan Africa are sustained by groundwater because it is probably the only realistic water supply option. However, some researchers have indicated that there are significant alterations in land use pattern and improper disposal of industrial effluents which are affecting groundwater, especially its quality (Makonjo and Calford, 2022). The scenario just described raises the question of whether the water from boreholes nationwide is comparable to WHO standard guidelines in terms of health implication of using the water for drinking and domestic purposes.

2.3 Physicochemical Quality of Borehole Water

Human beings like other animals require food, water, space and protection from diseases. The most urgent of all these is the need for water (Makonjo and Calford, 2022). Ehrlichs, (2014), in his work “Ethics of equilibrium” opined that one of the inalienable rights of man is the “right to drink pure (potable) water”. Humans need at least 2 liters of drinking water per day and this should be free of physical, chemical and biological impurities (Hanif *et al*., 2015). Our normal drinking water contains several minerals and salts like iron, manganese, nitrate, calcium, magnesium, sulphate, floride, as well as gasses such as carbon dioxide, oxygen and nitrogen; their proportions have to be carefully watched as indeed the possible content of bacteria (Newton, 2017).Water is essential to all forms of life and makes up to 50-95% of all plants and animals, and about 70% of human body (Buchholz, 2018). Water is also a vital resource for agriculture, manufacturing, transportation and many other human activities.

Agriculture is believed to be the largest user of the world’s freshwater resources, consuming 70% (Grafton and Hussey, 2011). Contamination of water sources leads to increased pH that affects mucous membranes, causes water to taste bitter and gives water a corrosive nature; increased dissolved oxygen, increases temperature of water and results in increased microbial activity (WHO, 2006). Nitrates can also soak into the ground and end up in drinking-water. All these can result into health problems that contribute to methemoglobinemia or blue baby syndrome disease which causes death in infants (WHO, 2016). In highly developed countries emphasis has shifted from concern over bacterial diseases to concern over water-borne diseases. Viral hepatitis for example has been found to occur more frequently in cities whose water supplies have comparatively high levels of water turbidity (Biswas and Sweetharam, 2018). Ionizing radiation from water polluted by water-soluble radioactive isotopes is capable of being concentrated in various tissues and organs as they pass through food chains and webs (Bartram *et al*; 2016).

In many cases, immediate environmental conditions are unfavourable; for instance, defecating in the nearby bushes, proximity of wells from latrines, the effects of oil exploration and exploitation in the zone coupled with the direct sewage – contamination of natural water bodies due to the cultural habit of defecating inside rivers, practiced in Niger Delta Regions of Nigeria have contributed greatly to water pollution (Wokem and Lawson-Jack, 2014).

2.4. Borehole Water Chemistry

The importance of groundwater quality has become increasingly recognized as the development of groundwater continues to expand in British Columbia. Monitoring of groundwater quality is becoming more important because of contamination concerns and development of new equipment and techniques for measuring contaminants in minute concentrations (Alonge, 2021). Although groundwater is generally less susceptible to contamination than surface waters it is usually more highly mineralized in its natural state. As water moves slowly through the ground it can remain for extended periods of time in contact with minerals present in the soil and bedrock and become saturated with dissolved solids from these minerals.

This dissolution process continues until chemical equilibrium is reached between the water and the minerals with which it is in contact (Adelegan, 2002). The types and relative concentrations of the chemical constituents in ground water provide information on the evolution of ground waters, age (residence time), and solubility, rates of movement, flow history and sources of recharge. Older ground waters, for example, are generally more mineralized than younger ground waters (Bello, 2009). Fresher ground waters are normally associated with recharge areas whereas ground waters in discharge areas are more mineralized. Groundwaters can be classified according to the most dominant percentage of cations and anions being present based on concentrations in equivalents per million [epm] (e.g. calcium-magnesium bicarbonate type) (Foster, 2015).

2.5 Mineral Constituents of borehole water

The greater part of the soluble constituents in groundwater comes from soluble minerals in soils and sedimentary rocks. The more common soluble constituents include calcium, sodium, bicarbonate and sulphate ions (Kelvin, 2020). Another common constituent is chloride ion derived from intruded sea water, connate water, evapotranspiration concentrating salts, and sewage wastes for example. Nitrate can be a natural constituent but high concentrations often suggest often cause pollution (WHO, 2014)

2.6 Quality Characteristics

The measure of Total Dissolved Solids (TDS) is a good indicator of the mineralized character of the water. Groundwater having less than 500 mg/L of total dissolved solids is generally satisfactory for domestic and industrial use while groundwater having greater than 1000 mg/L of total dissolved solids is generally unsatisfactory for these uses. High total dissolved solids are often indicative of other characteristics such as hardness (Okecha, 2000). Other properties that are especially useful in determining groundwater character are hardness, specific conductance and pH these constituents can be determined by simple procedures using field equipment (Dikka, 2011). In order to more precisely identify and measure the quality characteristics of groundwater, chemical, physical and biological analysis are usually required.

Chemical analysis requires the laboratory determination of the concentrations of common ions found in groundwater and is commonly reported in units of milligrams per liter (mg/L) (Longe, 2008). Concentrations may also be expressed as equivalents per million (epm) which is the moles of solute multiplied by the valence of the solute species in 10,000,000 g of water. Properties of groundwater often evaluated in a physical analysis include temperature, turbidity, odour, taste, and colour (Abattoir, 1988). The biological analysis includes a coliform bacteria test which indicates the sanitary quality of the water for human consumption. Additional parameters may be tested if a more detailed analysis is requested or where known or suspected sources of pollution exist. Some substances even in small concentrations can be troublesome (Ogboru, 2001). For example, iron concentrations of 1 to 5 mg/L in groundwater are common throughout British Columbia and can cause staining to plumbing fixtures and laundry, encrust well screens and clog pipes. Manganese in small concentrations can also cause staining and is even more objectionable as stains are harder to remove than those caused by iron (Okecha, 2000).

Chloride contamination is possible in wells located near the sea where pumping of these wells can move seawater into the freshwater aquifer making water not portable. Groundwater containing dissolved hydrogen sulphide gas is another common problem which imparts a characteristic "rotten egg" odour and taste to the water (Magaji, 2019). Hydrogen sulphide will combine with other impurities in the water to form iron sulphide (black water), calcium sulphide, and sodium sulphide. While TDS, specific conductance, hardness, and pH are good indicators of the character of groundwater, tritium (3H) and carbon 14 (14C) which are radioactive isotopes are good indicators of the age of groundwater. Between 1952 and 1962 large scale atmospheric testing of thermonuclear bombs were carried out and atmospheric contamination occurred. It is therefore apparent that groundwater from a location in the northern hemisphere, containing tritium at levels of hundreds or thousands of TU (tritium units), entered the groundwater zone after 1953 (Magaji, 2019). If the water has less than 5-10 TU it must have entered the groundwater zone prior to 1953. Two non-radioactive isotopes which occur in water are oxygen 18 (180) and deuterium (2H) and serve mainly as indicators of groundwater source areas and as evaporation indicators in surface water bodies (Osinbanjo, 2017).

2.7 Borehole water Pollution

Borehole water pollution (also called groundwater contamination) occurs when pollutants (effluents) are released to the ground and make their way down into groundwater. It can also occur naturally due to the presence of a minor and unwanted constituent, contaminant or impurity in the groundwater, in which in some cases it is more likely referred to as contamination rather than pollution. The pollutant creates a contaminant plume within an aquifer (Bello, 2019). Movement of water and dispersion within the aquifer spreads the pollutant over a wider area. Its advancing boundary, often called a plume edge, can intersect with groundwater wells into surface water such as seeps and spring, making the water supplies unsafe for humans and wildlife. The movement of the plume, called a plume front, may be analyzed through a hydrological transport model or groundwater models.

Analysis of groundwater pollution may focus on soil characteristics and site geology, hydrogeology, hydrology, and the nature of the contaminants (Kelvin, 2020). Pollution can occur from on-site sanitation systems, landfills, effluent from wastewater treatment plants, leaking sewers, petrol stations or from over application of fertilizers in agriculture. Pollution (or contamination) can also occur from naturally occurring contaminants, such as fluoride. Using polluted groundwater causes hazards to public health through poisoning or the spread of disease (Longe, 2018). Different mechanisms have an influence on the transport of pollutants, e.g. diffusion, absorption, precipitation, decay, in the groundwater. The interaction of groundwater contamination with surface waters is analyzed by use of hydrology transport models (Tebutt, 2023).

2.8 Types of water Pollutants

Contaminants found in groundwater cover a broad range of physical, inorganic chemical, organic chemical, bacteriological, and radioactive parameters. Principally, many of the same pollutants that play a role in surface water pollution may also be found in polluted groundwater, although their respective importance may differ (Makonjo and Calford, 2022). Pathogen Waterborne diseases can be spread via a groundwater well which is contaminated with faecal pathogens from pit latrines Pathogens contained in human or animal faeces can lead to groundwater pollution when they are given the opportunity to reach the groundwater, making it unsafe for drinking of the four pathogen types that are present in faeces (bacteria, viruses, protozoa, and helminths or helminth eggs), the first three can be commonly found in polluted groundwater, whereas the relatively large helminth eggs are usually filtered out by the soil matrix (UNESCO, 2006). Groundwater that is contaminated with pathogens can lead to fatal focal-oral transmission of diseases (e.g. cholera, diarrhoea). If the local hydrogeological conditions (which can vary within a space of a few square kilometres) are ignored, pit latrines can cause significant public health risks via contaminated groundwater (Robert, 2015).

2.9 Nitrate pollutants of borehole

, there is also the issue of nitrate pollution in groundwater from pit latrines, which has led to numerous cases of "blue baby syndromes" in children, notably in rural countries such as Romania and Bulgaria. Nitrate levels above 10 mg/L (10 ppm) in groundwater can cause "blue baby syndrome" (acquired methemoglobinemia) (Maduka, 2015). Nitrate can also enter the groundwater via excessive use of fertilizers, including manure. This is because only a fraction of the nitrogen-based fertilizers is converted to produce and other plant matter. The remainder accumulates in the soil or lost as run-off. High application rates of nitrogen-containing fertilizers combined with the high water-solubility of nitrate leads to increased runoff into surface water as well as leaching into groundwater, thereby causing groundwater pollution (Magaji, 2019). The excessive use of nitrogen-containing fertilizers (be they synthetic or natural) is particularly damaging, as much of the nitrogen that is not taken up by plants is transformed into nitrate which is easily leached. The nutrients, especially nitrates, in fertilizers can cause problems for natural habitats and for human health if they are washed off soil into watercourses or leached through soil into groundwater (Magaji, 2019).

2.9.1 Volatile organic compounds

Volatile organic compounds (VOCs) are a dangerous contaminant of groundwater. They are generally introduced to the environment through careless industrial practices. Many of these compounds were not known to be harmful until the late 1960s and it was some time before regular testing of groundwater identified these substances in drinking water sources (Akoteyon 2019).

2.10 Bacteria Contamination of Borehole

Foodborne diseases are considered to occur frequently and are usually associated with developing countries due to improper food handling, unhygienic practices, inadequate food safety legislation, weak regulatory systems, lack of financial resources for safety equipment and lack of awareness and/or training for butchers and other food handlers (Goja *et al*., 2013; Haileselassie *et al*., 2013). The US Centers for Disease Control and Prevention (CDC) also reported annual outbreaks of foodborne diseases from food of animal origin estimated at 76 million infections, 325,000 hospitalizations and 5,000 deaths each year in Nigeria (Aluko *et al*., 2014). The increase in the prevalence of communicable and zoonotic diseases such as tuberculosis, cysticercosis and trichinosis in communities is a further sign of the importance of abattoirs as disease surveillance points (Alton *et al*., 2015).

2.10.1 Total coliform bacteria

Total coliform bacteria are defined as aerobic or facultative anaerobic, Gram negative, non-spore forming, rod shaped bacteria, which ferments lactose and produce gas at 35°C (Standard Methods, 1995). Total coliforms include bacteria of known faecal origin such as *E. coli* as well as bacteria that may not be of faecal origin such as *Klebsiella* spp, *Citrobacter* spp, *Serratia* spp and *Enterobacter* spp which are found in nutrient rich water, soil decaying vegetation and drinking water with relatively high levels of nutrients (Ramteke *et al*., 2012; WHO, 2016a). The recommended test for the enumeration of total coliforms is membrane filtration using mEndo agar and incubation at 35°C to 37°C for 24 h to produce colonies with goldengreen metallic shine. In water quality studies, total coliform bacteria are used as a systems indicator, which provides information on the efficiency of water treatment (Makonjo and Calford, 2022).

The presence of total coliform in water samples are therefore, an indication that opportunistic pathogenic bacteria such as *Klebsiella* and *Enterobacter* which can multiply in water environments and pathogenic pathogens such as *Salmonella* spp, *Shigella* spp, *V. cholera, Campylobacter jejuni, Campylobacter coli, Yersinia enterocolitica* and pathogenic *E. coli* may be present. These pathogens and opportunistic microorganisms could cause diseases such as gastroenteritis, dysentery, cholera, typhoid fever and salmonellosis to consumers. In particular, individuals who suffer from HIV/AIDS related complications are more at risk of being infected by these microorganisms (Makonjo and Calford, 2022).

2.10.2 Faecal coliform bacteria

Faecal coliform bacteria are Gram negative bacteria, also known as thermotolerant coliforms or presumptive *E. coli*. The faecal coliform group includes other organisms, such as *Klebsiella* spp, *Enterobacter* spp and *Citrobacter* spp, which are not exclusively of faecal origin. *Escherichia coli* are specifically of faecal origin from birds, humans and other warm blooded animals (WHO, 1996a; Maier *et al*., 2000). Faecal coliform bacteria are therefore considered to be a more specific indicator of the presence of faeces (Maier *et al*., 2000). The recommended test for the enumeration of faecal coliforms is membrane filtration using mFC agar and incubation at 44.5°C for 24 h to produce blue colored colonies (Makonjo and Calford, 2022).

Faecal coliforms are generally used to indicate unacceptable microbial water quality and could be used as an indicator in the place of *E. coli* (SABS, 2001). The presence of faecal coliforms in a water sample indicates the possible presence of other pathogenic bacteria such as *Salmonella* spp, *Shigella* spp, pathogenic *E. coli*, *V. cholera*, *Klebsiella spp* and *Campylobacter* spp associated with waterborne disease. Unfortunately faecal coliform bacteria exhibit species to species variations in their respective stability and resistance to disinfection processes; do not distinguish between faeces of human and animals origin; have low survival rates and have been detected in water sources thought to be free of faecal pollution (Akoteyon 2019).

Escherichia coli bacteria Globally *E. coli* is used as the preferred indicator of faecal pollution (Edberg *et al*., 2000). It is a Gram negative bacterium and predominantly an inhabitant of the intestines of warm blooded animals and humans, which is used to indicate recent faecal pollution of water samples (Akoteyon 2019). Confirmation tests for *E. coli* include testing for the presence of the enzyme β-glucuronidase, Gram staining, absence of urease activity, production of acid and gas from lactose and indole production (Rice *et al*., 2021). Commercially available growth media containing the fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronidase (MUG) is used for the isolation and identification of *E. coli* from water samples (Shadix and Rice, 2021; Covert *et al*., 2022). The *E. coli* bacteria hydrolyse the MUG in the media, which then fluoresces under ultraviolet light (Shadix and Rice, 2021; Covert *et al*., 2022). However, false negative results on this media have been found due to injured cells, lack of expression of the gene which codes for the enzyme β-glucuronidase by the *E. coli* bacterium isolate, and non-utilization of the MUG reagent in the media by some *E. coli* strains (Akoteyon 2019).

2.10.3 Faecal enterococci bacteria

Faecal enterococci bacteria are found in the genus Enterococcus and include species like *Enterococcus faecalis, Enterococcus faecium, Enterococcus durans* and *Enterococcus hirae* (Standard Methods, 1995; WHO, 1996a). The genus *Enterococcus* are differentiated from the genus *Streptococcus* by their ability to grow in 6.5% sodium chloride, pH 9.6, temperatures of 45ºC and their tolerance for adverse growth conditions (Maier *et al*., 2000). Faecal enterococci are spherical, Gram positive bacteria, which are highly specific for human and animal faecal pollution (Standard Methods, 1995). Most of the species in the Enterococcus genus are of faecal origin and is regarded as specific indicators of human faecal pollution, although some species are found in the faeces of animals and plant material (Akoteyon 2019)

2.11 Water Pollution and Child Health

Diarrhea is a common disease in children. Diarrhoeal diseases (including cholera) kill 1.8 million people each year, 90 per cent of them children under the age of five, mostly in developing countries. 88% of diarrhoeal diseases are caused by inadequate water supply, sanitation and hygiene (Team, 2004). A large proportion of these are caused by exposure to microbially infected water and food, and diarrhea in infants and young children can lead to malnutrition and reduced immune resistance, thereby increasing the likelihood of prolonged and recurrent diarrhea (Marino, 2007). Pollution exposure experienced by children during critical periods of development is associated with height loss in adulthood (Zaveri *et al*., 2020). Diseases directly related to water and sanitation, combined with malnutrition, also lead to other causes of death, such as measles and pneumonia. Child malnutrition and stunting due to inadequate water and sanitation will continue to affect more than one-third of children in the world (Bartlett, 2013).

A study from rural India showed that children living in households with tap water had significantly lower disease prevalence and duration (Jalan and Ravallion, 2013). In conclusion, water pollution is a significant cause of childhood diseases. Air, water, and soil pollution together killed 940,000 children worldwide in 2016, two-thirds of whom were under the age of 5, and the vast majority occurred in low- and middle-income countries (Landrigan *et al*., 2018). The intensity of industrial organic water pollution is positively correlated with infant mortality and child mortality in less developed countries, and industrial water pollution is an important cause of infant and child mortality in less developed countries (Jorgenson, 2009). In addition, arsenic in drinking water is a potential carcinogenic risk in children (García-Rico *et al*., 2018). Nitrate contamination in drinking water may cause goiter in children (Vladeva *et al*.., 2000).

2.12 Multiple antibiotic-resistant isolates in borehole water

Seventy percent of the Earth's surface consists of water while the remaining is land which contains only 2% potable water (Lim *et al*. 1999). A major problem facing humanity, especially in an underdeveloped nation, is accessibility to adequate and quality water. With the increase in population in most towns and cities and the corresponding increase in demand for social amenities, it has become very challenging to me*et al*l the water requirements in terms of quantity, quality, and constancy. Nigeria is faced with many challenges in the drinking-water subsector (Akoteyon 2019). The public water supply in Nigeria is mostly non-existent and where available it is inaccessible, the supply is intermittent and unreliable and thus it has become increasingly difficult to me*et al*l the water requirements (Abubakar 2018); this has forced many households to resort to unwholesome water sources that are not potable, resulting in many digging personal boreholes or wells (Balogun *et al*. 2017).

Apart from the rapid population growth and urbanization, rising demand and falling supplies due to overexploitation and anthropogenic impacts remain some of the major challenges in the public water sector. In addition, low budget and poor investment in water infrastructure, poor policy implementation and lack of political will also contribute to the current low access to safe water supply in the country. The provision of potable water supply and management is one of the vital human needs for healthy living according to the sixth Sustainable Development Goal (SDG), which is geared towards ensuring the availability and sustainable management of water and sanitation for all. Therefore, it is expected that paying adequate attention to urban water supply should be prioritized in urban planning (Akoteyon 2019).

The failure of the government in providing safe drinking water led to people sourcing potable water by themselves by digging wells for household use. Ishaku *et al*. (2011) noted that the majority of the rural communities in Nigeria lack access to improved water supply. Generally, they rely on free water supply sources such as rivers, perennial streams, ponds and unprotected wells, which are susceptible to water-borne diseases. Pollution of groundwater is one of the major environmental challenges arising from improper and indiscriminate disposal of sewage, industrial and chemical waste. Findings from several studies are that groundwater is highly contaminated and clinically unsafe for human consumption (Mile *et al*. 2012). It has been reported that groundwater is easily contaminated by rainstorm overflows, runoff from farming areas and areas with septic systems and latrines that are improperly situated (Sparks 2005). Pathogens such as *Salmonella*, *Escherichia*, *Shigella*, *Vibrio* and *Campylobacter* have been identified in poorly treated water. However, a wide variety of opportunistic pathogens, such as *Aeromonas*, *Pseudomonas* and coliforms, are commonly found (Borchardt *et al*. 2003). Diarrhoeal infections are still a leading child-killer disease worldwide (Walker *et al*. 2013).

A great number of different species of bacteria have been isolated from water and many are potential causes of different types of diseases in human beings. The frequent presence of *Aeromonas* in drinking water raised the question of its role as an enteric pathogen because the production of enterotoxins and/or adhesins had been demonstrated. Bacillus spp. are often detected in drinking-water supplies, even supplies treated and disinfected by acceptable procedures. This is largely due to the resistance of spores to disinfection processes (Bartram *et al*. 2003). *Enterobacter sakazakii* is sensitive to disinfectants, and its presence in water can be prevented by adequate treatment (WHO/FAO 2004). *Klebsiella* spp. are natural inhabitants of many water environments. In drinking water distribution systems, the organisms can grow and colonize the taps. *Klebsiella* spp. are also excreted in the faeces of many healthy humans and animals, and they are readily detected in sewage-polluted water (Ainsworth 2004). The presence of *Shigella* spp. in drinking-water indicates recent human faecal pollution. Control measures that can be applied to manage potential risks include the protection of raw water supplies from human waste, adequate treatment, and protection of water during distribution. *Escherichia coli* (or, alternatively, thermotolerant coliforms) is a generally a reliable indicator for *Shigella* spp. in drinking-water supplies (Maleki *et al*., 2021).

The presence of the pathogenic *V. cholerae* O1 and O139 serotypes in drinking water is of major public health importance and can have serious health and economic implications in the affected communities (WHO 2002). *Salmonella* may be associated with all kinds of food and water. The incidence of typhoid fever decreases when the level of development of a country increases (i.e., controlled water sewage systems, pasteurization of milk and dairy products). Where these hygienic conditions are missing, the probability of faecal contamination of water and food remains high and so does the incidence of typhoid fever (Popoff and Le Minor 2005). *Pseudomonas. aeruginosa* is predominantly an environmental organism, and fresh surface water is an ideal reservoir. Pseudomonas aeruginosa is the most significant example of bacteria capable of multiplying in water, in contrast to most enterobacteria. This bacterium is frequently isolated from surface water and is also a major concern in mineral water bottling plants, because it is an opportunistic pathogen and can contaminate boreholes and bottling plants (Maleki *et al*., 2021).

Antibiotic resistance is one of the biggest threats to global health, food security, and development today. Antibiotic resistance can affect anyone, of any age, and in any country. Antibiotic resistance occurs naturally, but misuse of antibiotics in humans and animals is accelerating the process. A growing number of infections – such as pneumonia, tuberculosis, gonorrhoea, and salmonellosis – are becoming harder to treat as the antibiotics used to treat them have become less effective. Antibiotic resistance leads to longer hospital stays, higher medical costs and increased mortality (WHO 2018).

2.13 Classification of Waterborne Diseases

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. 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 (Abubakar 2018). Basically, waterborne diseases can be transmitted through four main routes: Waterborne route, Water-washed route, Water-based route and Insect vector route or water related route.

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 diarrhoea, which involves excessive stooling, often resulting to dehydration and possibly death. According to the World Health Organization, diarrheal disease 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. Further estimates suggest that 88% of that burden is attributable to unsafe water supply, sanitation and hygiene and is mostly concentrated on children in developing countries (Maleki *et al*., 2021). Most waterborne diseases are often transmitted via the fecal-oral route, and this occurs when human faecal material is ingested through drinking contaminated water or eating contaminated food which mainly arises from poor sewage management and improper sanitation. Faecal pollution of drinking-water may be sporadic and the degree of faecal contamination maybe low or fluctuate widely.

2.13.1 Water Pollution and Skin Diseases

Contrary to common sense that swimming is good for health, studies as early as the 1950s found that the overall disease incidence in the swimming group was significantly higher than that in the non-swimming group. The survey shows that the incidence of the disease in people under the age of 10 is about 100% higher than that of people over 10 years old. Skin diseases account for a certain proportion (Ansari and Akhmatov, 2020). A prospective epidemiological study of beach water pollution was conducted in Hong Kong in the summer of 1986–1987. The study found that swimmers on Hong Kong’s coastal beaches were more likely than non-swimmers to complain of systemic ailments such as skin and eyes. And swimming in more polluted beach waters has a much higher risk of contracting skin diseases and other diseases. Swimming-related disease symptom rates correlated with beach cleanliness (Ansari and Akhmatov, 2020).

A study of arsenic-affected villages in the southern Sindh province of Pakistan emphasized that skin diseases were caused by excessive water quality. By studying the relationship between excessive arsenic in drinking water caused by water pollution and skin diseases (mainly melanosis and keratosis), it was found that compared with people who consumed urban low-arsenic drinking water, the hair of people who consumed high-arsenic drinking water arsenic concentration increased significantly. The level of arsenic in drinking water directly affects the health of local residents, and skin disease is the most common clinical complication of arsenic poisoning. There is a correlation between arsenic concentrations in biological samples (hair and blood) from patients with skin diseases and intake of arsenic contaminated drinking water (Kazi *et al*., 2009). Another Bangladesh study showed that many people suffer from scabies due to river pollution (Hanif *et al*., 2020). Not only that, but water pollution from industry can also cause skin cancer (Arif *et al*., 2020). Studies using meta-analysis have shown that exposure to polluted Marine recreational waters can have adverse consequences, including frequent skin discomfort (such as rash or itching). Skin diseases in swimmers may be caused by a variety of pathogenic microorganisms (Yau *et al*., 2009). People (swimmers and non-swimmers) exposed to waters above threshold levels of bacteria had a higher relative risk of developing skin disease, and levels of bacteria in seawater were highly correlated with skin symptoms. Studies have also suggested that swimmers are 3.5 times more likely to report skin diseases than non-swimmers. This difference may be a "risk perception bias" at work on swimmers, who are generally aware that such exposure may lead to health effects and are more likely to detect and report skin disorders. It is also possible that swimmers exaggerated their symptoms, reporting conditions that others would not classify as true skin disorders (Fleisher and Kay. 2006).

2.13.2 Water Pollution and Cancer

According to WHO statistics, the number of cancer patients diagnosed in 2020 reached 19.3 million, while the number of deaths from cancer increased to 10 million. Currently, one-fifth of all global fevers will develop cancer during their lifetime. The types and amounts of carcinogens present in drinking water will vary depending on where they enter: contamination of the water source, water treatment processes, or when the water is delivered to users (Morris, 1995). From the perspective of water sources, arsenic, nitrate, chromium, etc. are highly associated with cancer. Ingestion of arsenic from drinking water can cause skin cancer and kidney and bladder cancer (Marmot *et al*., 2007). The risk of cancer in the population from arsenic in the United States water supply may be comparable to the risk from tobacco smoke and radon in the home environment. However, individual susceptibility to the carcinogenic effects of arsenic varies (Smith *et al*., 1992). A high association of arsenic in drinking water with lung cancer was demonstrated in a northern Chilean controlled study involving patients diagnosed with lung cancer and a frequency-matched hospital between 1994 and 1996. Studies have also shown a synergistic effect of smoking and arsenic intake in drinking water in causing lung cancer (Ferreccio *et al*., 2000). Exposure to high arsenic levels in drinking water was also associated with the development of liver cancer, but this effect was not significant at exposure levels below 0.64 mg/L (Lin *et al*., 2013). Nitrates are a broader contaminant that is more closely associated with human cancers, especially colorectal cancer. A study in East Azerbaijan confirmed a significant association between colorectal cancer and nitrate in men, but not in women (Maleki *et al*., 2021). The carcinogenic risk of nitrates is concentration-dependent. The risk increases significantly when drinking water levels exceed 3.87

mg/L, well below the current drinking water standard of 50 mg/L. Drinking water with nitrate concentrations lower than current drinking water standards also increases the risk of colorectal cancer (Schullehner *et al*., 2018).

2.13.3 Water Pollution and Diarrhea

Diarrhea is a common symptom of gastrointestinal diseases and the most common disease caused by water pollution. Diarrhea is a leading cause of illness and death in young children in low-income countries. Diarrhoeal diseases account for 21% of annual deaths among children under 5 years of age in developing countries (Waddington *et al*., 2009). Many infectious agents associated with diarrhea are directly related to contaminated water (Ahmed and Ismail, 2018). Parasitic worms present in non-purifying drinking water when is consumed by human beings causes diseases (Ansari and Akhmatov, 2020). It was found that treated water from water treatment facilities was associated with a lower risk of diarrhea than untreated water for all ages (Clasen *et al*., 2015). For example, in the southern region of Brazil, a study found that factors significantly associated with an increased risk of mortality from diarrhoea included lack of plumbed water, lack of flush toilets, poor housing conditions, and overcrowded households. Households without access to piped water had a 4.8 times higher risk of infant death from diarrhea than households with access to piped water (Victora *et al*., 2018) Enteroviruses exist in the aquatic environment. More than 100 pathogenic viruses are excreted in human and animal excreta and spread in the environment through groundwater, estuarine water, seawater, rivers, sewage treatment plants, insufficiently treated water, drinking water, and private wells (Fong and Lipp., 2005). A study in Pakistan showed that coliform contamination was found in some water sources. Improper disposal of sewage and solid waste, excessive use of pesticides and fertilizers, and deteriorating pipeline networks are the main causes of drinking water pollution. The main source of water-borne diseases such as gastroenteritis, dysentery, diarrhea, and viral hepatitis in this area is the water pollution of coliform bacteria (Khan *et al*., 2013).

3.0 CHAPTER THREE

MATERIALS AND METHODS

3.1 SAMPLE COLLECTION

Samples were collected from University of Benin hostels using sterile pet bottles and were transported aseptically to the microbiology laboratory for microbial evaluation and assessment.

3.2 Preparation and Sterilization of Culture Media

All culture media were prepared according to the manufacturer's instructions. Sterilization was at 121ºC at 15psi for 15 min unless otherwise stated by manufacturer.

Media Preparation

Media for microbiological analysis was weighed according to the manufacturer's specifications.

3.2.1 Nutrient agar

Twenty-eight grams (28 g) of nutrient agar was dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium was placed in an autoclave to sterilize it for 15 minutes at 121 °C. After sterilization, the flask was allowed to cool.

3.2.2 Potato Dextrose Agar (PDA)

PDA medium (39g) was dissolved in 1000ml of distilled water in a conical flask then closed with a cork stopper. The suspension was first dissolve completely by shaking and then sterilized by autoclaving at 121°c for 15 minutes. The medium was allowed to cool then dispensed aseptically into sterile petri dishes. The petri dishes was covered and allowed to solidify (Needam *et al*., 2005).

3.4 Fungal enumeration

Potato Dextrose Agar (PDA) with chloramphenicol (500mg/l) was prepared following the manufacturer's instructions and sterilized by autoclaving at 121^{o} C for 15 minutes, and allowed to cool. After cooling, prepared the medium was dispensed into petri-dishes and samples were inoculated onto the petridishes containing the prepared medium and incubated at room temperature for 5-7 days. After incubation, fungal colonies were purified by sub culturing onto freshly prepared PDA plates and incubated at room temperature for 5-7 days. Identification of pure fungal isolates was carried out by observing cultural characteristeristics such as pattern of growth and pigment production

3.5 cultural characteristics of Fungi from Samples

Following successful pour plate technique, isolation and culture will be made from a single colony and characterized using cultural features which include shape, size, colour of spores, reverse colour of fungus, texture of fungus (Needam *et al*., 2005)

3.6 Morphological identification of fungi

A drop of mounting fluid, lacto phenol cotton blue solution was placed on a grease free slide. Scrapings of the pure isolates were taken from the Potato Dextrose Agar (PDA) and transferred on the fluid using a sterilized, cooled wire needle. It was then pressed gently to enable it mix properly with the stain. A sterile forcep was used to place a cover slip over the slides and blotting paper was also used to wipe excess stain and then examined under low magnification (x10) and high magnification (x40) objectives (Akinola *et al*., 2011).

3.7 Enumeration and isolation of total heterotrophic bacterial count

Appropriate media was used for fungal and bacterial enumeration. Tryptone soy agar (supplemented with fluconazole) for bacteria Plates were cultured at 28±2ºC for 24 hours. The number of colony forming unit per milliliter (cfu/ml) was calculated using the formula below:

$$\frac{cfu}{ml} = \frac{number\ of\ colonies\ x\ dilution\ fold/series}{volume\ of\ inoculum}$$

(Willey *et al.*, 2008)

3.8 Phenotypic Identification of Bacteria from Samples

Following successful pour plate technique, isolation and culture was made from a single colony and characterized using cultural, morphological and biochemical methods using the Bergey's manual. Several tests such as Gram reaction, catalase, urease, indole, oxidase, sugar fermentation, citrate utilization, respective reaction on triple sugar iron agar tests were carried out to presumptively identify bacterial isolates (Holt *et al*., 1994).

3.9 Morphology identification

The morphological identity of each bacteria isolate was obtained by Gram staining so as to know the gram reaction, cell morphology and arrangement by viewing under the microscope. The Gram stain procedure is as follows:

A smear of the bacteria isolate was made on grease free slide and heat fix by passing over flame. The smear was flooded with crystal violet which is the primary stain for 1min then washed with distilled water.

Subsequently the slides was flooded with Lugol's iodine solution for 30sec and then washed off with distilled water.

95% alcohol was used for decolorization for 10sec and immediately washed off with distilled water.

Finally, the smear was counter stained with saffranin for 1min and washed off.

The slides were allowed to air dry before observing under the microscope using an oil immersion objective lens of ×100 magnifications to view the slides.

3.10 Biochemical identification

Biochemical test was carried out so as to help in the identification of the bacteria isolates as phenotypic (cultural) characteristics is not sufficient. The various biochemical test carried out are shown below;

3.10.1 Oxidase test

This is mainly used to differentiate between *pseudomonas* from other gram-negative rod bacteria. Oxidase test was carried out to identify bacteria species that will produce cytochtome oxidase enzyme. *Staphylococcus aureus* and *Escherichia coli* which are gram positive and gram negative respectively were employed as control. A piece of filter paper using sterilizes wire loop 2-3 drops of freshly prepared oxidase reagent (1% aqueous tetramethyl-3-phenyl nediamine dichloride) was added. A positive oxidase test is indicated by purple colouration within 10 seconds.

3.10.2 Urease test.

This is used to test organisms that have the abilities to produce the enzyme urease which catalyzes the breakdown of urea to produce ammonia. The test is usually used to differentiate organisms like *Proteus mirabilis* from other non-urease positive organism. A sterilized medium was dispensed into test tubes aseptically and the test bacteria isolated were inoculated into the medium and incubated at 37 degree centigrade for 24 hours. A change in colour from yellow to red-pink confirmed the presence of urease.

3.10.3 Indole production test

This test was used to determine which of the isolates has the ability to split indole from tryptophan present in peptone water. The best is usually used in differentiating gram-negative bacilli especially those of enterobacteriaceae. Five grams of commercially available peptone broth was dissolved in 1litre of distilled water. The medium was then sterilized by autoclaving at 121 degree centigrade for 15 minutes. The 4 ml of the medium was dispensed into sterile test tube and each of the bacteria isolates was inoculated into the peptone broth. The inoculated media was incubated 37 degree centigrade for 24 hours after which few drops of KOVAC reagent will be added. KOVAC reagents consist of 150ml of amylalcohol, 10g dimethylamino benzaldehyde and 150ml of concentrated hydrochloric acid. Positive test was indicated by the red colouration that occurs immediately at the upper part of the test tube.

3.10.4 Citrate utilization test

This test is used to identify which of the isolate can utilize citrate as the sole source of carbon for metabolism. The medium used for this test is simon`s citrate agar. In the preparation, 22 grams of commercially available simon`s citrate agar was dissolved in litre of distilled water and sterilized by autoclaving at 121 degree centigrade for 15 minutes. The medium is dispensed into test tubes and the test organism was inoculated by stabling the medium on the tubes using sterile straight inoculation wire containing culture. The tubes were incubated at 37 degree centigrade for about 24 hours. Positive result is indicated by a change in colour from green to bright blue colouration.

3.10.5 Catalase test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdowns of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive.

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

3.10.6 Sugar fermentation and production of gases using Triple sugar iron agar (TSI)

TSI was prepared following manufacturer's instruction and the prepared media was placed in a test tube and kept in a slant position for it to solidify. The slant and butt of the medium was inoculated with the test bacterium using a sterile loop and it was incubated for 18- 24 hr. The results were read on the basis of acid or alkaline production in the slant or butt region of the tube and gas production was confirmed by the presentence of crack or air bubbles in the slant or but region. More so, production of hydrogen sulphide was confirmed by the blackening of the medium. A prepared laboratory chat was used for result interpretation in line with microbiological standard protocol as well as other biochemical tests carried out on the isolates to confirm or ascertain their identity.

3.11 Statistical analysis

The data were analysed using the SPSS package version 21.0. All data are mean of three replicates. The mean, range and standard deviation of each parameter was determined.

4.0 CHAPTER FOUR

RESULTS

Figure 1, 2 and 3 represent the total heterotrophic bacterial counts, coliform count and fungal counts. The total heterotrophic bacterial count ranged from 4.46 $\log_{10}$ cfu/ml to 4.86 $\log_{10}$ cfu/ml, coliform ranges from 2.84 to 3.13 $\log_{10}$ cfu/ml and total fungal count ranged from 4.40 to 4.85 $\log_{10}$ cfu/ml. respectively.

Figure 4 represent the percentage occurrence of bacterial isolates obtained from stored water across the hostels: From the result obtained, four isolates were in the same percentage of 20% which was the highest. These isolates were *citrobacter sp*, *Enterobacter sp*, *Escherichia coli*, and *staphylococcus* sp.

Table 1 and 2 represents the cultural and morphological characterization of bacteria and fungal isolates respectively.

Table 3 shows phenotypic virulence test. To check for pathogenicity of the isolated bacterial, phynotypic virulence test was conducted for isolates obtained from the hostel water. This result shows the ability of some isolates to degrading DNase, liquefying gelatinase and also their reactions to hemolysin test.

Table 4: Antibiotic sensitivity test: There was a need to check if the isolates were multiple antibiotic resistant isolates or not and also to confirm which antibiotic was suitable to inhibit the growth of the isolated pathogens. To obtained this, antibiotic sensitivity test was conducted test conducted.

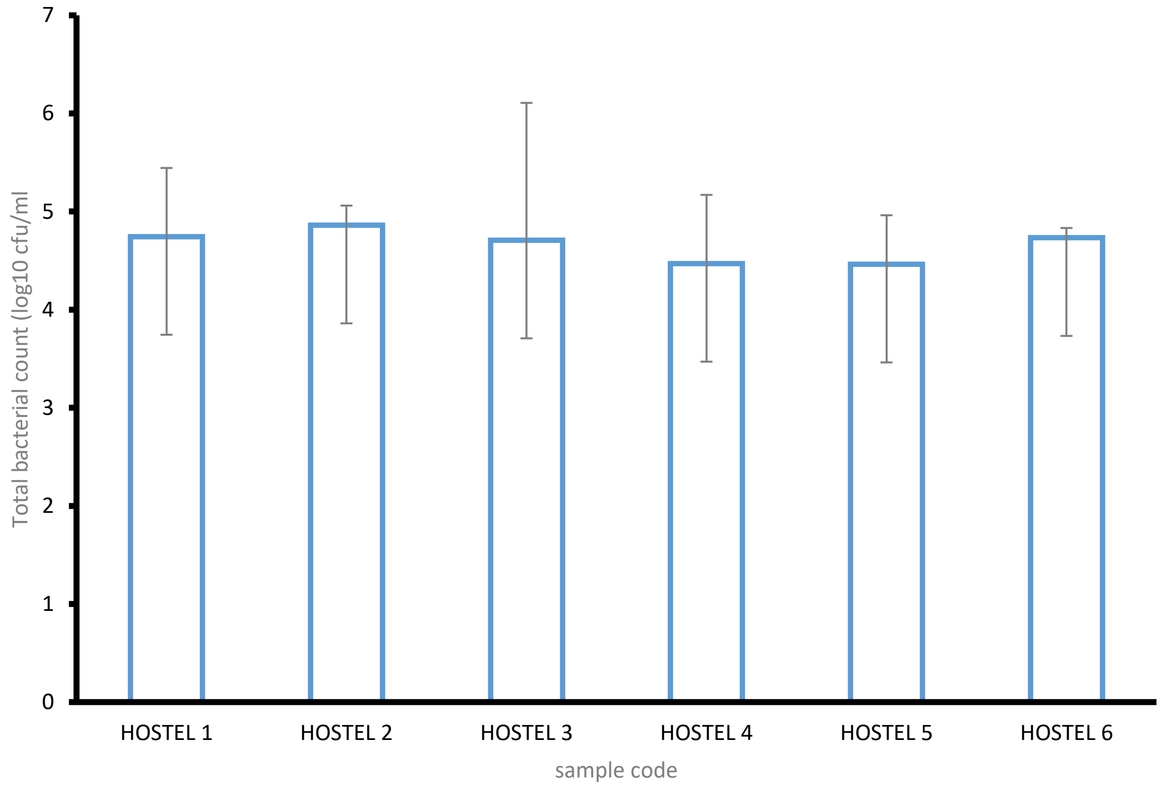

Figure 1: Total bacterial count (log_{10} cfu/ml)

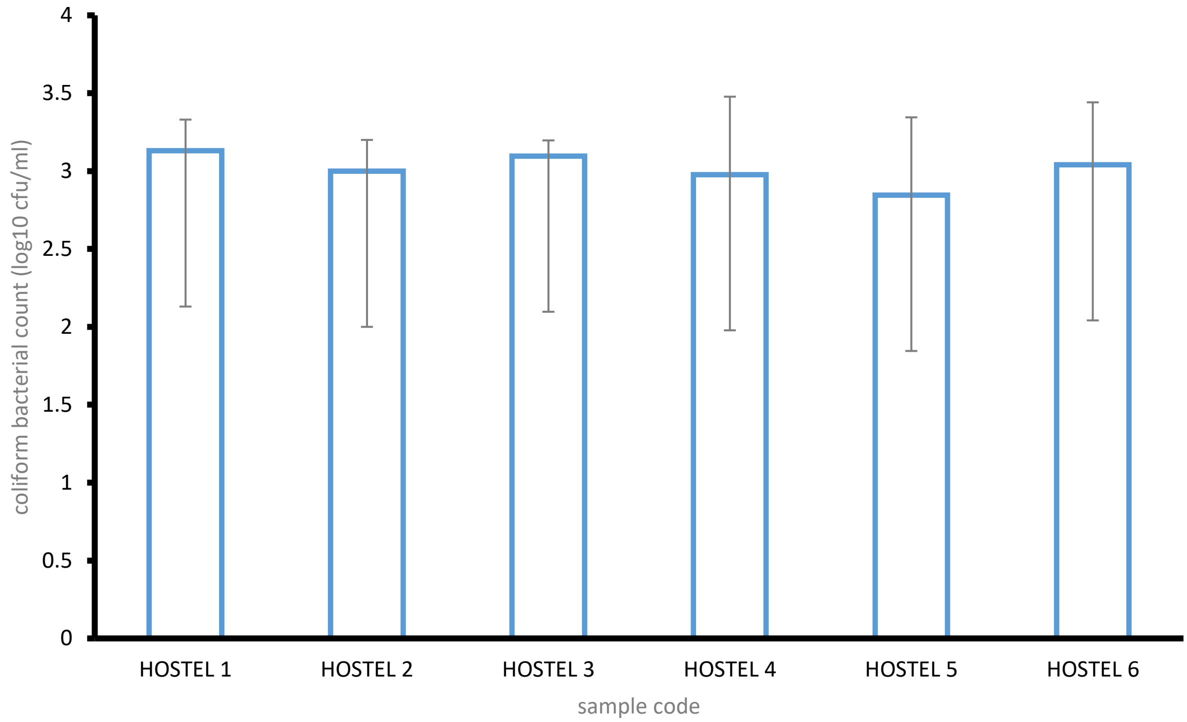

Figure 2: coliform bacterial counts

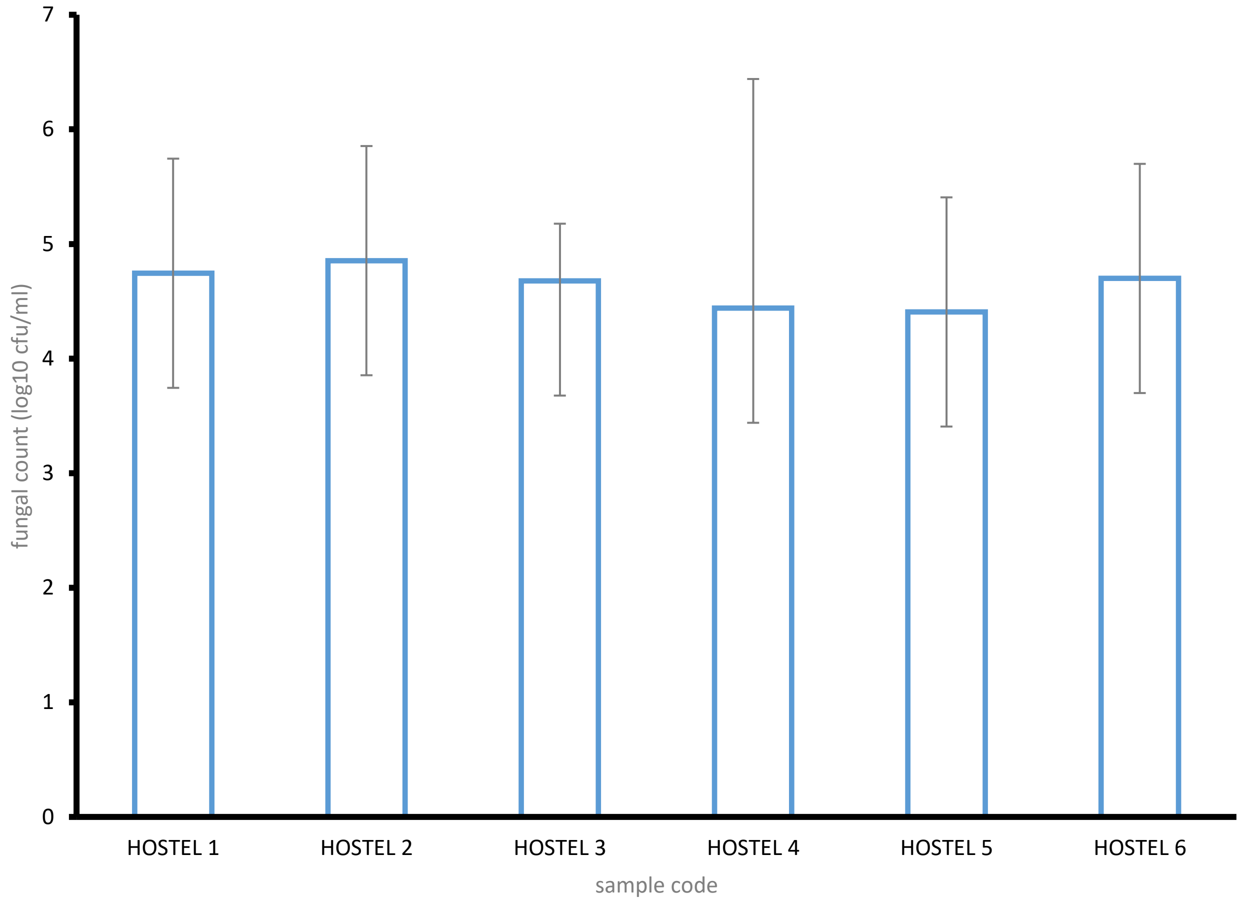

Figure 3: fungal count (log_{10} cfu/ml)

Table 1: cultural, morphological and biochemical characteristics of isolates

Shape	Circular	Circular	Circular	Circular	Circular	Circular
Elevation	Convex	Convex	Convex	Convex	Convex	Convex
Margin	Entire	Entire	Entire	Entire	Entire	Entire
Size	Small	Small	Small	Small	Small	Small
Morphological characteristics						
KOH	+	+	-	-	+	+
Gram stain	-	-	+	+	-	-
Cell morphology	Rod	Rod	Cocci	Rod	Rod	Rod
Cell arrangement	Single	Single	Clusters	Single	Chains	Clusters
Biochemical characteristics						
Catalase	+	+	+	+	+	+
Coagulase	-	-	+	-	-	-
Indole	+	-	-	-	-	-
Oxidase	-	-	-	-	-	-
Citrate	-	+	+	+	+	+
Urease	-	+	+	-	+	-
H_2S	-	-	-	-	+	-
Glucose	+	+	+	+	+	+
Lactose	+	+	+	-	+	-
Sucrose	+	+	+	+	+	+
Gr. Diff.	Green metallic		Yellow	Straw		Cream
	Sheen (EMB)		(MSA)	(BCA)		
Identity	*E. coli*	*Klebsiella* sp	S. *aureus*	*Bacillus* sp.	*Citrobacter* sp	*Enterobacter cloacae*

Kys: :+ = positive, - = negative

Table 2: Cultural and morphological characteristics of fungi isolates

Microscopic characteristics				
Nature of hyphae	Septate	Septate	Septate	Non- septate
Type of Spore	May produce rectangular arthroconidia, chlamydospores or may not spoulate	Conidiospore	Conidiospore	Sporangiophores
Spore structure/Attachment	May produce clamp connections (inset)	There are no distinct conidiophores. Conidia are colorless, generally ellipsoidal in shape but are very variable in size and shape.	Conidiogenous cells are cylindrical and give rise to annelides, producing slimy heads of one-celled oval to globose conidia	sporangiospores
Rhizoids	Absent	Absent	Absent	Absent
Appearance of special structure	Hyphae are colorless and septate, poorly branched.	Conidia are produced synchronously in dense groups on conidiogenous cells. There are no distinct conidiophores. Conidia are colorless, generally ellipsoidal in shape but are very variable in size and shape	Conidia, which become thick-walled and brown after liberation, Grows at 40C but not at 45C	sporangia are produced on the tips of sporangiophores. The sporangia contain spores, which are the reproductive units of Mucor
Class of fungi	Basidiomycetes	Ascomycetes	Ascomycete	Zygomycetes
Possible Identity	***Bjerkandera adusta***	***Aureobasidium pullulans***	***Scedosporium apiospermum***	*Mucor mucedo*

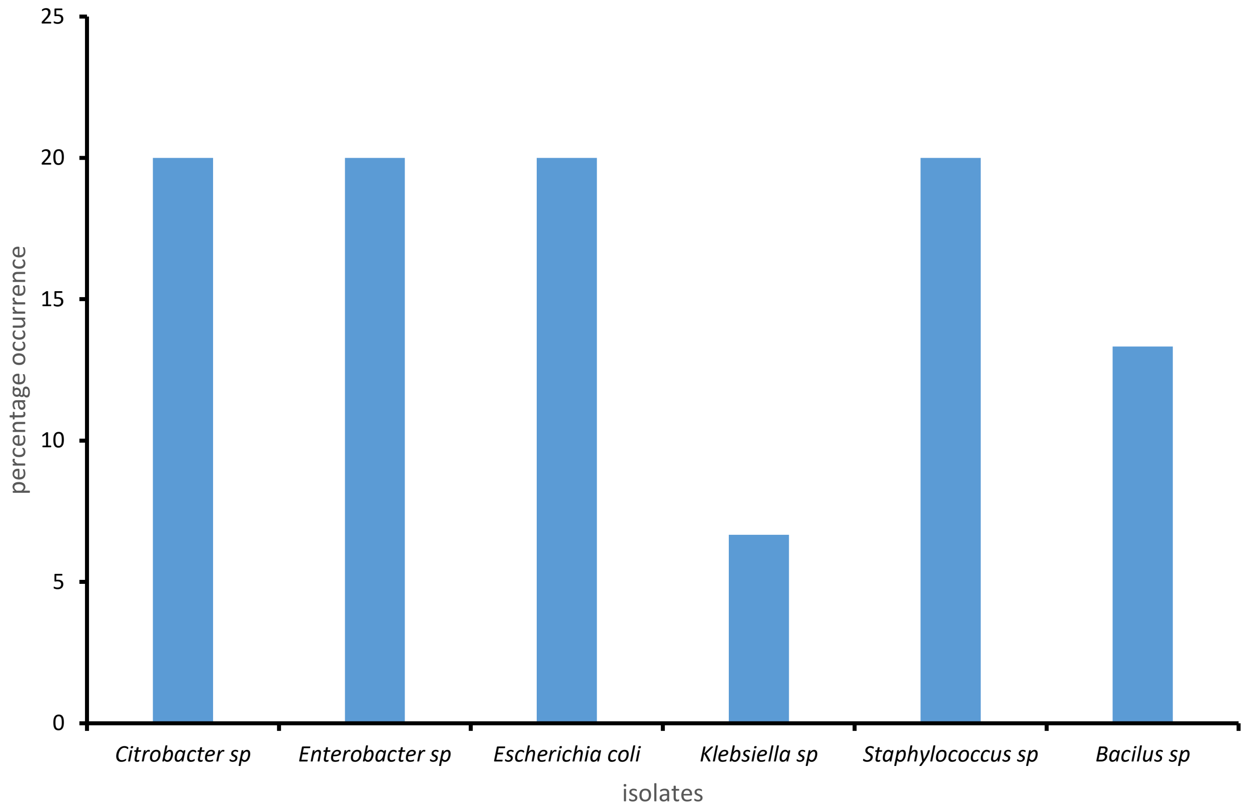

Figure 4: percentage occurrence of bacteria isolates

Table 3. Phenotypic virulence determinants of bacterial isolates

Bacterial Isolates	DNase	Hemolysin	Gelatinase
Escherichia coli	+	Beta	-
Staphylococcus aureus	+	Beta	+
Klebsiella sp	+	Gama	-
Enterobacter sp	+	Gama	-
Bacillus sp	+	Beta	-

Key: + = positive (present) - = negative (absent)

Table 4. antibiotic susceptibility test

ISOLATES	CS	CIP	GEN	E	TE	M	CD	AG
E.coli	0(R)	17(S)	12(I)	0(R)	0(R)	0(R)	9(R)	12(I)
Staphylococcus sp	0(R)	14(S)	15(S)	8(R)	0(R)	7(R)	11(I)	14(S)
Bacillus sp	0(R)	18(S)	15(S)	10(I)	14(S)	7(R)	0(R)	9(R)
Klebsiella sp	7(R)	22(S)	17(S)	10(I)	8(R)	0(R)	10(R)	15(S)
Citrobacter sp	0(R)	16(S)	14(S)	0(R)	0(R)	0(R)	0(R)	14(S)
Enterobacter sp	7(R)	24(S)	19(S)	10(I)	15(S)	0(R)	10(R)	15(S)

KEY

- R: RESISTANCE
- S: SUSCEPTIBLE
- CS: COLISTIN
- CIP: CIPROFLOXACIN
- GEN: GENTAMICIN
- E: ERYTHROMYCIN
- TE: TETRACYCLIN
- M: METRONIDAZOLE
- CD: CLINDAMYCIN
- AG: AUGMENTIN

5.0 CHAPTER FIVE

DISCUSSION

Bacterial and fungal contamination of borehole water in school hostels is a prevalent issue, particularly in regions where boreholes serve as primary water sources and hygiene practices may be challenging to maintain. Schools, especially in areas with limited infrastructure, often rely on boreholes to supply water for drinking, cooking, and cleaning, making the microbial quality of this water crucial for student health. From the result obtained in this study, the total heterotrophic bacterial count ranged from 4.46 log_{10} cfu/ml to 4.86 log_{10} cfu/ml, while coliform ranged from 2.84 to 3.13 log_{10} cfu/ml and fungal counts ranged from 4.40 to 4.85 log_{10} cfu/ml. However This result was in agreement with Akuffo *et al*.(2013) who carried out a study on bacteriological analysis on borehole water from different hostels.

Using the cultural, morphological and biochemical test, the isolates obtained in this study include, *Escherichia coli, Klebsiella sp, Staphylococcus sp, Bacillus sp, Citrobacter, Enterobacter* sp, *Bjerkandera sp, Aureobasidium sp, Scedosporium sp*, and *Mucor sp.* The achievement was in correspondent with study of (Adeyemi *et al*,. 2020) who isolated similar isolates from his borehole water samples. *Escherichia coli* (*E. coli*) is widely recognized as an indicator of fecal contamination and is commonly associated with gastrointestinal diseases. In school hostels, *E. coli* contamination may result from the proximity of boreholes to latrines or poorly managed waste disposal areas. This bacterium can cause symptoms such as diarrhea, nausea, and abdominal cramping, posing significant risks to students, especially in overcrowded environments (Nkansah *et al*., 2020). Other coliforms, such as *Klebsiella* sp. and *Citrobacter*, are also found in borehole water, indicating similar contamination routes. *Klebsiella* sp., for instance, is linked to urinary and respiratory infections, particularly in individuals with weakened immune systems (Oluduro *et al*., 2017).

Staphylococcus sp. and *Bacillus* sp. Obtained in this study are commonly present in soil and can infiltrate borehole water through ground seepage or when boreholes are poorly sealed. *Staphylococcus* sp., particularly *Staphylococcus aureus*, can cause skin and soft tissue infections, while *Bacillus* sp. are known to cause foodborne illnesses when they produce toxins in contaminated water sources (Anyam *et al*., 2021). *Enterobacter* sp., like other coliforms, indicate poor water quality and can lead to opportunistic infections, such as respiratory and wound infections, in students exposed to contaminated water (Omotayo *et al*., 2022).

Fungal contamination in borehole water, though often overlooked, is equally concerning in school hostels. *Bjerkandera adusta*, a wood-decay fungus, is known for its ability to degrade complex organic materials, which may enter borehole water systems through soil infiltration or biofilm formation. While this fungus does not typically cause infections in healthy individuals, it can pose health risks for those with compromised immune systems (Hageskal *et al*., 2009).

Aureobasidium pullulans is another fungus frequently detected in borehole water, where it forms biofilms on moist surfaces. This fungus can cause skin and respiratory issues, particularly in humid environments. It is notably resistant to disinfectants, complicating its removal from water sources used in hostels (Dai *et al*., 2018). *Scedosporium apiospermum*, a pathogenic mold, can lead to serious respiratory and systemic infections, especially in immunocompromised individuals. This fungus thrives in water systems with high organic loads, and outbreaks have been reported in communal water supplies (Novak Babič *et al*., 2020).

Mucor mucedo is a common waterborne fungus that, while generally saprophytic, can cause mucormycosis a serious fungal infection affecting the lungs, skin, and sinuses, particularly in individuals with weakened immunity. *Mucor* species often proliferate in boreholes with nutrient-rich conditions or insufficient chlorination, making it challenging to manage in communal water systems like school hostels (Sultan *et al*., 2018).

CONCLUSION

The contamination of borehole water in school hostels by various bacterial and fungal pathogens, such as *Escherichia coli, Klebsiella, Staphylococcus, Bacillus, Citrobacter, Enterobacter*, *Bjerkandera sp*, *Aureobasidium sp*, *Scedosporium sp*, and *Mucor sp*, presents serious public health risks. These microorganisms, arising from fecal contamination, soil infiltration, and environmental factors, can cause a range of infections, from gastrointestinal illnesses to skin, respiratory, and systemic diseases. Preventive measures, including borehole placement away from pollution sources, routine microbial testing, and advanced water treatment methods, are essential to protect students in communal living settings like school hostels. Additionally, education on sanitation and hygiene is vital to reduce the risk of waterborne infections. Implementing these strategies can help ensure safer water quality in school hostels, minimizing health risks and promoting a healthier living environment for students.

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