DETECTION AND ISOLATION OF *Escherichia coli* IN THE WASTEWATER FROM RESTAURANTS IN THE UNIVERSITY OF BENIN, NIGERIA

BY

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DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

NOVEMBER 2025

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE HONOURS (B.Sc. HONS) IN MICROBIOLOGY

NOVEMBER 2025

CERTIFICATION

This is to certify that this project was carried out by Emmanuella Oghosa DOMINIC (MISS) in the Department of Microbiology, Faculty of Life Sciences, University of Benin, under the supervision of Prof. E. O. Igbinosa, submitted to the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc.) degree in Microbiology.

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(PROJECT SUPERVISION)	

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Prof. E. O. IGBINOSA	**Date**
(Head of Department)	

DEDICATION

This work is duly dedicated to God Almighty, who has been and will always be my firm foundation. It is also dedicated to my wonderful parents, Mr. and Mrs. Dominic Agie, my very own superheroes.

ACKNOWLEDGEMENTS

I give thanks to God Almighty for His guidance, strength, and grace throughout my academic journey. His unwavering love and favour have carried me through every challenge and triumph.

I sincerely appreciate my supervisor, the Head of the Department of Microbiology—Prof. E. O. Igbinosa, for his constructive criticism and guidance throughout the course of this research. Special thanks to my course advisers, Mr. Oribhabor and Mrs. Agbowaneten, for being such lovely parental figures during my stay at the University of Benin, and to my lecturers, Dr. Chris Ajuzie and Prof. C. E. Oshoma, for always having kind words and titbits of helpful advice to share.

My heartfelt gratitude goes to my parents, Mr. and Mrs. Dominic Agie. Thank you so much, Mummy and Daddy, for your financial, moral, and emotional support. Your prayers and constant encouragement have been my greatest motivation. I love you both immensely. To my brothers, Marvellous and Wealth, my very own cheerleaders, your words of encouragement during my moments of weakness meant more than you can imagine.

To my extended family, Mr. and Mrs. Izokun, Aunty Edith, Mummy Jason, Mummy Gold, Aunty Jane, Aunty Elizabeth, Mummy Treasure, Aunty Ehimwema, Progress, and Uncle Moses, thank you for your calls, prayers, celebrations, and those unexpected credit alerts that came just when I needed them most. You all are the real MVPs.

To my dear friends: Adaobi, Tari, Salem, Precious, Gold, Gabriella, Nancy and Abah—thank you for always showing up for me, and for simply being in my corner. My fellow *Observers;* Tuoyo, Tiana, Alfonso, Ose, Precious, and Truth for your watchful eyes and for the sense of community we have shared thus far, I am grateful. My wonderful roommates thank you for being ever so supportive.

My senior colleagues: Jerry, Jibola, Favour, Merit, and Alex, thank you for helping me navigate the complexities of Microbiology, for answering my numerous questions, and for being present throughout my academic journey.

Finally, to myself. Emmanuella Oghosa Dominic, you are that girl. You are like a tree planted by the rivers of water, one that knows no dry season. Thank you for not giving up, for writing in spite of yourself and showing the beauty of your words. Remember, as you often do, “smile, don’t you know God loves you.” Rest assured that everything works together for your good. As James Alexander Malcolm MacKenzie Fraser would say, *“Je suis prest”*—you are ready, my love.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Wastewater generated from restaurants often contains a mixture of organic matter and microbial contaminants that may pose environmental and public health risks. This research focuses on the detection and isolation of *Escherichia coli (E. coli)* from wastewater collected from selected restaurants within the University of Benin, Benin City, Nigeria. For this study, wastewater samples were obtained from three restaurants: Helena's Kitchen, Home and Away, and Buka—during peak operation hours. The samples were collected aseptically and analysed using standard microbiological methods. The pour plate technique was employed for total heterotrophic bacterial counts, while selective media such as Eosin Methylene Blue (EMB) agar were used for the isolation of *E. coli*. Biochemical tests including indole, methyl red, citrate, urease, and triple sugar iron (TSI) were used to confirm the isolates. The results showed high microbial loads across all samples, with *E. coli* being consistently present, indicating faecal contamination of the wastewater. The identification of other bacterial species suggest contamination from multiple sources such as food residues, human handling, and the environment. The findings reveal poor wastewater management and hygiene practices in the studied restaurants. In conclusion, the consistent presence of *E. coli* in restaurant wastewater signifies potential health and environmental hazards within the University of Benin. It is therefore recommended that wastewater from restaurants be regularly monitored, and that adequate sanitation infrastructure and treatment systems be put in place to prevent contamination and safeguard public health.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Escherichia coli (*E. coli*) is a Gram-negative, rod-shaped bacterium commonly found in the intestines of humans and animals. While most *E. coli* strains are harmless and part of the normal gut flora, some pathogenic strains can cause a wide range of illnesses, including diarrhea, urinary tract infections, and even life-threatening conditions such as hemolytic uremic syndrome (Yu *et al.,* 2022). These pathogenic strains often enter the environment through human and animal waste and contaminate water sources if not properly treated. Water is a fundamental resource for human life and the functioning of food service establishments, including those located within tertiary institutions such as University of Benin. In the context of restaurant operations, water is used not only for cooking and cleaning but also for disposal of waste streams. Wastewater generated from food service operations is often rich in organic load and may carry microbial contaminants originating from food residues, utensils, human handling, and other sources of faecal and non-faecal contamination. Among the microbial contaminants of concern is *Escherichia coli* (*E. coli*), a Gram-negative, rod-shaped bacterium widely used as an indicator organism of faecal pollution in environmental water, and in some instances a direct pathogen causing gastrointestinal infections. Studies in Nigeria have indicated that *E. coli* and its diarrhoeagenic pathotypes are present in drinking-water wells, surface water and other environmental sources, signalling possible risks (Odetoyin, Ogundipe & Onanuga, 2022). Furthermore, wastewater discharged from restaurants may provide a route for *E. coli* to enter the wider environment and pose potential health risks to campus communities and downstream water users. Previous research in Nigeria showed that treated wastewater used for irrigation harbored enterotoxigenic *E. coli*, illustrating that even after treatment, *E. coli* may persist in effluent streams. (Onoh *et al.*, 2022). The detection and isolation of *E. coli* in wastewater from restaurants thus becomes relevant for both public health and environmental sanitation. On the campus of the University of Benin, with numerous restaurants and food outlets serving students, staff and visitors, the effective management of wastewater and its microbial load is especially pertinent. Understanding the extent to which *E. coli* is present in restaurant wastewater, the isolation of viable strains, and the implications for sanitation infrastructure and environment is paramount. This study therefore focuses on the detection and isolation of *E. coli* in wastewater from restaurants in the University of Benin, thereby contributing to the body of knowledge on environmental microbiology, food service sanitation and campus-based public health.

1.2 Aim and Objectives of the Study

The aim of this study is to detect and isolate *E. coli* in wastewater from restaurants in the University of Benin campus.

The objectives of this study were to:

(1) evaluate the total heterotrophic bacterial count of wastewater collected from restaurants in the University of Benin,

(2) isolate and identify *Escherichia coli* from wastewater samples collected from restaurants in the University of Benin,

(3) assess the public health significance of the occurrence of *Escherichia coli* in restaurants wastewater within the University of Benin campus.

CHAPTER TWO

2.0 LITERATURE REVIEW

Water is one of the most essential natural resources that supports all forms of life on earth. However, as populations increase and urbanization expands, water resources are increasingly threatened by contamination from human, industrial, and agricultural activities. Among the most concerning forms of contamination is microbial pollution, particularly by faecal indicator bacteria such as *Escherichia coli* (*E. coli*), which signifies the possible presence of disease-causing microorganisms. Wastewater generated from restaurants, food processing facilities, and households often carries large loads of organic materials, nutrients, and microorganisms, making it a critical environmental and public health concern.

The detection and isolation of *E. coli* in restaurant wastewater serve not only as an indicator of faecal contamination but also as a reflection of hygiene and waste management practices within such establishments. In developing countries, including Nigeria, restaurant wastewater is often discharged into open drains or surface water bodies without adequate treatment. This increases the risk of environmental pollution, disease transmission, and antimicrobial resistance proliferation (Akhigbe & Igbinosa, 2022).

The University of Benin, like many higher institutions in Nigeria, hosts a large number of food outlets and restaurants that serve thousands of students and staff daily. These outlets produce substantial quantities of wastewater containing organic matter and microbial contaminants. Investigating the microbial profile of such wastewater, especially the presence of *E. coli*, provides insights into potential health hazards, environmental impacts, and the effectiveness of waste management systems on campus.

2.1 Concept of Wastewater and Restaurant Wastewater

Wastewater refers to any water that has been adversely affected in quality by human use. It encompasses domestic sewage, industrial effluents, agricultural run-off, and commercial discharges (World Health Organization [WHO], 2022). The composition of wastewater typically includes organic and inorganic substances, nutrients (nitrogen and phosphorus), suspended solids, detergents, and pathogenic microorganisms. Depending on its source, wastewater may contain chemical pollutants, pharmaceuticals, and heavy metals (United Nations Environment Programme [UNEP], 2020).

Wastewater is generally classified into three main categories: domestic (or municipal) wastewater, industrial wastewater, and stormwater runoff. Domestic wastewater originates primarily from households, consisting of black water (toilet waste) and grey water (from sinks, showers, and kitchens). Industrial wastewater arises from manufacturing processes, while stormwater includes surface runoff during rainfall events that collect pollutants from roads, buildings, and land surfaces (Bitton, 2019).

Restaurant wastewater falls under the category of commercial wastewater, and its characteristics differ from domestic wastewater due to the high concentration of fats, oils, grease (FOG), food residues, and detergents used in cleaning. According to Zhang *et al.,* (2020), restaurant wastewater often contains high levels of biochemical oxygen demand (BOD) and chemical oxygen demand (COD), which create favourable conditions for microbial growth and biochemical activities. These conditions can encourage the increase in population of both harmless and pathogenic microorganisms.

In Nigeria, restaurant wastewater management remains largely informal and unregulated. Many small and medium-sized eateries, particularly those operating within academic institutions, lack proper wastewater treatment facilities. The wastewater is often released into open drainage channels or directly into the environment, where it stagnates and mixes with stormwater or groundwater. A study by Adebayo and Adesina (2021) found that restaurant effluents in urban areas of southwestern Nigeria contained high concentrations of *E. coli* and other enteric bacteria, exceeding the WHO permissible limits for safe discharge.

The University of Benin, situated within Benin City's urban area, shares similar challenges. With numerous food outlets serving a large student population, the volume of wastewater generated daily is significant. Wastewater from restaurants such as Helena's Kitchen, Buka, and Home and Away typically contains remnants of cooked and raw foods, dishwashing detergents, and organic debris. Without proper treatment, these effluents become nutrient-rich habitats for bacteria like *E. coli*, which can persist in the environment and spread through water channels (Nnaji & Chukwu, 2020).

The lack of structured wastewater management in university campuses contributes to potential environmental pollution and health risks. Open drainage systems serve as breeding grounds for mosquitoes, vectors, and rodents, while microbial contaminants may seep into nearby soil and groundwater systems. Therefore, understanding the microbial characteristics of restaurant wastewater is crucial for developing sustainable waste management and sanitation strategies within university environments.

2.2 Overview of *Escherichia coli*

Escherichia coli (*E. coli*) is a Gram-negative, rod-shaped, facultatively anaerobic bacterium belonging to the family *Enterobacteriaceae*. It was first described by Theodor Escherich in 1885 as a normal inhabitant of the intestinal tract of humans and warm-blooded animals (Madigan *et al.,* 2019). While most *E. coli* strains are non-pathogenic and play important roles in digestion and vitamin K production, certain strains have acquired virulence factors that make them pathogenic, capable of causing gastrointestinal and extraintestinal infections (Nataro & Kaper, 2019).

In microbiology, *E. coli* serves as a primary indicator organism for faecal contamination. This is because it is consistently present in human and animal feces at high concentrations, is relatively easy to detect, and does not multiply significantly outside the host under normal environmental conditions (Edberg *et al.,* 2021). The presence of *E. coli* in environmental samples such as water or wastewater indicates that faecal matter has entered the system, and thus there is potential for other pathogenic organisms to be present.

Pathogenic *E. coli* strains are classified based on their virulence mechanisms and clinical manifestations. These include:

- **Enteropathogenic *E. coli* (EPEC):** Causes diarrhea in infants, particularly in developing countries, through adherence to intestinal epithelial cells and destruction of microvilli.
- **Enterotoxigenic *E. coli* (ETEC):** Produces heat-labile (LT) and heat-stable (ST) toxins, responsible for travellers’ diarrhoea and outbreaks in areas with poor sanitation.
- **Enterohaemorrhagic *E. coli* (EHEC):** Notably *E. coli* O157:H7, produces Shiga toxin, causing haemorrhagic colitis and haemolytic uraemic syndrome (HUS).

- **Enteroinvasive *E. coli* (EIEC):** Invades intestinal mucosa, producing dysentery-like symptoms similar to *Shigella* infection.
- **Enteroaggregative *E. coli* (EAEC):** Forms biofilms and causes persistent diarrhoea, especially in children.
- **Diffusely adherent *E. coli* (DAEC):** Associated with diarrhoea in older children and adults (Nataro & Kaper, 2019).

The survival and persistence of *E. coli* in wastewater depend on environmental factors such as temperature, pH, organic matter concentration, and sunlight exposure. Studies have shown that *E. coli* can survive for several days to weeks in wastewater and surface water under favorable conditions (Bitton, 2019). High organic content, such as that found in restaurant effluents, provides nutrients that enhance bacterial survival.

Moreover, wastewater often serves as a reservoir for antimicrobial-resistant *E. coli*. The continuous discharge of antibiotics from domestic and clinical sources into wastewater facilitates the selection and propagation of resistant strains (Igbinosa *et al.,* 2023). Resistant *E. coli* from such environments can transfer resistance genes to other bacterial species through horizontal gene transfer, exacerbating the public health challenge of antimicrobial resistance (AMR).

In the University of Benin, where restaurant wastewater is frequently disposed of without treatment, the potential for both pathogenic and antibiotic-resistant *E. coli* to persist in the environment is high. This underscores the need for routine monitoring and microbiological assessment of such effluents.

2.3 Public Health Importance of *E. coli* in Wastewater

The presence of *E. coli* in wastewater holds significant implications for public health, as it serves as an indicator of faecal contamination and potential disease risk. Wastewater is a complex mixture of microorganisms, many of which are harmless. Other microorganisms, particularly enteric pathogens, pose serious risks when transmitted through water, food, or environmental contact. The detection of *E. coli* indicates the possible presence of enteric pathogens such as *Salmonella spp.*, *Shigella spp.*, *Vibrio cholerae*, and enteric viruses (WHO, 2022).

Direct exposure to contaminated wastewater can occur through skin contact, inhalation of aerosols, or accidental ingestion. Indirect exposure occurs when contaminated wastewater seeps into drinking water sources or irrigates crops consumed by humans (Jangid *et al.,* 2020). Both pathways contribute to the spread of waterborne diseases, especially in areas with inadequate sanitation infrastructure.

Globally, *E. coli* is among the leading causes of gastroenteritis, accounting for millions of diarrhoeal cases annually, especially in developing regions (CDC, 2023). The World Health Organization (2022) estimates that waterborne diseases result in over 1.7 billion cases of diarrhoea each year, with approximately 485,000 deaths, most of which occur in children under five years old. In Nigeria, the situation is exacerbated by poor wastewater management systems, overcrowding, and lack of access to safe water (Odetoyin *et al.,* 2022).

Several outbreaks of *E. coli* infections worldwide have been traced to contaminated water or food sources. For example, the 1993 *E. coli* O157:H7 outbreak in the United States linked to undercooked beef resulted in over 700 cases and several fatalities (CDC, 2023). Similarly, in

Africa, waterborne *E. coli* outbreaks have been documented in Kenya, Ghana, and Nigeria, often associated with untreated water or poor sanitation (Onoh *et al.,* 2022).

Beyond acute diarrhoeal illnesses, wastewater-borne *E. coli* also contributes to antimicrobial resistance (AMR) dissemination. Studies have demonstrated that wastewater acts as a reservoir for resistant bacteria and resistance genes. Igbinosa *et al.,* (2023) reported high frequencies of extended-spectrum β-lactamase (ESBL)-producing *E. coli* in untreated wastewater samples from southern Nigeria. The uncontrolled release of such effluents into the environment increases the risk of resistance gene transfer to environmental and commensal bacteria.

In the university setting, wastewater from restaurants and canteens often drains into open gutters and poorly maintained septic systems. Students and staff who come into contact with contaminated surfaces or use nearby water sources for domestic purposes face increased risk of infection. Furthermore, when this wastewater mixes with stormwater and eventually reaches public waterways, it poses risks to the surrounding communities.

The public health significance of *E. coli* in wastewater extends beyond immediate disease transmission. It reflects systemic lapses in sanitation, hygiene, and waste management. Regular monitoring of *E. coli* in restaurant wastewater, especially in institutional environments like the University of Benin, is therefore vital. It provides early warning signals for contamination, informs sanitation policy, and supports compliance with environmental safety standards such as those outlined by the WHO (2022) and Nigeria's National Environmental Standards and Regulations Enforcement Agency (NESREA, 2021)

2.4 Detection and Isolation Methods of *Escherichia coli* from Wastewater

The accurate detection and isolation of *Escherichia coli* from environmental samples such as wastewater are critical for assessing microbial quality and potential health risks. Different methods, both culture-based and molecular, have been developed to identify *E. coli* with varying degrees of sensitivity, specificity, and rapidity.

2.4.1 Culture-Based Methods

Culture-based detection remains the cornerstone of microbiological water quality testing, particularly in resource-limited settings. This method involves the use of selective and differential media that allow *E. coli* colonies to be visually distinguished based on characteristic growth patterns. Commonly used media include Eosin Methylene Blue (EMB) agar, MacConkey agar, and Nutrient agar (Cheesbrough, 2019).

On MacConkey agar, *E. coli* ferments lactose, producing acid that causes colonies to appear pink or red, while non-lactose fermenters appear colorless. On EMB agar, *E. coli* colonies typically exhibit a distinctive metallic green sheen due to strong acid production that precipitates the dyes (Holt *et al.,* 2020). Following isolation, colonies are subjected to a series of biochemical tests to confirm identity, including: Gram staining (to confirm Gram-negative rods), Indole Test (positive for *E. coli*), Methyl Red (MR) Test (positive),Voges–Proskauer (VP) Test (negative), and Citrate utilization Test (negative).Other confirmatory assays include Urease, Triple Sugar Iron (TSI), and Motility tests (Cheesbrough, 2019; Bartram & Pedley, 2020).

Culture-based methods are affordable and simple but have limitations in detecting viable but non-culturable (VBNC) cells and may require up to 48 hours for results. Nonetheless, they remain the standard in developing countries where molecular diagnostics are less accessible.

2.5.2 Most Probable Number (MPN) Technique

The Most Probable Number (MPN) method is a statistical estimation technique widely used to quantify coliform bacteria, including *E. coli*, in water and wastewater samples. It involves inoculating serial dilutions of the sample into lactose broth and incubating for gas production. Positive tubes are then confirmed using selective media such as EC broth and biochemical tests.

According to **APHA Standard Methods (2020)**, the MPN method is particularly useful for turbid samples (like restaurant wastewater) where direct plating might be impractical. However, it provides only an estimated bacterial count rather than an exact number. Despite this, it remains the benchmark method for faecal coliform analysis in many laboratories due to its reliability and adaptability.

2.4.3 Membrane Filtration Technique

The membrane filtration (MF) technique involves filtering a known volume of wastewater through a sterile membrane (0.45 µm pore size), which traps bacteria. The filter is then placed on selective media such as m-Endo agar or EMB agar and incubated. This method is rapid, accurate, and ideal for relatively clear water samples, although it may be less effective for highly turbid restaurant effluents (APHA, 2020).

2.4.4 Molecular and Advanced Detection Methods

With advances in biotechnology, molecular tools such as Polymerase Chain Reaction (PCR) have revolutionized the detection of *E. coli* and its pathogenic variants. PCR amplifies specific DNA sequences, allowing for precise identification of virulence genes such as *stx1*, *stx2* (Shiga toxin genes), *eae* (intimin), and *hlyA* (hemolysin) (Feng *et al.,* 2021). Other advanced methods include: Quantitative PCR (qPCR) for determining bacterial load, Fluorescence in situ Hybridization (FISH) for direct visualization, Enzyme-Linked Immunosorbent Assay (ELISA) for toxin detection, and Whole Genome Sequencing (WGS) for detailed molecular epidemiology.

While these methods provide higher sensitivity and specificity, their cost and equipment requirements limit their use in low-resource settings like Nigeria. Consequently, a combination of culture-based and biochemical methods remains the preferred approach for local wastewater microbiological analysis (Igbinosa *et al.,* 2023).

2.5 Empirical Studies on *E. coli* in Wastewater

2.5.1 Global Perspective

Globally, several studies have investigated the prevalence and characteristics of *E. coli* in wastewater. For instance, a study in India by Jangid *et al.,* (2020) detected *E. coli* in 92% of untreated wastewater samples from restaurants, with counts ranging between 10^4 and 10^7 CFU/mL. The isolates exhibited high resistance to antibiotics such as ampicillin and tetracycline, indicating environmental dissemination of resistant strains.

In China, *Zhang et al.,* (2020) analyzed effluents from catering establishments and observed *E. coli* concentrations exceeding WHO permissible limits. The researchers attributed this to improper waste segregation and low treatment efficiency. Similarly, studies from Europe have documented *E. coli* in treated effluents, highlighting the organism's persistence even after conventional treatment processes (Rizzo *et al.,* 2021).

A study by **Bitton (2019)** emphasized that *E. coli* can survive in wastewater treatment plants and in natural water bodies post-discharge, contributing to microbial pollution of aquatic ecosystems. Moreover, *E. coli* has been isolated from sludge and biofilms within treatment facilities, further complicating its removal.

2.5.2 African Context

In Africa, poor wastewater infrastructure and sanitation practices have exacerbated microbial contamination. **Okoh *et al.,* (2020)** in South Africa reported that *E. coli* counts in municipal wastewater often exceeded 10^5 CFU/mL even after secondary treatment. The persistence of faecal indicators in treated water suggested inefficiencies in treatment plants.

In Kenya, Muriuki *et al.,* (2021) found *E. coli* and coliform bacteria in 85% of wastewater samples collected from urban food joints, noting that wastewater discharge frequently entered surface water used for irrigation. Similar observations were reported in Ghana and Ethiopia, where wastewater reuse in agriculture was common despite the presence of faecal contaminants (Mensah *et al.,* 2020).

2.5.3 Nigerian Studies

In Nigeria, numerous studies have documented *E. coli* contamination in restaurant and domestic wastewater. **Adebayo and Adesina (2021)** investigated effluents from canteens in Ibadan and reported mean *E. coli* counts of 1.2 × 10^6 CFU/mL. The isolates showed high prevalence of multidrug resistance (MDR), particularly against β-lactam and sulfonamide antibiotics.

Similarly, **Nnaji and Chukwu (2020)** analyzed wastewater from university cafeterias in Enugu State and found that all samples tested positive for *E. coli*. Their findings suggested that unhygienic practices, such as direct disposal of wastewater into open drains, were key contributors to contamination.

In Benin City, **Igbinosa *et al.,* (2023)** identified *E. coli* as one of the predominant bacteria in untreated wastewater from eateries. The isolates exhibited extended-spectrum β-lactamase (ESBL) production, confirming the presence of antibiotic-resistant strains in the environment.

Another study by **Odetoyin *et al.,* (2022)** across southwestern Nigeria found *E. coli* in both treated and untreated wastewater samples, with 70% of isolates exhibiting resistance to at least three antibiotic classes. This pattern underscores the environmental dimension of antimicrobial resistance in Nigeria's wastewater systems.

These findings reveal a consistent pattern: restaurant and domestic wastewater in Nigeria contain high loads of *E. coli*, often exceeding international safety thresholds. The results also highlight poor enforcement of environmental regulations and limited public awareness about proper wastewater handling.

2.7 Summary and Research Gaps

The reviewed literature clearly establishes *E. coli* as a critical indicator of faecal contamination in wastewater systems globally. Its presence in restaurant effluents reflects the sanitary status of food establishments and the adequacy of local waste management systems.

Globally, studies have detailed the prevalence of *E. coli* in wastewater and have advanced molecular tools for its detection. In Africa and Nigeria, however, most studies rely on conventional microbiological methods due to limited access to molecular facilities. This underscores a technological gap in surveillance and diagnostic capability.

Specifically, gaps identified include:

1. Limited localized data on the microbiological quality of wastewater from university-based restaurants, particularly within the University of Benin.
2. Insufficient data on the biochemical characterization of *E. coli* isolates from such environments.
3. Scarce research on the correlation between wastewater *E. coli* levels and potential antimicrobial resistance profiles in institutional settings.
4. Lack of continuous monitoring frameworks for wastewater microbiological safety in Nigerian universities.

Addressing these gaps through systematic detection, isolation, and biochemical characterization of *E. coli* from restaurant wastewater in the University of Benin will provide valuable insights into the microbial risks associated with campus waste disposal practices. It will also contribute to the development of institutional hygiene policies and national environmental management strategies.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted within the premises of the University of Benin (UNIBEN), located in Benin City, Edo State, Nigeria. The University of Benin is one of Nigeria's foremost tertiary institutions, with a student population exceeding 40,000 and a wide range of commercial food outlets and restaurants that serve students, staff, and visitors. The University lies between latitude 6°24′N and longitude 5°37′E, within the tropical rainforest belt of southern Nigeria. The region experiences two main seasons—the wet season (April–October) and the dry season (November–March)—with annual rainfall ranging between 1,500 and 2,500 mm and an average temperature of about 27°C (Nigerian Meteorological Agency [NIMET], 2022).

The study focused on three major restaurants located within the Ugbowo campus: Helena's Kitchen, Buka (Food Court), and Home and Away Restaurant. These eateries were chosen based on their high patronage, accessibility, and proximity to student hostels. All three restaurants discharge their wastewater into nearby open drains without prior treatment, making them suitable sites for studying the presence and distribution of *Escherichia coli* in restaurant effluents. The study area thus provides an ideal model for assessing microbial contamination in wastewater within a university environment.

3.2 Sample Collection

A single sampling round was carried out to collect wastewater samples from the three restaurants. At each location, approximately 500mL of wastewater was collected directly from the main effluent discharge point into sterile screw-capped plastic bottles. The sampling bottles were pre-sterilized by autoclaving at 121°C for 15 minutes to ensure aseptic collection.

Samples were collected during peak restaurant operation hours (between 12:00 p.m. and 2:00 p.m.) when wastewater flow was highest, as this period is representative of typical bacterial load. Each bottle was carefully labeled with the sampling location, date, and time of collection.

Immediately after collection, the samples were placed in ice-packed coolers and transported to the Microbiology Laboratory, Faculty of Life Sciences, University of Benin, for analysis within two hours of collection. The short holding time minimized bacterial die-off and preserved sample integrity (Cheesbrough, 2010).

Standard precautions were followed to prevent cross-contamination during sampling and transportation. Sterile gloves, lab coats, and face masks were worn, and bottles were opened only during collection and immediately closed afterward.

3.3 Enumeration of Total Heterotrophic Bacteria

The total heterotrophic bacterial count (THBC) was determined to estimate the overall bacterial load present in each wastewater sample. Enumeration was carried out using the pour plate method, as described by Cheesbrough (2010).

Serial dilutions of each sample were prepared by transferring 1 mL of wastewater into 9 mL of sterile normal saline (0.85% NaCl), followed by serial tenfold dilutions up to 10^{-6}. From appropriate dilutions (usually 10^{-3} to 10^{-5}), 1 mL aliquots were inoculated into sterile nutrient agar plates in duplicates using the pour plate technique. The plates were gently swirled to ensure even distribution of the inoculum and allowed to solidify before incubation.

Plates were incubated inverted at 37°C for 24–48 hours, after which colonies were counted using a digital colony counter. The colony-forming units per milliliter (CFU/mL) were calculated using the formula:

CFU/mL = (Number of colonies × Dilution factor) / Volume of inoculum (mL)

Plates with colony counts between 30 and 300 were selected for enumeration to ensure statistical accuracy. Representative colonies exhibiting distinct morphological characteristics such as color, shape, and elevation were sub-cultured on Eosin Methylene Blue (EMB) agar to isolate presumptive *E. coli*.

Colonies showing metallic green sheen on EMB agar were recorded as presumptive *E. coli* isolates and subsequently subjected to a series of biochemical tests for confirmation (Holt *et al.,* 1994).

3.4 Biochemical Characterization of *Escherichia coli* Isolates

The identification of E. coli isolates was based on cultural, morphological, and biochemical characteristics following standard microbiological protocols (Cheesbrough, 2010; Bergey's Manual of Systematic Bacteriology, Holt *et al.,* 1994). The following biochemical tests were performed on each isolate obtained from EMB agar:

3.4.1 Gram Staining

Gram staining was carried out to determine the Gram reaction and morphology of bacterial isolates. A smear of the isolate was made on a clean glass slide, heat-fixed, and stained using the Gram staining technique (crystal violet, Gram's iodine, alcohol decolorizer, and safranin). Slides were observed under an oil immersion microscope (×100 objective lens). *E. coli* appeared as

Gram-negative, short rod-shaped (bacilli) cells that did not retain the crystal violet stain but appeared pink to red after counterstaining (Cheesbrough, 2010).

3.4.2 Oxidase Test

The oxidase test was used to detect the presence of the enzyme cytochrome c oxidase in the isolates. A sterile filter paper was soaked with 1% tetramethyl-p-phenylenediamine dihydrochloride reagent. A small portion of the test organism was smeared on the paper using a sterile applicator stick. The development of a purple color within 10–30 seconds indicated a positive reaction, while no color change denoted a negative result. *E. coli* typically yields an oxidase-negative reaction (Cheesbrough, 2010).

3.4.3 Indole Test

The indole test determines the ability of bacteria to decompose tryptophan to indole. The isolates were inoculated into tryptone broth and incubated at 37°C for 48 hours. After incubation, 5 drops of Kovac's reagent were added to each tube. A red-colored ring at the surface of the medium indicated a positive result, confirming indole production. *E. coli* characteristically gives an indole-positive reaction (Holt *et al.,* 1994).

3.4.4 Urease Test

The urease test identifies bacteria capable of hydrolyzing urea into ammonia and carbon dioxide through the enzyme urease. The isolates were inoculated into Christensen's urea agar slants and incubated at 37°C for 24–48 hours. A color change from yellow to pink indicated urease production. *E. coli* is typically urease-negative, showing no color change (Cheesbrough, 2010).

3.4.5 Citrate Utilization Test

This test detects the ability of bacteria to use citrate as the sole carbon source. Isolates were inoculated into Simmons citrate agar slants and incubated at 37°C for 48 hours. Growth accompanied by a color change from green to blue indicated a positive result. *E. coli* generally gives a negative citrate test, showing no growth and no color change (Holt *et al.,* 1994).

3.4.6 Triple Sugar Iron (TSI) Test

The TSI test differentiates bacteria based on the fermentation of glucose, lactose, and sucrose and the production of gas or hydrogen sulfide (H_2S). The isolates were inoculated by stabbing the butt and streaking the slant of the TSI agar and incubated at 37°C for 24 hours. A yellow butt and slant (acid/acid, A/A) indicated fermentation of both glucose and lactose/sucrose, while the presence of gas bubbles or cracks indicated gas production. *E. coli* typically exhibits acid/acid (A/A) reaction with gas but no H_2S production (Cheesbrough, 2010).

3.4.7 Motility Test

Motility was determined using semi-solid motility medium (0.4% agar). A straight stab inoculation was made in the center of the medium and incubated at 37°C for 24 hours. Diffuse or spreading growth from the line of inoculation indicated a motile organism, while growth restricted to the stab line indicated non-motility. *E. coli* is typically motile due to peritrichous flagella (Holt *et al.,* 1994).

3.4.8 Methyl Red and Voges-Proskauer (MR-VP) Test

The MR-VP tests determine bacterial fermentation patterns. Isolates were inoculated into glucose phosphate broth and incubated for 48 hours at 37°C. For the MR test, 5 drops of methyl red indicator were added; a red color indicated a positive result (mixed acid fermentation). For the VP test, Barritt's A and B reagents were added; a pink to red color after 15–30 minutes indicated acetoin production. *E. coli* typically gives a positive MR and negative VP reaction (Cheesbrough, 2010).

3.4.9 Sugar Fermentation Test

Sugar fermentation tests were conducted using peptone water supplemented with 1% of each sugar (glucose, sucrose, lactose, maltose, and mannitol) and phenol red indicator. Durham tubes were inserted to detect gas production. The media were inoculated with the test organisms and incubated at 37°C for 24–48 hours. A color change from red to yellow indicated acid production, while gas bubbles in the Durham tube signified gas formation. *E. coli* typically ferments glucose, lactose, mannitol, and sucrose with acid and gas production (Holt *et al.*, 1994; Cheesbrough, 2010).

3.5 Data Analysis

Data obtained from bacterial counts and biochemical tests were recorded, compiled, and analyzed using descriptive statistics. Colony counts were expressed as mean CFU/mL ± standard deviation for each sampling site. The frequency and percentage occurrence of *E. coli* isolates were calculated to compare contamination levels among the three restaurant wastewater samples.

Results were presented in tables and bar charts to illustrate the variation in bacterial load and the biochemical characteristics of the isolates. Statistical analyses were conducted using Microsoft Excel 2021, which facilitated computation and graphical representation.

The data interpretation focused on identifying patterns of microbial contamination and confirming the presence of *E. coli* based on standard diagnostic characteristics. Comparisons were made with established microbiological standards (e.g., WHO, 2022) to determine the public health implications of the results.

All procedures were carried out in strict compliance with biosafety guidelines and ethical standards applicable to environmental microbiological research within the University of Benin.

CHAPTER 4

4.0 RESULTS

Figure 4.1: Presents Heterotrophic bacterial counts (Log cfu/ml) of waste water samples collected from three restaurants (Helena's Kitchen, Home and Away, and Buka) in the University of Benin.

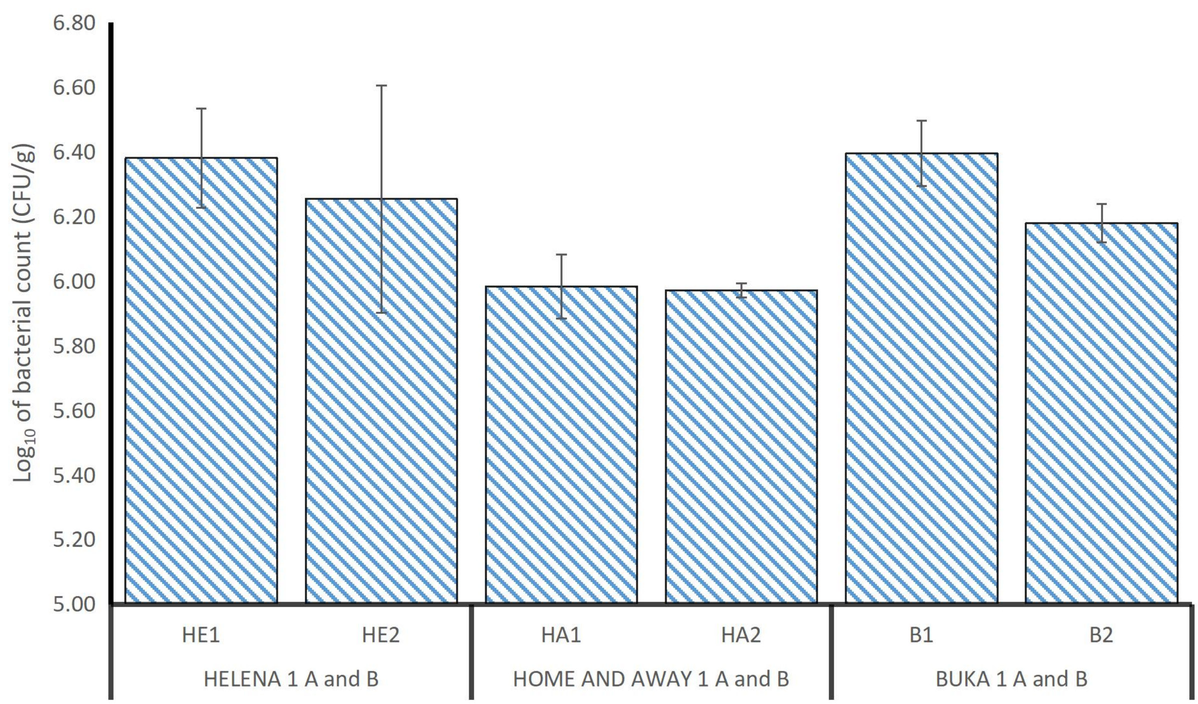
Figure 4.2: Presents the total coliform bacterial counts of waste water samples collected from the three restaurants.

Figure 4.3: Presents the frequency and percentage occurrence of bacterial isolates recovered from the waste water samples.

Table 4.1: Presents the cultural, morphological and biochemical characteristics of bacterial isolates recovered from the waste water samples.

Table 4.2: Shows the distribution of bacterial isolates across the waste water samples collected from the three restaurants.

Figure 4.1. Heterotrophic bacterial counts from the wastewater samples



Key:

HE1= Helena's Kitchen 1

HE2= Helena's Kitchen 2

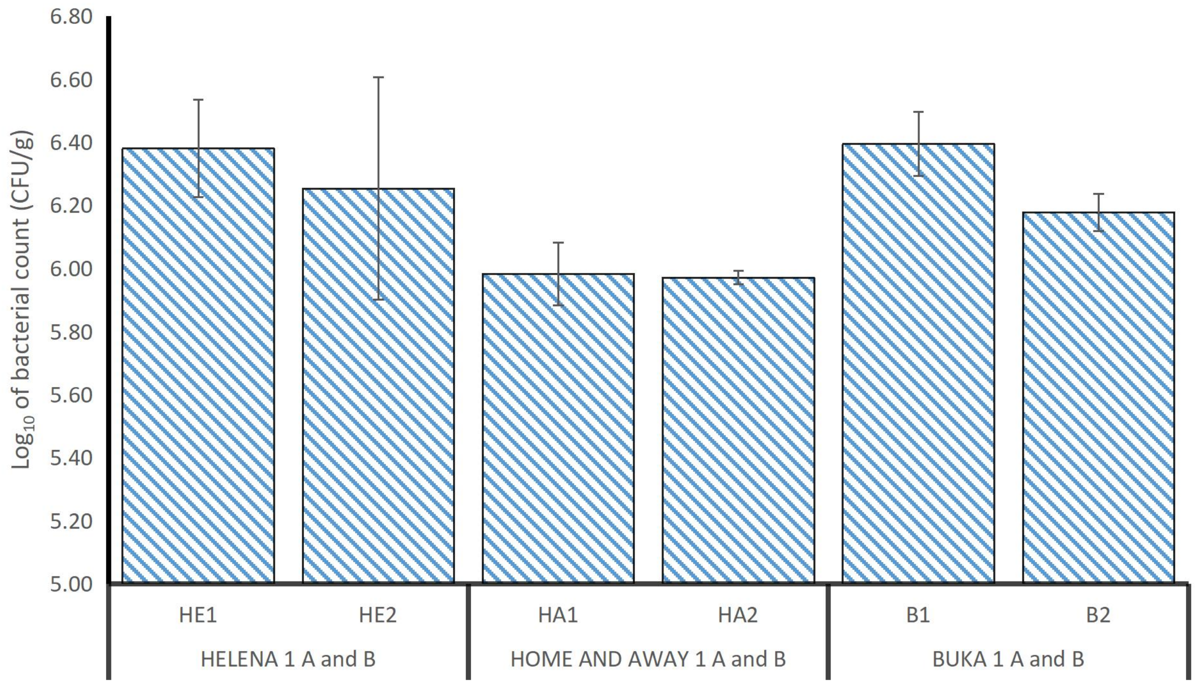
HA1= Home and Away 1

HA2= Home and Away 2

B1= Buka 1

B2= Buka 2

Figure 4.2. Total coliform counts from the wastewater samples of eateries in UNIBEN



Key:

HE1= Helena's Kitchen 1

HE2= Helena's Kitchen 2

HA1= Home and Away 1

HA2= Home and Away 2

B1= Buka 1

B2= Buka 2

Table 4.1. Cultural, morphological and microscopic characteristics of bacterial isolates obtained from samples

Morphological					
Elevation	Flat	Flat	Flat	Raised	Raised
Margin	Undulate	Undulate	Undulate	Entire	smooth
Color	Cream	Cream	Cream	lemon	Cream
Shape	Irregular	Irregular	Irregular	Circular	Irregular
Size	Large	Large	Large	Medium	Small
Gr. diff. agar	EMB	BCA	EMB	PCA	MSA
Colour	green	Straw	pink	green	Yellow
Staining					
Gram stain	-	+	-	-	+
cell type	Rod	Rod	Rod	rod	Cocci
Arrangement	disperse	disperse	disperse	disperse	clusters
Color	pink	purple	pink	pink	purple
Spore staining	-	+	-	-	-
Biochemical					
KOH String Test	+	-	+	+	-
Catalase	+	+	+	+	+
Indole	+	-	-	-	-
Citrate	-	+	+	+	+
Oxidase	-	-	-	+	-
Motility	+	+	+	+	-
Urease	-	-	+/-	+	+
Glucose	+	+	+	-	+
Sucrose	-	+	+	-	+
Lactose	+	+	+	-	+
Mannitol	-	+	+	-	-
Gas formation	+	-	+	-	-
H_2S formation	-	-	+	-	-
TSI (Slant/Butt) reaction	A/AG	A/A	A/AG	K/K	A/A*
Esculin Hydrolysis	-	-	-	-	-
Identity	*E. coli*	*Bacillus subtilis*	*Citrobacter freundii*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*

Key:

+ = Positive

- = Negative

A/AG = Acid/Acid with Gas (ferments glucose + lactose/sucrose and produces gas)

A/A = Acid/Acid (ferments glucose + lactose/sucrose, no gas)

K/K = Alkaline/Alkaline (no fermentation)

A/A*= Acid/ Acid with slight gas

Table 4.2: Distribution of bacterial isolates from samples

	HELENA 1 A and B	HELENA 1 A and B	HOME AND AWAY 1 A and B	HOME AND AWAY 1 A and B	BUKA 1 A and B	BUKA 1 A and B
Isolates	HE1	HE2	HA1	HA2	B1	B2
E. coli	+	-	-	+	+	+
Bacillus subtilis	+	+	+	+	+	+
Citrobacter freundii	+	-	-	+	+	+
Pseudomonas aeruginosa	-	-	+	+	-	-
Staphylococcus aureus	+	-	+	+	+	+

Key: Present (+), Absent (-)

HE1= Helena's Kitchen 1

HE2= Helena's Kitchen 2

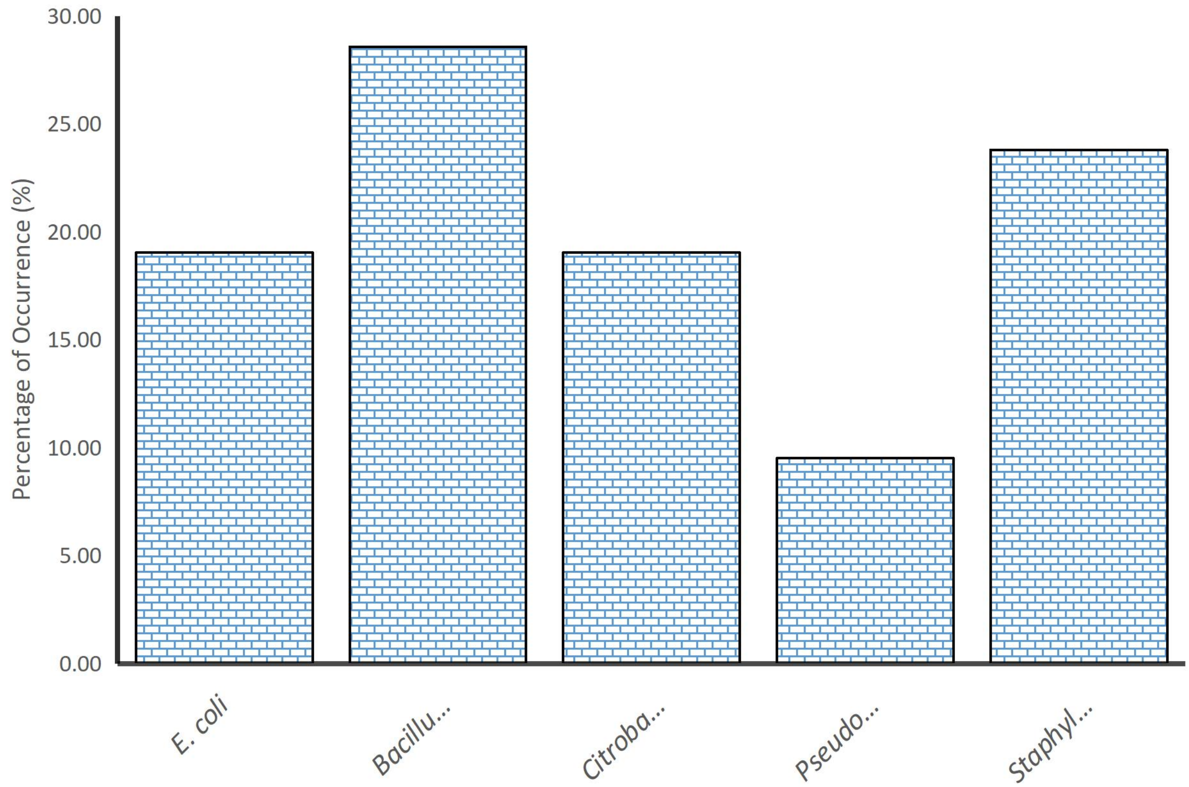
HA1= Home and Away 1

HA2= Home and Away 2

B1= Buka 1

B2= Buka 2

Figure 4.3. Frequency of bacterial occurrence obtained from the different samples



CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

The detection and isolation of *Escherichia coli* (*E. coli*) in wastewater from restaurants within the University of Benin campus provide significant insights into the microbial quality of effluents generated by food service establishments, as well as the broader implications for public health, environmental sanitation, and institutional waste management. The predominance of *E. coli* among the wastewater samples obtained affirms its role as an indicator organism for faecal contamination and a potential carrier of pathogenic and antimicrobial-resistant traits (Akhigbe & Igbinosa, 2022; Igbinosa, Uyi & Onoh, 2023).

The study demonstrated that wastewater samples collected from the three selected restaurants (Helena's Kitchen, Buka, and Home and Away), contained substantial microbial populations, with *E. coli* being among the most frequently isolated organisms. The presence of *E. coli* in all three sampling sites signifies a consistent pattern of faecal contamination across restaurant effluents. This finding is in line with the hypothesis that restaurant wastewater constitutes a potential vector for the dissemination of faecal microorganisms in campus environments.

In this study, the observed heterotrophic bacterial counts ranged from 5.85 to 6.40 Log_{10} cfu/ml, indicated substantial microbial presence across all sampling sites. The highest count was recorded at Buka point A (6.40 Log_{10} cfu/ml), while the lowest was obtained from Home and Away Point B (5.85 Log_{10} cfu/ml). This may be attributed to the accumulation of organic waste and inefficient drainage systems.

While *E. coli* is a typical inhabitant of the intestinal tract and serves as a reliable indicator of faecal pollution (Edberg *et al.,* 2021), organisms such as *P. aeruginosa* and *S. aureus* are often associated with environmental and opportunistic infections, implying the coexistence of both faecal and non-faecal contaminants (Bitton, 2019). This suggests contributions from soil, water, and kitchen surfaces, supporting earlier reports that restaurant wastewater is a complex matrix of mixed microbial origins (Zhang, Liu & Li, 2020; Adebayo & Adesina, 2021).

Overall, the results indicate poor hygienic and wastewater management practices within the sampled restaurants, consistent with observations in other Nigerian studies that identified high microbial loads in restaurant effluents due to inadequate sanitation and direct discharge into open drains (Nnaji & Chukwu, 2020; Adebayo & Adesina, 2021).

In environmental microbiology, *E. coli* is universally recognized as a sentinel organism for evaluating sanitary quality and potential pathogen presence (Edberg et al., 2021; WHO, 2022). Its detection suggests that wastewater generated in these restaurants likely originates from sources contaminated with human or animal feces, food residues, or improper cleaning of cooking utensils.

This finding is in line with previous Nigerian studies that reported *E. coli* in effluents from eateries, abattoirs, and domestic sewage. For instance, Igbinosa, Beshiru, and colleagues (2023) observed that untreated wastewater in Edo State contained high counts of *E. coli*, many of which were extended-spectrum β-lactamase (ESBL) producers, posing antimicrobial resistance risks. Similarly, Odetoyin, Ogundipe, and Onanuga (2022) found diarrhoeagenic *E. coli* in well water across Southwestern Nigeria, underscoring the pathogen's environmental persistence and transmission potential. Nnaji and Chukwu (2020) reported that all wastewater samples collected from university cafeterias in Enugu tested positive for *E. coli*, while Adebayo and Adesina (2021) observed microbial counts exceeding WHO permissible limits in restaurant wastewater in Ibadan. In South Africa, Okoh et al., (2020) documented *E. coli* counts exceeding 10^5 CFU/mL even after secondary treatment, revealing the limitations of conventional treatment facilities in removing faecal contaminants. Comparable studies in Kenya and Ghana reported *E. coli* in over 80% of restaurant and market wastewater samples, often associated with the reuse of untreated wastewater for irrigation (Muriuki, Njeru & Mwangi, 2021; Mensah, Adams & Boamah, 2020). These similarities suggest a systemic issue of poor wastewater management within institutional and urban food service establishments across Nigeria.

Globally, the occurrence of *E. coli* in restaurant wastewater has been linked to improper waste segregation, ineffective treatment systems, and poor hygiene compliance (Jangid, Patel & Sharma, 2020; Zhang *et al.,* 2020). In this study, the lack of pre-treatment mechanisms in the sampled restaurants, combined with the use of open drains for disposal, creates an ideal environment for *E. coli* survival and spread. The warm, nutrient-rich conditions of tropical wastewater, especially in Benin City's climate (NIMET, 2022), further support microbial proliferation and persistence.

The detection of *E. coli* and other isolates such as: *Citrobacter freundii, Bacillus subtilis, Pseudomonas aeruginosa, and Staphylococcus aureus* in wastewater is not merely indicative of contamination but also of potential health risk. Depending on the strain, *E. coli* can cause gastrointestinal infections, urinary tract infections, and, in severe cases, hemolytic uremic syndrome (Nataro & Kaper, 2019). This therefore points to a latent but serious health concern within the University of Benin environment, where wastewater may leak, stagnate, or flow into nearby soil and surface water bodies, increasing exposure potential among students and staff.

The detection of *E. coli* and other bacterial contaminants in wastewater from University of Benin restaurants holds profound implications for public health. Wastewater from these establishments is frequently discharged into open drains that run through campus areas, creating direct and indirect exposure risks. Contact with contaminated surfaces or aerosols can lead to infections, especially among individuals with compromised immunity (WHO, 2022).

Moreover, if such wastewater infiltrates groundwater or surface water systems, the contamination can extend beyond the university environment, affecting neighboring communities. Studies by Odetoyin *et al*., (2022) and Onoh *et al.,* (2022) confirmed that *E. coli* from environmental wastewater sources can enter irrigation systems, contaminating vegetables consumed raw. In this context, the findings of the present study represent not only an institutional sanitation issue but also a potential community health hazard.

Antimicrobial resistance (AMR) adds another layer of concern. Research in southern Nigeria has shown that wastewater often contains ESBL-producing *E. coli* and other multidrug-resistant bacteria (Igbinosa *et al.,* 2023). Even though this study did not include antibiotic susceptibility testing, the detection of *E. coli* from untreated effluents within the same geographical region suggests the possibility of similar resistance patterns. Continuous environmental exposure to resistant bacteria could facilitate horizontal gene transfer to commensal or pathogenic organisms, intensifying the spread of AMR (Rizzo, Manaia & Merlin, 2021).

Public health consequences of wastewater-borne pathogens also include outbreaks of diarrhoea diseases, which remain a major cause of morbidity in Nigeria (CDC, 2023). According to WHO (2022), over 1.7 billion diarrhoea cases occur globally each year, with significant proportions linked to unsafe water. The findings from this study thus reinforce the urgent need for improved wastewater monitoring and management in university settings.

From an environmental perspective, the presence of high microbial loads in restaurant wastewater contributes to the degradation of soil and aquatic ecosystems. Organic-rich effluents increase biochemical oxygen demand (BOD) and chemical oxygen demand (COD), leading to oxygen depletion and eutrophication when discharged into natural water bodies (UNEP, 2020).

On the University of Benin campus, the open drainage system provides a continuous pathway for wastewater to spread. During heavy rainfall, this wastewater may overflow into pedestrian walkways, and vegetation zones. Such conditions not only heighten disease transmission risks but also create aesthetic and odour problems. The recurring presence of *E. coli* thus serves as an early warning indicator for inadequate sanitation infrastructure.

The environmental implications extend beyond the campus. Effluents eventually drain into the broader Benin City stormwater system, potentially contaminating nearby watercourses. Without proper containment or treatment, this can lead to downstream microbial pollution, affecting agricultural lands and domestic water users. The objectives of this study were to (1) evaluate the total heterotrophic bacterial count of wastewater collected from restaurants in the University of Benin, (2) isolate and identify *Escherichia coli* from wastewater samples collected from restaurants in the University of Benin, (3) assess the public health significance of the occurrence of *Escherichia coli* in restaurants wastewater within the University of Benin campus.

The successful detection of *E. coli* and other bacterial species fulfills these objectives and provides empirical evidence that wastewater from university-based restaurants is microbiologically unsafe.

The biochemical tests conducted—including indole, citrate, and TSI assays—confirmed the identity of *E. coli*, aligning with standard diagnostic criteria outlined by Cheesbrough (2010) and Holt *et al.,* (1994). The positive indole and methyl red tests, combined with negative citrate and urease reactions, were characteristic of *E. coli*, validating the reliability of conventional biochemical methods for local environmental monitoring where molecular tools are unavailable.

These findings contribute baseline data on wastewater quality within the University of Benin and can inform institutional policies on sanitation, waste disposal, and environmental health management.

The absence of structured wastewater treatment or monitoring within the University of Benin highlights a broader institutional challenge common across Nigerian tertiary institutions. Many campus-based eateries lack proper effluent containment or pre-treatment systems. The results of this study underscores the necessity of establishing localized wastewater management protocols that include:

- **i.** Installation of grease traps and sedimentation systems at restaurants outlets.
- **ii.** Regular microbial surveillance of effluent discharge points.
- **iii.** Enforcement of hygiene and waste segregation policies.
- **iv.** Integration of waste management into campus sustainability and health programs.

Internationally, universities have implemented decentralized treatment systems such as constructed wetlands and biofiltration units to manage restaurant effluents sustainably (Bartram & Pedley, 2020). Adopting such approaches in Nigerian contexts could mitigate contamination and enhance environmental protection.

5.2 Limitations of the Study

Despite its significance, the study has certain limitations. First, the sampling design was cross-sectional, capturing only a snapshot of wastewater quality at a specific time. Seasonal variations in microbial load—linked to rainfall, temperature, and restaurant activity—were not assessed. Second, the study relied solely on culture and biochemical methods without molecular confirmation or antibiotic susceptibility profiling. Consequently, while the presence of *E. coli* was confirmed phenotypically, the pathogenic potential and resistance traits of the isolates remain undetermined.

Nevertheless, these limitations do not undermine the validity of the findings but instead point to future research directions involving molecular characterization and long-term monitoring.

5.3 CONCLUSION

This study confirms that wastewater from restaurants within the University of Benin is heavily microbiologically contaminated, with *E. coli* serving as a consistent indicator of faecal pollution. The presence of additional bacterial species such as *C. freundii*, *B. Subtilis*, *P. Aeruginosa*, and *S. Aureus* reflects a mixed microbial ecology arising from both human and environmental sources. The findings corroborate earlier research across Nigeria and other developing regions, demonstrating that restaurant wastewater is a significant vector for microbial dissemination when improperly managed.

From a public health standpoint, the occurrence of *E. coli* in untreated wastewater poses potential risks of enteric infections and environmental contamination. The implications extend beyond the campus, highlighting the urgent need for improved sanitation infrastructure, regulatory oversight, and environmental monitoring.

By generating baseline data on wastewater microbial quality, this study contributes valuable evidence for designing interventions that promote safer, more sustainable waste management practices within university environments and beyond.

5.4 RECOMMENDATION

1. Restaurant wastewater management should be improved. Restaurants within the University of Benin should be required to implement basic wastewater pre-treatment measures before discharge. A simple intervention such as routine drain cleaning can significantly reduce microbial load, including faecal indicators like E. coli.

2. Regular microbiological monitoring of restaurant effluents should be enforced. The university management, in collaboration with environmental health and microbiology units, should establish periodic testing of wastewater from food service outlets. This will help detect contamination early and prevent the spread of waterborne pathogens.

3. Food handlers and restaurant staff should receive routine hygiene and sanitation training. Many instances of faecal contamination originate from poor personal hygiene and improper waste handling. Regular training programmes on hand hygiene, waste disposal, and kitchen sanitation can reduce the introduction of pathogenic microorganisms into wastewater systems.

4. Improved campus drainage and sewage infrastructure are recommended. Old or poorly maintained drainage systems can act as reservoirs for pathogenic bacteria. Upgrading wastewater channels and ensuring proper connection to sewage treatment facilities will reduce environmental contamination and public health risks.

5. Public health awareness should be strengthened. Students, staff, and food vendors should be educated on the health implications of improper wastewater disposal. Awareness campaigns can foster shared responsibility for environmental hygiene within the university community.

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