MICROBIAL ANALYSIS OF DRINKING WATER IN ORHIONMWON LOCAL GOVERNMENT AREA

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR'S DEGREE (BSc). IN SCIENCE LABORATORY TECHNOLOGY, UNIVERSITY OF BENIN, BENIN CITY. EDO STATE, NIGERIA.

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CERTIFICATION

This is to certify that the research work titled "Microbial Analysis of Drinking Water in Orhiomwon LGA" was carried out by **ESEZOBOR PROSPER OSAYI** with matriculation number **LSC2007298** in the Department of Science Laboratory Technology, University of Benin, under my supervision in partial fulfilment of the requirements for the award of a Bachelor's Degree.

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DEDICATION

This research is dedicated to my family and mentors, whose steadfast support and encouragement were instrumental in making this work possible.

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LIST OF ABBREVIATIONS

WHO: World Health Organization

NIS: Nigerian Industrial Standards

HPC: Heterotrophic Plate Count

E. coli: Escherichia coli

THBC: Total Heterotrophic Bacteria Count

LGA: Local Government Area

UNICEF: United Nations International Children's Emergency Fund

APHA: American Public Health Association

SDG: Sustainable Development Goal

JMP: Joint Monitoring Programme

EMB: Eosin Methylene Blue

MPN: Most Probable Number

NA: Nutrient Agar

PDA: Potato Dextrose Agar

IMViC: Indole, Methyl Red, Voges-Proskauer, and Citrate tests

ANOVA: Analysis of Variance

PPE: Personal Protective Equipment

NSDWQ: Nigerian Standard for Drinking Water Quality

WSP: Water Safety Plan

SODIS: Solar Water Disinfection

CFU: Colony-Forming Unit

ABSTRACT

Access to safe drinking water remains a critical public health challenge in rural communities of Nigeria. This study assessed the microbial quality and potential health risks associated with drinking water from multiple sources in Orhionmwon Local Government Area (LGA), Edo State. A total of five water samples were collected, comprising two boreholes, two hand-dug wells, and Ikpe River Samples were analysed using the pour plate method for total heterotrophic bacterial count (THBC), total coliforms, *Escherichia coli*, and fungi, followed by biochemical identification of isolates. Results revealed bacterial loads ranging from 13 cfu/ml in borehole samples to 5.9×10^3 cfu/ml in river water, exceeding the World Health Organization (WHO, 2022) limit of 500 cfu/ml for drinking water. *E. coli* and coliforms were detected in the river and one well sample, indicating fecal contamination, while borehole water remained within permissible standards. Isolated bacterial species included *Bacillus spp., Pseudomonas aeruginosa, Proteus spp., Klebsiella spp., Enterobacter spp.,* and *E. coli,* while fungal isolates comprised *Aspergillus spp., Mucor spp., Penicillium spp.,* and *Rhodotorula spp*. The presence of these organisms, particularly fecal indicators and opportunistic pathogens, underscores potential risks of waterborne infections in the area. The study highlights the need for regular microbial surveillance, improved sanitation practices, and promotion of household water treatment methods such as boiling or chlorination to ensure safe drinking water and protect community health.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Water is universally acknowledged as one of the most essential resources for sustaining life. It is indispensable for human survival, economic development, agriculture, sanitation, and industrial operations. However, the availability of clean, safe drinking water remains a significant challenge globally, especially in many developing nations. According to the World Health Organization (WHO, 2022), at least 2.2 billion people globally lack safely managed drinking water services, while about 485,000 deaths are recorded annually from diarrheal diseases due to the consumption of contaminated water.

In Nigeria, access to potable water is a persistent public health concern, particularly in rural areas where communities often rely on unregulated water sources such as shallow wells, rainwater, and surface water bodies and borehole water without proper treatment facilities. These sources are frequently exposed to contamination from human and animal waste, agricultural runoff, and industrial discharges. Despite national and global water safety plans, the implementation at the community level remains weak (UNICEF, 2023).

Microbial contamination of drinking water is among the most dangerous and insidious threats to public health. Unlike physical contaminants that can often be detected visually or chemically, microbial contaminants, comprising bacteria, viruses, and protozoa, are microscopic and often require specialized laboratory procedures to detect. The presence of fecal indicator bacteria such as *Escherichia coli* and total coliforms serves as a proxy for recent fecal contamination and the potential presence of disease-causing pathogens such as *Salmonella spp.*, *Shigella spp.*, and *Vibrio cholerae* (Edokpayi *et al.,* 2021).

These pathogens are associated with gastrointestinal infections, including diarrhoea, cholera, typhoid, and dysentery.

Orhionmwon Local Government Area (LGA) in Edo State, Nigeria, is a predominantly agrarian and semi-urban community with a diverse population relying on multiple sources of drinking water. While some households may have access to boreholes and sachet water, a significant number still depend on traditional sources such as hand-dug wells, rainwater, and surface water from streams and rivers. Due to inadequate sanitation infrastructure, open defecation, and poor hygiene practices, these water sources are highly susceptible to microbial contamination (Arowolo *et al.,* 2022).

Field reports and local health records suggest periodic outbreaks of waterborne diseases, highlighting the need for a detailed microbial water quality assessment.

Understanding the microbial quality of water in Evbarue community in Orhionmwon is crucial not only for public health surveillance but also for planning effective water safety interventions and policies. Despite previous studies focusing on the physicochemical aspects of drinking water in the region, there is limited comprehensive research on the microbiological safety of drinking water in Evbarue community in Orhionmwon, particularly using standardized laboratory methods and benchmarking against international safety guidelines (Agbro *et al.,* 2021).

This study therefore aims to fill this gap by conducting a detailed microbial assessment of drinking water sources in Evbarue community in Orhionmwon LGA, identifying prevalent microbial contaminants, comparing findings with WHO and NIS standards, and recommending appropriate mitigation strategies to reduce the risk of waterborne diseases.

1.2 AIMS AND OBJECTIVES OF THE STUDY

To identify and quantify microbial contaminants, particularly total heterotrophic bacteria, total coliforms, and *E. coli*, in different drinking water sources in Evbarue community in Orhionmwon LGA.

1. To compare microbial loads across various water sources such as boreholes, hand-dug wells, and river water.
2. To assess the compliance of microbial concentrations in these water sources with WHO and Nigerian Industrial Standards (NIS).
3. To investigate potential environmental and anthropogenic factors contributing to microbial contamination in the study area.
4. To propose practical, community-driven interventions for reducing microbial contamination in drinking water

1.3 RESEARCH QUESTIONS

1. What types of microbial contaminants are present in drinking water sources in Evbarue community in Orhionmwon LGA?
2. What are the concentration levels of these microbial contaminants in relation to WHO and NIS safety limits?
3. How does the microbial quality differ among the various sources of drinking water in the study area?
4. What environmental and human factors are associated with microbial contamination in Orhionmwon?
5. What interventions can be recommended to improve the microbiological safety of drinking water in the area?

1.4 HYPOTHESIS

a. Null Hypothesis (H_0): There is no significant deviation between the microbial quality of drinking water sources (boreholes, wells, and river) in Orhionmwon LGA and the permissible limits set by WHO (2022) and NIS 554:2017.

b. Alternative Hypothesis (H_1): The microbial quality of drinking water sources (boreholes, wells, and river) in Orhionmwon LGA significantly deviates from the permissible limits set by WHO (2022) and NIS 554:2017, thereby posing potential public health risks.

1.5 SCOPE OF THE STUDY

This study will be conducted in Evbarue community in Orhionmwon LGA, Edo State. The investigation will focus on commonly used drinking water sources including boreholes, hand-dug wells, and river water. Only microbial parameters will be considered, specifically total heterotrophic bacteria count, total coliforms, and *Escherichia coli*. Physicochemical parameters, though relevant, fall outside the scope of this study. Sample collection, preservation, and analysis will follow standard laboratory protocols approved by WHO and APHA (2023).

CHAPTER TWO

LITERATURE REVIEW

2.1 WATER ANALYSIS

Access to safe and potable drinking water is a core public health necessity and a fundamental human right. The World Health Organization (WHO, 2022) emphasizes that safe drinking water should be free from pathogens and toxic substances, and should meet microbiological, physical, and chemical standards. Despite this, over 2.2 billion people globally lack access to safely managed drinking water services (UNICEF, 2023), and the problem is most acute in rural sub-Saharan Africa. In Nigeria, a significant proportion of the rural population depends on untreated surface and groundwater sources for domestic and drinking purposes, exposing them to microbial contamination and related health risks (Ibrahim *et al.,* 2023).

Microbial contamination of drinking water remains a major cause of disease outbreaks, especially among children and immunocompromised individuals. Waterborne diseases such as cholera, typhoid, and dysentery are primarily linked to the presence of pathogenic microorganisms in water. These diseases account for thousands of deaths annually in Nigeria alone (Uhunamure *et al.,* 2023). Therefore, routine microbial monitoring of drinking water sources, particularly in rural communities like Evbarue community in Orhionmwon LGA, is crucial for preventing the spread of infectious diseases and achieving Sustainable Development Goal 6, which is clean water and sanitation for all.

2.2 Waterborne Diseases and Public Health Significance

Waterborne illnesses are transmitted through ingestion of water contaminated with human or animal feces containing pathogenic bacteria, viruses, or protozoa. In low-income regions, including rural Nigeria, these diseases are common due to poor sanitation, lack of public health education, and inadequate infrastructure. Studies show that diarrhea, the most prevalent waterborne illness, results in the death of approximately 525,000 children under five each year globally (WHO, 2022). In Nigeria, outbreaks of cholera and typhoid fever remain a seasonal occurrence, particularly during the rainy season when flooding increases contamination through surface runoff (Onyekwere *et al.,* 2022).

The impacts of these diseases are not limited to health. Economically, they result in loss of productivity, school absenteeism, and a burdened healthcare system. Hence, the need for proactive water quality assessment, especially in informal settlements and rural water bodies like Ikpe River, is critical to safeguarding public health.

2.3 Microbial Contaminants in Water Sources: Biology, Health Effects, and Transmission Pathways

Drinking water sources are vulnerable to microbial contamination through various environmental, sanitary, and human activities. Common water sources like boreholes, rivers, and wells in low-income and developing regions are often exposed to fecal matter, decaying organic materials, and runoff from agricultural fields, resulting in the proliferation of diverse microbial populations (WHO, 2022). These microorganisms may include both harmless environmental species and harmful pathogenic bacteria, fungi, protozoa, and viruses.

2.3.1 Bacterial Pathogens

2.3.1.1. Escherichia coli (E. coli)

E. coli is a Gram-negative, rod-shaped, facultative anaerobic bacterium found naturally in the intestinal tracts of humans and animals. While most *E. coli* strains are harmless and serve beneficial roles in digestion, some pathogenic strains such as Enterohemorrhagic *E. coli* (EHEC) produce Shiga toxins and can cause severe gastrointestinal illnesses (Nwachukwu *et* *al.,* 2020). Infection often results in watery or bloody diarrhea, abdominal cramps, and in severe cases, hemolytic uremic syndrome, especially in children.

Contamination occurs through the direct discharge of feces into water sources, agricultural runoff, or cross-contamination from unhygienic handling of water. Its detection in drinking water serves as a definitive indicator of recent fecal contamination (WHO, 2022).

2.3.1.2. Salmonella spp.

Salmonella are Gram-negative, facultatively anaerobic bacilli capable of causing typhoid fever and gastroenteritis. They are typically transmitted via ingestion of water contaminated with the feces of infected individuals or animals. *Salmonella typhi*, the causative agent of typhoid fever, remains a significant cause of morbidity in Africa, particularly in rural and peri-urban communities with poor sanitation infrastructure (Akinbile *et al.,* 2023).

2.3.1.3. Shigella spp.

Shigella species are non-motile, Gram-negative bacteria responsible for bacillary dysentery, characterized by bloody diarrhea, fever, and abdominal pain. The organism has a low infectious dose (~10–100 organisms), making it highly transmissible through drinking contaminated water (Edokpayi *et al.,* 2021). Contamination typically arises in open wells and surface waters due to indiscriminate defecation and unhygienic practices.

2.3.1.4. Vibrio cholerae

V. cholerae is a curved, motile, Gram-negative bacterium that causes cholera, a disease marked by profuse watery diarrhea and severe dehydration. The organism thrives in warm, brackish water and is often linked to water bodies contaminated with sewage or human feces (Arowolo *et al.,* 2022). During outbreaks, cholera is frequently transmitted through the consumption of untreated river water or improperly washed vegetables.

2.3.1.5. Klebsiella spp.

Klebsiella are encapsulated, Gram-negative bacilli found in soil, water, and the intestinal tract. While part of the normal flora, some strains are opportunistic pathogens capable of causing pneumonia, urinary tract infections, and sepsis. Their presence in well and borehole water may result from contamination through cracked well linings or surface infiltration (Babalola and Osibanjo, 2023).

2.3.1.6. Enterobacter spp.

These bacteria are facultatively anaerobic, Gram-negative rods often present in water, sewage, and soil. They can cause wound infections, bacteremia, and respiratory tract infections. *Enterobacter* species are often isolated from polluted surface waters, particularly those exposed to wastewater and faecal discharge (Ibrahim *et al.,* 2023).

2.3.1.7. Proteus spp.

Proteus species, such as *Proteus mirabilis*, are motile, urease-positive, Gram-negative bacteria found in decomposing organic matter and fecal material. They are known for their swarming motility and are often implicated in urinary tract and wound infections. Their presence in well or river water indicates poor sanitary conditions and improper waste disposal (Nwachukwu *et al.,* 2020).

2.3.1.8. Pseudomonas aeruginosa

P. aeruginosa is a highly adaptable, Gram-negative bacterium known for its biofilm-forming ability and resistance to many disinfectants and antibiotics. It causes opportunistic infections, including otitis externa (“swimmer’s ear”), wound infections, and pneumonia in immunocompromised individuals. It is commonly found in poorly chlorinated borehole systems, storage tanks, and stagnant surface water (Uhunamure *et al.,* 2023).

2.3.1.9. Bacillus spp.

Bacillus are Gram-positive, spore-forming rods found widely in soil and water. Most species are non-pathogenic, but *Bacillus cereus* can cause foodborne illnesses and gastrointestinal upset. Their presence in borehole and well water reflects environmental contamination or poor storage hygiene, as spores resist harsh conditions and can survive treatment (Okoh *et al*., 2022).

2.3.2 Fungal Contaminants in Water

Although less frequently monitored than bacteria, fungi are increasingly recognized as emerging contaminants in drinking water. They may cause allergic reactions, respiratory illnesses, or systemic infections in immunocompromised individuals (WHO, 2020). Several fungal contaminants have been identified as potential threats to drinking water safety, including;

Aspergillus spp. Which are filamentous fungi that release airborne spores and are known to cause allergic reactions, aspergillosis, and systemic mycoses in susceptible individuals.

Penicillium spp., commonly found in soil and water, rarely cause infections but may produce harmful mycotoxins under certain environmental conditions.

Mucor spp. are rapid-growing fungi capable of causing mucormycosis, particularly in immunocompromised individuals.

Rhodotorula spp. are pigmented yeasts associated with opportunistic fungemia, especially in patients with indwelling medical devices such as catheters. These fungi typically infiltrate water systems through airborne spores, decaying vegetation near water collection points, or compromised infrastructure such as leaky wellheads and storage tanks (Edokpayi *et al.,* 2021).

2.3.3 Modes of Contamination and Transmission

The microbial quality of water is heavily influenced by the environmental conditions, human behavior, and infrastructure design of the area:

Fecal contamination remains one of the most common and dangerous sources of water pollution in developing regions. It typically arises when human or animal feces enter water bodies due to practices such as open defecation, the siting of pit latrines in close proximity to wells or streams, or leakages from poorly maintained sewage systems. The presence of fecal matter introduces a wide range of pathogenic microorganisms, including *Escherichia coli, Salmonella,* and *Vibrio cholerae*, which can cause severe gastrointestinal infections and outbreaks of waterborne diseases. This type of contamination poses a direct threat to public health, especially in rural communities where untreated water is often consumed directly.

Surface runoff is another major pathway through which contaminants enter rivers, streams, and open wells. During rainfall, water moves across agricultural fields, carrying fertilizers, pesticides, animal waste, and decaying organic matter into nearby water sources. Similarly, runoff from urban areas can transport industrial chemicals, oil residues, and waste from dumpsites into open water bodies. This process not only increases the microbial load in water but also introduces harmful chemicals and nutrients, which may trigger algal blooms and reduce water quality. The seasonal nature of rainfall makes surface runoff a recurring and sometimes unpredictable source of contamination.

Contamination of wells is a particularly serious concern in communities that depend on shallow or unprotected wells for drinking water. This problem often arises due to cracked or poorly constructed well linings that allow contaminants from surrounding soil or surface water to seep in. Shallow wells are especially vulnerable because they tap water that is closer to the surface and less filtered by geological layers. In addition, the absence of proper coverings or aprons around wells leaves them exposed to dust, insects, and direct entry of waste materials. These factors make unprotected wells a significant source of microbial and chemical contamination.

Another often-overlooked contributor to water contamination is the formation of biofilms. Certain bacteria such as **Pseudomonas** and **Bacillus** are capable of adhering to surfaces within storage tanks, borehole casings, and plumbing systems, forming protective biofilms. These biofilms act as a shield, allowing microorganisms to survive disinfection treatments such as chlorination. Once established, biofilms can serve as persistent reservoirs of pathogens, releasing them gradually into stored or distributed water. Their resistance to cleaning and chemical treatment makes them a critical challenge in maintaining water safety, particularly in systems that rely on long-term storage.

Improper water handling also plays a crucial role in contaminating drinking water, even after initial treatment. This occurs when water is stored in dirty containers, transported in unsanitary conditions, or exposed to unhygienic environments such as poorly maintained sachet water production facilities. Cross-contamination is common when clean water is poured into containers that previously held unclean water or when it is handled with unwashed hands. In many cases, water that is microbiologically safe at the point of collection becomes unsafe by the time it is consumed due to poor storage and handling practices. Addressing these issues requires both improved infrastructure and behavioral changes at the household and community levels.

2.3.4 Transmission Pathways to Humans

Waterborne pathogens primarily follow the fecal–oral route, where individuals ingest contaminated water directly or through food prepared with unsafe water. Infections may also occur via various methods;

Skin contact with contaminated river water is a common but often underestimated route of exposure to pathogens. Activities such as bathing, swimming, or washing clothes in rivers that receive untreated sewage, industrial effluents, or agricultural runoff expose the skin and mucous membranes to harmful microorganisms. Certain pathogens, including *Schistosoma* parasites and D*ermatophilic* fungi, can directly penetrate the skin, leading to infections without the need for ingestion. Even when pathogens cannot penetrate intact skin, small cuts, abrasions, or prolonged immersion in polluted water provide opportunities for microbial entry. This route of transmission is particularly prevalent in rural or peri-urban areas where rivers and streams double as recreational, domestic, and waste disposal sites.

Aerosol inhalation represents another important pathway of infection, particularly in poorly maintained water systems. When water becomes contaminated with bacteria such as *Legionella pneumophila*, it can generate aerosols through showers, cooling towers, or air-conditioning units. Inhalation of these fine water droplets allows pathogens to bypass the gastrointestinal tract and directly colonize the respiratory system. This is a well-documented cause of diseases such as Legionnaires' disease and Pontiac fever. Stagnation, inadequate disinfection, and the presence of biofilms in distribution pipes or storage tanks significantly increase the likelihood of aerosol-related transmission. This pathway highlights the need for routine monitoring and maintenance of water systems, especially in urban environments.

Contaminated hands or utensils during food handling constitute a major indirect route of waterborne disease transmission. When individuals use contaminated water for washing vegetables, cooking, or cleaning utensils, pathogens are easily transferred to food. Likewise, if hands are not washed properly after contact with unsafe water sources, they can carry infectious microorganisms to meals consumed by entire households. This transmission route is strongly associated with diarrheal diseases caused by *E. coli*, *Salmonella*, and *Shigella*. Cross-contamination is particularly problematic in settings where safe water is scarce and hygienic practices are difficult to maintain. Addressing this issue requires both improved access to clean water and the promotion of handwashing, safe food preparation, and hygienic storage practices.

Children under five, the elderly, and immunocompromised individuals are the most vulnerable to these infections (WHO, 2022).

2.3.5 Summary of Key Pathogens and Impacts

A variety of pathogenic microorganisms have been associated with drinking water contamination in developing regions. *Escherichia coli* (*E. coli*), frequently detected in rivers and wells, serves as a primary indicator of faecal pollution and is responsible for diarrheal diseases and other gastrointestinal infections. *Pseudomonas aeruginosa*, commonly isolated from boreholes and rivers, thrives in aquatic biofilms and poses a risk of opportunistic infections, particularly in immunocompromised individuals. *Klebsiella spp.*, often found in wells and river water, are associated with urinary tract infections and pneumonia, and their occurrence is frequently linked to poor water storage and handling practices. Fungal contaminants such as *Aspergillus spp.* are also prevalent in river and well water, where they contribute to respiratory illnesses, often introduced through decaying organic matter. *Vibrio cholerae*, typically present in rivers during cholera outbreaks, is a major public health concern as it causes acute watery diarrhea linked to sewage infiltration into water sources. Similarly, *Salmonella spp.* have been reported in wells and river water, causing typhoid fever and other enteric infections, usually as a result of poor sanitation and hygiene practices.

2.4 Common Microbial Indicators in Water Quality Assessment

Microbial water quality assessment typically focuses on indicator organisms rather than testing for all known pathogens. The rationale is that the presence of these indicators suggests possible contamination including faecal contamination and the likelihood of other pathogens being present.

The most commonly monitored microbial indicators include:

Total Heterotrophic Bacteria Count (THBC) is a broad measure of microbial presence, encompassing aerobic and facultatively anaerobic bacteria capable of growing on nutrient-rich media. Elevated THBC levels may indicate nutrient availability or conditions favoring microbial growth, potentially signaling poor water quality or inadequate treatment (Bicudo *et al.,* 2021). THBC is widely used to assess microbial loads in drinking water systems and detect changes during storage or distribution.

Total Coliforms serve as general indicators of water sanitation quality. These gram-negative, rod-shaped bacteria are found in environmental and intestinal sources. Their presence suggests potential contamination or inadequate treatment, though not all coliforms are fecal in origin (Saxena *et al.,* 2015). Total coliform testing remains a standard due to its simplicity and ability to provide a broad assessment of water hygiene.

Faecal Coliforms, a subset of total coliforms, are specifically associated with fecal contamination from humans or animals. These bacteria thrive at elevated temperatures (e.g., 44.5°C), distinguishing them from environmental coliforms. Their detection indicates recent fecal pollution, posing health risks due to potential pathogens (WHO, 2022). Faecal coliform testing is essential for evaluating the safety of drinking and recreational waters.

Escherichia coli (E. coli) is a key fecal indicator within the coliform group, strongly linked to recent fecal contamination. Almost exclusively derived from human and animal intestines, *E.*

coli is a reliable marker for confirming fecal pollution and potential pathogen presence (Odonkor and Ampofo, 2020). Its detection in water is a critical indicator of health risks, making *E. coli* testing a cornerstone of global water quality standards.

Enterococcus spp. are gram-positive bacteria used alongside *E. coli* as fecal contamination indicators, particularly in brackish and marine waters. Their resilience in high-salinity environments makes them ideal for assessing coastal or estuarine water quality (Byappanahalli *et al.,* 2018). *Enterococcus* spp. are associated with fecal pollution and are used to establish safety guidelines for recreational waters.

Clostridium perfringens spores indicate long-term fecal contamination due to their environmental persistence. These spores can survive water treatment processes like chlorination, making them valuable for assessing disinfection efficacy and detecting historical contamination (Ahmed *et al*., 2020). Their presence in treated water may highlight deficiencies in filtration or disinfection systems.

Heterotrophic Plate Count bacteria (HPCs) measure bacteria growing on low-nutrient media, reflecting opportunistic microorganisms in water systems. While not inherently harmful, elevated HPCs may indicate biofilm formation or poor storage hygiene (Chowdhury *et al*., 2023). Monitoring HPCs is useful for evaluating water distribution system integrity and detecting bacterial regrowth post-treatment.

In summary, these microbial indicators collectively provide a robust framework for assessing water quality. By integrating tests for THBC, total coliforms, fecal coliforms, E. coli, Enterococcus spp., C. perfringens spores, and HPCs, water quality professionals can evaluate both immediate and long-term contamination risks, ensuring the safety of drinking and recreational waters.

These indicators help water managers and public health officers estimate the level of microbial pollution and evaluate potential risks to consumers (Akintola *et al.,* 2023).

.2.5 Pour Plate Method for Microbial Enumeration

The pour plate technique is a standard microbiological method used to estimate the total viable microbial count in water. In this method, 1 ml of the water sample is pipetted into a sterile Petri dish, after which molten agar, typically nutrient agar cooled to about 45°C, is poured in and mixed thoroughly. The plates are allowed to solidify and are then incubated at 35 to 37°C for 24 to 48 hours. Microbial colonies are then counted and reported as colony-forming units per milliliter (cfu/ml).

This method is particularly effective for enumerating total heterotrophic bacteria, and it allows for both surface and embedded colony development, which improves sensitivity (Akinbile *et al.,* 2023). While the technique is not used for strict anaerobes or fastidious organisms, it remains one of the most appropriate low-cost options for rural water monitoring in Nigeria.

2.5.1. Literature Using Pour Plate Method for Microbial Enumeration:

Edokpayi *et al*. (2021) applied the pour plate technique to evaluate the microbial quality of groundwater in Limpopo Province, South Africa. Their study revealed that bacterial loads were particularly elevated during the rainy season, which they attributed to surface runoff carrying fecal material, organic debris, and other contaminants into shallow aquifers. The study further highlighted that unprotected wells and poorly constructed boreholes were more susceptible to seasonal fluctuations in microbial load. This finding is significant because it demonstrates the vulnerability of groundwater sources to environmental and anthropogenic influences, particularly in rural communities where sanitary infrastructure is inadequate.

In a related study, Agbro *et al.* (2021) employed the pour plate method to enumerate heterotrophic bacteria in groundwater samples collected across Edo Central, Nigeria. Their results showed that more than 60% of the sampled wells and boreholes contained bacterial counts exceeding the permissible limits recommended by WHO. The authors linked this contamination primarily to the lack of sanitary protection around wells, including unlined well shafts, uncovered openings, and proximity to refuse dumps and pit latrines. They emphasized that such practices increase the likelihood of fecal contamination and microbial proliferation. This study underscores the importance of proper sanitary design and regular monitoring in ensuring water safety, particularly in rural and peri-urban settlements of Nigeria.

Similarly, Babalola and Osibanjo (2023) investigated the microbial quality of borehole water in southwest Nigeria, using standard culture methods including the pour plate technique. They reported microbial loads that consistently exceeded WHO safe limits, with isolates including *E. coli*, *Klebsiella spp.*, and *Pseudomonas spp.* The study concluded that domestic activities such as washing, indiscriminate waste disposal, and proximity of boreholes to septic tanks and soak-away pits were the primary drivers of contamination. Importantly, the authors stressed that even boreholes, often assumed to be safer than wells, could be compromised by poor siting, shallow drilling, and inadequate casing. Their findings highlight the critical role of environmental planning and public awareness in preventing groundwater contamination.

Collectively, these studies demonstrate that both groundwater and surface water sources are vulnerable to microbial contamination, particularly when sanitary barriers are weak or absent. They also illustrate the reliability of the pour plate method for enumerating heterotrophic bacteria and identifying contamination trends. By drawing parallels to the present study in Orhionmwon LGA, it becomes clear that local water sources, whether boreholes, wells, or rivers, are similarly at risk, especially in communities where sanitary infrastructure is inadequate and human activities occur close to water points.

These studies support the pour plate method as a practical and reliable tool for evaluating microbial water quality.

2.6 Review of Studies in Related Areas

Several studies have assessed microbial contamination in water sources across Edo State and Nigeria at large.

Agbro *et al.* (2021) carried out a comprehensive assessment of borehole and well water in Esan communities, Edo State, using both the pour plate and Most Probable Number (MPN) techniques. Their results showed bacterial counts ranging from 1.2×10^3 to 4.5×10^4 CFU/mL, far exceeding WHO permissible limits. The study attributed this high microbial burden to unsealed wellheads, unlined latrines, and poor environmental protection of water points, which created pathways for microbial infiltration. This highlights how poor infrastructure and proximity to sanitation hazards remain critical factors influencing groundwater safety in rural Nigerian communities.

Similarly, Uhunamure *et al*. (2023) evaluated the bacteriological quality of the Ikpoba and Ossiomo Rivers, which are hydrologically connected to the Ikpe River, one of the focal sources in this present study. Their analysis revealed the presence of *E. coli*, *Salmonella spp.*, and total coliforms, with contamination levels highest in downstream locations, where agricultural runoff, industrial effluents, and human activities were most concentrated. The researchers employed the pour plate method, serial dilution, and selective culture on Eosin Methylene Blue (EMB) agar for confirmation. Their findings demonstrate that rivers in Edo State are highly vulnerable to fecal and pathogenic contamination, posing significant risks to communities dependent on them for drinking and domestic use.

In northern Nigeria, Egbere *et al.* (2021) investigated the microbial quality of sachet water in Jos using pour plate and membrane filtration methods. Their results showed that 43% of samples exceeded WHO microbial limits, with *E. coli* and coliforms frequently detected. The contamination was linked to unhygienic production processes, poor handling, and unsafe storage conditions during transportation and retail. This finding is particularly relevant given the reliance on sachet water in many Nigerian communities as an alternative to unsafe wells and rivers, yet it underscores that sachet water itself may pose microbial risks if quality control measures are not strictly enforced.

In southeastern Nigeria, Onyekwere *et al.* (2022) studied microbial levels in domestic wells and harvested rainwater in Anambra State. They used both MPN and pour plate methods and reported that contamination levels were significantly higher during the rainy season. The primary causes were surface runoff, soil erosion, and animal droppings near water collection points. This seasonal trend corroborates the findings of Edokpayi *et al*. (2021) in South Africa and indicates that rainfall and environmental runoff strongly influence microbial water quality in tropical climates.

In another related study, Ibrahim *et al.* (2023) analyzed river water used for irrigation and domestic activities in Nasarawa State, north-central Nigeria. Their investigation revealed heavy microbial loads, particularly during the wet months, with confirmed isolates including *E. coli*, *Shigella spp.*, and fecal streptococci. The researchers employed pour plate techniques alongside biochemical confirmation for identification. Their findings not only demonstrate the vulnerability of surface waters to contamination but also highlight the dual risk posed to communities: direct exposure through domestic use and indirect exposure via consumption of irrigated crops contaminated with pathogens.

Together, these studies demonstrate that microbial contamination of water sources is a nationwide and regional challenge in Nigeria and Sub-Saharan Africa. Whether in sachet water, wells, boreholes, or rivers, contamination is often linked to poor sanitary protection, unhygienic practices, seasonal runoff, and infrastructural lapses. They also reinforce the reliability of the pour plate method, often combined with MPN or selective media, in detecting and quantifying bacterial loads across different water sources. Importantly, the findings resonate with the present study in Orhionmwon, where boreholes were found to be relatively safe compared to wells and river water, which exhibited significant microbial contamination.

Although studies have been done on major rivers in Edo State, little literature exists on microbial assessments of Ikpe River at Evbarue community in Orhionmwon LGA. The river's proximity to industrial facilities, including oil refineries and gas pipelines, as well as its use for daily domestic purposes, makes it a critical site for microbial analysis.

2.7 Prevalent Factors Contributing to Microbial Contamination in Evbarue community in Orhionmwon LGA

The lifestyle and environmental conditions of communities in Orhionmwon LGA area contribute significantly to microbial pollution of the Ikpe River. The river is used by residents for multiple purposes, including bathing, washing, cooking, and even drinking. Several key factors contribute to its contamination:

Open defecation remains a prevalent practice in many areas, particularly along riverbanks and in bushes near water sources. This practice introduces fecal pathogens, such as *Escherichia coli* and *Salmonella*, directly into water bodies, increasing the risk of waterborne diseases like cholera and dysentery (UNICEF and WHO, 2023). Open defecation near rivers undermines water quality and poses significant health risks to communities relying on these water sources for drinking and domestic use.

Wastewater from homes, including water used for bathing, cooking, and cleaning, is frequently discharged directly into rivers without treatment. This untreated wastewater contains organic matter, pathogens, and chemical pollutants, such as detergents, which degrade water quality and disrupt aquatic ecosystems (Mateo-Sagasta *et al.,* 2018). The absence of wastewater treatment infrastructure exacerbates contamination, particularly in densely populated areas near rivers.

Livestock farming, common in surrounding villages, contributes to water contamination through animal waste runoff during rainfall. Faecal matter from livestock introduces pathogens and nutrients, such as nitrogen and phosphorus, into water bodies, leading to eutrophication and harmful algal blooms (Bouwman *et al.,* 2013). This runoff significantly impairs water quality and increases the risk of zoonotic diseases in communities using affected water sources.

Inadequate waste disposal infrastructure results in solid wastes, including plastics, being dumped into rivers or their tributaries. These wastes not only physically pollute water bodies but also release microplastics and chemical pollutants, which harm aquatic life and contaminate drinking water supplies (Li *et al.,* 2021). Improper waste management exacerbates the accumulation of debris in rivers, hindering natural flow and ecosystem health.

Lack of community awareness about water treatment methods leads to the consumption of untreated surface water, increasing exposure to microbial and chemical contaminants. Without knowledge of simple treatment techniques, such as boiling or filtration, communities remain vulnerable to waterborne diseases (Odonkor and Mahami, 2020). Educational interventions are critical to improving water safety practices and reducing health risks in affected areas.

Oil-related activities and industrial expansion contribute to surface water contamination by introducing hydrocarbons, heavy metals, and other pollutants into rivers. These activities disrupt natural purification processes, such as microbial degradation and sedimentation, reducing the ecosystem's ability to self-cleanse (Nriagu *et al.,* 2016). Industrial pollution poses long-term risks to both human health and aquatic biodiversity, necessitating stringent regulatory measures.

In conclusion, open defecation, untreated wastewater, livestock runoff, poor waste disposal, lack of awareness, and industrial activities collectively degrade river water quality, posing significant public health and environmental challenges. Addressing these issues requires integrated approaches, including improved sanitation infrastructure, community education, and stricter pollution controls, to safeguard water resources and protect ecosystems.

These conditions create an environment where pathogenic microorganisms can thrive, increasing the risk of disease outbreaks within the community.

2.8 Mitigation Strategies for Microbial Pollution

Effective control of microbial contamination requires multi-level interventions:

Household-level treatment methods, such as boiling, chlorination, and filtration, are effective in reducing microbial and chemical contaminants in drinking water. Promoting these methods through public health campaigns can empower communities to ensure safe water access, particularly in areas lacking centralized treatment systems. Studies show that household water treatment, when combined with safe storage, significantly reduces waterborne disease incidence (Clasen *et al.,* 2015). Campaigns should focus on affordability, accessibility, and proper use of these methods to maximize their impact.

Safe sanitation facilities must be provided and their use encouraged to curb open defecation, a major source of fecal contamination in water bodies. Community-led total sanitation programs have proven effective in reducing open defecation by promoting the construction and use of latrines (Pickering *et al*., 2019). Incentives, subsidies, and awareness campaigns can further encourage adoption, particularly in rural and peri-urban areas where open defecation is prevalent, thereby protecting water sources from fecal pathogens.

Public education on hygiene, safe water collection, and storage practices is essential to minimize post-collection contamination. Improper handling and storage of water can reintroduce pathogens, undermining treatment efforts. Educational programs that teach handwashing, proper container cleaning, and safe storage techniques have been shown to reduce contamination risks (Rufener *et al.,* 2020). Community-based workshops and school curricula can enhance awareness and foster sustainable hygiene behaviors.

Periodic water quality monitoring by environmental agencies provides critical data for identifying contamination sources and designing targeted interventions. Regular testing for microbial indicators, such as *Escherichia coli* and fecal coliforms, can serve as an early warning system for pollution events (Bain *et al.,* 2021). By integrating monitoring with geographic information systems, agencies can prioritize high-risk areas and allocate resources effectively to mitigate water quality threats.

Regulatory enforcement on industrial effluent discharge and community waste disposal is vital to prevent further degradation of water bodies. Strict regulations on industrial wastewater treatment and solid waste management can reduce the release of pollutants, such as heavy metals and microplastics, into rivers and lakes (Schwarzenbach *et al.,* 2010). Enforcement mechanisms, including fines and compliance audits, ensure accountability and protect aquatic ecosystems from industrial and community-driven contamination.

2.9 Summary of Literature Gaps

The literature consistently shows that microbial contamination is widespread in rural water systems in Nigeria. However, limited studies have focused specifically on drinking water sources like boreholes, hand dug well, and water bodies like the Ikpe River at Evbarue community in Orhionmwon LGA, despite its connection to the Ossiomo River and its exposure to industrial and domestic pollution. This study addresses these gaps and provides evidence that can support public health policy, community awareness, and long-term water safety interventions.

CHAPTER THREE

METHODOLOGY

3.1 Study Area

This chapter presents the research design and methodological procedures adopted for assessing the microbial quality of drinking water from multiple sources in Orhionmwon Local Government Area (LGA), Edo State, Nigeria. The study evaluated water samples from rivers, wells, boreholes, with Ikpe River serving as the representative source for river water, while the well and borehole water sample were collected from Evbarue community in Orhionmwon LGA. It outlines the study area, sample collection methods, analytical techniques (particularly the pour plate method), quality control measures, and the statistical methods used for data analysis.

3.2 Study Population

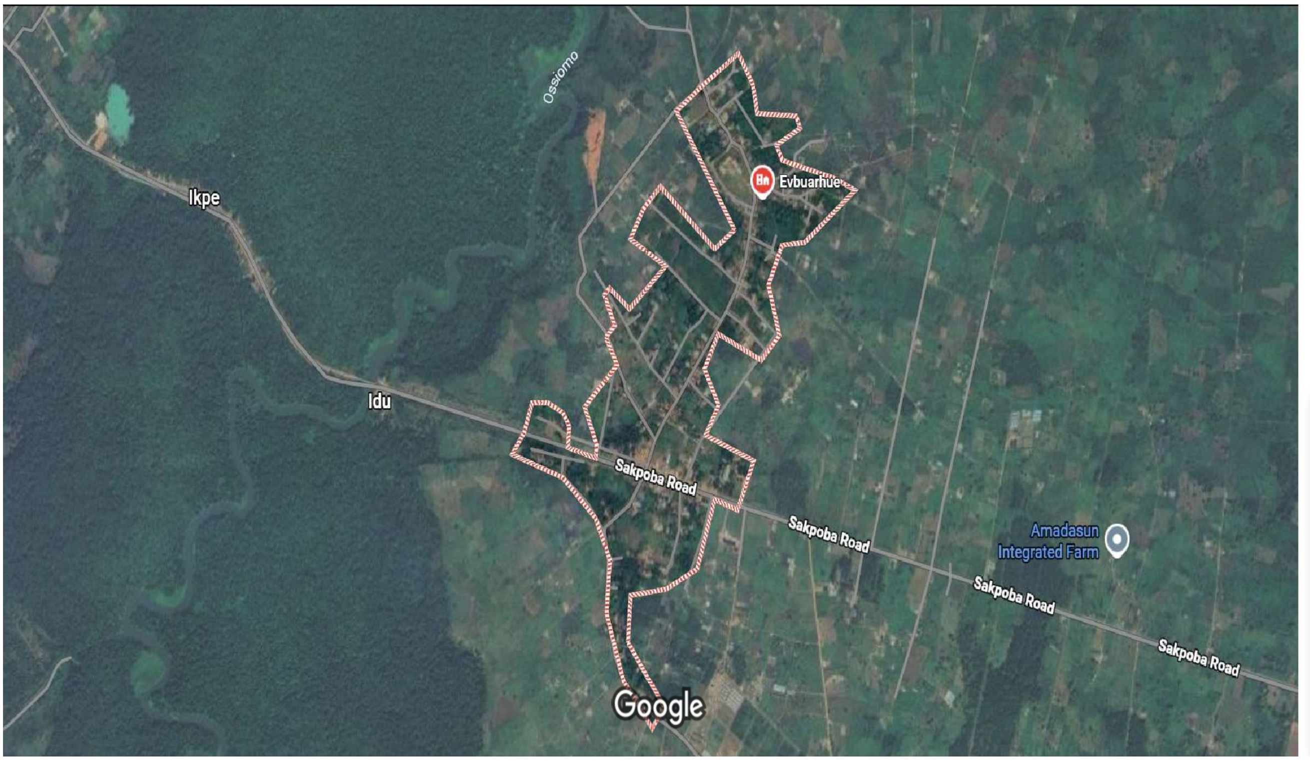

Figure 3.1: Map of study area showing Evbuarhue community in Orhionmwon LGA

The study was carried out in Evbuarhue community in Orhionmwon LGA, located in southern Edo State, Nigeria with GPS coordinate of Latitude 6° 15' 13" N, Longitude 5° 59' 3" E. It comprises a mixture of rural and peri-urban communities, many of which rely on varied sources of water for daily consumption.

Orhionmwon Local Government Area (LGA), located in southern Edo State with its headquarters in Abudu town, is one of Nigeria's largest rural administrative units, covering approximately 2,382 km^2 (NPC, 2006). According to the 2006 national census, the population of Orhionmwon was recorded at 206,717 residents, making it a moderately populated region compared to urban LGAs like Oredo (NPC, 2006). Local estimates from the Orhionmwon LGA Council suggest a higher figure, closer to 342,000, reflecting population growth driven by rural-urban migration and natural increase (Orhionmwon LGA, 2020). Based on Nigeria's annual population growth rate of approximately 2.6% (World Bank, 2021), projections for 2021 estimate the population to exceed 300,000, with potential growth to around 350,000 by 2025. This growth is fueled by the region's agricultural productivity, particularly in yams, cassava, and cocoa, as well as oil and gas activities, which attract labor migration (Akinyemi and Adebayo, 2019). The demographic composition is diverse, predominantly consisting of the Bini (Edo) ethnic group, alongside significant Yoruba, Igbo, and Hausa communities (Omoera, 2017). The youth population (under 35 years) constitutes the majority, supporting Orhionmwon's role as Edo State's "food basket" (Akinyemi and Adebayo, 2019). However, challenges such as limited infrastructure, including water and sanitation facilities, strain resources and exacerbate public health risks (UNICEF and WHO, 2023). Addressing these issues is critical to sustaining the growing population and ensuring community well-being.

Common sources of drinking water include surface water (Ikpe River), groundwater sources (boreholes and hand-dug wells), and commercially packaged sachet water. Ikpe River flows through Ologbo community and connects to the Ossiomo River, serving as a primary surface water source for some residents. Orhionmwon is characterized by heavy rainfall during the wet season, unregulated waste disposal, and limited water treatment infrastructure, all of which contribute to microbial contamination risks. The area is home to both industrial and domestic activities, including farming, fishing, open defecation, and petroleum refining.

3.3 Research Design

The study employed a descriptive cross-sectional research design, which involved collecting and analysing water samples from four major sources, river water (Ikpe River), borehole water, well water, and sachet water, within selected communities in Orhionmwon LGA. The design allowed for point-in-time assessment and comparison of microbial quality across these sources.

3.4 Sample Collection

3.4.1 Sampling Sites and Sources

Water samples were collected from:

a) **Ikpe River** (surface water): 2 locations (upstream, midstream, downstream)

b) **Boreholes**: 2 different functional boreholes across the LGA

c) **Hand-dug wells**: 2 separate wells in different residential zones

This provided a total of 6 sampling points, ensuring representation from each water source commonly consumed in the LGA.

3.4.2 Sampling Procedure

a) All samples were collected using sterile 500 mL glass or plastic bottles.

b) Bottles were pre-rinsed three times with the respective water source (except sachet).

c) For river, borehole, and well water, samples were taken approximately 30 cm below the surface to avoid contamination from debris or surface films.

d) Sachet water was collected aseptically from unopened sachets.

e) Samples were labeled appropriately, stored in an ice-packed cooler, and transported to the laboratory within 6 hours to ensure sample integrity.

3.5 Microbial Analysis

3.5.1 Method Used: Pour Plate Technique

The pour plate method was used to determine the Total Heterotrophic Bacterial Count (THBC) and presence of coliforms and fungi/mould.

Materials and Reagents

a) Nutrient agar (NA) for total bacteria

b) MacConkey agar or Eosin Methylene Blue (EMB) agar for coliforms

c) Potato Dextrose Agar for fungi/mould

d) Sterile Petri dishes

e) Micropipettes and tips

f) Sterile distilled water

g) Incubator set at 35–37°C

Procedure

1. Serial dilutions of the water samples were prepared (e.g., 10^{-1} to 10^{-3}).
2. **1 mL of the appropriate dilution** was transferred into sterile Petri dishes.

3. About **15–20 mL of molten agar** (cooled to 45°C) was poured into each dish and gently swirled to mix.
4. Plates were allowed to solidify and incubated at **37°C for 24–48 hours**.
5. After incubation, colonies were counted using a **manual colony counter** or magnifying lens.
6. Colony counts were recorded as **cfu/mL** and samples were tested in **triplicates** for accuracy.

3.5.2 Biochemical Confirmation

Suspected *E. coli* or *Salmonella* colonies was sub-cultured and subjected to Gram staining and biochemical tests (e.g., IMViC tests) for confirmatory identification.

3.6 Quality Control and Assurance

To ensure reliability and validity of the microbial results:

a) All glassware and media were sterilized by autoclaving at 121°C for 15 minutes.

b) Negative controls (blanks) were included in each batch to check for contamination.

c) All experiments were done in triplicates.

d) Media were quality-tested by incubating uninoculated plates.

e) Proper personal protective equipment (PPE) was worn during sample handling.

3.7 Data Analysis

a) Microbial data (cfu/mL) were recorded for each sampling point and expressed as mean ± standard deviation.

b) Descriptive statistics such as mean counts and frequency of contamination were calculated.

c) Data were compared with WHO and Nigerian Standard for Drinking Water Quality (NSDWQ) limits.

d) Results were presented in tables and charts.

e) Statistical comparisons among sites were performed using Analysis of Variance (ANOVA) or t-tests, depending on sample size and distribution.

3.8 Ethical Considerations

Although this study involved environmental sampling and no human or animal subjects, ethical best practices were observed, including:

a) Community engagement and permission from local authorities before sampling.

b) Proper handling and disposal of biological waste in accordance with biosafety protocols.

c) No identifiable personal data was collected.

3.9 Limitations of the Study

a) The study was limited to culture-based methods and did not employ molecular techniques like PCR.

b) Seasonal variation was not captured, as samples were collected during a single period.

CHAPTER FOUR

RESULTS

4.1 Results of Microbial Analysis

The results obtained from the microbial analysis of five drinking water samples collected from different sources in Orhionmwon Local Government Area, Edo State are presented in table below. The water sources include two boreholes, two hand-dug wells, and the Ikpe River (as a representative surface water source). Additionally, common microbial isolates from the samples were identified and compared. Access to safe drinking water is a fundamental requirement for public health, yet many communities in rural areas, such as Orhionmwon Local Government Area in Edo State, Nigeria, face challenges related to water quality. Contamination of drinking water sources with microbial pathogens poses significant health risks, including waterborne diseases such as cholera, typhoid fever, and diarrheal illnesses (WHO, 2022). To assess the microbial quality of drinking water in Orhionmwon, a comprehensive analysis was conducted on five water samples collected from diverse sources, including two boreholes (S1 and S2), two hand-dug wells (S3 and S4), and the Ikpe River (S5), which serves as a representative surface water source.

Table 4.1: Total Bacterial Count in Water Samples

Sample	10^{-1}	10^{-2}	10^{-3}	Mean Count (cfu/mL)
S1	23	2	NG	22
S2	16	1	NG	13
S3	38	6	NG	490
S4	54	9	1	810
S5	TNC	68	5	5900

Table 4.1 shows the total bacteria count in the water sample with different serial dilution 10^1, 10^2, $10^{3,}$ from the table S2 had the lowest microbial growth with S5 had the highest microbial load.

Key To The Table:

S1- Borehole 1

S2 – Borehole 2

S3 – Well 1

S4 – Well 2

S5 – River Sample

Table 4.2: Total Fungal Count in Water Samples

Sample	10^0	10^{-1}	10^{-2}	Mean Count (cfu/mL)
S1	1	NG	NG	1
S2	NG	NG	NG	0
S3	8	NG	NG	8
S4	12	1	NG	11
S5	98	8	NG	89

Table 4.2 showed total fungal count in sample s1-s5, while S2 had no detected fungal, S1, S3,S4 and S5 all showed fungal growth with S5 being the highest.

Table 4.3: Total Coliform Count in Water Samples

Sample	10^0	10^{-1}	Mean Count (cfu/mL)
S1	NG	NG	0
S2	NG	NG	0
S3	4	NG	4
S4	NG	NG	0
S5	10	2	15

Table 4.3 shows the total coliform count in water samples S1-S5, S1 and S2 had zero coliform count while S3 and S5 had coliform count above limit.

Table 4.4: E. coli Count in Water Samples

Sample	10^0	10^{-1}	Mean Count (cfu/mL)
S1	NG	NG	0
S2	NG	NG	0
S3	2	NG	2
S4	NG	NG	0
S5	8	1	9

Table 4.4 shows the E. Coli count in the water sample S1-S4 , with E. Coli present only in S3 and S5

Table 4.5: Microbial Isolates in Water Samples

Sample	Bacterial Isolates	Fungal Isolates
S1	*Bacillus spp.*, *P. aeruginosa*, *Proteus spp.*	*Aspergillus spp.*
S2	*Bacillus spp.*, *P. aeruginosa*	None
S3	*Bacillus spp.*, *P. aeruginosa*, *Proteus spp.*, *Klebsiella spp.*, *E. coli*	*Aspergillus spp.*, *Rhodotorula spp.*
S4	*Bacillus spp.*, *P. aeruginosa*, *Proteus spp.*, *Klebsiella spp.*	*Aspergillus spp.*, *Mucor spp.*, *Rhodotorula spp.*
S5	*Bacillus spp.*, *P. aeruginosa*, *Proteus spp.*, *Klebsiella spp.*, *E. coli*, *Enterobacter spp.*	*Aspergillus spp.*, *Mucor spp.*, *Penicillium spp.*, *Rhodotorula spp.*

Table 4.5 Summarizes the Bacterial and Fungal isolates Found in each Water Source

Table 4.6: WHO and Nigerian Industrial Standard for drinking water and health significance/remarks

Parameter	WHO Guideline Value	NIS Guideline Value (NIS 554:2015)	Health Significance / Remarks
Total Coliforms	0 CFU/100 ml	0 CFU/100 ml	Indicator of general water quality and potential contamination; presence suggests possible pathway for pathogens.
Faecal Coliforms (Thermotolerant Coliforms)	0 CFU/100 ml	0 CFU/100 ml	Indicates faecal contamination and possible presence of enteric pathogens.
Escherichia coli (E. coli)	0 CFU/100 ml	0 CFU/100 ml	Specific indicator of recent faecal contamination; its presence indicates potential pathogenic microorganisms.
Enterococci (Faecal Streptococci)	0 CFU/100 ml	0 CFU/100 ml	Indicates contamination from human or animal faeces; used as a secondary indicator.
Clostridium perfringens (including spores)	0 CFU/100 ml	0 CFU/100 ml	Resistant spores indicate remote or persistent contamination; used for assessing water treatment effectiveness.
Heterotrophic Plate Count (HPC)	No health-based guideline; typically <500 CFU/ml (operational limit)	≤500 CFU/ml	Reflects general microbial growth; high counts may indicate regrowth in distribution systems.
Total Fungi Count (Yeasts and Moulds)	0 CFU/100ml	0 CFU/100ml	Indicate organic contamination and poor hygiene; some fungi produce toxins or cause allergic and optimistic infections.

4.2 Total Bacterial Count

Table 4.1 shows the total heterotrophic bacterial counts in cfu/mL for each sample.

Observations:

All samples showed varying levels of bacterial presence.

River water had the highest count (5900 cfu/mL).

Borehole samples had the lowest bacterial loads, well below WHO's heterotrophic bacteria guideline of <500 cfu/mL.

4.3 Total Fungal Count

Table 4.2 presents the total fungal count (cfu/mL) across the samples.

Observations:

River water again showed the highest fungal load.

Borehole 2 had no fungal growth, while borehole 1 had minimal growth.

Well water fungal counts exceeded WHO safe thresholds, indicating possible environmental contamination.

4.4 Total Coliform Count

Coliform presence is a key indicator of fecal or environmental contamination.

Observations:

Coliforms were detected in river and one well sample.

Borehole water samples complied with WHO/NIS limits of 0 cfu/100 mL.

River and well 1 samples exceeded permissible limits, indicating likely fecal contamination.

4.5 E. coli Count

E. coli is the most specific indicator of recent fecal pollution and public health risk.

Observations:

E. coli was only detected in well 1 and river water, both exceeding WHO/NIS limit of 0 cfu/100 mL.

Boreholes and well 2 showed no growth, suggesting better microbial safety.

4.6 Microbial Isolates Identified

Table 4.5 summarizes the bacterial and fungal isolates found in each water source.

The microbial analysis of the different water sources in Orhionmwon revealed varying distributions of bacterial and fungal isolates.

Borehole 1 yielded *Bacillus spp.*, *Pseudomonas aeruginosa*, and *Proteus spp.*, with *Aspergillus spp.* as the only fungal isolate.

Borehole 2 contained *Bacillus spp.* and *Pseudomonas aeruginosa*, but no fungal isolates were detected.

In contrast, Well 1 showed a wider range of microbial contamination, including *Bacillus spp.*, *Pseudomonas aeruginosa*, *Proteus spp.*, *Klebsiella spp.*, and *Escherichia coli*, while fungal isolates identified were *Aspergillus spp.* and *Rhodotorula spp.*

Similarly, Well 2 harbored *Bacillus spp.*, *Pseudomonas aeruginosa*, *Proteus spp.*, and *Klebsiella spp.*, alongside fungal isolates such as *Aspergillus spp.*, *Mucor spp.*, and *Rhodotorula spp.*

The Ikpe River recorded the highest diversity of contaminants, with bacterial isolates including *Bacillus spp.*, *Pseudomonas aeruginosa*, *Proteus spp.*, *Klebsiella spp.*, *Escherichia coli*, and

Enterobacter spp., while fungal contaminants consisted of *Aspergillus spp.*, *Mucor spp.*, *Penicillium spp.*, and *Rhodotorula spp.*

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The results of this study revealed varying degrees of microbial contamination in drinking water sources across Orhionmwon Local Government Area, with surface water (Ikpe River) and well water showing significantly higher microbial loads than borehole and sachet water samples. These findings are consistent with numerous studies conducted in rural Nigeria, which have identified surface and shallow groundwater sources as the most susceptible to microbial pollution (Akinbile *et al*., 2023).

5.1.1 Total Bacterial Count

The total heterotrophic bacterial count (THBC) is an indicator of the general microbial load in water and reflects the sanitary condition of the source. According to WHO guidelines, heterotrophic plate counts should ideally not exceed **500 cfu/mL** in treated water (WHO, 2022). In this study, the Ikpe River sample recorded a mean bacterial count of 5900 cfu/mL, which is more than ten times the permissible limit, indicating heavy microbial contamination likely caused by surface runoff, domestic waste discharge, and open defecation practices commonly observed in the area (Uhunamure *et al.,* 2023).

Well 2 (810 cfu/mL) and Well 1 (490 cfu/mL) also recorded high bacterial loads, which may be attributed to their shallow depth, proximity to latrines, and use of uncovered or poorly maintained water storage structures. Borehole 1 and Borehole 2, by contrast, showed significantly lower counts (22 and 13 cfu/mL respectively), which are within acceptable limits. This suggests that properly constructed and protected boreholes offer better microbial safety, aligning with findings by Edokpayi *et al.* (2021) and Agbro *et al.* (2021).

5.1.2 Total Fungal Count

Fungi in drinking water can result from organic pollution and environmental exposure, especially in surface water and shallow wells. The river sample recorded a fungal load of 89 cfu/mL, far exceeding counts in other samples. This agrees with observations from Ibrahim *et al.* (2023), who reported that rivers exposed to human and animal activities often harbor fungal spores such as *Aspergillus*, *Penicillium*, and *Mucor*. The presence of fungi in well samples also suggests inadequate sanitary protection and possible biofilm formation in stagnant water containers.

5.1.3 Total Coliform Count

Coliform bacteria are used as indicators of water sanitation quality. According to both WHO and NIS standards, coliforms must not be detectable in 100 mL of drinking water. The Ikpe River had a coliform count of 15 cfu/mL, clearly indicating fecal pollution, possibly from human and animal waste entering the river. Well 1 also showed coliform presence (4 cfu/mL), suggesting environmental contamination possibly due to proximity to refuse dumps, pit latrines, or surface runoff during rainfall. Similar contamination patterns were reported by Egbere *et al.* (2021) in their study on well water in Jos and by Onyekwere *et al.* (2022) in Anambra.

Interestingly, borehole samples showed no coliform growth, confirming the effectiveness of depth and proper construction in protecting groundwater sources from surface microbial infiltration.

5.1.4 E. coli Presence

The presence of *Escherichia coli* (E. coli) is a direct indicator of recent fecal contamination. In this study, *E. coli* was detected in well 1 (4 cfu/mL) and river water (9 cfu/mL), both of which violate the WHO/NIS standard of 0 cfu/100 mL. This raises significant health concerns, as *E. coli* is associated with diarrheal diseases, urinary tract infections, and other gastrointestinal illnesses (WHO, 2022). The detection of *E. coli* confirms poor hygiene practices and inadequate protection of these sources. In contrast, boreholes and well 2 showed no presence of *E. coli*, reaffirming their relative safety.

5.1.5 Identified Microbial Isolates

A variety of bacterial and fungal isolates were detected, with *Bacillus spp.*, *Pseudomonas aeruginosa*, and *Proteus spp.* being present in all water types. These organisms are opportunistic pathogens that may cause infections, especially in immunocompromised individuals. The presence of *Klebsiella spp.*, *Enterobacter spp.*, and *E. coli* in surface and well water further emphasizes the risk of gastrointestinal infections from untreated water. Similar findings were reported by Babalola and Osibanjo (2023), who noted these pathogens in river and well water used for domestic purposes in southwestern Nigeria.

Fungal isolates such as *Aspergillus spp.*, *Mucor spp.*, *Penicillium spp.*, and *Rhodotorula spp.* were also detected, particularly in surface water. These fungi can be allergenic or pathogenic under certain conditions and indicate organic contamination, high nutrient load, or decaying material (Arowolo *et al.,* 2022).

These organisms are known for causing a wide range of illnesses:

- ***E. coli***: Diarrhea, gastroenteritis, hemolytic uremic syndrome.
- ***Pseudomonas aeruginosa***: Opportunistic infections, especially in hospitals and immunocompromised persons.
- ***Klebsiella spp.*** and ***Enterobacter spp.***: UTIs, pneumonia, septicemia.
- ***Fungi***: Respiratory infections and allergies (WHO, 2022).

5.1.6 Implications for Public Health in Orhionmwon

Given that many residents of Orhionmwon LGA rely on these water sources without treatment, the results highlight a significant public health risk. Ikpe River, in particular, is a multipurpose water body used for drinking, bathing, washing, and fishing. Without adequate community education, sanitary infrastructure, and point-of-use water treatment options, the risk of disease outbreaks remains high.

The microbial safety of borehole water in this study underscores the importance of investing in protected groundwater development. However, the overall findings suggest a pressing need for water safety planning, including routine water quality monitoring, awareness campaigns, and affordable household treatment methods like boiling or chlorination.

5.2 CONCLUSION

This study investigated the microbial quality of drinking water sources in Orhionmwon Local Government Area of Edo State, focusing on five distinct sources: two boreholes, two hand-dug wells, and a surface water body (Ikpe River). The analysis was conducted using the pour plate method to enumerate microbial colonies and identify bacterial and fungal species.

Results revealed that:

a) Borehole 1 and Borehole 2 met the World Health Organization (2022) and Nigerian Industrial Standard (NIS 554:2017) microbial requirements, with *E. coli* and total coliforms absent and heterotrophic plate counts (HPC) well below 500 CFU/mL.

b) Well 1 was found to be contaminated with *E. coli* and total coliforms, indicating recent fecal pollution and making it unfit for drinking without treatment.

c) Well 2 exceeded the acceptable HPC limit (810 CFU/mL), though it did not contain fecal indicators, suggesting biofilm development or organic pollution.

d) Ikpe River was heavily contaminated, with high counts of *E. coli*, coliforms, and fungi. This surface water is unsafe for drinking and poses significant public health risks.

e) Several pathogenic and opportunistic microorganisms were isolated, including *E. coli*, *Klebsiella spp.*, *Pseudomonas spp.*, *Bacillus spp.*, *Proteus spp.*, and fungal isolates like *Aspergillus*, *Mucor*, and *Penicillium* species.

These findings indicate that groundwater (particularly boreholes) remains the most microbiologically reliable source when properly constructed and maintained. In contrast, surface water and shallow wells are highly vulnerable to contamination, especially in areas with open defecation, poor sanitation infrastructure, and agricultural runoff.

The presence of *E. coli* and coliforms in some sources implies a public health hazard, especially to vulnerable groups such as children, the elderly, and immunocompromised individuals. Regular microbial monitoring, community health education, and strategic water treatment interventions are essential to safeguard drinking water safety in the study area.

5.3 RECOMMENDATIONS

Based on the findings and conclusions of this study, the following recommendations are made:

53.1 For Government and Public Health Authorities

1. Enforce regular microbial monitoring of drinking water sources at the community level using WHO and NIS guidelines.
2. Rehabilitate or decommission unsafe water sources, especially hand-dug wells and surface water systems without protection or treatment infrastructure.
3. Promote sanitation programs aimed at eradicating open defecation, especially near water collection points.
4. Implement community-based Water Safety Plans (WSPs) as advocated by WHO, to reduce risks from catchment to consumer.

5.3.2 For Communities and Households

1. Avoid direct consumption of untreated river or well water; boiling or chlorination should be mandatory.
2. Protect wells and boreholes with proper linings, covers, and fences to minimize contamination.
3. Maintain hygienic storage practices, such as using clean, closed containers and avoiding hand contact with water during retrieval.

5.3.3 For Water Producers (e.g., Sachet Water Operators)

1. Adhere strictly to NIS microbial water quality specifications and maintain clean processing environments.

2. Train staff in good hygiene practices and conduct routine quality control using certified laboratories.

5.3.4 For Future Researchers

1. Conduct seasonal microbial profiling to evaluate contamination trends during dry and rainy periods.
2. Investigate protozoan and viral contaminants, which are not covered in this study but pose serious waterborne risks.
3. Explore cost-effective disinfection technologies, such as solar water disinfection (SODIS) and biosand filters, suitable for rural communities.

5.4 Contribution to Knowledge

This research provides updated data on the microbial quality of diverse drinking water sources in Orhionmwon LGA, highlighting the persistent risk posed by untreated surface and shallow groundwater. It supports the implementation of microbial surveillance and affordable treatment technologies to improve water safety in underserved Nigerian communities.

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