PHYSICOCHEMICAL AND BACTERIOLOGICAL ANALYSIS ON WATER SOURCES IN UNIVERSITY OF BENIN EKENWAN CAMPUS BENIN CITY, EDO STATE, NIGERIA

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Frank Erere ORUGBO

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MICROBIOLOGY TECHNIQUES

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES. IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE (B.Sc.) IN SCIENCE LABORATORY TECHNOLOGY

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CERTIFICATION

This is to certify that this project work carried out by Frank Erere ORUGBO with the matriculation number, LSC2007345 of the department of Science Laboratory Technology (Microbiology Techniques), Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

This book is dedicated to God Almighty whose unwavering love, guidance, direction and strength have fueled my journey of discovery.

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ABSTRACT

Groundwater and borehole water remain a vital source of drinking water in many Nigerian cities, yet their quality is often compromised by physicochemical and microbial contamination. This study assessed the physicochemical and bacteriological analysis on water sources in University of Benin Ekenwan Campus Benin City, Edo State, Nigeria. A total of eighteen water samples were collected from six hostel locations including Notre Dame, Uniben Table Water, Bore-hole source Water, Boy's hostel, Postgraduate hostel, and Girl's hostel, and analysed using the method of A.O.A.C. The microbiological analysis was analysed using standard microbiological methods. The results revealed that physicochemical analysis revealed that pH values of the water ranged between 4.58 and 6.38, falling below the FEPA acceptable limit of 6.5–8.5. Most physicochemical parameters analysed were below permissible limits, however, iron (0.56–1.06 mg/L) and chromium (0.10–0.25 mg/L) exceeded the FEPA limits of 0.3 mg/L and 0.05 mg/L respectively. Microbiological results showed high contamination across hostel water samples. Presumptive coliform counts ranged from 6.67×10^{3} cfu/ml (Notre Dame) to 2.83×10^{4} cfu/ml (Boys hostel), aerobic bacterial counts ranged from 6.67×10^{3} cfu/ml (Notre Dame) to 8.67×10^{4} cfu/ml (Postgraduate hostel), while fungal counts ranged from 1.00×10^{1} sfu/ml (Notre Dame) to 2.00×10^{2} sfu/ml (Boys hostel). The microbial results exceeded WHO and FEPA permissible standards. Identified bacteria include *Staphylococcus aureus, Corynebacterium* spp., *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Klebsiella aerogens*, *Enterobacter aerogens* and *Chromobacterium violaceum*, *Penicillium notatum*, *Aspergillus flavus*, *Aspergillus niger* and *Microsporum audouinii*. Among bacteria, *K. aerogens* had the highest occurrence (21.9 %) while *A. hydrophila* had the least occurrence (9.4 %). Among fungi, *A. flavus* (35.7%) was the most frequently occurring, while *M. audouinii* (14.3 %) had the lowest occurrence. Antibiotic sensitivity testing before plasmid curing showed multidrug resistance across isolates. *Staphylococcus aureus* displayed resistance to amoxicillin, augmentin, pefloxacin, azithromycin, and ceftriaxone, with the highest multidrug resistant index (MDRI) of 0.8. *Pseudomonas fluorescens* and *Enterobacter aerogens* each had an MDRI of 0.5, while *Corynebacterium* spp. and *Chromobacterium violaceum* exhibited MDRI values ranging from 0.2 to 0.3. After plasmid curing, resistance was lost in several isolates, with *A. hydrophila*, *S. aureus*, *Corynebacterium* spp., *P. fluorescens*, *K. aerogens* and *C. violaceum* showing susceptibility to tested antibiotics. This study demonstrated that while physicochemical parameters of water sources in Ekenwan Campus were largely within permissible limits except for iron and chromium, the microbiological quality was grossly compromised. The detection of multidrug resistant organisms, both plasmid-mediated and chromosomal, highlights the public health risk associated with these water sources. The findings confirm that water supplied in these hostels is unsafe for direct consumption and requires adequate treatment and disinfection to reduce risks of waterborne infections and antibiotic-resistant outbreaks in the student community.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Water is widely regarded as a fundamental resource essential for life, human health, agriculture and economic development, yet its quality has been a serious concern globally due to increasing contamination from both natural and anthropogenic activities (Sila, 2019; Achahbar, 2020). As water makes up a large portion of the human body and is critical in all biological processes, the safety of water sources is crucial to prevent disease outbreaks and promote public health (Gadiso *et al*., 2020). In many rural and urban communities, the direct consumption of untreated water has been linked to waterborne diseases, which continue to pose significant health risks especially in developing nations (Atiku *et al*., 2018; Aleru *et al*., 2020).

In different parts of Africa, numerous studies have consistently shown the presence of bacteriological contaminants in water sources, pathogens like *Escherichia coli* including Salmonella, Pseudomonas species and Vibrio cholerae (Aleru *et al.,* 2020; Iduh *et al.,* 2018; Sila, 2019). These microorganisms enter water bodies through improper sewage disposal, runoff from agricultural activities and faecal contamination, posing threats to communities relying on such sources (Achahbar, 2020; Ndjouhoua *et al*., 2022). A study in Sokoto State, Nigeria, identified high counts of total coliforms and faecal coliforms in well and river water, indicating severe microbial contamination that exceeds WHO standards (Iduh *et al.,* 2018). Similar findings in Abuja, Nigeria, highlighted high bacterial loads in river and well water samples, with isolated pathogens including Escherichia coli and Pseudomonas species, reinforcing the health dangers of consuming untreated water (Atiku *et al.,* 2018).

In addition to bacteriological hazards, physicochemical parameters such as pH, turbidity, dissolved oxygen and nitrate concentrations play a vital role in assessing water safety and quality (Duressa et al., 2019). Deviations in these parameters often indicate contamination and poor water management practices (Achahbar, 2020; Ndjouhoua *et al.,* 2022). For instance, high turbidity can reduce the efficiency of disinfection processes and may protect microorganisms from treatment agents, making them more resistant and harmful (Benaissa et al., 2024). Similarly, elevated nitrate levels, often resulting from fertiliser runoff, can cause methemoglobinemia, especially in infants (Achahbar, 2020). Studies across several regions, including Ethiopia and Morocco, have highlighted significant seasonal and spatial variations in physicochemical properties of water sources, which are influenced by rainfall patterns, land use changes and urbanization (Achahbar, 2020; Duressa *et al.,* 2019). In Tetouan, Morocco, water sources in urban areas were found to have higher levels of hardness, magnesium, and sulphates compared to rural areas where nitrate and nitrite levels were higher (Achahbar, 2020). These differences show the importance of continuous monitoring and context-specific interventions to improve water quality (Gadiso *et al.,* 2020).

The problem of microbial contamination is not limited to rural or low-income areas; urban centres also face challenges related to ageing infrastructure, improper waste disposal and industrial effluents (Akani et al., 2021). In Port Harcourt, Nigeria, water from storage tanks showed variable pH, high conductivity, and the presence of bacteria such as *Citrobacter* and *Klebsiella* species, illustrating that even supposedly safe water sources can harbour harmful microorganisms if not properly maintained (Akani *et al.,* 2021). These findings emphasise the need for improved water hygiene and maintenance of storage facilities to ensure safety (Akani *et al.,* 2021).

Globally, the convergence analysis of improved water sources has shown progress in expanding access, but disparities still exist, particularly in sub-Saharan Africa and parts of Asia (Chang et al., 2018). While the Millennium Development Goals aimed to improve water access, the quality aspect remains critical as many people still rely on unimproved or contaminated sources (Chang *et al.,* 2018). The increasing urbanisation and industrial activities have exacerbated water pollution, making treatment more complex anBWS-Borehole Water Source,d expensive (Chowdhury, 2018). Access to safe water remains a pressing challenge in many communities worldwide, with bacteriological and physicochemical contamination posing serious health threats. Despite improvements in water supply systems, studies have shown the persistent presence of harmful bacteria such as *Escherichia coli* and *Salmonella* in various water sources, including wells, rivers, and storage tanks (Iduh *et al.,* 2018; Sila, 2019). Physicochemical issues like high turbidity, abnormal pH, and chemical pollutants can further reduce water quality, compromising disinfection effectiveness and increasing the risk of disease outbreaks (Achahbar, 2020; Duressa *et al.,* 2019).

Poor waste disposal, agricultural runoff, and inadequate maintenance of water infrastructure exacerbate these problems, affecting both rural and urban populations. In many cases, even when chemical standards are met, microbial contamination persists, highlighting the need for integrated monitoring and management strategies (Gadiso *et al.,* 2020).

Amidst this global context, it becomes important to focus on specific localities to understand unique challenges and propose solutions that are both practical and effective. The University of Benin Ekenwan Campus in Benin City, Edo State, Nigeria, serves as a significant case study due to its rapidly expanding student population, urban development and reliance on various water sources. As students and staff depend on these sources for daily activities,

understanding the bacteriological and physicochemical quality of these water sources is necessary to prevent health risks and ensure a safe learning environment. This study will help in identifying potential contaminants, guiding campus authorities on proper water treatment and storage practices, and ultimately safeguarding public health within the university community.

The dependence on multiple water sources without thorough, regular quality assessment increases the risk of waterborne diseases among students and staff. The lack of updated data on the bacteriological and physicochemical state of campus water sources makes it difficult for administrators to ensure safe water supply. This gap not only threatens health but also undermines academic productivity and overall well-being. Thus, there is a need to analyse and address these water quality challenges to create a healthier and safer learning environment.

1.2 Aim and Objectives of the Study

The aim of this study was to investigate the bacteriological and physicochemical analysis of water sources in the University of Benin, Ekenwan campus, Benin city, Edo State, Nigeria.

The specific objectives of the study were to:

1. determine the physiochemical properties of water sources in hostels at Ekenwan Campus, University of Benin.
2. enumerate, isolate and characterize microbial isolates in the water samples.
3. determine the antibacterial susceptibility pattern of the bacteria isolates from the water sources.
4. determine if the antibiotic susceptibility pattern of the bacterial isolated lies in the plasmid DNA or chromosomal DNA.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Water Sources in Nigeria

Water is considered a vital resource for human survival, social development and economic growth, and in Nigeria, its availability and quality continue to influence livelihoods across rural and urban areas (Omokaro *et al.,* 2024; Egbueri *et al.,* 2024). The landscape of water sources in Nigeria is broad and can be understood through a careful examination of surface water, groundwater and alternative sources like rainwater harvesting. These sources are not only important for daily consumption but also essential for agriculture, industry and environmental sustainability (Nouban *et al.,* 2020; Omokaro *et al.,* 2024). Before discussing each type of water source, it is important to note that access to safe and clean water in Nigeria is still a challenge for millions despite the country's abundant natural water resources (Enyidi, 2017; Omokaro *et al.,* 2024). Multiple studies highlight that only a fraction of the population has regular access to potable water, pushing communities to depend on any available sources irrespective of their quality (Dangana and Muhammad, 2016; Iwuala *et al.,* 2020).

2.1.1 Surface Water Sources

Surface water in Nigeria consists mainly of rivers, lakes and streams that flow across different regions of the country. These sources are widespread and contribute significantly to household, agricultural and industrial water needs (Umoren and Onianwa, 2012; Omokaro *et al.,* 2024). The Niger and Benue rivers, along with their tributaries, form the major surface water

systems supporting various uses, including irrigation and fishing activities that sustain many rural economies (Enyidi, 2017). Pollution of surface water remains a major issue, especially in regions like the Niger Delta, where oil spills and industrial discharges have caused widespread contamination (Omokaro *et al.,* 2024). Studies on the Qua Iboe River system show that surface water in Nigeria often contains heavy metals and organic pollutants, which pose severe health risks (Umoren and Onianwa, 2012). Research also shows that runoff from agricultural lands and urban waste contribute significantly to water quality deterioration, which compromises the safety of these water sources (Egbueri *et al.,* 2024). The dependence on surface water is higher in southern parts of Nigeria due to the presence of larger river systems, yet pollution levels have forced many to seek alternative sources where possible (Egbueri *et al.,* 2024; Dangana *et al.,* 2015). While these water bodies have potential for sustainable use, poor management and lack of enforcement of environmental laws have continued to threaten their viability (Omokaro *et al.,* 2024).

2.1.2 Groundwater Sources

Groundwater in Nigeria is accessed mainly through wells and boreholes, which are popular in both urban and rural settings (Haruna and Garba, 2019; Moses and Ishaku, 2020). Boreholes, in particular, are regarded as more reliable due to their deeper reach and perceived protection from surface contaminants (Iwuala *et al.,* 2020). However, studies have shown that groundwater is not always free from pollution. Research conducted in Maiduguri revealed that groundwater samples contained heavy metals like lead, cadmium and mercury beyond safe limits, which can lead to long-term health complications such as kidney and liver damage (Haruna and Garba, 2019). The risk is higher in areas with extensive mining and industrial activities, which often result in toxic substances seeping into aquifers (Egbueri *et al.,* 2024).

In regions like Niger State and Imo State, boreholes have been identified as the major water source for many households (Iwuala *et al.,* 2020; Dangana and Muhammad, 2016). However, accessibility challenges persist as some households travel long distances or spend significant time fetching water due to limited availability of functional boreholes (Dangana and Muhammad, 2016). This has a direct impact on hygiene and general well-being as it limits water use for cleaning and personal care (Iwuala *et al.,* 2020).

2.1.3 Rainwater Harvesting and Other Alternative Sources

Rainwater harvesting has emerged as an important supplementary water source, especially in areas where surface and groundwater sources are unreliable or heavily polluted (Owoade, 1989). The potential of rainwater harvesting in Nigeria is significant, given the country's seasonal rainfall pattern that provides opportunities to collect and store large quantities of water (Owoade, 1989).

Despite its potential, the practice is not yet widespread, mostly due to lack of awareness and infrastructural constraints (Iwuala *et al.,* 2020). In rural communities like Akinima in Rivers State, rainwater harvesting is among the few available options during dry periods when river levels are low and boreholes dry up (Nnodu *et al.,* 2010). However, studies indicate that rainwater collected without proper systems often contains microbiological and chemical contaminants, which can cause waterborne diseases (Nnodu *et al.,* 2010). Some urban households also rely on packaged water, popularly known as sachet water, especially where municipal supply systems fail to meet demands (Haruna and Garba, 2019). While sachet water offers convenience, concerns about its quality remain as regulatory oversight is often weak (Haruna and Garba, 2019).

2.1.4 Distribution and Accessibility

Water distribution in Nigeria faces significant challenges that affect both rural and urban dwellers. Despite efforts by different government agencies like the River Basin Development Authorities, equitable distribution of water resources has not been fully realised (Enyidi, 2017; Omokaro *et al.,* 2024). In many communities, especially in the southern parts of Niger State and parts of Imo State, accessibility is influenced by factors such as distance to the water source, time spent fetching water and financial constraints to install boreholes or water storage facilities (Dangana and Muhammad, 2016; Iwuala *et al.,* 2020). Households in these areas often have to rely on hand-dug wells and streams, which are vulnerable to contamination from human and animal activities (Moses and Ishaku, 2020). The situation in urban areas is not much better as rapid urbanisation and poor infrastructure have led to erratic municipal water supply, forcing residents to depend on private boreholes and water vendors, which increases costs and further restricts equitable access (Omokaro *et al.,* 2024).

2.1.5 Quality Considerations

Water quality in Nigeria varies widely depending on the source and local conditions. In urban centres like Osun State, studies on mini-water schemes revealed that even governmentapproved sources can harbour pathogenic bacteria, including multiple antibiotic-resistant strains (Fakayode and Ogunjobi, 2018). This reality underlines the risk faced by communities depending on these systems for daily water needs. In rural areas, chemical pollutants from agricultural and industrial activities contribute to unsafe water quality. The presence of nitrates, heavy metals and organic pollutants in both surface and groundwater is well documented and often exceeds safe limits recommended by international and national standards (Egbueri *et al.,* 2024; Haruna and Garba, 2019). Public health implications are severe as unsafe

water contributes to diarrhoeal diseases, kidney failure and other chronic conditions, especially among vulnerable populations like children and the elderly (Fakayode and Ogunjobi, 2018; Egbueri *et al.,* 2024).

2.1.6 Management and Policy Framework

While Nigeria has several water-related policies and regulatory agencies, implementation and enforcement remain weak (Enyidi, 2017). The Federal Ministry of Water Resources and associated bodies like the River Basin Development Authorities and National Water Research Institute are responsible for developing and managing water resources but overlapping roles and poor coordination undermine effectiveness (Enyidi, 2017). Integrated Water Resource Management (IWRM) has been identified as a necessary approach to improve water use efficiency and ensure sustainable supply but practical application remains limited (Nouban *et al.,* 2020). The need for community involvement and education on water safety and hygiene is also critical, as many households lack awareness of contamination risks and proper handling practices (Iwuala *et al.,* 2020). The water sources in Nigeria are diverse and essential for sustaining life but they face challenges related to pollution, accessibility and poor management. Both surface and groundwater play significant roles, while alternative sources like rainwater harvesting and sachet water continue to supplement supply amidst growing demand. Addressing these issues requires a combined effort of policy reform, infrastructural investment and public education to ensure that safe and sufficient water is accessible to all Nigerians.

2.2 Water Quality and Public Health

Water quality plays a crucial role in maintaining good health as contaminated water can transmit a wide range of diseases affecting communities across the world (Peiyue et al., 2019). Clean water is essential for hydration, cooking and sanitation and the absence of safe water

contributes to increased incidences of illnesses such as diarrhoea, cholera, typhoid and various parasitic infections which remain significant causes of mortality particularly in developing regions (Saini and Shrivastava, 2024). Researchers have highlighted that poor water quality is closely linked to the occurrence of waterborne diseases and poses severe public health risks especially among children and vulnerable populations (Ocheo et al., 2017). In many urban and rural areas, the presence of microbial contaminants like E. coli, coliform bacteria and viruses has been found in drinking water supplies which signifies faecal contamination and poses immediate health threats (Khalid *et al.,* 2023). When water sources are exposed to untreated sewage, agricultural runoff or industrial waste, the concentration of harmful pathogens rises significantly and communities relying on such water face an increased burden of disease (Temam et al., 2019). The study conducted in Adama City in Ethiopia revealed that a high percentage of water samples tested positive for total coliform bacteria and faecal coliform bacteria which demonstrated the risk of waterborne infections among residents (Temam *et al*., 2019). Chemical contamination is another critical aspect of water quality that affects public health. High levels of nitrates, heavy metals such as arsenic and lead and pesticides have been reported in several water sources worldwide which can lead to chronic health conditions including cancers and developmental issues in children (Otorkpa *et al*., 2024). Public health experts stress that monitoring and regulating drinking water is fundamental to disease prevention as poor water quality affects not only physical health but also has psychological consequences among populations that become anxious about their daily water consumption (Khalid *et al.,* 2023). In Karachi, Pakistan, the majority of water supplies were found unsuitable for drinking due to microbial and chemical contamination which led to significant health risks and reduced public trust (Khalid *et al.,* 2023). This sense of distrust and fear can contribute to mental stress as people worry about potential

infections and long-term health impacts (Khalid *et al.,* 2023). In many parts of Africa, inadequate infrastructure and poor water treatment systems contribute to unsafe drinking water which continues to be a major public health challenge (Otorkpa, *et al*., 2024). Lack of funds, ageing pipes and unregulated borehole constructions further complicate the issue leading to a higher incidence of waterborne diseases (Otorkpa *et al*., 2024). This situation mirrors similar patterns observed in rural parts of Asia where traditional beliefs and reliance on untreated water sources such as holy wells expose communities to dangerous pathogens (Wiwanitkit, 2017). In some areas, residents believe that water from certain natural sources possesses spiritual or healing properties and consume it without any form of purification which heightens the risk of infection (Wiwanitkit, 2017). The spread of diseases like cholera and hepatitis A through contaminated water highlights the pressing need for robust water safety management systems (Peiyue *et al*., 2019). In Ireland, public water supplies failed to meet safety standards for several harmful chemicals including arsenic, lead and trihalomethanes which placed communities at continuous risk despite the presence of regulations (Liu and Wu 2019). Similar challenges were seen in regions like Tomsk in Russia where groundwater chemical analysis revealed potential health risks though the noncarcinogenic risk was found acceptable, underscoring the need for comprehensive and continuous monitoring (Yankovich *et al.,* 2016).

Wastewater-based epidemiology has become an increasingly valuable tool for monitoring community health through viral tracking in sewage which supports early outbreak detection and offers real-time insights into public health trends (Al-Daim, 2025). The integration of virological surveillance in water safety regulations has been recommended to protect communities from emerging viral threats as current wastewater and water treatment systems remain insufficient for eliminating all persistent toxic substances and microbes (Al-Daim, 2025). Public perception

plays a vital role in water safety as it affects community engagement in health interventions and policy support. A study in Newfoundland, Canada revealed a disconnect between perceived and actual water quality as many residents reported high satisfaction despite measurable contamination in their water sources (Ocheo *et al*., 2017). The influence of education and awareness on public perception was highlighted in Algeria where people with higher education levels demonstrated greater trust in water safety, suggesting that education campaigns can significantly enhance community resilience to waterborne diseases (Benameur *et al.,* 2021).

Ensuring safe water supply involves not only technical interventions but also active community participation and policy enforcement. In New South Wales, Australia, the implementation of drinking water quality assurance programmes under public health laws has significantly improved risk management and awareness in regional and Aboriginal communities (Byleveld *et al.,* 2016). These measures include regular testing, public reporting and immediate corrective actions which reduce contamination risks and improve public confidence in water services (Byleveld *et al.,* 2016). The quality of water is also impacted by physical parameters like turbidity, temperature and pH which influence microbial growth and chemical reactions. High turbidity and the presence of nutrients promote the growth of harmful organisms while temperature variations can affect chlorine residuals in water treatment, compromising disinfection effectiveness (Saini and Shrivastava, 2024). Water quality indexes have been developed to guide health assessments though limitations exist regarding accuracy and applicability in different contexts (Thomas *et al.,* 2018). Pollution from agricultural activities remains a significant challenge for water safety worldwide. Fertiliser runoff introduces nitrates and phosphates into water bodies leading to eutrophication and harmful algal blooms which further compromise water quality and increase health risks through toxin exposure (Otorkpa et al.,

2024). Industrial discharges containing heavy metals and persistent organic pollutants also contribute to chronic health problems such as liver and kidney diseases, cancers and reproductive issues (Liu and Wu 2019).

Safe water access is a fundamental human right and an essential component of sustainable development. Public health interventions must address water security at multiple levels including source protection, infrastructure improvement and community education to mitigate risks effectively (Watt, 2017). Policies must prioritize the integration of health considerations into water resource management to ensure that quality standards are upheld consistently and equitably (Watt, 2017).

2.3 Physicochemical Parameters of Water

Water is a crucial natural resource whose quality is determined by a range of physicochemical parameters. These parameters play an essential role in defining water's suitability for human consumption, agricultural purposes, industrial applications and supporting aquatic life (Raji *et al.,* 2020; Ibrahim, 2025). Each parameter reflects different aspects of water quality, providing information on possible contamination, natural mineral content and overall health safety

(Al-Zurfi *et al.,* 2019).

2.3.1 pH and Temperature

pH measures the degree of acidity or alkalinity in water and is vital as it affects biological and chemical reactions. Water with pH levels below 6.5 is generally considered acidic, while values above 8.5 are regarded as alkaline (Raji *et al.,* 2020). Maintaining a neutral to slightly alkaline pH is essential because extreme values may corrode pipes and affect taste, potentially

leading to leaching of heavy metals (Maishanu and Muhammad, 2024). The optimal pH for most natural waters falls between 6.5 and 8.5, ensuring minimal risk to consumers and infrastructure (Sharma and Singh, 2016). Temperature influences chemical solubility and reaction rates in water. Higher temperatures can reduce dissolved oxygen levels, leading to potential stress on aquatic organisms (Ibrahim, 2025). Elevated temperatures also enhance microbial activity and accelerate chemical reactions, which can alter taste and odour (Sampson *et al.,* 2020). Cooler water tends to hold more oxygen, making it preferable for supporting aquatic ecosystems (Al-Zurfi *et al.,* 2019).

2.3.2 Turbidity and Total Dissolved Solids

Turbidity refers to the cloudiness or haziness of water caused by suspended particles. High turbidity can harbour pathogens and shield them from disinfection processes, making water unsafe for consumption (Maishanu and Muhammad, 2024). Clear water typically indicates fewer suspended solids, which suggests lower microbial contamination risks (Sharma & Singh, 2016).

Total dissolved solids (TDS) represent the combined content of all inorganic and organic substances dissolved in water. High TDS levels can affect water taste and contribute to hardness (Raji *et al.,* 2020). TDS values within acceptable limits ensure that water remains palatable and safe for long-term use. The acceptable limit for TDS is generally below 500 milligrams per litre, as higher concentrations might lead to gastrointestinal irritation (Al-Zurfi *et al.,* 2019).

2.3.3 Electrical Conductivity and Hardness

Electrical conductivity (EC) measures water's ability to conduct an electric current, directly linked to the concentration of dissolved ions. Higher EC indicates greater mineral content and possible pollution from agricultural or industrial activities (Sharma and Singh, 2016).

Monitoring EC is useful in assessing salinity and overall ionic balance in water sources (Sampson *et al.,* 2020).

Hardness results from the presence of calcium and magnesium ions and affects the usability of water in households and industries. Hard water may form scales in pipes and reduce soap efficiency (Ibrahim, 2025). While not a direct health hazard, hardness above 200 milligrams per litre can be undesirable for domestic use. Maintaining moderate hardness ensures water remains suitable for daily activities without damaging plumbing systems (Raji *et al.,* 2020).

2.3.4 Dissolved Oxygen and Biological Oxygen Demand

Dissolved oxygen (DO) is crucial for the survival of aquatic organisms and indicates water's self-purification ability. Low DO levels suggest organic pollution and reduced capacity to support fish and other organisms (Al-Zurfi *et al.,* 2019). Adequate DO levels, typically above 5 milligrams per litre, are necessary to maintain balanced aquatic ecosystems (Ibrahim, 2025). Biological oxygen demand (BOD) measures the amount of oxygen needed by microorganisms to decompose organic matter. High BOD indicates significant organic pollution, which can deplete oxygen levels and lead to anaerobic conditions harmful to aquatic life (Sampson *et al.,* 2020). Monitoring BOD is vital in evaluating organic contamination and wastewater impact (Sharma and Singh, 2016).

2.3.5 Chemical Oxygen Demand and Nutrients

Chemical oxygen demand (COD) assesses the total amount of oxygen required to chemically oxidise both biodegradable and non-biodegradable substances. Higher COD values suggest the presence of pollutants that are not easily broken down biologically (Maishanu & Muhammad, 2024). Tracking COD helps in understanding overall pollution loads and planning effective treatment methods (Al-Zurfi *et al.,* 2019). Nutrients like nitrates and phosphates are

essential for plant growth but excessive levels can trigger algal blooms, reducing oxygen availability and harming aquatic life (Raji *et al.,* 2020). Elevated nitrate levels in drinking water can pose health risks such as methemoglobinemia in infants, commonly known as blue baby syndrome (Ibrahim, 2025). Ensuring nutrient levels remain within permissible limits is necessary to prevent eutrophication and maintain ecosystem balance
(Sharma and Singh, 2016).

2.3.6 Heavy Metals

Heavy metals including lead, cadmium, chromium and zinc pose significant health risks even at low concentrations. Lead exposure can impair neurological development in children, while cadmium can cause kidney damage and skeletal disorders (Sampson *et al.,* 2020). Chromium is known for its carcinogenic properties and zinc, although an essential nutrient, can cause gastrointestinal distress in excess (Al-Zurfi *et al.,* 2019). The presence of heavy metals often originates from industrial discharges, mining activities and agricultural runoff. Regular monitoring is essential to prevent bioaccumulation in food chains and safeguard public health (Raji *et al.,* 2020). Adhering to strict regulatory standards for heavy metals ensures that water remains safe for human consumption and environmental integrity is maintained (Ibrahim, 2025).

2.3.7 Salinity and Alkalinity

Salinity refers to the concentration of dissolved salts in water, which influences taste and usability for irrigation and drinking purposes. High salinity levels can reduce water suitability for irrigation by affecting soil structure and plant health (Sharma and Singh, 2016). Maintaining low salinity levels is crucial for agricultural sustainability and potable water standards (Al-Zurfi *et al.,* 2019). Alkalinity measures water's capacity to neutralise acids, largely governed by bicarbonates, carbonates and hydroxides. Adequate alkalinity provides a buffering capacity, protecting aquatic

life from sudden pH changes (Maishanu and Muhammad, 2024). It also indicates the ability of water to resist acidification, which is important for maintaining chemical stability (Raji *et al.,* 2020).

2.3.8 Chlorides and Sulphates

Chlorides in water come from natural sources, sewage and industrial effluents. While essential in small quantities, high chloride levels can impart a salty taste and cause corrosion in metal pipes (Sampson *et al.,* 2020). The permissible limit for chlorides in drinking water is usually around 250 milligrams per litre to prevent taste and corrosion issues (Ibrahim, 2025).

Sulphates are naturally occurring anions that can affect taste and have laxative effects when present in high concentrations (Raji *et al.,* 2020). The acceptable limit for sulphates in drinking water is generally below 250 milligrams per litre. Elevated levels can lead to dehydration and digestive discomfort, especially in sensitive populations (Al-Zurfi *et al.,* 2019).

2.3.9 Total Suspended Solids and Total Solids

Total suspended solids (TSS) represent particles suspended in water, which can include silt, decaying plant matter and industrial residues. High TSS levels can reduce light penetration, affecting photosynthesis in aquatic plants and clogging fish gills (Sharma & Singh, 2016). Keeping

TSS within acceptable levels is crucial for maintaining clear water and supporting aquatic habitats (Maishanu and Muhammad, 2024). Total solids encompass both suspended and dissolved materials. Elevated total solids may affect water clarity and reduce its suitability for drinking and industrial uses (Raji *et al.,* 2020). Monitoring total solids provides an overall indication of water cleanliness and the effectiveness of filtration processes (Ibrahim, 2025).

2.4 Bacteriological Quality of Water

Bacteriological quality of water is a fundamental aspect of public health because water can serve as a vehicle for transmitting many infectious diseases to humans. Water contaminated with bacteria poses serious health threats to communities especially where access to treated and safe water is limited (Srivastava *et al.,* 2018; Gadiso *et al.,* 2020). Bacteria found in water are commonly used as indicators of faecal contamination since they reveal the possible presence of pathogenic microorganisms that can cause diseases such as cholera, typhoid fever, and diarrhoea (Tarqui-Mamani *et al.,* 2016; Naghipour *et al.,* 2016). Coliform bacteria, particularly *Escherichia c*oli, are widely recognised as important indicators of water quality because their presence signals direct faecal contamination (TarquiMamani *et al.,* 2016). According to Srivastava *et al*. (2018), water samples from hospitals and residential water supplies often exceeded permissible limits for coliform counts, raising serious concerns about the hygiene standards in those environments. When water sources are contaminated with faecal matter, they may carry pathogenic bacteria that can cause gastroenteritis, hepatitis A, and other severe infections (Martone-Rocha *et al.,* 2014).

In a study conducted by Gadiso *et al*. (2020), high levels of total *coliform* and faecal coliform were reported in multiple water samples, indicating that untreated or poorly managed water sources can be a direct risk to health. The findings highlighted that water containing faecal *coliforms* typically implies the presence of enteric pathogens, which are responsible for waterborne outbreaks (Tarqui-Mamani *et al.,* 2016). The bacteriological assessment of water quality generally involves the detection of indicator organisms rather than every possible pathogenic bacterium since indicator organisms are easy to detect and usually present in higher numbers (Naghipour *et al.,* 2016). Total coliform counts and *E. coli* are among the most commonly tested bacteria during water quality assessments because they provide a reliable

measure of contamination levels and potential health hazards (Srivastava *et al.,* 2018). Research from multiple global studies has shown that in areas with inadequate sanitation infrastructure, bacterial contamination of water is more widespread and persistent, contributing to a high burden of waterborne diseases (Tarqui-Mamani *et al.,* 2016; AlHmani *et al.,* 2024). In Yemen, after the collapse of sanitation activities, microbial pollution in water sources nearly doubled, showing a strong relationship between water infrastructure and microbial quality (Al-Hmani *et al.,* 2024). Naghipour *et al*. (2016) also examined water coolers in hospitals and found that improper maintenance of distribution systems and neglect of cleaning practices significantly contributed to bacterial contamination (Naghipour *et al.,* 2016). The accumulation of biofilms inside pipes and tanks can provide a breeding ground for bacteria, allowing them to persist even in treated water (Srivastava *et al.,* 2018). In many studies, heterotrophic bacteria have also been used as indicators of general microbial activity in water (Martone-Rocha *et al.,* 2014). High heterotrophic plate counts suggest poor maintenance or secondary contamination after treatment. In a study on student housing water supplies, high levels of heterotrophic bacteria, E. coli, and Pseudomonas aeruginosa were detected, indicating failures in sanitation and hygiene (Martone-Rocha *et al.,* 2014). A study in Peru found that more than 70 percent of water samples in certain regions contained total coliforms and over 60 percent contained *E. coli*, confirming that a significant portion of the population relied on unsafe drinking water (Tarqui-Mamani *et al.,* 2016). This high prevalence of bacteria often results from poor handling, storage practices, and the use of untreated water sources (Tarqui-Mamani *et al.,* 2016).

In Brazil, a similar pattern was observed where nearly 68 percent of water samples tested did not meet bacteriological standards, with high contamination levels attributed to unprotected water sources and inadequate household water storage (Miranda *et al.,* 2018). The lack of

protective covers on wells and improper sewage disposal were identified as key factors contributing to bacterial contamination (Miranda *et al.,* 2018). In some settings, like refill water stations, operator hygiene practices have a critical impact on water quality (Enjelina *et al.,* 2017). The study highlighted that even when equipment and facilities meet technical sanitation standards, poor hygiene practices by personnel can lead to bacterial contamination, making operator training and certification crucial for safeguarding water safety. Bacterial contamination is not only limited to small rural areas but can also be significant in urban and institutional settings. For example, in Klang Valley, Malaysia, even swimming pool water showed high bacterial counts despite regular chlorination, indicating that inadequate maintenance and hygiene behaviour of swimmers can override disinfection efforts (Rosli, 2020). The health implications of consuming bacteriologically contaminated water are severe. Pathogenic bacteria can cause acute gastrointestinal infections, which are a leading cause of morbidity and mortality, especially among children under five (Gadiso *et al.,* 2020; Tarqui-Mamani *et al.,* 2016). Long-term exposure to contaminated water can lead to chronic health issues, malnutrition, and hinder educational and economic opportunities due to repeated illness episodes (Al-Hmani *et al.,* 2024).

The presence of bacteria like *Pseudomonas aeruginosa* and *Aeromonas species* also indicates potential risks of opportunistic infections, particularly in immunocompromised individuals (Martone-Rocha *et al.,* 2014). Inadequate water treatment and storage practices increase the chance for these bacteria to proliferate, posing further health hazards (Naghipour *et al.,* 2016). Studies emphasise the importance of community education and awareness to reduce bacterial contamination. Good hygiene practices such as regular cleaning of storage tanks, safe handling, and proper treatment methods like boiling or chlorination are crucial measures to ensure bacteriological safety (Miranda *et al.,* 2018; Enjelina *et al.,* 2017). Maintaining

bacteriological quality requires continuous monitoring, proper sanitation infrastructure, and enforcement of water safety regulations (Srivastava *et al.,* 2018; Al-Hmani *et al.,* 2024). Regular bacteriological testing helps identify contamination early and supports prompt interventions to protect public health (Tarqui-Mamani *et al.,* 2016).

2.4.1 Types of Bacteria Found in Water

2.4.1.1 *Coliform Bacteria*

Coliform bacteria are often used as indicator organisms to assess the sanitary quality of water. These bacteria are naturally found in the intestines of humans and warm-blooded animals and their presence in water indicates possible faecal contamination which can lead to serious health is more directly linked to faecal material (Spence and Taylor, 2016). Studies issues (Shukla, 2015). Total coliforms include a broad group while faecal coliforms represent a subgroup that conducted in China showed high levels of coliforms and potential E. coli in river and reservoir water sources while groundwater generally had lower or undetectable levels (Liu *et al.,* 2022). This variation highlights the role of environmental factors and human activities in bacterial contamination of water sources (Liu *et al.,* 2022). The study further showed that the presence of coliform bacteria often coincided with increased levels of pathogenic bacteria which can cause gastrointestinal infections (Liu *et al.,* 2022). In Calabar, Nigeria, researchers found that *Escherichia coli* accounted for more than half of the bacterial isolates from different water sources confirming widespread faecal contamination (Agbo *et al.,* 2017). These findings suggest that areas with poor sanitation infrastructure and improper waste disposal practices are at higher risk of waterborne diseases (Agbo *et al.,* 2017).

2.4.1.2 *Escherichia coli*

Escherichia coli, commonly known as *E. coli*, is considered one of the most critical indicators of recent faecal pollution in water. Certain strains can cause severe diarrhoeal diseases and other complications if ingested (Hour et al., 2016). In Mymensingh, Bangladesh, a study found that *E. coli* was the most frequently isolated bacteria from municipal water sources confirming continuous faecal contamination which poses a serious threat to community health (Chakraborty *et al.,* 2024). The study also noted high antibiotic resistance among these isolates, indicating additional risks of treatment failure when infections occur (Chakraborty *et al.,* 2024). *Gastropods* were also identified as unexpected sources of E. coli contamination since they carry and release bacteria into water environments (Leister *et al.,* 2023). This highlights that *E. coli* contamination may not only come from human and animal faeces but can also be spread through vectors like snails which makes controlling contamination more complex (Leister *et al.,* 2023).

2.4.1.3 Enteric Pathogenic Bacteria

Enteric bacteria such as *Salmonella*, *Shigella* and *Vibrio species* are often isolated from contaminated water and are responsible for causing typhoid fever, dysentery and cholera respectively (Chakraborty *et al.,* 2024). In a recent study in Bangladesh, Salmonella and Shigella were detected in significant proportions which indicates direct faecal input into the water systems (Chakraborty *et al.,* 2024). Vibrio species were also found which underscores the risk of cholera outbreaks in areas dependent on untreated water sources (Chakraborty *et al.,* 2024). Researchers in China also detected *Clostridioides* difficile, a bacterium commonly associated with severe diarrhoea, in river and groundwater samples which stresses the importance of continuous monitoring and advanced treatment before human consumption (Liu *et al.,* 2022).

2.4.1.4 Opportunistic Pathogenic Bacteria

Bacteria such as *Legionella*, *Pseudomonas* and *Mycobacterium* are considered opportunistic pathogens as they primarily affect immunocompromised individuals but can still pose risks to healthy people (Gomez-Alvarez *et al.,* 2015). In a metropolitan distribution system study, significant levels of these bacteria were found in both groundwater and surface water samples which indicates that water treatment processes must address these microorganisms effectively to prevent outbreaks (Gomez-Alvarez *et al.,* 2015). Studies have shown that these bacteria often persist in biofilms within distribution systems where they are protected from disinfectants which complicates their control (Liu *et al.,* 2018). This biofilm association means that even if the water appears clean at the source, bacteria can proliferate during distribution leading to exposure risks at the tap (Liu *et al.,* 2018).

2.4.1.5 Cyanobacteria

Cyanobacteria, also known as blue-green algae, are photosynthetic bacteria found in freshwater sources and can produce toxins harmful to humans and animals (Han *et al.,* 2020). Researchers in China highlighted that these bacteria were categorized as resistant taxa and often persisted through treatment processes especially in reservoirs and surface water sources (Han *et al.,* 2020). This persistence is concerning because the toxins they release can cause liver damage, neurological effects and gastrointestinal illnesses (Han *et al.,* 2020). Their growth is often encouraged by high nutrient levels and warm temperatures, which makes them a common concern during certain seasons, particularly in tropical and subtropical regions (Han *et al.,* 2020).

2.4.1.6 Actinobacteria

Actinobacteria are common in both surface and groundwater and are often responsible for earthy and musty odours in water (Gomez-Alvarez *et al.,* 2015). While not all species are

pathogenic, some can cause opportunistic infections particularly in people with weakened immune systems (Gomez-Alvarez *et al.,* 2015). Studies from China showed that *Actinobacteria* were among the dominant bacteria in treated and untreated water systems highlighting the need for proper control measures throughout the supply chain (Liu *et al.,* 2018). *Actinobacteria* have also been identified in biofilm communities which adds another layer of risk due to their ability to survive and persist within distribution pipelines (Liu *et al.,* 2018).

2.4.1.7 Other Indicator and Non-Indicator Bacteria

Besides the major groups, studies have detected bacteria like *enterococci*, *Clostridium perfringens* and *Staphylococcus* ***aureus*** in various water sources indicating different forms and sources of contamination (Liu *et al.,* 2022). *Enterococci* are especially significant as indicators of faecal contamination and have been widely used in microbial water quality assessments (Leister *et al.,* 2023). *Clostridium perfringens* was found in river water and groundwater, signifying possible long-term or intermittent contamination events (Liu *et al.,* 2022). Rare bacteria, sometimes referred to as the rare biosphere, also play roles in shaping microbial communities in water sources and can affect water quality through biogeochemical cycles (Dang *et al.,* 2022). These rare taxa can mediate environmental changes and influence the succession of more dominant bacteria making them important even if present in low abundances
(Dang *et al.,* 2022).

2.5 Antibiotic Resistance in Waterborne Pathogens

Antibiotic resistance in waterborne pathogens has become one of the most pressing challenges affecting both environmental safety and human health across the world, a situation that continues to worsen each year as a result of excessive and inappropriate use of antibiotics (Grobelak et al., 2020; Jackson et al., 2020). Pathogens found in water sources have developed

resistance mechanisms that allow them to survive even in the presence of antibiotics that were previously effective (Kaushik *et al.,* 2019). The main drivers of this resistance include indiscriminate antibiotic use in human medicine, animal farming, and the release of pharmaceutical residues into water bodies (Adefisoye and Olaniran, 2022). It has been confirmed that water environments act as reservoirs and mixing grounds for antibiotic-resistant bacteria and genes, which can then spread rapidly to humans and animals through multiple exposure routes (Sanganyado and Gwenzi, 2019). The presence of antibiotic-resistant bacteria in water systems is greatly facilitated by the genetic adaptability of microbes, including the use of mobile genetic elements such as plasmids and integrons, which aid in the horizontal transfer of resistance genes between different bacterial species (Kaushik *et al.,* 2019). When bacteria acquire these genetic elements, they can withstand different antibiotics, leading to the emergence of multidrug-resistant strains, a phenomenon that has been reported globally (Farkas et al., 2016). Recent metagenomic studies have revealed that waterborne bacteria often carry multiple resistance genes simultaneously, making them difficult to control and treat if infections occur (Ghadigaonkar and Rath, 2023).

Water distribution systems, especially in healthcare settings, have been shown to harbour biofilms that act as protective shelters for bacteria. Within these biofilms, bacteria can survive standard disinfection processes and continue to exchange resistance genes (Gholipour *et al.,* 2024). Biofilm formation on pipe surfaces and taps creates ideal conditions for the persistence and proliferation of antibiotic-resistant bacteria, posing a serious risk to patients in hospitals and communities relying on these water systems (Gholipour *et al.,* 2024). Chlorination, a common method used for water disinfection, has paradoxically been implicated in promoting antibiotic resistance among waterborne pathogens. A study by Adefisoye and Olaniran (2022) explained

that exposure to chlorine can create selective pressure, allowing resistant bacteria to thrive while susceptible ones are killed. This selective survival results in an increased proportion of resistant strains in treated water (Adefisoye and Olaniran, 2022). In addition, the chemical reactions induced by chlorination may facilitate the expression and activation of resistance genes, adding another layer of complexity to the challenge (Adefisoye and Olaniran, 2022).

Extended-spectrum beta-lactamase (ESBL) producing bacteria have been frequently detected in various water samples, illustrating the alarming spread of resistance to critical antibiotics such as cephalosporins and carbapenems (Salamandane et al., 2022). In Mozambique, a high level of resistance was recorded in Klebsiella and Aeromonas species isolated from street water, revealing significant resistance to amoxicillin, cefoxitin, and even imipenem (Salamandane *et al.,* 2022). The presence of ESBL and AmpC beta-lactamase genes among these isolates highlights the risk of severe infections in humans consuming or contacting contaminated water (Salamandane *et al.,* 2022). Integrons play a critical role in the spread of antibiotic resistance, acting as gene capture systems that can acquire and express resistance genes from various sources (Kaushik *et al.,* 2019). Class 1 integrons have been widely identified in Enterobacteriaceae from water samples, indicating anthropogenic contamination and suggesting that wastewater effluent is a significant contributor to the spread of resistance (Farkas *et al.,* 2016). These integrons often carry multiple resistance gene cassettes, which enable bacteria to resist different classes of antibiotics, further complicating treatment options (Kaushik *et al.,* 2019).

A recent review by Yu *et al*. (2022) discussed the unexpected role of protists in facilitating antibiotic resistance transfer in aquatic environments. Protists can act as hosts and vectors, providing an environment for bacterial growth and gene exchange, which accelerates the

dissemination of resistance genes (Yu *et al.,* 2022). This complex ecological interaction underscores the fact that controlling antibiotic resistance requires not only targeting bacteria but also considering their relationships with other microorganisms (Yu *et al.,* 2022).

Drinking water systems have also been identified as routes for exposure to antibioticresistant bacteria and genes. Sanganyado and Gwenzi (2019) reviewed data showing that both treated and untreated water supplies may contain resistant strains, highlighting gaps in water treatment processes. Even when advanced treatments like UV irradiation and oxidative processes are employed, there is evidence that disinfection can sometimes promote horizontal gene transfer, making bacteria even more resilient (Sanganyado and Gwenzi, 2019). In Cameroon, Enterobacter cloacae, Citrobacter freundii, Salmonella typhi and Shigella sonnei were isolated from various water sources and showed high levels of resistance to several antibiotics including β-lactams, aminoglycosides and quinolones (Njoya *et al.,* 2021). The multidrug resistance observed among these isolates indicates a serious health risk to communities relying on these water sources (Njoya *et al.,* 2021). The study further revealed that bacteria isolated from wastewater and surface water exhibited higher resistance levels compared to those from groundwater, suggesting that contamination from human and animal waste plays a significant role in resistance spread (Njoya *et al.,* 2021). In an editorial by Suzuki *et al*. (2017), it was emphasized that aquatic systems serve as both reservoirs and conduits for antibiotic-resistant bacteria and genes. The release of treated and untreated wastewater into rivers and lakes introduces large quantities of resistant bacteria into natural ecosystems, where they can persist and interact with indigenous microbial communities (Suzuki *et al.,* 2017). Agricultural runoff containing antibiotic residues from livestock further exacerbates this situation, intensifying selective pressures in water environments (Suzuki *et al.,* 2017). Advanced research has started

exploring new technologies to control antibiotic resistance in water systems. A recent study by Yu *et al*. (2024) introduced a piezoelectric membrane strategy combined with peroxymonosulfate activation, which showed promising results in significantly reducing antibiotic-resistant bacteria and genes from water samples (Yu *et al.,* 2024). This approach uses reactive oxygen species generated through piezoelectric activation to disrupt resistance mechanisms, representing a novel direction in water treatment (Yu *et al.,* 2024).

Despite these technological advancements, the persistence of resistant bacteria remains a concern because resistance genes can linger in the environment even after bacterial cells are inactivated (Ghadigaonkar and Rath, 2023). Residual DNA carrying resistance traits can be taken up by other bacteria, a process known as transformation, which ensures the continuation of resistance within microbial communities (Ghadigaonkar and Rath, 2023). This phenomenon highlights the importance of targeting both live bacteria and free-floating genetic materials in water treatment strategies (Ghadigaonkar and Rath, 2023). Studies have also shown that fish and other aquatic organisms can act as reservoirs for antibiotic-resistant bacteria, posing additional risks to humans who consume seafood (Jackson *et al.,* 2020). The transfer of resistant pathogens through food chains not only threatens human health but also has economic implications due to restrictions on fish trade and increased healthcare costs (Jackson *et al.,* 2020). Aquaculture practices that involve the use of antibiotics for disease prevention contribute further to the problem, creating environments where resistant strains can develop and spread (Jackson *et al.,* 2020). The role of biofilms cannot be overstated, as these microbial communities provide a haven where bacteria can survive harsh conditions and evade both physical and chemical treatments (Gholipour *et al.,* 2024). Biofilms in water distribution systems, industrial pipelines

and natural aquatic environments contribute to the persistence and spread of resistant bacteria, making their management crucial for public health protection (Gholipour *et al.,* 2024).

2.6 Regulatory Standards for Drinking Water Quality

Regulatory standards for drinking water quality are frameworks established to ensure that water supplied to the public is safe, acceptable and consistent with public health goals (Scholz, 2016; Van Winckel *et al.,* 2021). One of the core elements is the determination of maximum admissible concentrations for various contaminants. These concentrations, often referred to as guide levels and maximum admissible levels, serve as benchmarks for safe water and act as preventive measures against both short-term and chronic health effects (Scholz, 2016). Standards for drinking water set strict limits on microbiological content to prevent diseases like cholera and typhoid that are often spread through contaminated water (Baylis, 2016). In most regions, regulatory frameworks focus on both chemical and microbial safety, taking into account the potential presence of heavy metals such as lead, arsenic and cadmium, which can cause significant health problems even at low levels over time (Shi, 2023; Han *et al.,* 2023). In the European Union, for instance, the Drinking Water Directive sets standards for 48 parameters, including microbiological, chemical and indicator parameters, to ensure water is safe throughout distribution systems (Laaninen, 2018). The directive allows member states to implement additional national measures, provided these are stricter and designed to protect public health (Laaninen, 2018).

Globally, the World Health Organization guidelines often serve as a reference point for national standards, offering limits for a range of chemical substances, including nitrates, fluoride and pesticides (Van Winckel *et al.,* 2021). In many countries, the adoption of WHO guidelines is modified to reflect local environmental conditions, existing infrastructure and risk assessments

(Gokçekuş *et al.,* 2021). The Philippines revised its national standards to classify parameters into mandatory, primary and secondary, allowing for context-specific monitoring that prioritises both health and aesthetic concerns, such as taste and odour (Lomboy *et al.,* 2017). The approach to defining standards involves not only setting permissible limits but also outlining operational procedures for treatment plants, including disinfection methods and residual disinfectant levels, to control microbial contamination effectively (Bozorg-Haddad *et al.,* 2021). There is also a requirement for continuous monitoring, with defined frequencies and analytical methods specified to detect and manage non-compliance quickly (Scholz, 2016; Kriyt *et al.,* 2019).

Some countries, like China, have introduced extensive revisions to their national drinking water standards to address emerging contaminants and adapt to new scientific knowledge. The latest Chinese standards, updated in 2022, now include a broader range of biological, chemical and physical risk factors, reflecting growing awareness of complex environmental and industrial pollutants (Shi, 2023; Han *et al.,* 2023). This update also involves more detailed management requirements across the entire water supply process, highlighting a shift towards a more holistic, preventative approach rather than relying only on end-point testing (Han *et al.,* 2023).

Temporary deviations from standard limits are sometimes permitted during infrastructure upgrades or emergencies, but these deviations must be strictly controlled and justified to avoid jeopardising public health (Novikov *et al.,* 2021). In Russia, temporary deviations were systematically assessed to allow for a phased improvement in water treatment facilities, while ensuring that health risks remain minimal during transitional periods (Novikov *et al.,* 2021).

Harmonisation of standards remains a challenge due to differing national priorities and technical capacities. While some regions enforce stringent standards, others adopt more flexible measures influenced by economic and technological constraints (Van Winckel *et al.,* 2021;

Gokçekuş *et al.,* 2021). Studies have highlighted that even within Europe, variations in the strictness of chemical standards are common, which can impact the level of health protection afforded to citizens (Van Winckel *et al.,* 2021). The importance of aesthetic parameters such as colour, turbidity and taste has also been increasingly recognised, as these factors influence consumer trust and acceptance of water supplies (Yu *et al.,* 2024). While these parameters do not typically pose direct health risks, their control is vital for ensuring that people continue to use safe water sources rather than turning to potentially unsafe alternatives (Yu *et al.,* 2024).

Recent studies have emphasised the need for a risk-based approach to water safety, integrating regular hazard assessments and adaptive management plans into regulatory frameworks (Shi, 2023; Zhao *et al.,* 2018). This involves updating standards dynamically to account for new contaminants and changing environmental conditions, rather than relying on fixed lists of parameters (Zhao *et al.,* 2018). In the context of chemical contaminants, countries like Russia have developed harmonised hygienic standards that integrate both national and international experiences, addressing inconsistencies that could undermine effective water quality assessments (Kriyt *et al.,* 2019). Comparative evaluations of different national standards show that some countries, like Australia and Japan, maintain very strict drinking water limits, reflecting a strong precautionary stance, while others follow WHO guidelines more closely with minor adjustments (Nnaji *et al.,* 2016). The inclusion of socio-economic factors in standard setting, especially in developing regions, has been increasingly discussed. In India, the regulatory process for bottled water standards revealed the influence of large industries and the limited inclusion of consumer and environmental considerations, which can lead to standards that do not fully reflect public health needs (Sharma, 2023). Technical and operational standards also include requirements for materials used in distribution systems to prevent contamination through

corrosion or leaching, which has been an area of concern under the European Drinking Water Directive revisions (Laaninen, 2018). This aspect extends beyond water treatment and focuses on maintaining quality until the point of use, a critical step in water safety management (Laaninen, 2018).

While many national frameworks have improved compliance rates over recent decades, there are still challenges related to enforcement and monitoring, especially in areas with fragmented regulatory oversight (Elbakidze and Beeson, 2021). In the United States, state-level heterogeneity in water quality regulations has sometimes led to temporary increases in violations, although these generally decline once systems adapt to new standards (Elbakidze and Beeson, 2021). Additionally, the need for public transparency and consumer information has been underscored by various studies, which recommend clearer communication about water quality parameters and potential health risks (Laaninen, 2018; Lomboy *et al.,* 2017). When consumers are informed about the sources and quality of their water, they are more likely to support investments in infrastructure improvements and comply with conservation measures (Lomboy *et al.,* 2017).

The global push towards harmonisation of water standards aims to create a more unified understanding of safe drinking water, while allowing flexibility to address local conditions and emerging threats such as microplastics and pharmaceuticals (Van Winckel *et al.,* 2021; Fawell, 2015). However, disparities in resources and technical capabilities continue to challenge full standardisation efforts, highlighting the importance of adaptive and context-sensitive regulatory frameworks (Van-Winckel *et al.,* 2021).

2.7 Empirical Review of Related Studies

Aleru *et al*. (2020) assessed borehole water sources in Gokana Local Government Area, Rivers State, Nigeria, to determine if they were safe for drinking and domestic use. Using in-situ and laboratory tests, they analysed 60 samples from 20 boreholes. Physicochemical results showed pH values between 6.3 and 7.7, temperatures from 27 to 30 degrees Celsius and low turbidity, all meeting acceptable standards. However, bacteriological findings revealed high counts of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*, which exceeded WHO limits of zero per 100 ml. Analytical proof confirmed these bacteria could cause severe gastrointestinal diseases. The authors recommend immediate treatment interventions and strict monitoring to ensure the water becomes safe for consumption. Atiku *et al*. (2018) conducted their study in Jabi, Abuja, Nigeria, aiming to compare the safety of river, well, borehole and sachet water sources. They collected samples and analysed them using standard laboratory methods. Physicochemical results showed that all water sources except the river had parameters within WHO limits, though river water had high turbidity and total dissolved solids. Bacteriological analysis detected *Escherichia coli, Pseudomonas* spp and *Aeromonas* spp in river and well water, with total coliform counts up to 103 cfu/ml, well above acceptable standards. Analytical tests confirmed sachet water as safest. They recommend prioritising proper treatment of river and well water and promoting public education on water hygiene. Akani *et al*. (2021) studied water stored in tanks across a tertiary institution in Port Harcourt, Rivers State, Nigeria, aiming to evaluate its safety for student use. Eighty samples were collected from 16 locations and examined using standard physicochemical and bacteriological tests. Physicochemical results showed pH ranged from 4.15 to 7.16, with conductivity values up to 364 µs/cm, and dissolved oxygen between 3.04 and 7.36 mg/l,

revealing some variations but mostly safe. Bacteriological findings included *Citrobacter* spp, *Klebsiella* spp, *Vibrio* spp and *Salmonella* spp, indicating contamination likely from poor maintenance. Analytical evidence confirmed bacterial counts surpassed safety limits. The authors recommend regular cleaning, strict maintenance schedules and covering tanks to protect water quality. Ndjouhoua *et al*. (2022) carried out their research in Angondjentom, Libreville suburb, Gabon, focusing on well water used by local residents. Twelve wells were examined through physicochemical and bacteriological analyses. Results showed pH levels within WHO limits, and conductivity increased during rainy seasons. Despite safe physicochemical parameters, bacteriological tests revealed high levels of pathogenic germs like coliforms, likely due to proximity to latrines and showers. Analytical proofs showed contamination risks increased significantly during rains. The authors recommend relocating wells away from contamination sources and implementing community sensitisation on hygiene and maintenance.

Sila (2019) evaluated rural water sources in Kenya, including rivers, springs and boreholes, to check compliance with Kenya Bureau of Standards and WHO guidelines. Using microbiological culturing and chemical assays, samples revealed mean turbidity of 0.78 NTU for boreholes and up to 23,830 CFU/100 ml coliforms in rivers. Boreholes showed low contamination while rivers had *Shigella*, *Salmonella* and *Vibrio cholerae*. Physicochemical parameters like pH and colour were mostly acceptable. Analytical proofs linked high bacterial loads to upstream pollution and poor sanitation. The author recommends increasing borehole access, promoting water treatment at home and improving sanitation infrastructure. Baba *et al*. (2022) conducted their study in Lapai, Niger State, Nigeria, to assess water safety for domestic use. Using microbial culture methods and physicochemical assays, they found total viable counts up to 2.45 x 10^7 cfu/mg and coliform counts exceeding 1100 MPN index/100 ml. Dominant

bacteria included *E coli* and *Klebsiella* spp. Physicochemical tests showed varied pH, high conductivity and fluctuating nitrate levels, reflecting agricultural runoff and waste disposal issues. Analytical findings indicated severe health risks. The authors recommend urgent upgrades to water treatment systems, routine monitoring and educating residents on safe storage practices. Dunia *et al*. (2018) analysed water from Buhama and Kalimbi springs in South Kivu, Democratic Republic of Congo, to understand its safety for community use. Physicochemical results generally fell within WHO guidelines, including stable pH and low turbidity. However, bacteriological analysis found significant coliform and pathogenic bacteria presence in certain sites, indicating faecal contamination likely from runoff and sanitation failures. Analytical proofs from sanitary surveys supported contamination risks. The authors recommend establishing local water committees, strengthening water treatment measures and running community health education campaigns. Redouane (2020) investigated water from wells in Bas Cheliff area, Algeria, aiming to determine drinking safety. Chemical analysis showed high conductivity (up to 1293 μs/cm) and high total dissolved solids due to fertiliser runoff. pH values were relatively stable. Bacteriological tests confirmed presence of faecal bacilli, suggesting contamination from agricultural activities and domestic waste. Analytical proofs highlighted increased risks during rainy seasons. The author recommends reducing fertiliser use near wells and improving construction to prevent surface runoff entry. Tyagi *et al*. (2015) studied drinking water sources across Uttarakhand, India, to assess health risks. They examined both raw and supply water. Raw sources showed high total and faecal coliform counts, while supply water showed reduced contamination, reflecting treatment success. Physicochemical findings such as pH and turbidity mostly met WHO standards, but some areas exceeded safe turbidity levels. Analytical proofs

confirmed disparities in treatment efficiency between regions. The authors recommend enhancing water treatment coverage and ongoing microbial monitoring to ensure safe supply.

Onivefu *et al*. (2024) examined sachet water sold in Sagamu, Ogun State, Nigeria, to check if it met safety standards. They used physicochemical tests and microbial culture methods. Findings showed acidic pH values (4.73 to 6.10) and bacterial counts near acceptable limits, with total enteric bacteria ranging from 280 to 380 cfu/100 ml. Physicochemical results mostly aligned with WHO standards, except for pH. Analytical evidence suggested contamination could occur during production or distribution. The authors recommend stricter monitoring of sachet water producers and reinforcing hygiene practices among handlers to improve consumer safety.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Sampling Location

This study was carried in hostels at Ekenwan Campus, University of Benin, Benin City, Edo State. Edo State is located in Southwestern part of Nigeria at longitude 6.6°N and 5.9°E. It is about 40 miles from the Gulf of Guinea.

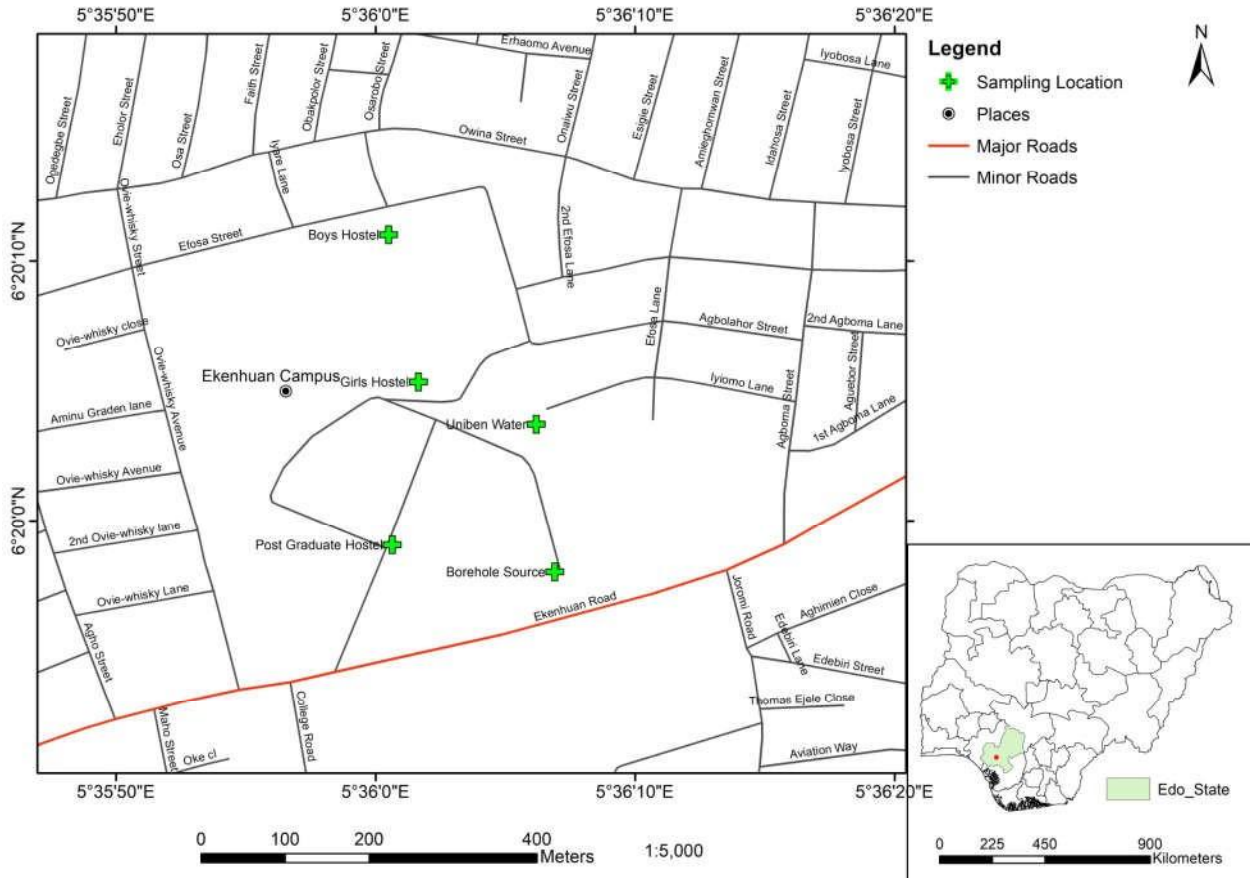

Figure 1.0: Map of Study Location (Ekenwan Campus, University of Benin, Benin City, Edo State).

3.1 Sample Collection

Eighteen water samples, collected in triplicate from six locations including Notre Dame table water, Uniben table Water, Uniben Borehole water source, Boy's hostel, Postgraduate hostel and Girl's hostel, were obtained between 7.00 am and 9.00 am on the day of sampling. Borehole nozzles were sterilised with 70% acetone, flushed for two minutes, and samples collected in sterile containers. All were labelled and transported in ice coolers for laboratory analysis within four hours (Ekhaise and Omavwoya, 2008; Thompson *et al*., 2012; Tsegahun *et al*., 2017).

3.2 Physicochemical Analysis

The water samples from all sampling points were analysed using standard methods of (AOAC, 2000)..

3.2.1 pH

The pH of water samples was measured at the sampling point using a calibrated pH meter (model 300408.1, Denver Instrument Company, USA). Calibration was done with buffers of pH 4.0 and 7.0. The probe was immersed in each sample until readings stabilised, expressed in pH units as hydrogen ion concentration. After each measurement, the probe was rinsed with deionised water to prevent cross contamination (Singh *et al*., 2020).

3.2.2 Electrical Conductivity

The electrical conductivity of the water samples was measured on site using a multi-parameter analyser (Hach model C0150). The meter was stabilised for 10 minutes, then calibrated with a potassium chloride standard solution. The probe was immersed in each sample, and readings taken once the stability indicator disappeared. After every measurement, the probe was rinsed with deionised water to prevent cross contamination (AOAC, 2000).

3.2.3 Total Dissolved Solids (TDS)

The TDS was determined using multiparameter analyzer (Hach model C0150). TDS was calibrated by pressing "TDS" and immersing probe in the Potassium Chloride solution provided. Then, the probe was rinsed and immersed in the sample solution. The TDS was read by pressing "TDS" (WHO, 1991)

Calculations:

The meter reads TDS directly in mg/l (APHA, 2005).

3.2.4 Turbidity

Twenty-five milliliters (25 ml) of water sample was poured into the cuvette and read at zero in the spectrophotometer at 450nm. Twenty-five milliliters (25 ml) of filtered water sample was poured into another cuvette and read in the spectrophotometer (PG 550 model) at 450nm (AOAC, 2000).

Calculation

Turbidity (NTU) = instr. Reading × Slope Reciprocal.

Where:

Instr. Reading = optical density (Absorbance); Slope Recip. =Standard reading.

3.2.5 Alkalinity Test

Hundred millilitres (100ml) of water was measured into a conical flask and phenolphthalein indicator was added (colour unchanged). A methyl orange indicator was added which changed the colour to yellow, 0.1M HCl was titrated against the mixture in conical flask up to the attainment of reddish coloration which marks the end point of the first titration where the reading was noted.

Then, the mixture in the conical flask was boiled and allowed to cool where the same 0.1M HCl again be titrated against the mixture (that is the second titration which gives a faint yellow coloration) and the second reading was noted (APHA, 1999).

Calculation:

The sum of first reading and second reading multiplied by 50 (which is the approved conversion factor) gives the total alkalinity of the final water.

3.2.6 Salinity as Chloride (Cl)

100ml of water sample was quantitatively measured into a 250-ml of conical flask, followed by the addition of 1ml of K_2CrO_4 indicator, and titrated with 0.014N $AgNO_3$. The mixture was fitrated from yellow to reddish colour, the colour changes from yellow to reddish brown at the end-point was observed and recorded. Thus, calculated as:

$$\text{Chloride } (Cl^-) \quad \frac{35.5 \times C_b \times V_b \times 1000}{\text{Vol. sample}}$$

Where C_b, = Concentration of AgNO, (Normality)

V_b = Volume of AgNO, (Consumed)

3.2.7 Total Hardness

50ml of water sample was quantitatively measured into 150ml capacity conical flask, added were 2ml of buffer solution and 2-drops of Eriochrome Black T indicator after which the mixture was titrated with 0.01N EDTA from wine colour to blue end-point. Total hardness was calculated as follows:

$$\text{Total Hardness as } CaCO_3, \text{ mg/l} = \frac{\text{ml of (EDTA) (0.01) 50 x 1000}}{\text{Vol of sample (ml)}}$$

3.2.8 Calcium Hardness

50ml of sample was measured into 150 ml conical flask followed by addition of 1ml of 8. O N KOH. 4-drops of calcium indicator and titrated with 0.01N EDTA from wine colour to blue endpoint. The calcium hardness was calculated as follows:

$$\text{Ca as } CaCO_3 \text{ (mg/l)} = \frac{\text{(ml of EDTA) (0.01) 50 x 1000}}{\text{Vol of Sample}}$$

3.2.9 Magnesium Hardness

Mg as $CaCO_3$ = Total Hardness - Calcium Hardness (mg/l)

3.2.10 Sulphate (mg/l)

This was determined by Turbidimetric Method. A filtered quantity of sample was measured into conical flask and made up to 100ml with distilled water.5ml of conditioning reagent was added and stirred. 0.5g of barium chloride crystal was then added and stirred again. after one minute the absorbance was read at 420nm.

3.2.11 Nitrate (mg/1)

Fifty (50.0) ml of filtered water sample was measured into an evaporating dish and evaporated to dryness, After cooling, 1ml phenoidisulphonic acid was added. the content of the evaporating dish was transferred into 50ml volumetric flask with 25- 35ml of distilled water. 4ml of ammonium hydroxide was added to develop the colour and diluted to a volume with distilled water. The blank was also carried out. The nitrate content in the sample was measured at 410 nm using the UV vis spectrophotometer.

3.2.12 Total Suspended Solid (TSS)

Dry a clean dish of suitable size at 103-105 ˚C in an oven until constant weight is achieved. Cool to room temperature in a desiccator. Note the weight, pipette after mixing thoroughly 100 ml of the samples accurately into a dish and evaporate to dryness on a steam bath. Wipe the outside of the dish and dry the residue in an oven for about 1 hour at 103-105 °C Transfer quickly the dish to a desiccator, cool to room temperature and weigh. Return the dish to the oven, dry further for 10-20 minutes, reweigh after cooling to room temperature Repeat until the weight of the dish plus residue is constant to within 0.05 mg, Subtract the weight of the dish to obtain the weight of the total solids

3.2.13 Phosphate (PO) APHA 425C

Measure 40ml of the sample, add 5ml of Antimony Molybdate to the solution, followed by2ml ascorbic acid. The blank solution is subjected to the same treatment as the sample. After about 1020 mins, measure the absorbance both sample and blank solution with UV-vis spectrophotometer at a wavelength of 880nm

3.3 Heavy Metal Analysis

The triple acid digestion method of Sahrawat *et al.* (2002) was employed. The sample (1ml) was weighed into a micro-kjeldahl digestion flask to which 20cm^3 of mixture of concentrated HNO_3, H_2SO_4, and 60 % $HClO_4$ (9:2:1 v/v) were added. The flask was put on a heating block and digested to a clear solution, cooled and the content transferred into a 100cm^3 volumetric flask and made-up to the volume mark with water. The solution was used for determination of mineral elements (lead, iron, manganese, copper, and silicon) (Sahrawat *et al.*2002).

3.4 Media Preparation

The media used for the culturing of bacteria were prepared according to the manufacturer's specifications. They include nutrient agar, MacConkey agar, eosine-methylene blue agar, *Salmonella-Shigella* agar and Potato Dextrose Agar (Cheesbrough, 2000).

3.4.1 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 then placed in an autoclave to sterilize for 15 minutes at 121

°C. After sterilization, the flask was allowed to cool (Cheesbrough, 2000) and then poured into sterilized petri-dishes.

3.4.2 McConkey Agar

A 52.0 g of MacConkey agar (MAC) was weighed and poured into a serial conical flask. One thousand milliliter (1000ml) of distilled water was added and the mixture was sterilized at 121°c for 15 minutes at 15 psi in the autoclave for even distribution of the agar. Twenty millilitres (20 ml) of the agar was poured in each sterilized petri-dishes.

3.5.3 Eosine-methylene Agar

Eosine-methylene blue agar was prepared by dissolving 500 ml of Eosin is a dye in 250 mls of distilled water and boil to completely dissolve agar. The media was sterilized for 15 minutes at 15 psi (121 ^{o}C). It was then cool to 45^{o}C and before pouring into sterile Petri dishes (Cheesbrough, 2006).

3.5.4 Potato Dextrose Agar

Potato Dextrose Agar (39 g) was dissolved in 1000 ml of distilled water in a conical flask. It was mixed thoroughly and sterilized by autoclaving at 121 ^{o}C for 15 minutes. The medium was cooled to 40^{o}C and then dispensed aseptically into sterile Petri dishes

3.6 Bacteriological Analysis of Water samples

3.6.1 Total heterotrophic bacteria count

The spread plate method was used for the determination of total heterotrophic bacteria count. Ten- fold serial dilution of each water sample was prepared aseptically in physiological saline 10^{-1} up to 10^{-4} and 0.1ml aliquot of each dilution was placed on Nutrient Agar plates. All incubation was conducted at 28- 37 ^{o}C for 24 hrs-48 hrs under aerobic conduction. After incubation the number of discrete colonies was counted in terms of colony forming units. Also, subculture was carried out on MacConkey agar, eosine-methylene blue agar and *Salmonella-Shigella* Agar for identification of bacteria species (Cheesebrough, 2006).

$$\frac{\text{Total Count}}{\text{Volume of Aliguot}} \times \frac{\text{Dilution Factor}}{1}$$

3.6.2 Identification of Organisms

Isolated bacteria species were identified based on their cultural, morphological and biochemical characteristics. Biochemical test such as, Gram staining, mortality, indole, citrate, oxidase, catalase, coagulase and sugar fermentation test were used in the characterization and identification of the isolates based on the guidelines in Bergey's manual of systematic Bacteriology.

3.7 Microscopic Identification of Fungal Isolates

Potato dextrose agar plates were incubated at 28±2°C for 3 to 5 days, after which fungal colonies were enumerated and identified based on morphological and colonial characteristics following Pitt *et al*. (1992). A portion of each colony was mounted on slides with lactophenol cotton blue, covered and examined microscopically to observe hyphal structures for proper identification.

3.8 Antibiotic Sensitivity Testing

The susceptibility test was conducted following the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2006) using the agar diffusion method on Mueller-Hinton agar with the Kirby-Bauer modified disc diffusion technique. Ten antibiotic discs corresponding to drugs commonly applied in human and animal infections were used, including pefloxacin (10 µg), gentamycin (25 µg), ciprofloxacin (10 µg), azithromycin (30 µg), levofloxacin (20 µg) and ceftriaxone (20 µg). Pure colonies of pathogenic bacterial isolates from selective agar were suspended in 1 ml sterile normal saline and adjusted to a 0.5 McFarland standard. Sterile Mueller Hinton agar plates were inoculated by spreading 0.1 ml of this suspension over the surface, air dried, and antibiotic discs applied within 15 minutes at 15 mm spacing. Plates were incubated at 37°C for 24 hours, after which inhibition zones were measured and interpreted following CLSI guidelines (CLSI, 2017), with results classified as susceptible, intermediate or resistant (Lauderdale, 2003).

3.9 Plasmid Profile of Bacterial Isolation

Five millilitres (5 ml) of bacterial culture were centrifuged for 15–20 seconds at full speed, after which the supernatant was discarded and 200 µl of Pl buffer (red) added. The pellet was resuspended by vortexing before 200 µl of P2 buffer (green) was introduced and mixed by gentle

inversion until the solution became purple and viscous, indicating complete lysis. Neutralization was achieved with 400 µl of P3 buffer (yellow), producing a yellow solution which was incubated at room temperature for 1–2 minutes before centrifugation for 2 minutes. The supernatant was transferred into a Zymo-spin IIN column and centrifuged for 30 seconds. Following disposal of the flow through, 200 µl of Endo-wash buffer (P4) and 200 µl of plasmid wash buffer were applied in sequence, with centrifugation steps of 30 seconds and 1 minute respectively. The column was transferred into a sterile 1.5 ml tube and 300 µl of DNA elution buffer was added for 30 seconds to elute plasmid DNA, which was analysed on 0.8 % agarose gel, visualised with a UV transilluminator, and recorded using the Gel Documentation System (Model G:BOX, Syngene).

3.10 Plasmid Curing

The plasmid curing study was performed for highly resistant isolate by physical method (treating cells at 45°C), as described by Fortina and Silva (1996). The isolate was inoculated in Luria broth

(Hi-Media) in duplicate. One flask was incubated at 37°C while the other at elevated temperature (45°C) overnight for plasmid curing. The curing was confirmed by loss of plasmid and antibiotic susceptibility testing using antibiotics to which organisms were resistant.

3.11 Data Analysis

The data generated were analyzed by one –way ANOVA (analysis of variance) using Genstat 12th edition analytical package as well as non-parametric t. test. Differences in mean were compared by Duncan's multiple range tests.

CHAPTER FOUR

4.0 RESULTS

4.1 Physicochemical Parameter

Physicochemical parameter of water samples from various hostels in University of Benin is shown in Table 4.1. pH values of the water samples ranged from 4.58±0.03 to 6.38±0.16, electrical conductivity (EC) ranged from 39.3±0.33 to 112.7±1.67 µs/cm, total dissolved solid ranged from 19.3±.033 to 56.0±1.00 mg/l, total suspended ranged from 19.3±0.33 to 56.0±1.00 mg/l, hardness ranged from 1.40±0.17 to 2.77±0.12 (mg/l), Alkalinity ranged from 0.83±0.05 to 1.58±0.19mg/l, Chloride ranged from 9.97±0.22 to 34.3±0.21 mg/l, Phosphate ranged from 0.11±0.01 to 0.27±0.01 mg/l, nitrate ranged from 0.08±0.00 to 0.66±0.01 mg/l, Sulphate ranged from 0.20±0.01 to 0.38±0.02 mg/l, Calcium ranged from 0.04±0.01 to 0.06±0.00mg/l, Magnesium ranged from

3.56±0.47 to 5.54±0.46 mg/l).

4.2 Heavy Metal

Sodium ranged from 0.02±0.00 to 0.03±0.00 mg/l, Potassium ranged from 0.34±0.0 to 0.61±0.03mg/l, Iron ranged from 0.56±0.01 to 1.06±0.05 mg/l, Zinc ranged from 0.31±0.03 to 0.56±0.03mg/l, Copper ranged from 0.08±0.00 to 0.26±0.03 mg/l while Chromium ranged from 0.10±0.04 to 0.25±0.02mg/l. pH, E.C., TDS, Hardness, Alkalinity, Chloride, Phosphate, Nitrate, Calcium, Magnesium, Sodium, Potassium, Zinc and Copper were within FEPA acceptable limit for drinking water while Iron and Chromium were above FEPA acceptable limit in drinking water respectively. Tested parameters such as pH, E.C., TDS, S.S, T.S, TUR, Hardness,

Alkalinity, Chloride, Phosphate, Nitrate, Sulphate, Potassium, Iron, Copper, Lead and Cadmium were significantly different ($P<0.05$) in all the water samples while Calcium, Magnesium, Sodium and

Chromium were not significantly different ($P>0.05$) (Table 4.2).

Table 4.1: Physicochemical parameter of water samples from various hostels in University of Benin

Parameter	Borehole water Source	GH	PG	Uniben	BH	NT	P value	FEPA limit
pH	4.64±0.07	4.58±0.03	5.26±0.16	5.97±0.05	4.68±0.05	6.38±0.16		6.5-8.5
E.C (µS/cm)	73.7±0.33	74.0±0.00	65.0±1.53	112.7±1.67	72.3±0.88	39.3±0.33	0.00	1000
TDS (mg/l)	37.0±0.00	37.0±0.00	32.3±0.88	56.0±1.00	35.7±0.67	19.3±.033	0.00	500
S.S (mg/l)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00	5
T.S (mg/l)	37.0±0.00	37.0±0.00	32.3±0.88	56.0±1.00	35.7±0.67	19.3±0.33	0.00	5
TUR (NTU)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00	1-5
Hardness (mg/l)	1.97±0.09	2.43±0.06	2.15±0.07	2.77±0.12	1.87±0.03	1.40±0.17	0.00	150
Alkalinity (mg/l)	0.90±0.04	1.38±0.07	1.15±0.03	1.58±0.19	0.97±0.03	0.83±0.05	0.00	100
Chloride (mg/l)	27.6±0.20	28.2±0.38	23.9±0.80	34.3±0.21	24.5±1.64	9.97±0.22	0.00	250
Phosphate(mg/l)	0.11±0.01	0.23±0.02	0.19±0.01	0.23±0.01	0.27±0.01	0.11±0.01	0.00	0.5
Nitrate (mg/l)	0.16±0.02	0.66±0.01	0.48±0.05	0.14±0.01	0.17±0.01	0.08±0.00	0.00	50
Sulphate (mg/l)	0.26±0.01	0.31±0.02	0.23±0.01	0.38±0.02	0.27±0.01	0.20±0.01	0.00	100
Calcium (mg/l)	0.04±0.01	0.04±0.01	0.06±0.00	0.05±0.01	0.04±0.00	0.05±0.00	0.24	75
Magnesium (mg/l)	5.54±0.46	3.56±0.47	3.85±0.11	5.45±0.14	5.09±0.49	4.77±0.69	0.09	50

KEYS:

BWS-Borehole Water Source, GH- girls hostel, PG- postgraduate hostel, NT-Notre Dame Table water, B- Uniben Table Water, BH-Boys hostel, EC- Electrical conductivity. TDS- Total dissolved solid, S.S. – Suspended solid, T.S.-Total solid, TUR- Turbidity, $P \geq 0.05$ = not significant, $P \leq 0.05$ = significant, FEPA = Federal Environmental Protection Agency

Table 4.2: Heavy metal parameter of water samples from various hostels in University of Benin

Parameter	Borehole Water Source	GH	PG	Uniben	BH	NT	P value	FEPA limit
Sodium (mg/l)	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.03±0.00	0.18	200
Potassium (mg/l)	0.57±0.03	0.61±0.03	0.41±0.03	0.43±0.03	0.42±0.06	0.34±0.03	0.00	10
Iron (mg/l)	1.06±0.05	0.76±0.01	0.64±0.02	0.98±0.03	0.56±0.01	0.69±0.02	0.00	0.3
Zinc (mg/l)	0.56±0.03	0.44±0.02	0.36±0.02	0.53±0.03	0.39±0.01	0.31±0.03	0.00	5.0
Copper (mg/l)	0.21±0.02	0.11±0.02	0.12±0.02	0.26±0.03	0.08±0.00	0.08±0.00	0.00	1.0
Lead (mg/l)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00	0.01
Cadmium (mg/l)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00	0.003
Chromium (mg/l)	0.23±0.10	0.25±0.02	0.23±0.10	0.25±0.02	0.10±0.04	0.19±0.01	0.27	0.05

KEYS:

BWS-Borehole Water Source, GH- girls hostel, PG- postgraduate hostel, NT-Notre Dame Table water, B- Uniben Table Water, BH-Boys hostel, EC- Electrical conductivity. TDS- Total dissolved solid, S.S. – Suspended solid, T.S.-Total solid, TUR- Turbidity, $P \geq 0.05$ = not significant, $P \leq 0.05$ = significant, FEPA = Federal Environmental Protection Agency

Table 4.3 shows the presumptive coliform bacteria count. Bacteria count from Notre Dame water ranged from $6.67\pm0.16\times10^3$ to $1.67\pm0.72\times10^4$ cfu/ml, Uniben Water ranged from $1.15\pm0.57\times10^4$ to $1.67\pm0.44\times10^4$ cfu/ml, Boys hostel water samples ranged from $1.33\pm0.17\times10^4$ to $2.83\pm0.17\times10^4$ cfu/ml, water samples from postgraduate hostel ranged from $1.10\pm0.57\times10^4$ to $2.02\pm0.93\times10^4$ cfu/ml while girls hostel water samples ranged from $2.24\pm0.56\times10^4$ to $2.70\pm0.15\times10^4$ cfu/ml. Coliform bacteria count in water samples from Notre Dame, Uniben Water and Girls hostel were not significantly different ($P>0.05$) while water samples from Source, Boys hostel and Postgraduate hostel were significantly different ($P<0.05$).

Table 4.4 shows the aerobic bacteria count in Notre Dame water ranged from $6.67\pm0.17\times10^3$ to $1.67\pm0.72\times10^4$ cfu/ml, Uniben Water samples ranged from $2.00\pm0.29\times10^4$ to $2.83\pm0.17\times10^4$ cfu/ml, water source samples ranged from $1.67\pm0.44\times10^4$ to $6.67\pm0.17\times10^4$ cfu/ml, water samples from Boys hostel ranged from $1.10\pm0.57\times10^4$ $2.83\pm0.17\times10^4$ cfu/ml, Postgraduate hostel water samples ranged from $1.83\pm0.17\times10^3$ to $8.67\pm0.19\times10^4$ cfu/ml while Girls hostel water samples $1.30\pm0.15\times10^4$ to $1.67\pm0.17\times10^4$ cfu/ml. Aerobic bacterial count in water samples from Uniben and Girls hostel were not significantly different ($P>0.05$) while aerobic bacterial counts Notre Dame, Source, Boys hostel and Postgraduate hostel water sources were significantly different ($P<0.05$).

Table 4.5 show the results of Fungal count in water samples from various hostels in University of Benin. Fungal count in Notre Dame water ranged from $1.00\pm0.00\times10^1$ to $2.33\pm0.33\times10^1$ cfu/ml, Uniben water samples ranged from $2.67\pm0.67\times10^1$ to $3.33\pm0.33\times10^1$ cfu/ml, Water source samples ranged from $1.00\pm0.58\times10^1$ to $2.00\pm0.00\times10^1$ cfu/ml, water samples from Boys hostel ranged from $1.00\pm0.00\times10^1$ to $2.00\pm0.12\times10^2$ cfu/ml, Postgraduate hostel samples ranged from

$1.33\pm0.67\times10^1$ to $1.00\pm0.12\times10^2$ cfu/ml while Girls hostel water samples ranged from $2.67\pm0.67\times10^1$ to $3.33\pm0.33\times10^1$ cfu/ml. Fungal counts in water samples from all hostels sampled were significantly different ($P<0.05$)

Table 4.6 represent the cultural, morphological and biochemical characterization of bacteria isolates. Bacterial isolated were two (2) Gram positive bacteria; *Staphylococcus aureus* and *Corynebacterium* spp and five (5) Gram negative bacteria; *Aeromonas hydrophila, Pseudomonas fluorescens, Klebsiella aerogens, Enterobacter aerogens* and *Chromobacterium violaceum.* Fungal isolated include *Penicillium notatum, Aspergillus flavus, Aspergillus niger* and *Microsporum audouinii*

Table 4.7 The percentage occurrence of bacterial isolates from water samples in the different hostels is shown in Table 4.8. Out of the 96 total number of bacterial isolated from the water samples, 13(13.5 %) were from Notre Dame, 24(25 %) were from Uniben Water, 21(21.9 %) were from Source, 6(6.25 %) were from Boys hostel, 19(19.8%) were from Postgraduate hostel while 13(13.5 %) were from Girl's hostel. *Klebsiella aerogens* (21.9 %) was the most occurring bacterial isolate while *Aeromonas hydrophila* was the least occurred bacterial isolate (9.4 %). The percentage occurrence of fungal isolates from water samples in the different hostels shows that 3(21.4 %) out of the total fungal isolates (14) were from Notre Dame, 3(21.4 %) were from Uniben Water, 2(14.3 %) were from Source, 2(14.3 %) were from Boys hostel, 2(14.3 %) were from postgraduate hostel while 2(14.3 %) were from girl's hostel. *Aspergillus flavus* (35.7 %) was the most occurring fungi isolate while *Microsporum audouinii* was the least occurred fungi isolate (14.3%)

Table 4.3: Presumptive coliform bacteria colony count (cfu/ml**) of water samples from various hostels in University of Benin**

Location	Sample 1	Sample 2	Sample 3
A	$1.67\pm0.72\times10^{4a}$	$8.33\pm0.16\times10^{3a}$	$6.67\pm0.16\times10^{3a}$
B	$1.67\pm0.44\times10^{4a}$	$1.67\pm0.44\times10^{4a}$	$1.15\pm0.57\times10^{4a}$
C	$0.00\pm0.00\times10^{4a}$	$0.00\pm0.00\times10^{4b}$	$0.00\pm0.00\times10^{4c}$
D	$1.33\pm0.17\times10^{4a}$	$2.83\pm0.17\times10^{4b}$	$2.00\pm0.29\times10^{4c}$
E	$1.33\pm0.17\times10^{4a}$	$1.10\pm0.57\times10^{4b}$	$2.02\pm0.93\times10^{4c}$
F	$2.24\pm0.56\times10^{4a}$	$2.60\pm0.30\times10^{4a}$	$2.70\pm0.15\times10^{4a}$

KEY

A- Notre Dame Table water, B- Uniben Table Water, C – Borehole Water Source, D- Boy's hostel, E- Postgraduate hostel and F- Girl's hostel.

Values are expressed as Mean ± Standard Error of triplicate experiments. Mean values with similar superscript within row are not significantly different from each other ($P>0.05$). Mean values with different superscript within rows are significantly different from each other ($P<0.05$).

Table 4.4: Aerobic bacterial count (cfu/ml**) of water samples from various hostels in University of Benin**

Location	Sample 1	Sample 2	Sample 3
A	$8.33\pm0.17\times10^{3a}$	$6.67\pm0.17\times10^{3b}$	$1.67\pm0.17\times10^{4c}$
B	$2.00\pm0.29\times10^{4a}$	$2.83\pm0.17\times10^{4a}$	$2.67\pm0.17\times10^{4a}$
C	$0.00\pm0.00\times10^{4a}$	$1.67\pm0.44\times10^{4b}$	$6.67\pm0.17\times10^{4c}$
D	$1.10\pm0.57\times10^{4a}$	$2.50\pm0.29\times10^{4b}$	$2.83\pm0.17\times10^{4c}$
E	$1.83\pm0.17\times10^{3a}$	$2.50\pm0.29\times10^{4b}$	$8.67\pm0.19\times10^{4c}$
F	$1.30\pm0.15\times10^{4a}$	$1.32\pm0.66\times10^{4a}$	$1.67\pm0.17\times10^{4a}$

KEY

A- Notre Dame, B- Uniben Water, C – Source, D- Boy's hostel, E- postgraduate hostel and F- girl's hostel, cfu- coliform forming unit

Values are expressed as Mean ± Standard Error of triplicate experiments. Mean values with similar superscript within row are not significantly different from each other ($P>0.05$). Mean values with different superscript within rows are significantly different from each other ($P<0.05$).

Table 4.5: Fungal count (sfu/ml) **of water samples from various hostels in University of Benin**

Location	Sample 1	Sample 2	Sample 3
A	$1.00\pm0.00\times10^{1a}$	$2.33\pm0.33\times10^{1b}$	$0.00\pm0.00\times10^{1c}$
B	$2.67\pm0.67\times10^{1a}$	$2.67\pm0.67\times10^{1a}$	$3.33\pm0.33\times10^{1a}$
C	$0.00\pm0.00\times10^{1a}$	$1.00\pm0.58\times10^{1b}$	$2.00\pm0.00\times10^{1c}$
D	$0.00\pm0.00\times10^{1a}$	$2.00\pm0.12\times10^{2b}$	$1.00\pm0.00\times10^{1c}$
E	$1.00\pm0.12\times10^{2a}$	$0.00\pm0.00\times10^{1b}$	$1.33\pm0.67\times10^{1c}$
F	$3.33\pm0.33\times10^{1a}$	$0.00\pm0.00\times10^{1b}$	$2.67\pm0.67\times10^{1c}$

KEY

A- Notre Dame, B- Uniben Water, C – Source, D- Boys hostel, E- postgraduate hostel and F- girls hostel, sfu- spore forming unit

Values are expressed as Mean ± Standard Error of triplicate experiments. Mean values with similar superscript within row are not significantly different from each other ($P>0.05$). Mean values with different superscript within rows are significantly different from each other ($P<0.05$).

Table 4.6: Characterization and Identification of Bacterial Isolates

PARAMETERS	ISO 1	ISO 2	ISO 3	ISO 4	ISO 5	ISO 6	ISO 7
Cultural Shape	Circular	Circular	Irregular	Circular	Circular	Circular	Circular
Colour	Greyishwhite to creamy	Cream	White/grayish	Greenish	Cream	Cream	Purple
Size	Medium	Medium	Small	Medium	Medium	Medium	Medium
Elevation	Raised	Convex	Raise	Flat	Flat	Flat	Raised
Transparency	Opaque	Opaque	Translucent	Opaque	Raised	Raised	Opaque
Morphological							
Gram stain	Negative	Positive	Positive	Negative	Negative	Negative	Negative
Cell type	Rod	Cocci	Road	Rod	Rod	Rod	Rod
Cell arrangement	Single	Clusters	Palisade	Single	Single	Single	Single
Biochemical Urease	-	-	+	-	-	-	+
Indole	-	-	-	-	+	-	-
Citrate	+	-	-	+	-	+	+
Catalase	+	+	-	-	-	+	+
Coagulase	-	+	ND	ND	ND	ND	ND
Oxidase	+	-	-	+	+	-	+
Oxidative Fermentation Test							
Glucose	A	A	-	NR	A	A	A
Sucrose	A	A	-	NR	NR	NR	NR
Lactose	NR	A	NR	NR	NR	A	NR
Maltose				NR	NR	A	NR
Manitol			A	A		NR	NR
Isolates	*Aeromonas hydrophila*	*Staphylococcus aureus*	*Corynebacterium spp*	*Pseudomonas fluorescens*	*Klebsiella aerogens*	*Enterobacter aerogens*	*Chromobacterium violaceum*

KEY: + = POSITIVE, - = NEGATIVE, A=ACID

Table 4.7: Cultural and microscopic characterization of fungal isolates

Cultural	Green flat colony with Greenish white periphery colony	Black fluffy colony with yellow reverse side yellow	Cottony white colony with reverse side dirty white
Morphological			
Nature of hyphae	Septate	Non-septate	Septate
Colour of spore	Green	Brownish	Army green
Type of spore	Conidiophore Conidiophore	Conidiophore	Conidiophores
Appearance of special structure	Brush-like Branching Conidia	conidia	Foot cells Unbranching conidia
Fungal isolates	*Penicillium notatum* *Aspergillus flavus*	*Aspergillus niger*	*Microsporum audouinii*

Table 4.8: Percentage Occurrence of Bacterial Isolates from various Hostels in University of Benin

Bacteria isolates	A	B	C	D	E	F	Total	Occurrence (%)
Aeromonas hydrophila	1	2	4	0	2	0	9	9.4
Staphylococcus aureus	1	6	3	1	3	2	16	16.7
Corynebacterium spp	3	3	4	1	1	1	13	13.5
Pseudomonas fluorescens	0	3	3	0	4	3	13	13.5
Klebsiella aerogens	3	4	1	2	6	5	21	21.9
Enterobacter aerogens	2	5	2	1	3	1	14	14.6
Chromobacterium violaceum	3	1	4	1	0	1	10	10.4
Total	13(13.5 %)	24(25 %)	21(21.9%)	6(6.25%)	19(19.8%)	13(13.5%)	96	**100**

Key:

A- Notre Dame
B- Uniben Water
C – Source
D- Boy's hostel
E- Postgraduate hostel
F- Girl's hostel

Table 4.9: Percentage occurrence of fungal isolates from various hostels in University of Benin

Fungal isolates	**A**	**B**	**C**	**D**	**E**	**F**	**Total**	Occurrence (%)
Penicillium notatum	2	0	0	0	1	0	3	21.4
Aspergillus flavus	1	0	0	1	1	2	5	35.7
Aspergillus niger	0	2	1	1	0	0	4	28.6
Microsporum audouinii	0	1	1	0	0	0	2	14.3
Total	3(21.4%)	3(21.4%)	2(14.3%)	2(14.3%)	2(14.3%)	2(14.3%)	14	100

Key:

A- Notre Dame
B- Uniben Water
C – Source
D- Boy's hostel
E- Postgraduate hostel
F- Girl's hostel

Table 4.10 shows the antibiotic susceptibility pattern of isolated bacterial before plasmid curing. *Staphylococcus aureus* and *Pseudomonas fluorescens* were resistant to Amoxicillin, Augumentin, Pefloxacin, Azithromycin and Ceftriaxone. *Corynebacterium* spp was resistant to amoxicillin, azithromycin and ceftriaxone while *Aeromonas hydrophila* was resistant to amoxicillin and gentamycin. *Aeromonas hydrophila, Staphylococcus aureus, Corynebacterium* spp *Pseudomonas fluorescens, Klebsiella aerogens, Enterobacter aerogens* and *Chromobacterium violaceum* were susceptible to ciprofloxacin, pefloxacin, ofloxacin, levofloxacin and sparfloxacin. *Staphylococcus aureus* had the highest multidrug resistant index (0.8) followed by *Pseudomonas fluorescens* (0.5) and *Enterobacter aerogens* (0.5)

Table 4.11 shows the antibiotic susceptibility pattern of isolated bacterial after plasmid curing. *Aeromonas hydrophila, Staphylococcus aureus, Corynebacterium* spp*, Pseudomonas fluorescens, Chromobacterium violaceum* and *Klebsiella aerogens* isolated from Notre Dame, Uniben Water, water Source, Boys hostel and postgraduate hostel were all susceptible to the tested antibiotics while *Staphylococcus aureus, Pseudomonas fluorescens*, *Enterobacter aerogens*, *Chromobacterium violaceum* and *Aeromonas hydrophila* isolated from postgraduate and girls hostels were resistant to Amoxicillin, Augumentin, Pefloxacin and Ceftriaxone. *Staphylococcus aureus* and *Enterobacter aerogens* had the highest multidrug resistant index (0.5) followed by *Pseudomonas fluorescens* (0.4).

Table 4.10 shows the antibiotic susceptibility pattern of isolated bacterial before plasmid curing. *Staphylococcus aureus* and *Pseudomonas fluorescens* were resistant to Amoxicillin, Augumentin, Pefloxacin, Azithromycin and Ceftriaxone. *Corynebacterium* spp was resistant to amoxicillin, azithromycin and ceftriaxone while *Aeromonas hydrophila* was resistant to amoxicillin and gentamycin. *Aeromonas hydrophila, Staphylococcus aureus, Corynebacterium* spp *Pseudomonas*

fluorescens, Klebsiella aerogens, Enterobacter aerogens and *Chromobacterium violaceum* were susceptible to ciprofloxacin, pefloxacin, ofloxacin, levofloxacin and sparfloxacin. *Staphylococcus aureus* had the highest multidrug resistant index (0.8) followed by *Pseudomonas fluorescens* (0.5) and *Enterobacter aerogens* (0.5)

Table 4.11 shows the antibiotic susceptibility pattern of isolated bacterial after plasmid curing. *Aeromonas hydrophila, Staphylococcus aureus, Corynebacterium* spp*, Pseudomonas fluorescens, Chromobacterium violaceum* and *Klebsiella aerogens* isolated from Notre Dame, Uniben Water, water Source, Boys hostel and postgraduate hostel were all susceptible to the tested antibiotics while *Staphylococcus aureus, Pseudomonas fluorescens*, *Enterobacter aerogens*, *Chromobacterium violaceum* and *Aeromonas hydrophila* isolated from postgraduate and girls hostels were resistant to Amoxicillin, Augumentin, Pefloxacin and Ceftriaxone. *Staphylococcus aureus* and *Enterobacter aerogens* had the highest multidrug resistant index (0.5) followed by *Pseudomonas fluorescens* (0.4).

Table 4.10: **Antibiotic sensitivity patterns of bacterial isolates before plasmid curing**

Samples	Isolates	CPX	AM	AU	CN	PEF	PFX	AZ	LEV	CF	SP	MDR
A1	*Aeromonas hydrophila*	32(S)	18(S)	12(R)	20(S)	30(S)	30(S)	30(S)	30(S)	26(S)	28(S)	0.1
A2	*Staphylococcus aureus*	30(S)	19(S)	20(S)	28(S)	30(S)	30(S)	30(S)	34(S)	34(S)	34(S)	0.0
A3	*Corynebacterium spp*	22(S)	9(R)	18(S)	13(R)	22(S)	22(S)	20(S)	22(S)	15(I)	20(S)	0.2
B1	*Aeromonas hydrophila*	22(S)	11(R)	19(S)	10(R)	22(S)	24(S)	20(S)	20(S)	15(I)	20(S)	0.2
B2	*Pseudomonas fluorescens*	32(S)	18(S)	10(R)	14(I)	20(S)	24(S)	30(S)	30(S)	22(S)	30(S)	0.1
B3	*Aeromonas hydrophila*	22(S)	10(R)	19(S)	8(R)	20(S)	22(S)	20(S)	20(S)	15(I)	30(S)	0.2
C1	*Corynebacterium* spp	30(S)	13(R)	20(S)	24(S)	26(S)	24(S)	11(R)	26(S)	12(R)	28(S)	0.3
C2	*Corynebacterium spp*	30(S)	10(R)	22(S)	24(S)	26(S)	24(S)	11(R)	28(S)	11(R)	28(S)	0.3
C3	*Aeromonas hydrophila*	34(S)	30(S)	32(S)	26(S)	30(S)	32(S)	32(S)	32(S)	32(S)	34(S)	0.0
D1	*Klebsiella aerogens*	28(S)	14(I)	19(S)	20(S)	20(S)	22(S)	24(S)	24(S)	18(S)	20(S)	0.0
D2	*Aeromonas hydrophila*	26(S)	11(R)	10(R)	28(S)	16(I)	10(R)	28(S)	28(S)	13(R)	20(S)	0.4
D3	*Enterobacter aerogens*	34(S)	28(S)	13(R)	20(S)	34(S)	34(S)	34(S)	34(S)	34(S)	34(S)	0.5
E1	*Chromobacterium violaceum*	34(S)	28(S)	13(R)	20(S)	34(S)	34(S)	34(S)	34(S)	34(S)	34(S)	0.1
E2	*Staphylococcus aureus*	11(R)	11(R)	8(R)	13(R)	12(R)	10(R)	10(R)	16(I)	12(R)	16(I)	0.8
E3	*Pseudomonas fluorescens*	26(S)	12(R)	12(R)	28(S)	10(R)	14(I)	10(R)	26(S)	12(R)	34(S)	0.5
F1	*Enterobacter aerogens*	34(S)	20(S)	16(I)	13(R)	10(R)	30(S)	34(S)	34(S)	34(S)	30(S)	0.2
F2	*Chromobacterium violaceum*	22(S)	13(R)	10(R)	26(S)	10(R)	26(S)	24(S)	28(S)	24(S)	28(S)	0.3
F3	*Aeromonas hydrophila*	34(S)	22(S)	28(S)	28(S)	22(S)	26(S)	34(S)	34(S)	34(S)	34(S)	0.0

PEF: Pefloxacin, CN: Gentamycin, CPX: Ciprofloxacin, PFX: Pefloxacin, AZ, Azithromycin, LEV: Levofloxacin and CF: Ceftriaxone AM = Amoxicillin, SP = Sparfloxacin, AU = Augumentin S = Sensitive, R = Resistance, I = Intermediate R = 0-13, I = 14-17, S = 18 and above MDRI = Multiple drug Resistance index.
MDR index ≥ 0.2 (public health significance)

Table 4.11: Antibiotic sensitivity patterns of bacterial isolates after plasmid curing

Samples	Isolates	CPX	AM	AU	CN	PEF	PFX	AZ	LEV	CF	SP	MDR
A1	*Aeromonas hydrophila*	34(S)	22(S)	20(S)	24(S)	30(S)	30(S)	30(S)	30(S)	28(S)	30(S)	0.0
A2	*Staphylococcus aureus*	26(S)	20(S)	24(S)	26(S)	18(S)	28(S)	26(S)	28(S)	28(S)	30(S)	0.0
A3	*Corynebacterium spp*	24(S)	20(S)	22(S)	20(S)	22(S)	24(S)	22(S)	24(S)	20(S)	26(S)	0.0
B1	*Aeromonas hydrophila*	24(S)	20(S)	24(S)	19(S)	24(S)	24(S)	22(S)	26(S)	19(S)	26(S)	0.0
B2	*Pseudomonas fluorescens*	34(S)	22(S)	24(S)	19(S)	26(S)	26(S)	24(S)	22(S)	20(S)	28(S)	0.0
B3	*Aeromonas hydrophila*	24(S)	20(S)	20(S)	20(S)	26(S)	26(S)	36(S)	36(S)	24(S)	36(S)	0.0
C1	*Corynebacterium spp*	34(S)	20(S)	20(S)	21(S)	26(S)	24(S)	28(S)	28(S)	20(S)	22(S)	0.0
C2	*Corynebacterium spp*	36(S)	18(S)	24(S)	26(S)	28(S)	24(S)	19(S)	28(S)	19(S)	28(S)	0.0
C3	*Aeromonas hydrophila*	32(S)	19(S)	28(S)	26(S)	28(S)	24(S)	21(S)	28(S)	18(S)	28(S)	0.0
D1	*Klebsiella aerogens*	36(S)	32(S)	32(S)	28(S)	30(S)	32(S)	34(S)	34(S)	36(S)	36(S)	0.0
D2	*Aeromonas hydrophila*	30(S)	24(S)	30(S)	28(S)	28(S)	28(S)	34(S)	36(S)	36(S)	32(S)	0.0
D3	*Enterobacter aerogens*	34(S)	20(S)	20(S)	24(S)	34(S)	34(S)	34(S)	34(S)	30(S)	22(S)	0.0
E1	*Chromobacterium violaceum*	30(S)	22(S)	22(S)	26(S)	26(S)	26(S)	24(S)	28(S)	24(S)	24(S)	0.0
E2	*Staphylococcus aureus*	26(S)	11(R)	8(R)	28(S)	10(R)	14(I)	10(R)	26(S)	12(R)	20(S)	0.5
E3	*Pseudomonas fluorescens*	26(S)	8(R)	10(R)	28(S)	8(R)	15(I)	14(I)	18(S)	12(R)	20(S)	0.4
F1	*Enterobacter aerogens*	28(S)	9(R)	10(R)	28(S)	10(R)	16(I)	8(R)	28(S)	10(R)	24(S)	0.5
F2	*Chromobacterium violaceum*	26(S)	20(S)	19(S)	30(S)	22(S)	24(S)	20(S)	28(S)	20(S)	26(S)	0.0
F3	*Aeromonas hydrophila*	34(S)	30(S)	20(S)	24(S)	34(S)	36(S)	36(S)	36(S)	34(S)	34(S)	0.0

PEF: Pefloxacin, CN: Gentamycin, CPX: Ciprofloxacin, PFX: Pefloxacin, AZ, Azithromycin, LEV: Levofloxacin and CF: Ceftriaxone AM = Amoxicillin, SP = Sparfloxacin, AU = Augumentin S = Sensitive, R = Resistance, I = Intermediate R = 0-13, I = 14-17, S = 18 and above MDRI = Multiple drug Resistance index.
MDR index ≥ 0.2 (public health significance)

CHAPTER FIVE

5.0: DISCUSSION

From Table 4.1, the pH values of all water samples, ranging from 4.58 to 6.38, fall below the FEPA recommended limit of 6.5 to 8.5 for drinking water. This indicates an acidic environment in all the sources assessed, which raises several public health and environmental concerns. Acidic water is naturally corrosive and enhances the solubility of toxic metals such as lead, copper, and zinc, increasing their concentrations in water and elevating the risks of metal toxicity and water pipe corrosion. These reactions can contribute to the metallic taste of water and infrastructural degradation, especially in plumbing systems (Arhin *et al*., 2023). Exposure to such water, especially over time, may lead to adverse symptoms including gastrointestinal irritation, diarrhoea, and organ damage due to heavy metal ingestion (Nordberg, 1990). Acidic pH has also been linked to the mobilization of metals like chromium, cadmium, and aluminium, potentially compounding toxicity risks and making water unsafe without treatment (Song *et al*., 2024). Electrical conductivity values recorded across all samples ranged from 39.3 to 112.7 µS/cm, remaining well within the FEPA threshold of 1000 µS/cm. This indicates a relatively low ionic concentration in the water, suggesting limited presence of dissolved salts and minimal mineralization. Electrical conductivity directly correlates with the total ionic content, reflecting the presence of inorganic ions such as calcium, sodium, chloride, and sulphate (Selvaraju *et al*., 2022). While these values appear non-threatening from a regulatory perspective, extremely low conductivity, as seen in the Notre Dame sample (39.3 µS/cm), could imply insufficient essential minerals, which may affect the palatability and physiological usefulness of the water (Yang *et al*., 2021). Research has shown that conductivity values below 100 µS/cm often indicate poor

buffering capacity and vulnerability to pH fluctuations, which may worsen corrosivity and lead to metal leaching in plumbing systems (Gonsor and Tomasik, 2017). The Total Dissolved Solids (TDS) concentrations recorded across the sampled water sources ranged from 19.3 mg/L in Notre Dame to 56.0 mg/L in the Boys Hostel. All values fall significantly below the FEPA maximum permissible limit of 500 mg/L for potable water. While this may indicate a relatively low presence of inorganic salts and dissolved minerals, extremely low TDS can result in flat taste and lack of essential minerals, which may affect palatability and long-term health if consumed continuously (Pushpalatha *et al*., 2022; Samuel, 2022). TDS values under 30 mg/L have also been associated with insufficient buffering capacity, which can make the water more vulnerable to pH fluctuations and corrosivity (Boyd, 2019). Water in this range might also be considered "too pure" by WHO standards, as drinking water with TDS levels below 100 mg/L has been found to contribute less to daily mineral intake and may even have a diuretic effect (Grafton *et al*., 2012). The observed low TDS in all hostel samples could be a result of excessive filtration, possibly reverse osmosis processes, or natural sources with low mineral content (Pushpalatha *et al*., 2022; Wang, 2021). Although health risks at these levels are minimal, the long-term consumption of such low-mineral water warrants monitoring. The suspended solids (S.S) values recorded across all hostel water samples were consistently 0.00 ± 0.00 mg/L, falling well below the FEPA guideline of 5 mg/L. This indicates an absence of visible or measurable particulate matter retained during filtration, suggesting relatively good clarity of the water sources. Although zero values may imply effective sediment removal or absence of pollution, such measurements must be interpreted cautiously, especially if sampling and analytical methods had limited sensitivity (Snazelle, 2019). Absence of suspended solids reduces risks of microbial attachment and sediment accumulation in distribution systems.

Suspended solids often serve as carriers of pathogens, heavy metals, and nutrients, elevating health and environmental risks when present (Roy and Majumder, 2018). Thus, the absence of suspended particles signifies a reduced likelihood of microbial shielding or turbidity-induced interference with disinfection. However, zero values across all locations may warrant method verification to ensure reliable detection. Total solids (T.S) measurements, which represent the sum of suspended and dissolved solids, ranged from 19.3 ± 0.33 mg/L (Notre Dame) to 56.0 ± 1.00 mg/L (Uniben borehole), all of which fall significantly below the FEPA permissible limit of 500 mg/L. These figures correspond closely with the TDS values reported, due to the absence of measurable suspended solids across all samples. Low T.S levels typically indicate clean water with minimal inorganic or organic contaminants and are generally desirable in potable supplies (Bhandari and Sapkota, 2023). However, excessively low total solids might suggest reduced mineral content, which can lead to corrosion in plumbing systems and poor taste perception (Boyd, 2019). The parallel between T.S and TDS values in this dataset reflects consistency in the absence of particulates and low ionic concentration. Water with such characteristics often originates from aquifers with minimal interaction with surrounding geology or from systems with thorough filtration. Turbidity readings across all samples were 0.00 ± 0.00 NTU, which is within the FEPA range of 1–5 NTU for drinking water. Turbidity serves as an important indicator of water clarity and potential contamination by particulate matter, including microbes. A turbidity value of zero suggests high visual clarity and minimal suspended particles (Sefa-Ntiri *et al*., 2014). Although this can reflect good water quality, particularly in relation to pathogen removal, it may also point to the limitations of detection if the instrument's sensitivity was low or improperly calibrated (Snazelle, 2019; Stevenson and Bravo, 2019). Low or undetectable

turbidity generally correlates with reduced microbial risk, improved disinfection efficacy, and enhanced aesthetic quality (Boyd,

2019). Nevertheless, persistent zero turbidity readings across multiple sources may necessitate cross-verification to ensure analytical accuracy. Realistically, trace turbidity is often present in natural waters, even if below detection thresholds. Hardness in the analysed water samples is considerably low, ranging between 1.40 mg/L and 2.77 mg/L, which is far below the FEPA limit of 150 mg/L. Water with such low hardness is typically termed as soft, and although not harmful, it can be corrosive to plumbing systems (Koçak *et al*., 2011). Hard water, within permissible levels, has been shown to provide essential minerals like calcium and magnesium that aid cardiovascular function (Karbasdehi *et al*., 2018; Koçak *et al*., 2011). In contrast, water with extremely low hardness, such as observed in the hostels, lacks these beneficial minerals and may increase the risk of metal leaching from pipes due to its corrosive nature (Pourfaraj and Mokhtari, 2021). The consistent softness across all hostels indicates limited contact with mineral-rich substrates or overtreatment through reverse osmosis which can strip water of minerals (Makwana *et al*., 2012). Alkalinity values in the water samples lie between 0.83 mg/L and 1.58 mg/L, significantly lower than the FEPA guideline of 100 mg/L. Such low alkalinity implies a limited buffering capacity, which means the water is less capable of neutralising acids, leading to potential pH fluctuations that can affect taste and metal solubility (Karbasdehi *et al*., 2018). It also reflects a deficiency of bicarbonates and carbonates, often tied to reduced geological interaction with carbonate-rich rocks (Sutcliffe, 2010). Continuous consumption of low-alkaline water might not have direct adverse health effects but increases vulnerability to acidic contaminants and pipe corrosion (Pourfaraj & Mokhtari, 2021). Similar low alkalinity patterns in rural drinking sources were reported in groundwater samples exposed to excessive purification

(Makwana *et al*., 2012), suggesting overtreatment or naturally low-mineral environments may be contributing factors here. Chloride levels across the hostel samples range from 9.97 mg/L to 34.3 mg/L, all well within the FEPA limit of 250 mg/L. These values are typical of fresh groundwater sources and pose no direct health risk (Karbasdehi *et al*., 2018). However, elevated chloride concentrations beyond natural background levels often signal contamination from human waste, agricultural runoff, or improper waste disposal (Muththamizh *et al*., 2023). The highest value recorded in the boys' hostel may reflect localised anthropogenic influences or differences in plumbing material corrosion. Sustained exposure to high chloride concentrations, while not a health hazard per se, could impact infrastructure by increasing water corrosiveness (Makwana *et al*., 2012). The phosphate concentrations range between 0.11 mg/L and 0.27 mg/L, remaining well below the permissible limit of 0.5 mg/L. These values are generally considered safe, yet their presence, even in trace quantities, usually points towards input from detergents, decaying organic matter, or fertiliser residues (Rajkumar *et al*., 2024). Although phosphate is not directly toxic, it promotes eutrophication if discharged into surface waters, disrupting aquatic ecosystems (Muththamizh *et al*., 2023). The relatively elevated value in the Notre Dame hostel may suggest nearby greywater infiltration or domestic effluent discharge, which aligns with studies that associate phosphate surges with urbanised residential areas (Shah & Trivedi, 2006). Nitrate concentrations across the water sources are very low, ranging from 0.08 mg/L to 0.66 mg/L, far below the FEPA and WHO limit of 50 mg/L. These low levels indicate minimal agricultural or sewage contamination, as elevated nitrates are typically associated with nitrogen-based fertiliser runoff or leachate from latrines (Muththamizh *et al*., 2023). However, higher concentrations in the postgraduate and Uniben samples, though not problematic, might be early indicators of leaching due to soil permeability or improper waste disposal practices (Rajkumar *et al*., 2024).

Regular monitoring is advisable, as nitrates pose significant risks to infants, potentially leading to methaemoglobinaemia or "blue baby syndrome" when concentrations exceed safe limits (Muththamizh *et al*., 2023).

Sulphate levels in the samples range between 0.20 mg/L and 0.38 mg/L, which is well below the 100 mg/L guideline. These concentrations are indicative of unpolluted groundwater and are unlikely to cause any odour, taste, or health issues. High sulphate levels, when present, have been known to produce laxative effects and contribute to gastrointestinal discomfort (Makwana *et al*., 2012). The low levels observed here suggest limited exposure to sulphate minerals or industrial discharges. Similar values were observed in decentralized municipal desalination systems, indicating either limited natural sulphate content or efficient removal during water treatment (Karbasdehi *et al*., 2018). Calcium concentrations in the samples range from 0.04 mg/L to 0.06 mg/L, which is considerably below the WHO acceptable guideline of 75 mg/L. Such low values reflect a deficiency in calcium-bearing minerals like limestone in the aquifer systems or overfiltration during water treatment (Koçak *et al*., 2011). While low calcium in water does not directly threaten health, it limits a source of dietary calcium intake, which plays a vital role in bone health and cellular functions (Makwana *et al*., 2012). Prolonged exposure to water with very low calcium can also impact the palatability and increase corrosion of distribution systems due to reduced mineral balance (Pourfaraj and Mokhtari, 2021). Magnesium concentrations in the samples range from 3.56 mg/L to 5.54 mg/L, remaining within the FEPA permissible limit of 50 mg/L. These values are adequate and reflect a natural balance in groundwater composition. Magnesium, similar to calcium, plays a protective role against cardiovascular diseases and contributes to enzyme activation and metabolic processes (Koçak *et al*., 2011). The moderate levels recorded here are not a cause for concern, though the boys' and girls' hostel samples

exhibited slightly higher values, potentially indicating contact with mafic or ultramafic rocks (Sutcliffe, 2010). Low magnesium waters have previously been associated with reduced health benefits, particularly among populations relying solely on such sources (Karbasdehi *et al*., 2018).

Table 4.2 presents concentrations of selected heavy metals in water samples from various hostels within the University of Benin, comparing them with FEPA limits to assess compliance and potential health risks associated with exposure through drinking water across different sources. The sodium concentration across all water sources in the sampled hostels remained constant at 0.02 mg/l, significantly below the FEPA limit of 200 mg/l. This uniformity suggests either a naturally low sodium presence in the aquifer or effective treatment that reduces sodium accumulation. Sodium is essential for fluid balance and nerve function, but excess intake through water can increase hypertension risks, particularly among salt-sensitive populations (Ferrante *et al*., 2013; Virkutyte and Sillanpää, 2006). Water with sodium below 20 mg/l is generally considered acceptable for people on low-sodium diets (Sharrett *et al*., 1982). In these samples, the negligible levels observed pose no health concern. Previous studies reported sodium concentrations in groundwater ranging from 13 to 45 mg/l in industrially influenced areas, where pollution and mineral dissolution elevate levels beyond the ideal range (Ibidunni, 2003). No such trend is evident here. The absence of anthropogenic sodium contributors, such as road salt runoff or domestic sewage, may also explain these consistent and low values. Potassium values ranged from 0.34 to 0.61 mg/l across all sources, with the highest concentration observed in the postgraduate hostel (PG). These levels are well below the FEPA guideline of 10 mg/l. Although potassium is a necessary micronutrient involved in cellular function, excess intake via drinking water is not typically a concern due to low concentrations and efficient renal excretion in healthy individuals (Ferrante *et al*., 2013). Elevated potassium in water, however, can be problematic for

individuals with kidney dysfunction (Basheer, 2020). In this case, the values reported are safe for all populations. Higher values in PG and GH might indicate minor contamination from organic matter decay or slight fertilizer infiltration, as potassium leaching is common in urban and peri-urban areas with vegetation or landscaped environments (Brima, 2017). Nonetheless, the low range affirms compliance with both WHO and FEPA standards, reflecting minimal risk and indicating no immediate anthropogenic interference. Iron concentrations spanned from 0.56 to 1.06 mg/l, with the highest levels found in GH and BH. These exceed the FEPA limit of 0.3 mg/l, indicating a potential contamination concern. Iron in drinking water, although not typically toxic, can cause aesthetic issues like metallic taste and staining of plumbing and laundry. Chronic exposure at elevated levels may also contribute to iron overload disorders in susceptible individuals (Krasnopyorova *et al*., 2024). Studies have identified iron concentrations up to 1.9 mg/l in untreated waters near mineral springs, suggesting natural geological leaching as a primary source (Virkutyte and Sillanpää, 2006). However, iron levels above permissible limits in urban groundwater have also been attributed to corrosion of old iron pipes and leaching from iron-rich soils (Ashaolu, 2020; Nnaji and Omotugba, 2014). Given that both GH and BH recorded the highest concentrations, pipe infrastructure or well lining materials may be contributing factors. This situation calls for monitoring and the use of iron-removal filtration systems. Zinc concentrations ranged from 0.31 to 0.56 mg/l, all within the FEPA limit of 5.0 mg/l. These values are considered safe and may even contribute beneficially to human health, as zinc is essential for immune function, enzymatic activity and wound healing. Drinking water typically contributes only a small portion of daily zinc intake, which mainly comes from food (Ferrante *et al*., 2013). Concentrations under 3 mg/l are not associated with any taste or colour issues, making these values acceptable from both health and aesthetic perspectives (Sharrett *et al*.,

1982). Sources of zinc in groundwater include natural mineral dissolution, corrosion of galvanized plumbing, and stormwater runoff (Lee *et al*., 2005). However, the lower values in NT and BH compared to GH and BH may point to variances in pipe materials or maintenance. These findings align with previous reviews showing zinc concentrations under regulatory thresholds in non-industrial regions (Boamah, 2018), confirming the absence of contamination concern in these samples. Copper levels in the water samples varied between 0.08 and 0.26 mg/l, all comfortably within the FEPA limit of 1.0 mg/l. The peak concentration recorded in BH might be attributed to leaching from copper piping, which is a known contributor to copper in domestic water (Sharrett *et al*., 1982). While copper is necessary for cardiovascular health and neurological function, concentrations exceeding 1.3 mg/l can cause gastrointestinal irritation, particularly in children (Ferrante *et al*., 2013). These values do not suggest any immediate health threats. However, slight elevation in some sources might still be reflective of mild pipe corrosion or the presence of copper-containing alloys in plumbing infrastructure (Basheer, 2020). The lowest values recorded in NT and PG could be linked to newer piping materials or less interaction with metallic components. All readings remain below threshold, indicating water quality is acceptable in terms of copper content. All water samples reported undetectable levels of lead (0.00 mg/l), which is a highly positive finding considering the FEPA limit of 0.01 mg/l. Lead exposure, even at low concentrations, has been linked to irreversible neurological damage, reduced cognitive performance in children, and increased cardiovascular risks in adults (Boamah, 2018; Krasnopyorova *et al*., 2024). The absence of lead across all sources suggests that plumbing systems in the sampled hostels are likely free from lead-containing materials, or the water chemistry does not promote lead leaching. These results contrast with earlier findings in urban settings where old plumbing systems led to levels exceeding permissible limits (Adeniyi *et al*.,

2016). The complete non-detection here offers strong assurance of safety, reinforcing the effectiveness of either infrastructure choices or corrosion control practices within the water supply system of the university hostels. Cadmium was not detected in any water sample, indicating a complete absence across all hostel sources. This is significant, as cadmium is a toxic metal with no known physiological function in the human body. Long-term exposure, even at low levels, can result in renal dysfunction, skeletal damage, and is classified as a human carcinogen (Ferrante *et al*., 2013). The FEPA limit of 0.003 mg/l is highly stringent due to its toxicity, and these results show complete compliance. Previous studies identified cadmium in urban waters primarily due to industrial discharge, battery disposal and deteriorating pipes (Bilal and Rahman, 2015). The non detection here aligns with findings in relatively unpolluted environments, implying minimal risk from anthropogenic activity and confirming that these water sources are safe from cadmium related health hazards. Chromium values ranged from 0.10 to 0.25 mg/l, all exceeding the FEPA permissible limit of 0.05 mg/l. This is concerning, as high chromium intake is associated with liver and kidney damage, and chromium VI in particular is a recognized carcinogen (Ferrante *et al*., 2013; Krasnopyorova *et al*., 2024). The elevated levels may stem from natural sources such as chromium-bearing rocks, or anthropogenic inputs including corrosion of metal fittings or nearby industrial discharge. Studies on surface water in mining areas have recorded chromium levels far above safe thresholds, attributing them to ore processing and waste discharge (Lee *et al*., 2005). While the exact oxidation state is not specified here, any chromium level above 0.05 mg/l should warrant attention. These readings may indicate contamination, and advanced treatment methods like reverse osmosis or ion exchange could be necessary to ensure long-term safety. The variations across sources suggest differences in plumbing or environmental exposure that need investigation. Table 4.3 presents the presumptive

coliform bacteria colony counts across six locations representing water sources from different hostels in the University of Benin. Values are reported in colony-forming units per millilitre (cfu/ml) and vary across sample replicates. The presence of coliform bacteria in drinking water is a recognized indicator of faecal contamination and microbial water quality deterioration. The WHO guideline for coliform bacteria in potable water is zero cfu per 100 ml, meaning any detectable presence indicates potential contamination and poses a health risk. The data in Table 4.3 shows that coliform bacteria were detected in nearly all water samples from the hostels, except those collected from location C (labelled as the source). Across the other locations, coliform counts ranged between 6.67×10^{3} and 2.24×10^{4} cfu/ml, which far exceeds the World Health Organization's acceptable limit of zero coliforms in 100 ml of drinking water (Brown *et al*., 2008; Pal, 2014). The consistently high values in samples from Notre Dame (A), Uniben water (B), and hostel sites (D, E, F) are indicative of contamination likely due to faecal intrusion, plumbing defects, or poor water storage conditions (Thom *et al*., 2024; LeChevallier, 2018). The water from location C, considered the primary source, showed no detectable coliforms across all samples, which points to contamination occurring during post-source handling or within the distribution systems (Nedelkova *et al*., 2019). Presence of coliforms has been directly linked to increased risks of gastrointestinal infections and other waterborne illnesses in previous investigations (Syamsussabri *et al*., 2019; Ogamba, 2013). The significantly high counts in location F (girl's hostel), peaking at 2.70×10^{4} cfu/ml in Sample 3, underline a critical microbial quality failure, which reflects poorly managed infrastructure or cross-contamination from sanitary sources (Jazrawi *et al*., 1988). Recent research has confirmed that the survival and multiplication of coliforms in distribution systems are often influenced by temperature, organic nutrients, and stagnation of water in pipelines (Camper *et al*., 1991; LeChevallier *et al*., 1991).

Therefore, the observed trends in this dataset suggest urgent attention to localised infrastructural and environmental factors contributing to post-treatment contamination. Given the public health implications of coliform presence, particularly in institutional settings, implementing stringent surveillance and water quality assurance is essential (Vergaray *et al*., 2007; Kutz, 1984).

Table 4.3 presents the presumptive coliform bacteria count in water samples from different hostel locations, highlighting the extent of microbial contamination and its potential health implications. The results show that coliform counts in Notre Dame water ranged from 6.67×10^{3} to 1.67×10^{4} cfu/ml, Uniben Water from 1.15×10^{4} to 1.67×10^{4} cfu/ml, Boys hostel from 1.33×10^{4} to 2.83×10^{4} cfu/ml, postgraduate hostel from 1.10×10^{4} to 2.02×10^{4} cfu/ml, and Girls hostel from 2.24×10^{4} to 2.70×10^{4} cfu/ml. The Source samples recorded 0.00 cfu/ml across all replicates, showing no detectable contamination. WHO and FEPA guidelines require potable water to be completely free of coliforms, meaning that all the hostel water sources except the Source exceed permissible limits and pose significant health risks (Pal, 2014). High coliform counts in Boys hostel and Girl's hostel water suggest faecal contamination likely linked to pipe leakages, poor sanitation, or infiltration of sewage into supply systems (Sari *et al*., 2024). The presence of coliforms is associated with diarrhoeal diseases, gastrointestinal infections, and waterborne outbreaks (Khan & Gupta, 2020). Persistent contamination at levels above 10^{3} cfu/ml indicates chronic exposure risks, especially in environments with dense populations like student hostels. This highlights the urgent need for infrastructural improvements, regular monitoring, and disinfection protocols to reduce contamination.

Table 4.4 presents aerobic bacterial counts in water samples from hostels at the University of Benin, reflecting general microbial load and the sanitary status of the supply. The results show that aerobic bacterial counts in the hostel water sources were considerably high, ranging from

6.67±0.17×10³ cfu/ml in Notre Dame to as much as 8.67±0.19×10⁴ cfu/ml in the postgraduate hostel. WHO recommends that heterotrophic plate counts should not exceed 500 cfu/ml in drinking water, and while they are not direct indicators of faecal contamination, elevated levels signify microbial regrowth, biofilm formation, or inadequate disinfection (WHO, 2017). Uniben water recorded consistently high values between 2.00±0.29×10⁴ and 2.83±0.17×10⁴ cfu/ml, while Girls hostel showed relatively stable counts of 1.30±0.15×10⁴ to 1.67±0.17×10⁴ cfu/ml. Postgraduate hostel water exhibited the highest variation, with values escalating from 1.83±0.17×10³ to

8.67±0.19×10⁴ cfu/ml, reflecting possible intermittent contamination or poor storage practices. Elevated aerobic bacteria count at such levels have been linked to increased survival of opportunistic pathogens including *Pseudomonas fluorescens*, *Aeromonas hydrophila*, and *Staphylococcus aureus*, which thrive in distribution systems with deteriorating infrastructure (Rusin *et al*., 1997; Yang, 2010). High microbial loads impair disinfection efficiency, promote antibiotic resistance, and increase risks of gastrointestinal infections, respiratory complications, and secondary microbial contamination (Ashbolt, 2015; Gong *et al*., 2023). The significant differences observed ($P<0.05$) in Notre Dame, Borehole Water Source, Boys hostel, and Postgraduate hostel samples highlight inconsistency in treatment and water handling. Such levels far exceed permissible standards, underscoring the urgent need for intervention through improved chlorination, infrastructure maintenance, and routine microbial monitoring.

Table 4.5 presents fungal counts in hostel water samples, measured in spore forming units (sfu/ml), which highlight the extent of fungal contamination in drinking water sources. The results indicate widespread fungal contamination across the hostel water supplies, with counts ranging from

1.00±0.00×10¹ sfu/ml in Notre Dame to as high as 2.00±0.12×10² sfu/ml in Boys hostel water. WHO has not set definitive fungal limits for potable water, yet fungal presence above 1–10 cfu/ml is regarded as indicative of contamination from distribution or storage systems (Mirshekar *et al*., 2019). Uniben water samples recorded stable fungal counts between 2.67±0.67×10¹ and 3.33±0.33×10¹ sfu/ml, while postgraduate hostel samples fluctuated between 1.33±0.67×10¹ and 1.00±0.12×10² sfu/ml, highlighting irregular contamination patterns. Boys hostel water showed the highest peak contamination (2.00×10² sfu/ml), which far exceeds values typically reported in safe drinking systems and signals possible biofilm growth or infiltration from plumbing infrastructure (Amadi-Ikpa *et al*., 2021). The most frequently isolated fungi in such environments include *Aspergillus flavus*, *Aspergillus niger*, *Penicillium notatum*, and *Candida* species, many of which are resistant to chlorine and capable of producing mycotoxins harmful to humans (Ma and Bibby, 2017; Mhlongo *et al*., 2020). These fungi can impair water taste, trigger allergic reactions, and present serious health risks to immunocompromised individuals, including aspergillosis and systemic fungal infections (Olawale *et al*., 2024). The significant differences across samples ($P<0.05$) demonstrate uneven contamination, pointing towards inconsistent maintenance of distribution and storage systems. Persistent fungal loads in potable water systems represent both a public health risk and a technical challenge, as conventional chlorination alone is insufficient for inactivation (Ali *et al*., 2017).

Table 4.6 presents the biochemical and morphological characterization of bacterial isolates, which revealed both Gram-positive and Gram-negative organisms including *Staphylococcus aureus*,

Corynebacterium spp., *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Klebsiella aerogenes*, *Enterobacter aerogenes* and *Chromobacterium violaceum*. The occurrence of these organisms in

drinking water represents a significant health concern since many are known pathogens or opportunistic bacteria. The isolation of *Staphylococcus aureus* and *Corynebacterium* spp. reflects contamination that may originate from human handling or poor sanitary conditions, and their presence has been linked to outbreaks of water-related diseases when untreated water is consumed (Agwaranze *et al*., 2017). The detection of *Aeromonas hydrophila* and *Pseudomonas fluorescens* is critical as these are waterborne organisms associated with gastrointestinal infections and opportunistic diseases, and both have been found frequently in biofilms of distribution systems where they persist despite disinfection (September *et al*., 2007; Papadopoulou *et al*., 2008). Similarly, *Klebsiella aerogenes* and *Enterobacter aerogenes* belong to the coliform group and are recognised indicators of faecal contamination, while their antibiotic resistance patterns raise further public health concerns (Bonso *et al*., 2023; Amoah *et al*., 2006). The presence of *Chromobacterium violaceum*, although rare, is alarming since it has been associated with severe systemic infections in immunocompromised individuals (Adeoyo and Omaku, 2022). These findings align with reports that untreated or poorly maintained water systems harbour multiple pathogenic species, often displaying resistance to common antibiotics, which complicates treatment outcomes (Gholipour *et al*., 2023; Onajobi *et al*., 2015). The isolates in Table 4.6 confirm that the water sources under investigation may pose risks of waterborne outbreaks, particularly among vulnerable groups, unless adequate treatment and monitoring strategies are enforced.

Table 4.7 presents the cultural and microscopic characterization of fungal isolates identified from the analyzed water samples. The fungal isolates identified in the water samples included *Penicillium notatum*, *Aspergillus flavus*, *Aspergillus niger* and *Microsporum audouinii*. These organisms are known contaminants of drinking water and have implications for human health,

environmental safety and water quality. The presence of *Penicillium notatum*, which produces brush-like conidia, has been widely reported in water systems and is associated with biofilm formation that enhances its persistence in distribution networks (Amadi-Ikpa *et al*., 2021). This organism can produce metabolites linked with musty odours in water, raising quality concerns (Zhao *et al*., 2024). *Aspergillus flavus*, identified through branching conidia and coffee-black spores, is more concerning as it is known to produce aflatoxins which are potent carcinogens, raising toxicological risks in consumers (Mhlongo *et al*., 2020). The detection of *Aspergillus niger*, characterized by black fluffy colonies and non-septate hyphae, is significant as this species is both an opportunistic pathogen and a contributor to organoleptic deterioration of drinking water, with mycotoxins that can affect liver and kidney function (Wen *et al*., 2021). The isolation of *Microsporum audouinii*, a dermatophyte with army green spores and unbranching conidia, indicates contamination that may have originated from human or environmental sources and raises dermatological health risks, especially in immunocompromised individuals (Mirshekar *et al*., 2019). The diversity observed in this water source reflects the increasing recognition of fungi as emerging waterborne contaminants capable of surviving treatment processes and disseminating through distribution systems (Oliveira *et al*., 2016). Their persistence highlights gaps in conventional treatment methods, as fungi are often resistant to chlorination and can form resilient spores (Ali *et al*., 2017). The presence of these isolates demonstrates a potential link between microbial water contamination and health outcomes, aligning with growing evidence that fungi in drinking water are underestimated contributors to waterborne diseases and aesthetic deterioration (Olawale *et al*., 2024).

Table 4.8 reveal the occurrence of bacterial isolates which 4.8 reflects a level of contamination, with a total of 96 isolates distributed across the hostels. *Klebsiella aerogens* accounted for the

highest proportion at 21.9% (21 isolates), showing strong presence in Uniben Water (4 isolates), Postgraduate hostel (6 isolates) and Girl's hostel (5 isolates). Such dominance is consistent with reports that *Klebsiella* species are common in contaminated water systems and can serve as indicators of faecal pollution, with potential to cause pneumonia, urinary tract infections, and gastrointestinal diseases (Abass *et al*., 2022; Mannapperuma *et al*., 2013). In contrast, *Aeromonas hydrophila* recorded the lowest occurrence at 9.4% (9 isolates), though its presence is still notable as *Aeromonas* species have been associated with diarrhoeal illness and wound infections in humans

(September *et al*., 2007). The moderate prevalence of *Staphylococcus aureus* (16.7%), *Corynebacterium spp.* (13.5%), and *Pseudomonas fluorescens* (13.5%) is significant because these organisms, while opportunistic, may form persistent biofilms in water systems that support survival and resistance to disinfection (Suthar *et al*., 2009; Vaz-Moreira *et al*., 2017). *Enterobacter aerogens* represented 14.6% of isolates, an observation aligned with reports of Enterobacteriaceae dominance in polluted water sources where poor sanitation is a major contributing factor (Choudhury *et al*., 2021). The identification of *Chromobacterium violaceum* (10.4%) also raises concern, as this rare organism can be opportunistic and cause systemic infections in immunocompromised individuals (Omari & Yeboah-Manu, 2012). The results indicate that

Uniben Water accounted for the highest total isolates (25%), followed by the Source (21.9%) and Postgraduate hostel (19.8%), suggesting multiple routes of contamination. Such microbial loads exceed permissible WHO and FEPA standards which require absence of pathogenic bacteria in potable water. The health implications include heightened risks of gastrointestinal infections,

opportunistic systemic infections, and antibiotic-resistant outbreaks linked to waterborne pathogens (Kamdar *et al*., 2025; Kumar *et al*., 2013).

Table 4.9 outlines the occurrence of fungal isolates across different hostel water samples, highlighting variations in prevalence and potential health implications. The fungal isolates identified in Table 4.9 reveal significant diversity, with *Aspergillus flavus* recorded as the most prevalent (35.7 %), followed by *Aspergillus niger* (28.6 %), *Penicillium notatum* (21.4 %), and *Microsporum audouinii* (14.3 %). The predominance of *Aspergillus* species in drinking water is concerning, as these fungi are well known for producing mycotoxins and for causing respiratory and systemic infections, particularly in immunocompromised individuals (Mhlongo *et al*., 2020). The high proportion of *A. flavus* is critical, given its ability to produce aflatoxins that have been associated with carcinogenic and hepatotoxic effects in humans (Mirshekar *et al*., 2019). The occurrence of *Penicillium notatum* at 21.4 % indicates its role as a persistent contaminant in water systems, with studies linking *Penicillium* species to biofilm formation in storage and distribution systems, thereby reducing water quality and increasing risk of opportunistic infections (AmadiIkpa *et al*., 2021). The detection of *Microsporum audouinii* (14.3%), though less frequent, is also relevant as this dermatophyte has been implicated in skin infections, suggesting contamination from anthropogenic sources. The World Health Organization does not set specific permissible limits for fungi in water but emphasizes that their presence poses indirect health risks, particularly through allergenic reactions, opportunistic infections, and mycotoxin exposure (Olawale *et al*., 2024). The fungal distribution observed here reflects inadequate water treatment and possible contamination from distribution pipelines or storage containers. The widespread occurrence of *Aspergillus* and *Penicillium* aligns with findings in multiple surveys of drinking water systems where these genera were dominant contaminants (Oliveira *et al*., 2016).

The high prevalence of potentially pathogenic fungi in these hostel water supplies signals risks to both water quality and public health, underscoring the need for improved monitoring and disinfection protocols.

The antibiotic susceptibility profiles of bacterial isolates before and after plasmid curing provide insight into the resistance mechanisms of organisms contaminating the water sources within the University of Benin Ekenwan Campus. From Table 4.10 it can be seen that before plasmid curing, *Staphylococcus aureus* demonstrated resistance to amoxicillin, augmentin, pefloxacin, azithromycin and ceftriaxone with a multiple drug resistance index (MDRI) of 0.8. *Pseudomonas fluorescens* showed resistance to the same antibiotics with an MDRI of 0.5. *Corynebacterium* spp resisted amoxicillin, azithromycin and ceftriaxone, recording an MDRI of 0.3, while *Aeromonas hydrophila* resisted amoxicillin and gentamycin with MDRI values between 0.1 and 0.4 depending on the sample source. *Enterobacter aerogens* also recorded an MDRI of 0.5, while *Chromobacterium violaceum* showed resistance patterns with MDRIs between 0.1 and 0.3. These values surpass the 0.2 MDRI threshold of public health concern, confirming the significance of multidrug resistance in these isolates. The high resistance of *S. aureus* is consistent with the increasing emergence of multidrug resistant staphylococci globally, which has been strongly linked to plasmid-borne β-lactamase genes and the misuse of antibiotics (Onifade & Palmer, 2018; Adesoji *et al*., 2016).

Table 4.11 shows the after plasmid curing where it is observed that, the sensitivity pattern changed substantially. *Aeromonas hydrophila, Staphylococcus aureus, Corynebacterium* spp, *Pseudomonas fluorescens, Klebsiella aerogens* and *Chromobacterium violaceum* became susceptible to most of the antibiotics tested, with many isolates recording an MDRI of 0.0. However, isolates recovered from postgraduate and girls' hostels retained resistance. In these

cases, *S. aureus*, *Enterobacter aerogens*, *P. fluorescens* and *C. violaceum* remained resistant to amoxicillin, augmentin, pefloxacin and ceftriaxone. Following curing, *S. aureus* and *Enterobacter aerogens* each retained an MDRI of 0.5, while *P. fluorescens* showed 0.4. This indicates that while resistance in several isolates were plasmid-mediated and lost after curing, others possessed chromosomal resistance or integron-associated traits, which cannot be eliminated by plasmid curing. These outcomes agree with the findings of Okoye et al., (2022), who showed that plasmid curing restores susceptibility in some bacteria, while others with chromosomal resistance remain unaffected.

The observed resistance of *S. aureus* and *P. fluorescens* before curing reflects the widespread presence of plasmid-borne resistance among staphylococci and pseudomonads in both environmental and clinical settings. In Nigeria, *S. aureus* and *Pseudomonas* species have been consistently documented as key multidrug resistant organisms in water distribution systems and hospital effluents (Onifade and Palmer, 2018; Adesoji et al., 2015). These bacteria frequently harbour integrons and plasmids encoding resistance to β-lactams and fluoroquinolones, explaining their resistance to amoxicillin, augmentin and ceftriaxone as seen in this study. Narrative evidence also indicates that resistance in pseudomonads is often mediated by efflux pumps in addition to plasmid-carried resistance genes, which may explain why *P. fluorescens* maintained significant resistance even after curing (Adesoji *et al*., 2015). The resistance of *Aeromonas hydrophila* before curing, particularly to amoxicillin and gentamycin, also agrees with documented findings that *Aeromonas* species are commonly resistant to β-lactams due to chromosomally encoded β-lactamases (Omoruyi *et al*., 2023). The improvement in antibiotic susceptibility of *A. hydrophila* following curing highlights the plasmid-borne contribution to resistance. This corroborates earlier reports that plasmid elimination enhances antibiotic efficacy

against environmental isolates of *Aeromonas* and *Enterobacter* (Iheanacho and Antai, 2023). The persistence of resistance in *S. aureus* and *Enterobacter aerogens* after curing is consistent with studies documenting non-plasmid resistance mechanisms, including chromosomal mutations and integrons (Adesoji *et al*., 2016). This highlights the complexity of antimicrobial resistance mechanisms in waterborne pathogens, showing that plasmid curing alone cannot eliminate all resistance traits. The implications of these findings are considerable for public health. The presence of isolates with MDRI values as high as 0.8, such as *S. aureus*, indicates significant multidrug resistance capable of limiting treatment options. This is consistent with observations in Nigerian groundwater, where more than 99 % of isolates were multidrug resistant and resistant to at least five antibiotic classes (Aromolaran *et al*., 2022). The detection of such resistance in drinking water sources raises concerns of community transmission, as consumption of contaminated water can result in hard-to-treat infections. The pattern observed in this study reflects broader environmental trends where untreated wastewaters and abattoir effluents serve as reservoirs for multidrug resistant organisms. In Benin City, multidrug resistant *Pseudomonas* and *Enterobacter* species have been isolated from abattoir environments, many of which carried plasmid-borne colistin resistance genes (Omoruyi *et al*., 2023). The similarity between those findings and the present results shows that the spread of plasmid-mediated resistance across environmental reservoirs is a growing public health issue.

Narrative comparisons with the work of Otokunefor et al., (2019) also confirm that plasmid curing decreases multidrug resistance in *Escherichia coli* and other non-clinical isolates, but that chromosomal resistance can still persist, which reflects what is observed here with *Enterobacter* and *S. aureus*. The pattern of resistance persistence after curing is therefore consistent with

documented evidence of integron-mediated antibiotic resistance in waterborne bacteria (Adesoji *et al*., 2016).

The findings emphasize the role of water systems as reservoirs and vehicles for multidrug resistant bacteria. Studies of off-campus hostels in Nigeria have reported similar risks, where multidrug resistant *Salmonella, Pseudomonas* and *Staphylococcus* species were recovered from domestic water sources used by students, increasing the likelihood of waterborne disease outbreaks (Uguomore and Igbonezu, 2025). This study confirms those risks in the University of Benin setting, where untreated or inadequately treated water supplies are a major concern for student health. The fact that plasmid curing eliminated resistance in several isolates but not all, underlines the need for integrated surveillance strategies that account for both plasmid-mediated and chromosomal mechanisms. The widespread resistance observed here aligns with the systematic review of multidrug resistant bacteria in foods and drinks across Nigeria, which concluded that water and beverages are major carriers of resistant organisms and thus critical in resistance gene transmission (Mola *et al*., 2021). The antibiotic susceptibility profiles show that water sources in the study area are contaminated with multidrug resistant organisms, many of which harbour plasmid-borne resistance genes. The persistence of resistance in isolates like *S. aureus* and *E. aerogens* even after curing demonstrates the involvement of chromosomal and integron-mediated mechanisms, making treatment more difficult. These findings highlight the urgent need for safe water treatment, strict antimicrobial stewardship, and continuous surveillance of resistance determinants in environmental reservoirs.

5.1 CONCLUSION

Water remains a vital resource for human health, development and environmental stability, yet its safety is often threatened by chemical contamination and microbial pollution. Access to clean

water is not only essential for hydration but also for preventing infectious diseases and reducing long-term exposure to harmful substances that affect organs and overall wellbeing. The presence and spread of antimicrobial resistance in aquatic environments further complicate the challenge, as resistant organisms move between natural ecosystems, animals and humans, creating risks that extend beyond local boundaries. Ensuring safe water supplies therefore requires continuous monitoring, improved treatment technologies, and strict management of environmental pollutants. Effective policies, sustainable practices and community engagement remain central to protecting water resources and safeguarding public health across generations.

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APPENDIX I

Oneway ANOVA

Physicochemical Results

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
pH	1.00	3	4.5833	.05774	.03333	4.4399	4.7268	4.55	4.65
	2.00	3	5.2567	.27791	.16045	4.5663	5.9470	4.94	5.46
	3.00	3	5.9733	.08505	.04910	5.7621	6.1846	5.91	6.07
	4.00	3	4.6433	.11676	.06741	4.3533	4.9334	4.54	4.77
	5.00	3	4.6767	.08505	.04910	4.4654	4.8879	4.59	4.76
	6.00	3	6.3833	.27791	.16045	5.6930	7.0737	6.18	6.70
	Total	18	5.2528	.73638	.17357	4.8866	5.6190	4.54	6.70
Electrical conductivity	1.00	3	74.0000	.00000	.00000	74.0000	74.0000	74.00	74.00
	2.00	3	65.0000	2.64575	1.52753	58.4276	71.5724	63.00	68.00
	3.00	3	112.6667	2.88675	1.66667	105.4956	119.8378	111.00	116.00
	4.00	3	73.6667	.57735	.33333	72.2324	75.1009	73.00	74.00
	5.00	3	72.3333	1.52753	.88192	68.5388	76.1279	71.00	74.00
	6.00	3	39.3333	.57735	.33333	37.8991	40.7676	39.00	40.00
	Total	18	72.8333	22.16847	5.22516	61.8092	83.8575	39.00	116.00
Total disolved solid	1.00	3	37.0000	.00000	.00000	37.0000	37.0000	37.00	37.00
	2.00	3	32.3333	1.52753	.88192	28.5388	36.1279	31.00	34.00
	3.00	3	56.0000	1.73205	1.00000	51.6973	60.3027	55.00	58.00
	4.00	3	37.0000	.00000	.00000	37.0000	37.0000	37.00	37.00
	5.00	3	35.6667	1.15470	.66667	32.7982	38.5351	35.00	37.00
	6.00	3	19.3333	.57735	.33333	17.8991	20.7676	19.00	20.00
	Total	18	36.2222	11.09613	2.61538	30.7042	41.7402	19.00	58.00
suspended solid	1.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	2.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	3.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	4.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	5.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	6.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	18	.0000	.00000	.00000	.0000	.0000	.00	.00
T.S	1.00	3	37.0000	.00000	.00000	37.0000	37.0000	37.00	37.00
	2.00	3	32.3333	1.52753	.88192	28.5388	36.1279	31.00	34.00
	3.00	3	56.0000	1.73205	1.00000	51.6973	60.3027	55.00	58.00
	4.00	3	37.0000	.00000	.00000	37.0000	37.0000	37.00	37.00
	5.00	3	35.6667	1.15470	.66667	32.7982	38.5351	35.00	37.00
	6.00	3	19.3333	.57735	.33333	17.8991	20.7676	19.00	20.00
	Total	18	36.2222	11.09613	2.61538	30.7042	41.7402	19.00	58.00
turbidity	1.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	2.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	3.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	4.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	5.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	6.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	18	.0000	.00000	.00000	.0000	.0000	.00	.00
Hardness	1.00	3	2.4267	.09866	.05696	2.1816	2.6717	2.36	2.54
	2.00	3	2.1500	.11533	.06658	1.8635	2.4365	2.06	2.28
	3.00	3	2.7667	.20984	.12115	2.2454	3.2879	2.53	2.93

	4.00	3	1.9700	.15524	.08963	1.5844	2.3556	1.82	2.13
	5.00	3	1.8767	.06028	.03480	1.7269	2.0264	1.82	1.94
	6.00	3	1.4033	.29670	.17130	.6663	2.1404	1.18	1.74
	Total	18	2.0989	.46545	.10971	1.8674	2.3304	1.18	2.93
	1.00	3	1.3800	.12767	.07371	1.0628	1.6972	1.27	1.52
	2.00	3	1.1500	.05568	.03215	1.0117	1.2883	1.10	1.21
	3.00	3	1.5767	.33471	.19325	.7452	2.4081	1.20	1.84
Alkalinity	4.00	3	.9033	.06506	.03756	.7417	1.0650	.84	.97
	5.00	3	.9667	.05686	.03283	.8254	1.1079	.92	1.03
	6.00	3	.8333	.09504	.05487	.5972	1.0694	.74	.93
	Total	18	1.1350	.30494	.07188	.9834	1.2866	.74	1.84
	1.00	3	28.1667	.65064	.37565	26.5504	29.7829	27.50	28.80
	2.00	3	23.9000	1.38924	.80208	20.4489	27.3511	22.30	24.80
	3.00	3	34.3000	.36056	.20817	33.4043	35.1957	33.90	34.60
Chloride	4.00	3	27.6333	.35119	.20276	26.7609	28.5057	27.30	28.00
	5.00	3	24.5333	2.83608	1.63741	17.4881	31.5785	22.70	27.80
	6.00	3	9.9667	.37859	.21858	9.0262	10.9071	9.70	10.40
	Total	18	24.7500	7.71974	1.81956	20.9111	28.5889	9.70	34.60
	1.00	3	.2300	.03000	.01732	.1555	.3045	.20	.26
	2.00	3	.1900	.02000	.01155	.1403	.2397	.17	.21
	3.00	3	.2267	.02082	.01202	.1750	.2784	.21	.25
Phosphate	4.00	3	.1167	.01155	.00667	.0880	.1454	.11	.13
	5.00	3	.2667	.02082	.01202	.2150	.3184	.25	.29
	6.00	3	.1133	.01528	.00882	.0754	.1513	.10	.13
	Total	18	.1906	.06197	.01461	.1597	.2214	.10	.29
	1.00	3	.6567	.02082	.01202	.6050	.7084	.64	.68
	2.00	3	.4800	.09539	.05508	.2430	.7170	.39	.58
	3.00	3	.1400	.01732	.01000	.0970	.1830	.13	.16
Nitrate	4.00	3	.1600	.02646	.01528	.0943	.2257	.14	.19
	5.00	3	.1667	.02517	.01453	.1042	.2292	.14	.19
	6.00	3	.0767	.00577	.00333	.0623	.0910	.07	.08
	Total	18	.2800	.22131	.05216	.1699	.3901	.07	.68
	1.00	3	.3067	.03055	.01764	.2308	.3826	.28	.34
	2.00	3	.2333	.02517	.01453	.1708	.2958	.21	.26
	3.00	3	.3833	.03512	.02028	.2961	.4706	.35	.42
Sulphate	4.00	3	.2633	.02082	.01202	.2116	.3150	.24	.28
	5.00	3	.2733	.02082	.01202	.2216	.3250	.25	.29
	6.00	3	.2033	.01528	.00882	.1654	.2413	.19	.22
	Total	18	.2772	.06276	.01479	.2460	.3084	.19	.42
	1.00	3	.04333	.013204	.007623	.01053	.07613	.029	.055
	2.00	3	.06033	.004163	.002404	.04999	.07068	.057	.065
	3.00	3	.05167	.018583	.010729	.00550	.09783	.039	.073
Calcium	4.00	3	.03900	.012767	.007371	.00728	.07072	.025	.050
	5.00	3	.03867	.008327	.004807	.01798	.05935	.032	.048
	6.00	3	.04667	.004619	.002667	.03519	.05814	.044	.052
	Total	18	.04661	.012410	.002925	.04044	.05278	.025	.073
	1.00	3	3.5600	.81185	.46872	1.5433	5.5767	2.67	4.26
	2.00	3	3.8533	.18583	.10729	3.3917	4.3150	3.64	3.98
	3.00	3	5.4500	.26153	.15100	4.8003	6.0997	5.27	5.75
Magnesium	4.00	3	5.5400	.80299	.46361	3.5453	7.5347	4.78	6.38
	5.00	3	5.0867	1.44091	.83191	1.5072	8.6661	4.22	6.75
	6.00	3	4.7700	1.19528	.69010	1.8008	7.7392	3.94	6.14
	Total	18	4.7100	1.08840	.25654	4.1687	5.2513	2.67	6.75
	1.00	3	.01767	.005774	.003333	.00332	.03201	.011	.021
	2.00	3	.02467	.005774	.003333	.01032	.03901	.018	.028
	3.00	3	.01667	.004726	.002728	.00493	.02841	.013	.022
Sodium	4.00	3	.02233	.008327	.004807	.00165	.04302	.013	.029
	5.00	3	.01700	.005196	.003000	.00409	.02991	.014	.023
	6.00	3	.02733	.003512	.002028	.01861	.03606	.024	.031
	Total	18	.02094	.006412	.001511	.01776	.02413	.011	.031
Potassium	1.00	3	.6100	.05292	.03055	.4786	.7414	.57	.67

	2.00	3	.4100	.05196	.03000	.2809	.5391	.38	.47
	3.00	3	.4300	.04583	.02646	.3162	.5438	.39	.48
	4.00	3	.5667	.04619	.02667	.4519	.6814	.54	.62
	5.00	3	.4267	.09609	.05548	.1880	.6654	.34	.53
	6.00	3	.3367	.04933	.02848	.2141	.4592	.28	.37
	Total	18	.4633	.10938	.02578	.4089	.5177	.28	.67
	1.00	3	.7567	.02517	.01453	.6942	.8192	.73	.78
	2.00	3	.6433	.03512	.02028	.5561	.7306	.61	.68
	3.00	3	.9833	.05132	.02963	.8559	1.1108	.94	1.04
Iron	4.00	3	1.0633	.08021	.04631	.8641	1.2626	.98	1.14
	5.00	3	.5633	.02517	.01453	.5008	.6258	.54	.59
	6.00	3	.6867	.03512	.02028	.5994	.7739	.65	.72
	Total	18	.7828	.19020	.04483	.6882	.8774	.54	1.14
	1.00	3	.4367	.03786	.02186	.3426	.5307	.41	.48
	2.00	3	.3600	.02646	.01528	.2943	.4257	.34	.39
	3.00	3	.5300	.04583	.02646	.4162	.6438	.48	.57
Zinc	4.00	3	.5633	.06028	.03480	.4136	.7131	.50	.62
	5.00	3	.3933	.02082	.01202	.3416	.4450	.37	.41
	6.00	3	.3133	.05508	.03180	.1765	.4501	.26	.37
	Total	18	.4328	.09869	.02326	.3837	.4819	.26	.62
	1.00	3	.11067	.029006	.016746	.03861	.18272	.082	.140
	2.00	3	.12333	.041633	.024037	.01991	.22676	.090	.170
	3.00	3	.26000	.050000	.028868	.13579	.38421	.210	.310
Copper	4.00	3	.20667	.025166	.014530	.14415	.26918	.180	.230
	5.00	3	.07667	.007371	.004256	.05836	.09498	.071	.085
	6.00	3	.07900	.004000	.002309	.06906	.08894	.075	.083
	Total	18	.14272	.074584	.017580	.10563	.17981	.071	.310
	1.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	2.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	3.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
Lead	4.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	5.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	6.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	18	.0000	.00000	.00000	.0000	.0000	.00	.00
	1.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	2.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	3.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
Cadmuium	4.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	5.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	6.00	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	18	.0000	.00000	.00000	.0000	.0000	.00	.00
	1.00	3	.2533	.03512	.02028	.1661	.3406	.22	.29
	2.00	3	.2300	.17436	.10066	-.2031	.6631	.03	.35
	3.00	3	.2500	.03606	.02082	.1604	.3396	.21	.28
Chromium	4.00	3	.2300	.06000	.03464	.0810	.3790	.17	.29
	5.00	3	.0967	.07572	.04372	-.0914	.2848	.01	.15
	6.00	3	.1900	.02000	.01155	.1403	.2397	.17	.21
	Total	18	.2083	.09005	.02122	.1636	.2531	.01	.35