ANTIBACTERIAL RESISTANCE PROFILE OF *KLEBSIELLA* SPECIES FROM MALE HOSTEL DRAINS IN UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

BY

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DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF BENIN
BENIN CITY

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A RESEARCH PROJECT 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 DEGREE OF B. sc (HONS) IN MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY.

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CERTIFICATION

This is to certify that this project was carried out by ABDULATEEF IGENEGBA SALISU in the Department of Microbiology under the supervision of PROF. E. O. IGBINOSA at University of Benin, Benin city, Edo state, Nigeria.

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PROF. E. O. IGBINOSA **DATE**

(HEAD OF DEPARTMENT)

DEDICATION

This work is dedicated to God Almighty, for His strength and grace during this academic journey and also to my parents, family members and loved ones for their continuous support and encouragements.

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A very special appreciation goes to my beloved mother, Mrs. Charity Salisu, and my father, Mr. Musa Salisu, for the unwavering love, sacrifices, patience, care, and support they have given me. I pray that God will continue to bless you father and mother, and that you will eat the fruit of your labour in good health and sound mind.

My heartfelt gratitude goes to my group “Be guilded” for the support and care. Thank you all for being part of my story and for your support in one way or another.

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ABSTRACT

Wastewater drains within university hostels can serve as reservoirs for various microorganisms, including opportunistic pathogens that pose significant health and environmental risks. Among these microbes, *Klebsiella* species—especially *Klebsiella oxytoca*—are notable for their ability to survive in damp environments and their resistance to multiple antibiotics. This study aimed to isolate and identify *Klebsiella* spp. from wastewater drains in male hostels (Hall 3 and Hall 4) at the University of Benin, Edo State, Nigeria. Wastewater samples were aseptically collected from four designated drain points—two each from Hall 3 and Hall 4—using sterile 500 mL bottles. The samples were transported in ice-packed coolers to the Microbiology Laboratory, Faculty of Life Sciences, for bacteriological examination. Standard microbiological methods were used to enumerate, isolate, and identify bacterial species based on their cultural, morphological, and biochemical features. The total heterotrophic bacterial counts ranged from 4.82 to 4.87 Log_{10} CFU/mL, reflecting high microbial loads in all the sampled drains. Total *Klebsiella* counts varied between 5.75 to 6.00 Log_{10} CFU/mL, with the highest found at Hall 4 Point A and the lowest at Hall 3 Point A. The identified bacterial isolates included *Escherichia coli*, *Klebsiella oxytoca*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Notably, *Klebsiella oxytoca* appeared consistently across all sampling points, indicating its strong adaptability to wastewater environments. The findings underscore that hostel drainage systems can act as reservoirs and transmission routes for potentially pathogenic and environmentally persistent bacteria. Regular sanitation and effective wastewater management are therefore essential to minimize environmental contamination and associated public health risks.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Water pollution continues to pose one of the most serious global environmental and public health threats. Wastewater, especially from residential settings like student hostels, contains a complex mix of organic substances, chemicals, and microorganisms, some of which are disease-causing. In many developing nations, including Nigeria, poor sanitation infrastructure and ineffective wastewater management often result in the direct release of untreated wastewater into open drains and nearby surroundings (Adegoke *et al*., 2018; Odonkor and Mahami, 2020). Such conditions favor microbial persistence, multiplication, and spread, thereby endangering the health of residents and surrounding communities.

Wastewater is a major reservoir of enteric bacteria such as *Escherichia coli, Salmonella, Shigella, and Klebsiella* species, all of which can cause opportunistic or severe infections (Rizzo *et al*., 2013). Among these, Klebsiella species have gained global attention due to their rising prevalence in both environmental and clinical contexts. These organisms, belonging to the family Enterobacteriaceae, are widespread in soil, water, sewage, and plants, and also inhabit the gastrointestinal tracts of humans and animals (Podschun and Ullmann, 1998).

The most clinically relevant member, *Klebsiella pneumoniae*, is responsible for a wide range of infections including urinary tract infections, pneumonia, bloodstream infections, septicemia, and wound infections (Pitout *et al*., 2015; Paczosa and Mecsas, 2016). Its presence in wastewater is expected since it is excreted in feces and other bodily fluids, making hostel drainage systems—

collecting waste from toilets, bathrooms, kitchens, and laundries—suitable reservoirs for its persistence.

A critical health concern linked to *Klebsiella* species in wastewater is their ability to serve as carriers of antimicrobial resistance (AMR). These bacteria are known to produce extended-spectrum β-lactamases (ESBLs) and carbapenemases, including New Delhi Metallo-β-lactamase (NDM) and *Klebsiella pneumoniae* carbapenemase (KPC), which provide resistance to last-resort antibiotics (Nordmann *et al*., 2012; Wyres and Holt, 2018). Recognizing this threat, the World Health Organization (WHO) in 2017 classified carbapenem-resistant *K. pneumoniae* as a top-priority pathogen for antibiotic research and development (WHO, 2017). Wastewater environments act as hubs for horizontal gene transfer, enabling the dissemination of resistance genes among pathogenic and non-pathogenic bacteria (Rizzo *et al*., 2013; Manaia *et al*., 2018).

In Nigeria, insufficient sewage treatment in universities frequently results in hostel wastewater being directly discharged into drains and surrounding soils, raising the risk of exposure for students and staff. Several studies have confirmed the occurrence of multidrug-resistant Gram-negative bacteria, including *Klebsiella* species, in wastewater from urban areas, abattoirs, and hospitals across the country (Olowe *et al*., 2015; Igbinosa *et al*., 2017). However, little is known about the bacterial composition of wastewater from hostel settings, particularly in female hostels where high population density, shared sanitation, and diverse wastewater sources may encourage bacterial persistence and transmission.

Investigating the occurrence and characteristics of *Klebsiella* species in hostel wastewater is therefore essential. Such research not only provides baseline data on environmental reservoirs of pathogenic bacteria but also identifies risks associated with untreated wastewater exposure and contributes to global AMR monitoring. This aligns with the One Health framework, which emphasizes the interconnectedness of human, animal, and environmental health in combating infectious diseases and antimicrobial resistance (Robinson *et al*., 2016; Hernando-Amado *et al*., 2019).

1.2 Aim and Objectives

The aim of this study is to evaluate the bacteriological presence of *Klebsiella* species in wastewater from male hostel drains at the University of Benin.

The specific objectives were to;

1. evaluate the total heterotrophic bacterial count of wastewater collected from male hostel drains at the University of Benin;
2. isolate and identify *Klebsiella* species from wastewater samples collected from male hostel drains.
3. evaluate the antibiotic susceptibility profile of *Klebsiella* species isolated from the wastewater.

CHAPTER TWO

2.0 LIERATURE REVIEW

2.1 Concept of Wastewater

Wastewater refers to water whose quality has been negatively altered by human activities, making treatment necessary before it can be safely discharged into the environment or reused. It is an inevitable byproduct of daily life, arising whenever water is used for domestic, institutional, agricultural, or industrial purposes (Tchobanoglous *et al*., 2014). According to the World Health Organization (WHO, 2006), wastewater is a composite liquid waste stream originating from households, commercial and institutional establishments, industries, and at times, stormwater runoff.

The major categories of wastewater include:

Domstic wastewater: Produced in homes and residences, it consists of blackwater (toilet waste containing feces and urine) and greywater (effluents from bathing, laundry, and kitchens). In urban areas, domestic wastewater typically represents the largest share (Corcoran *et al*., 2010).

Institutional wastewater: Generated from schools, hospitals, markets, hostels, and other community institutions. Hostel wastewater, in particular, combines discharges from toilets, bathrooms, kitchens, and laundries, resulting in a highly complex mixture rich in microbes and chemical pollutants (Odonkor and Mahami, 2020).

Industrial wastewater: Originating from industrial activities such as food processing, textile production, pharmaceuticals, chemical industries, and abattoirs. These effluents often contain hazardous substances, including chemicals, heavy metals, and microbial contaminants (Jurado *et al*., 2010).

Agricultural wastewater: Runoff from farmlands containing fertilizers, pesticides, animal excreta, and crop residues. This is a key contributor to nutrient pollution and microbial contamination in surface waters.

2.1.1. Characteristics of Domestic and Institutional Wastewater

The composition of wastewater is highly variable and depends on its origin, the characteristics of the contributing population, lifestyle patterns, and environmental conditions. Domestic and institutional wastewater is typically rich in organic matter, nutrients, and diverse microorganisms.

From a physical perspective, wastewater is characterized by turbidity, suspended solids, odor, color, and temperature. Suspended particles provide surfaces for microbial attachment and biofilm development, which enhance bacterial persistence. Elevated turbidity further limits sunlight penetration, creating favorable conditions for the survival of bacteria such as *Klebsiella* species (Bitton, 2011).

Its chemical properties include high levels of organic carbon, nitrogen compounds (ammonia, nitrates), phosphorus, fats, oils, and detergents. Wastewater also exhibits high biological oxygen demand (BOD) and chemical oxygen demand (COD), both indicators of abundant degradable organic material (Tchobanoglous *et al*., 2014). These nutrient-rich conditions promote microbial growth. Moreover, antimicrobial residues from human medications and cleaning agents, frequently found in hostel and institutional wastewater, can shape microbial communities and drive the development of antimicrobial resistance (Rizzo *et al*., 2013).

The biological composition of wastewater is particularly important for public health. It harbors a wide range of microorganisms, including bacteria, viruses, protozoa, *and helminths. Common bacterial groups include Escherichia coli, Enterococcus, Salmonella, Shigella, Vibrio,* and *Klebsiella* species (Okoh *et al*., 2007; Rizzo *et al*., 2013). Microbial loads vary with population density and sanitation conditions, with hostel wastewater generally containing higher counts due to the large number of individuals sharing facilities.

Institutional wastewater—such as that from male hostel drains—is especially complex. In addition to human waste, it contains residues from food, sanitary products, cosmetics, and detergents. This combination produces a nutrient-rich and selective environment that supports microbial persistence and often favors opportunistic pathogens like *Klebsiella pneumoniae* (Odonkor and Mahami, 2020).

2.1.2. Wastewater as an Environmental Reservoir for Pathogens

Wastewater is widely recognized as both a reservoir and a transmission route for pathogenic organisms. Its moist, nutrient-rich environment enables the survival and persistence of enteric bacteria, viruses, and parasites. Research has shown that untreated wastewater often contains high concentrations of bacteria such as *Escherichia coli, Enterococcus, Salmonella, Shigella, Vibrio cholerae,* and *Klebsiella* species (Ashbolt, 2015; Manaia *et al*., 2018).

Among these, *Klebsiella* species are particularly well-suited to wastewater habitats because of their metabolic adaptability and ability to thrive within biofilms. They are commonly recovered from municipal sewage, hospital effluents, and urban drainage systems (Podschun and Ullmann, 1998; Wyres and Holt, 2018). Their presence in wastewater is concerning since they act not only as opportunistic pathogens but also as significant reservoirs of antimicrobial resistance (AMR) genes (Nordmann *et al*., 2012).

Wastewater systems further promote horizontal gene transfer (HGT) among bacteria via plasmids, integrons, and transposons, accelerating the spread of resistance determinants (Rizzo *et al*., 2013; Manaia *et al*., 2018). Consequently, untreated wastewater is often described as a "hotspot" for the emergence and dissemination of AMR. Notably, carbapenemase-producing *Klebsiella pneumoniae* has been detected in untreated sewage in both developed and developing countries (Zhou *et al*., 2017).

The health risks increase when untreated wastewater contaminates surface and groundwater sources, irrigated crops, or when individuals come into direct contact with drains and effluents. In hostel environments, where sanitation infrastructure is often overstretched and population density is high, the likelihood of exposure and disease transmission is particularly elevated. Male hostel drains may pose additional risks, as the disposal of waste products (e.g. urinating) can create selective pressures on microbial populations, encouraging the persistence of resistant strains.

2.2 Wastewater in Developing Countries

Wastewater management is a major environmental and public health concern in developing nations, where rapid population growth, urban expansion, and industrial activities often exceed the pace of infrastructural development. In contrast to high-income countries, where wastewater treatment systems are generally well established and strictly regulated, many low- and middle-income countries (LMICs), including Nigeria, face significant gaps in infrastructure, policy, and resources needed for safe wastewater collection, treatment, and disposal. As a result, untreated or inadequately treated wastewater is frequently released into the environment, leading to the contamination of water, soil, and air. This not only promotes the spread of infectious diseases but also accelerates the development and spread of antimicrobial resistance (AMR) (Kumar *et al*., 2022; Odonkor and Mahami, 2020).

2.2.1. Sanitation Challenges in Nigeria and Other Low- and Middle-Income Countries

In Nigeria, as in many other low- and middle-income countries (LMICs), access to adequate sanitation remains limited, with a large segment of the population lacking safely managed facilities. The World Health Organization (WHO) and UNICEF (2021) estimate that around 46 million Nigerians still engage in open defecation, while less than 20% of wastewater receives proper treatment before disposal. Contributing factors include poorly planned urban development, insufficient funding, weak regulatory enforcement, and heavy dependence on pit latrines and septic tanks (Akpor and Muchie, 2011).

In urban centers, inadequate sewage infrastructure often results in wastewater overflow into streets and residential areas, creating ideal conditions for the proliferation of pathogens, vectors, and parasites. These circumstances are strongly linked to diarrheal illnesses, cholera epidemics, typhoid fever, and helminth infections, which continue to be major causes of sickness and death in many developing regions (Prüss-Ustün *et al*., 2019). Industrial effluents, frequently released untreated, add further complexity by introducing heavy metals and pharmaceutical residues into the environment, thereby worsening pollution and driving the emergence of drug-resistant bacteria in water and soil systems (Osiemo *et al*., 2019).

In rural and peri-urban communities, the problem is compounded by dependence on surface water sources such as streams, rivers, and ponds, which are commonly contaminated by human and animal waste. Climate change, particularly flooding, further intensifies this challenge by spreading untreated wastewater into populated areas (Odonkor and Mahami, 2020). The lack of affordable wastewater treatment options and limited awareness of safe sanitation practices underscore the urgent need for innovative, sustainable, and locally appropriate solutions in LMICs.

2.2.2. Hostel Drainage Systems and Their Microbiological Implications

Hostel settings, especially within universities and similar institutions in developing countries, pose significant challenges for wastewater management. Drainage systems in these environments are often poorly designed, inadequately maintained, and prone to blockages caused by improper disposal of solid waste, leftover food, and waste products. This frequently results in the accumulation of stagnant wastewater in open drains, creating ideal conditions for microbial survival and proliferation (Olorunfemi *et al*., 2019).

Microbiological investigations of hostel drainage have repeatedly shown high concentrations of pathogenic bacteria, including *Escherichia coli, Salmonella* spp., *Shigella* spp., *Staphylococcus aureus,* and *Pseudomonas aeruginosa* (Igbinosa *et al*., 2017). These pathogens serve as indicators of fecal contamination and are commonly associated with gastrointestinal illnesses and skin infections. Furthermore, hostel drains often act as reservoirs of multidrug-resistant bacteria, fueled by the indiscriminate disposal of antibiotics and personal care products by students (Njoku-Obi, 2024). The warm, nutrient-rich, and moist conditions in stagnant wastewater further enhance bacterial persistence and facilitate the horizontal exchange of resistance genes among microbial populations.

The close proximity of hostel drainage systems to kitchens, bathrooms, and living areas increases the likelihood of cross-contamination through direct human contact, insect vectors, or aerosolization during rainfall. In addition, poorly managed drains create breeding sites for mosquitoes, linking wastewater mismanagement to vector-borne diseases such as malaria. Leakages from hostel drainage into groundwater or nearby surface waters also contribute to broader environmental pollution and raise the risk of waterborne disease outbreaks among students and surrounding communities (Obioma and Onuoha, 2021).

2.3 Bacteriological Quality of Wastewater

The bacteriological quality of wastewater is a key measure used to determine its level of contamination, potential health hazards, and suitability for discharge or reuse. Wastewater contains a wide range of microorganisms such as bacteria, fungi, protozoa, and viruses, with bacteria being the most prevalent group because of their adaptability and ability to thrive in diverse environments (Akpor *et al*., 2014). The bacterial composition is largely shaped by the origin of the wastewater, which may stem from domestic, industrial, hospital, or agricultural sources. Evaluating wastewater quality therefore involves measuring the total heterotrophic bacterial count, identifying specific microbial indicators of pollution, and analyzing environmental factors that affect microbial load and diversity.

2.3.1. Total Heterotrophic Bacterial Count in Wastewater

The total heterotrophic bacterial count (THBC) is a commonly used microbiological parameter for estimating the overall bacterial load in wastewater. It measures the number of viable and culturable bacteria capable of utilizing organic and inorganic carbon compounds under aerobic conditions (Tortora *et al*., 2020). Due to the high organic content of wastewater, heterotrophic bacteria are usually present in large numbers, as the nutrient-rich environment supports rapid microbial growth.

Research from developing countries has shown that THBC levels in untreated domestic wastewater typically range from 10^5 to 10^8 CFU/mL, depending on the degree of organic pollution and the quality of local sanitation infrastructure (Igbinosa and Okoh, 2009). Elevated counts are often associated with inadequate sewage systems, indiscriminate waste disposal, and the direct release of untreated effluents into surface waters. High THBC values not only reflect heavy organic contamination but also suggest the possible presence of pathogenic bacteria such as *Escherichia coli, Salmonella* spp., and *Vibrio cholerae* (Odonkor and Mahami, 2020).

Regular monitoring of THBC is essential in wastewater quality assessment, as it provides a useful index of the overall microbial burden. This information is critical for the design, operation, and performance evaluation of wastewater treatment facilities (Nasseri et al., 2018). Where wastewater remains untreated or inadequately treated, high bacterial loads pose significant public health risks, particularly when such water is used for irrigation, discharged into rivers, or infiltrates groundwater supplies.

2.3.2. Factors Influencing Bacterial Load in Wastewater

The bacterial concentration in wastewater is largely determined by environmental and physicochemical conditions such as organic matter content, pH, temperature, dissolved oxygen, and nutrient levels.

1. Organic Matter Content

Organic matter, typically measured as biochemical oxygen demand (BOD) or chemical oxygen demand (COD), serves as the primary food source for heterotrophic bacteria. Elevated organic loads stimulate microbial growth and diversity, resulting in higher total heterotrophic bacterial counts (THBC). Wastewater from industrial discharges, food processing plants, and densely populated communities often contains significant amounts of organic material, thereby supporting large bacterial populations.

2. pH

The pH of wastewater strongly influences bacterial viability and metabolism. Most species thrive at neutral to mildly alkaline pH levels (6.5–8.5). Acidic effluents, often from industrial sources, tend to suppress many bacterial groups but support acid-tolerant organisms, whereas alkaline conditions favor the persistence of alkaliphilic and spore-forming species (Samer, 2015).

3. Temperature

Temperature regulates bacterial growth dynamics and community composition. Mesophilic bacteria, with an optimal growth range of 20–40°C, are predominant in most wastewater systems, particularly in tropical environments like Nigeria (Okoh *et al.*, 2010). Warmer conditions can enhance microbial metabolism and speed up organic matter breakdown, while extreme temperatures—either high or low—can limit survival.

4. Dissolved Oxygen (DO)

The amount of dissolved oxygen determines which bacterial groups dominate. Aerobic species flourish in oxygen-rich waters, whereas anaerobic and facultative bacteria are more abundant in oxygen-deficient areas such as septic tanks or stagnant drains. Low-oxygen conditions also favor the growth of sulfate-reducing and methanogenic bacteria, which are responsible for odor generation and methane emissions (Bitton, 2014).

5. Nutrient Availability

Nutrients such as nitrogen, phosphorus, and trace minerals are essential for microbial growth. Excess nutrient input from agriculture or domestic wastewater often causes eutrophication, which encourages the overgrowth of bacteria and algae in receiving water systems (Kumar and Chopra, 2012).

2.3.3. Microbial Diversity in Wastewater Environments

Wastewater contains a broad spectrum of bacterial groups, ranging from harmless environmental species to opportunistic and pathogenic strains. The diversity of microorganisms present is strongly influenced by the wastewater source: domestic wastewater is typically rich in fecal-associated bacteria, whereas industrial and hospital effluents often harbor environmental microbes alongside multidrug-resistant clinical isolates (Vaz-Moreira *et al.*, 2014).

Indicator Bacteria

Escherichia coli and other coliforms are widely recognized as indicators of fecal pollution in wastewater. Their presence signals possible contamination with enteric pathogens such as *Salmonella* spp., *Shigella* spp., and *Vibrio cholerae*, which are commonly transmitted through unsafe water (Ashbolt, 2015).

Opportunistic Pathogens

Opportunistic organisms, including *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Enterococcus* spp., are often detected in wastewater. These microbes pose infection risks, particularly to immunocompromised individuals, and are increasingly linked to antimicrobial resistance (Rizzo *et al.*, 2013).

Environmental and Resistant Strains

Environmental bacteria such as *Acinetobacter* spp., *Aeromonas* spp., and *Bacillus* spp. are also commonly recovered from wastewater and drainage systems. Hospital and pharmaceutical discharges introduce multidrug-resistant strains, establishing wastewater as a critical reservoir of antibiotic resistance genes (Kümmerer, 2009).

Anaerobic and Specialized Bacteria

In oxygen-deprived sections of wastewater, anaerobic bacteria like *Clostridium* spp. and methanogenic archaea play key roles in degrading organic matter. Sulfate-reducing bacteria additionally produce hydrogen sulfide, contributing to foul odors and structural corrosion (Bitton, 2014).

Overall, the diverse bacterial communities in wastewater underscore its ecological significance while also presenting serious health threats. Although some microbes aid in nutrient cycling and organic matter breakdown, the coexistence of pathogens and resistant bacteria makes untreated wastewater a considerable public health concern.

2.4 The Genus *Klebsiella*

The genus *Klebsiella,* part of the family Enterobacteriaceae, comprises Gram-negative bacteria of both medical and environmental importance. Taxonomically, it is placed within the domain Bacteria, phylum Pseudomonadota (formerly Proteobacteria), class Gammaproteobacteria, order Enterobacterales, and family Enterobacteriaceae (Podschun and Ullmann, 1998; Holt *et al*., 2015). The genus was first introduced by Trevisan in 1885 and named after the German microbiologist Edwin Klebs.

Several species have been identified within this genus, including K*lebsiella pneumoniae, Klebsiella oxytoca, Klebsiella variicola, Klebsiella granulomatis, Klebsiella michiganensis,* and *Klebsiella aerogenes* (Brisse *et al*., 2006; Wyres *et al*., 2020). Among these, *K. pneumoniae* and *K. oxytoca* are the most clinically significant, frequently implicated in hospital-associated infections. Additionally, *K. variicola* and *K. quasipneumoniae* have gained recognition as emerging pathogens in both humans and animals (Rodríguez-Medina *et al.*, 2019).

Advances in molecular techniques, particularly multilocus sequence typing (MLST) and whole-genome sequencing, have refined the taxonomy of *Klebsiella*. These approaches have uncovered cryptic species within the *K. pneumoniae* complex and highlighted their importance in epidemiology and clinical infections (Wyres and Holt, 2018).

2.4.1. Morphological and Biochemical Characteristics

Klebsiella species are Gram-negative, non-motile, facultatively anaerobic bacilli, generally ranging from 0.3–1.0 µm in width to 0.5–5.0 µm in length. A defining characteristic is their thick polysaccharide capsule, visible as a mucoid layer around colonies and responsible for the positive "string test" result (Podschun and Ullmann, 1998; Paczosa and Mecsas, 2016). This capsule plays a central role in virulence by shielding the bacteria from phagocytosis and complement attack, while also facilitating biofilm development.

From a biochemical standpoint, *Klebsiella* are oxidase-negative, catalase-positive, ferment glucose with acid and gas production, and reduce nitrate to nitrite. They are lactose fermenters, forming pink colonies on MacConkey agar due to acid release. Species differentiation is aided by indole testing: *K. pneumoniae* is typically indole-negative, whereas *K. oxytoca* is indole-positive. Additional features include being urease-positive, citrate-positive, and Voges–Proskauer (VP) positive, distinguishing them from other members of the Enterobacteriaceae family (Barbier *et al*., 2013). Their capacity to utilize citrate as a sole carbon source and ferment a wide range of sugars highlights their metabolic adaptability.

2.4.2. Ecological Distribution (Environmental, Animal, and Human Reservoirs)

Klebsiella species are widely distributed organisms that occupy a variety of ecological niches. They are common in the environment, occurring in soil, surface waters, sewage, and plants, where they contribute to nitrogen fixation and the breakdown of organic matter (Bagley, 1985; Podschun and Ullmann, 1998). Their persistence in both terrestrial and aquatic systems is supported by their metabolic flexibility and capacity to form biofilms on surfaces.

In animals, *Klebsiella* has been detected in the intestinal tracts of mammals, birds, and insects, as well as on the mucosal surfaces of livestock. For instance, *K. pneumoniae* has been isolated from cattle, horses, and poultry, where it may act as an opportunistic pathogen responsible for mastitis, septicemia, and respiratory infections (Gharrah *et al.*, 2017). This zoonotic potential underscore its relevance within the One Health framework, linking human, animal, and environmental health.

In humans, *Klebsiella* normally exists as a commensal organism in the gastrointestinal tract, oropharynx, and skin, but under certain conditions—such as immunosuppression, hospitalization, or antibiotic exposure—it can shift to a pathogenic role. Clinically, *K. pneumoniae* is particularly associated with hospital-acquired infections, including pneumonia, urinary tract infections, bacteremia, wound infections, and liver abscesses (Paczosa and Mecsas, 2016). The rise of multidrug-resistant strains, especially carbapenem-resista*nt K. pneumoniae* (CRKP), has made this pathogen a major global public health concern (Navon-Venezia *et al.*, 2017; Wyres and Holt, 2018).

In summary, *Klebsiella* represents a genus with a broad ecological presence, functioning as an environmental saprophyte, animal colonizer, and important human pathogen. Its ability to persist across multiple reservoirs enhances its transmission potential, highlighting the importance of continuous surveillance of its epidemiology and antimicrobial resistance trends.

2.5 Clinical and Public Health Importance of *Klebsiella* Species

*Klebsiella species, e*specially *Klebsiella pneumoniae* and *Klebsiella oxytoca*, are recognized as significant opportunistic pathogens linked to a wide spectrum of clinical infections. Although they are normal residents of the human gastrointestinal tract, these bacteria can cause severe disease in immunocompromised individuals, hospitalized patients, and those with underlying health conditions such as diabetes, chronic pulmonary disease, or liver disorders (Paczosa and Mecsas, 2016).

Among the most serious infections caused by *K. pneumoniae* is pneumonia, often presenting as a necrotizing form with high mortality. A characteristic feature is the production of thick, blood-stained sputum, commonly described as “currant jelly sputum” (Podschun and Ullmann, 1998). In hospital settings, *Klebsiella* is also a leading pathogen in ventilator-associated pneumonia (VAP), posing major challenges in intensive care units.

Klebsiella is a frequent cause of urinary tract infections (UTIs), particularly in catheterized patients. It is often isolated from cases of complicated UTIs, cystitis, and pyelonephritis. Its pathogenicity is partly due to fimbriae-mediated adhesion, which enables colonization of uroepithelial cells (Podschun and Ullmann, 1998; Gupta *et al.*, 2011).

These bacteria are also implicated in septicemia and bacteremia, especially among patients with invasive medical devices or weakened immune systems. Infections caused by multidrug-resistant strains, notably carbapenem-resistant *K. pneumoniae* (CRKP), are linked to extended hospital stays, higher treatment costs, and elevated mortality rates (Navon-Venezia *et al.*, 2017).

Additional infections include wound infections, liver abscesses, meningitis, and endophthalmitis, with hypervirulent strains particularly associated with pyogenic liver abscesses (Shon *et al*., 2013). In neonatal care units, *Klebsiella* is a major cause of outbreaks of sepsis and meningitis in infants, conditions that continue to drive significant morbidity and mortality in low- and middle-income countries (Zaidi *et al*., 2005).

2.5.1. Virulence Factors

The pathogenicity of *Klebsiella* species arises from a diverse range of virulence factors that enable colonization of host tissues, evasion of immune defenses, and survival in hostile environments. The capsule (K antigen) is considered the most critical determinant of virulence. Composed of thick polysaccharide layers, the capsule shields the bacterium from phagocytosis and complement-mediated killing, ensuring persistence within the host. Over 80 capsular serotypes have been identified, with K1 and K2 serotypes strongly linked to hypervirulent strains that cause invasive diseases such as meningitis and liver abscesses (Paczosa and Mecsas, 2016).

Another key factor is the presence of fimbriae (adhesins), especially type 1 and type 3 fimbriae. These filamentous appendages facilitate adhesion to epithelial cells, colonization of indwelling devices, and biofilm formation. Biofilms play a central role in respiratory and urinary tract infections by providing a protective environment for bacterial communities, which also contributes to antibiotic tolerance (Struve *et al*., 2009).

To overcome iron restriction within the host, *Klebsiella* species secrete siderophores, small molecules that scavenge iron from host proteins. Common siderophores include enterobactin, yersiniabactin, salmochelin, and aerobactin. Notably, aerobactin is strongly associated with hypervirulent strains and serves as a key marker distinguishing them from classical *Klebsiella* pathogens (Russo *et al*., 2011).

The endotoxin lipopolysaccharide (LPS), an integral component of the outer membrane, also contributes to disease severity. LPS stimulates strong immune responses that may result in septic shock, while its O-antigen side chain enhances resistance to complement-mediated lysis and promotes bloodstream infections (Hsieh *et al*., 2012).

Additional virulence traits include outer membrane proteins, efflux pumps, and hydrolytic enzymes such as urease, which is particularly important in urinary tract infections. By generating ammonia, urease increases urinary pH, fostering bacterial persistence and facilitating urinary stone formation (Podschun and Ullmann, 1998).

Collectively, these virulence determinants illustrate the multifactorial strategies employed by *Klebsiella* to establish infections, evade host defenses, and adapt to various niches. Their synergistic effects explain why *Klebsiella* remains a highly significant pathogen in both clinical and public health contexts.

2.5.2. Emergence of Hypervirulent *Klebsiella pneumoniae* Strains

Over the last three decades, a distinct pathotype known as hypervirulent *Klebsiella pneumoniae* (hvKp) has emerged as a significant clinical concern. Unlike the classical variant (cKp), which predominantly causes hospital-acquired infections in immunocompromised individuals, hvKp has the remarkable ability to induce severe community-acquired infections in otherwise healthy hosts (Shon *et al*., 2013).

Infections caused by hvKp are commonly characterized by pyogenic liver abscesses that can progress to metastatic complications such as endophthalmitis, meningitis, and necrotizing fasciitis, often associated with high morbidity rates. These strains are primarily linked to capsular serotypes K1 and K2 and are defined by their excessive production of capsule material and siderophores—particularly aerobactin and salmochelin—which significantly enhance their virulence potential (Russo and Marr, 2019).

A growing public health concern is the emergence of hvKp strains that have acquired multidrug resistance determinants, including extended-spectrum β-lactamases (ESBLs) and carbapenemases. These hybrid strains, termed hypervirulent multidrug-resistant Klebsiella pneumoniae (hmKp), combine aggressive pathogenicity with extensive antimicrobial resistance, posing a formidable global health challenge due to limited treatment options (Gu *et al*., 2018; Lam *et al*., 2018).

The worldwide spread of hvKp is facilitated by factors such as increased international mobility, clonal dissemination, and plasmid-mediated co-transfer of virulence and resistance genes. This underscores the urgent need for enhanced global surveillance, rapid diagnostic approaches, and the development of novel therapeutic interventions to effectively control hvKp infections (Wyres and Holt, 2018).

2.6 *Klebsiella* Species in Wastewater Environments

Klebsiella species are Gram-negative, facultatively anaerobic members of the Enterobacteriaceae family. They are ubiquitously distributed across natural environments, including soil, water, sewage, vegetation, and the gastrointestinal tracts of humans and animals. Within wastewater systems, these bacteria play a dual role—as opportunistic pathogens and as indicators of fecal contamination—making them particularly significant from a public health standpoint. Their widespread occurrence in such environments primarily results from inputs associated with human and animal excreta, hospital effluents, and community wastewater discharges (Podschun and Ullmann, 1998; Janda and Abbott, 2006).

The persistence of *Klebsiella* species in wastewater ecosystems is largely attributed to their remarkable ecological adaptability. They are capable of thriving in both nutrient-rich and nutrient-limited aquatic environments, supported by their protective polysaccharide capsules that shield them from environmental stressors such as desiccation and protozoan grazing. Additionally, their ability to form biofilms enhances their environmental endurance, as biofilms provide a barrier against antimicrobial compounds and disinfectants commonly employed in wastewater treatment processes (Vuotto *et al.*, 2014).

Moreover, *Klebsiella* exhibits tolerance to a wide range of environmental fluctuations, including changes in pH, temperature, and organic load, enabling their survival in sewage and treated effluents alike (Adeleke *et al.*, 2021). This high level of adaptability explains their frequent isolation from raw sewage, secondary treatment stages, and even post-treatment wastewater effluents.

2.6.1. Occurrence in Domestic, Hospital, and Municipal Wastewater

The occurrence of *Klebsiella* species across various wastewater sources has been widely documented. In domestic wastewater, their presence is primarily attributed to fecal excretion from asymptomatic human carriers, residual food matter, and surface runoff (Leclerc *et al*., 2001). Hospital wastewater, however, presents a more critical concern since it frequently contains multidrug-resistant (MDR) *Klebsiella* strains derived from infected patients, contaminated medical instruments, and the intensive use of antibiotics in healthcare environments (Chitnis *et al*., 2004). These effluents often harbor high levels of *Klebsiella pneumoniae* and related species producing extended-spectrum β-lactamases (ESBLs) and carbapenemases, rendering them resistant even to last-line antibiotics (Kümmerer, 2009).

Municipal wastewater—comprising discharges from domestic, healthcare, industrial, and commercial sources—serves as another significant reservoir of *Klebsiella.* Their frequent detection in both raw sewage and treated effluents underscores their environmental persistence and contribution to the dissemination of antimicrobial resistance (Barguigua *et al*., 2011). Despite the intended role of wastewater treatment plants (WWTPs) in reducing microbial loads, these facilities often fail to completely eliminate *Klebsiella*, allowing viable cells and resistance genes to persist and contaminate receiving surface waters (Manaia *et al*., 2018). This persistence poses a substantial risk to human health through contact with contaminated irrigation water, recreational sites, and drinking water systems.

Across Africa—including Nigeria—numerous studies have confirmed the widespread occurrence of *Klebsiella* in wastewater environments. In Nigeria, isolates have been recovered from abattoir effluents, hospital discharges, municipal sewage, and surface waters contaminated by untreated wastewater (Adeleke *et al*., 2021; Osińska *et al.*, 2019). For instance, Adekanmbi and Falodun (2015) detected high concentrations of *K. pneumoniae i*n hospital effluents in Ibadan, demonstrating the significant role of medical wastewater in environmental pollution. Similarly, (Oladipo *et al*., 2019) reported the isolation of *Klebsiella* species from wastewater and adjoining rivers in Lagos, with observed resistance to multiple antibiotic classes, including cephalosporins and fluoroquinolones.

Comparable trends have been observed across other parts of Africa. In Morocco, Barguigua *et al.* (2011) identified ESBL-producing *K. pneumoniae* in hospital effluents, while Adefisoye and Okoh (2017) in South Africa reported *Klebsiella* among the dominant MDR bacteria in municipal wastewater. Collectively, these findings emphasize that African wastewater systems serve as important reservoirs for *Klebsiella* persistence and antimicrobial resistance propagation—particularly in regions lacking adequate wastewater treatment infrastructure.

In Nigeria, the challenge is especially severe, as most hospital and municipal wastewaters are discharged untreated into rivers, drainage systems, and surface waters that are often used by local populations (Oluyege *et al*., 2015). This practice exacerbates environmental contamination, facilitates horizontal gene transfer, and increases the risk of human exposure. Without significant improvement in wastewater management capacity, *Klebsiella* is likely to remain a critical source of antimicrobial resistance within aquatic environments.

2.7 Antibiotic Resistance in *Klebsiella* Species

Antibiotic resistance among *Klebsiella* species has become a major global public health concern, primarily due to their remarkable ability to acquire, maintain, and disseminate resistance genes across diverse environments. *Klebsiella pneumoniae* is especially worrisome and has been designated by the World Health Organization (WHO) as a critical priority pathogen owing to its multidrug-resistant (MDR) nature and its role in causing both hospital-acquired and community-associated infections (WHO, 2017). This challenge is further intensified by environmental reservoirs—particularly wastewater—which serve as hubs for resistant strains and facilitate horizontal gene transfer, thereby accelerating the spread of antimicrobial resistance.

2.7.1. Mechanisms of Resistance in *Klebsiella* Species

2.7.1.1. Extended-Spectrum Beta-Lactamases (ESBLs)

One of the primary mechanisms of antibiotic resistance in *Klebsiella* species is the production of extended-spectrum β-lactamases (ESBLs). These enzymes hydrolyze a wide spectrum of β-lactam antibiotics—including penicillins, third-generation cephalosporins, and aztreonam—thereby neutralizing their therapeutic efficacy (Paterson and Bonomo, 2005). The genes encoding ESBLs, such as bla_{CTX-M}, bla_{SHV}, and bla_{TEM}, are typically plasmid-borne, which facilitates their horizontal transfer among bacterial strains and species. The global prevalence of ESBL-producing *K. pneumoniae* has increased significantly in recent years, often leaving carbapenems and a few other last-resort antibiotics as the only viable treatment options (Pitout and Laupland, 2008).

2.7.1.2. Carbapenemases

Carbapenems, once considered the most reliable treatment for infections caused by ESBL-producing bacteria, have become increasingly ineffective due to the emergence of carbapenemase-producing *Klebsiella* strains. The most prevalent enzymes responsible for this resistance include *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), Verona integron-encoded metallo-β-lactamase (VIM), and Oxacillinase-48 (OXA-48) (Nordmann et al., 2011). These carbapenemases hydrolyze nearly all β-lactam antibiotics, severely limiting available therapeutic options. Infections caused by carbapenemase-producing *K. pneumoniae* are particularly concerning in healthcare environments, as they are often linked to elevated rates of morbidity and mortality (Logan and Weinstein, 2017).

2.7.1.3. Efflux Pumps and Porin Modifications

In addition to enzymatic mechanisms, *Klebsiella* species exhibit resistance through non-enzymatic strategies such as efflux pump overexpression and alterations in membrane porins. Efflux systems, particularly the AcrAB-TolC complex, actively expel a wide range of antibiotics—including fluoroquinolones, tetracyclines, and aminoglycosides—thereby reducing their intracellular concentrations and effectiveness (Li and Nikaido, 2009). Furthermore, mutations or loss of outer membrane porins like OmpK35 and OmpK36 lead to decreased cell permeability. When these porin modifications occur alongside ESBL or AmpC β-lactamase production, they synergistically enhance carbapenem resistance (Doumith *et al.*, 2009).

2.7.1.4. Plasmid-Mediated and Mobile Genetic Elements

In *Klebsiella* species, many antibiotic resistance genes are carried on mobile genetic elements such as plasmids, integrons, and transposons, which facilitate their rapid spread across bacterial species and environments. The coexistence of multiple resistance determinants—such as carbapenemase genes combined with aminoglycoside-modifying enzymes and colistin resistance genes like mcr-1—has been increasingly reported, thereby compounding treatment challenges and limiting available therapeutic options (Liu *et al*., 2016).

2.7.2. Global and Regional Trends in Antimicrobial Resistance in *Klebsiella*

Globally, the incidence of ESBL-producing *Klebsiella* species has reached concerning proportions. Surveillance data reveal that more than 50% of *Klebsiella pneumoniae* isolates in parts of Asia, Africa, and South America exhibit ESBL production (Jean *et al*., 2016). In contrast, Europe and North America are witnessing a growing prevalence of carbapenemase-producing strains, particularly those harboring KPC and NDM enzymes, which have been implicated in numerous hospital outbreaks (Munoz-Price *et al*., 2013).

Across Africa, antimicrobial resistance surveillance remains inadequate; however, available evidence suggests a widespread presence of both ESBL- and carbapenemase-producing *Klebsiella* in clinical and environmental settings. In Nigeria, several studies have documented multidrug-resistant *K. pneumoniae* in hospital wastewater, effluents, and clinical isolates, indicating concurrent transmission between healthcare and environmental reservoirs (Aibinu *et al*., 2012; Ogbolu *et al.*, 2018). This situation is particularly concerning in low- and middle-income countries (LMICs), where limited access to advanced antimicrobial therapies amplifies the risks associated with resistant *Klebsiella* infections.

2.7.3. *Klebsiella* in Wastewater as a Hotspot for Resistance Gene Transfer

Wastewater systems particularly those receiving discharges from hospitals and municipal sources serve as critical hotspots for the proliferation and exchange of antimicrobial resistance genes.

Klebsiella species, frequently isolated from hospital effluents, municipal sewage, and industrial wastewater, act as important reservoirs of resistance determinants (Rizzo *et al.*, 2013). The combination of dense microbial populations, residual antibiotics, and disinfectants within these environments creates intense selective pressure, enabling resistant strains to survive and multiply.

Horizontal gene transfer mechanisms such as conjugative plasmids, integrons, and bacteriophages are highly active in wastewater ecosystems. Studies have demonstrated that *Klebsiella* strains isolated from wastewater often carry plasmids encoding ESBLs, carbapenemases, and other resistance determinants, which can be transferred to other members of the Enterobacteriaceae family, including *Escherichia coli* (Manaia *et al.*, 2018). In Nigeria, investigations have identified multidrug-resistant *Klebsiella* in wastewater from tertiary hospitals and abattoirs, highlighting the contamination of rivers and agricultural lands used for irrigation (Osadebe and Okounim, 2020; Atta *et al.*, 2022).

Thus, wastewater not only serves as a reservoir of resistant *Klebsiella* but also as an active hub for genetic exchange, accelerating the environmental spread of antimicrobial resistance. This situation presents a major public health concern, as untreated or poorly treated effluents can contaminate surface and groundwater, facilitating the transmission of resistance genes to humans, animals, and other environmental microorganisms.

2.8 Public Health Implications of Wastewater-Associated *Klebsiella*

The occurrence of *Klebsiella* species in wastewater represents a significant public health concern, particularly in areas with limited or poorly maintained sanitation infrastructure. Wastewater serves not only as a reservoir but also as a conduit for the transmission of pathogenic and multidrug-resistant bacteria, increasing the potential for human exposure. This risk is particularly elevated in densely populated environments such as hostels, communities, and healthcare facilities, where inadequate hygiene practices, overcrowding, and ineffective wastewater management systems facilitate the spread of these microorganisms.

2.8.1. Risks of Human Exposure in Hostel and Community Environments

Hostel and community settings constitute high-risk environments for exposure to *Klebsiella*-contaminated wastewater, largely because of shared sanitation facilities and inconsistent hygiene practices. Inadequately maintained drainage systems can lead to wastewater overflow into residential spaces, resulting in the contamination of surfaces, fomites, and even potable water supplies. Consequently, students and community residents may become exposed to pathogenic *Klebsiella* species through direct contact with wastewater, inhalation of contaminated aerosols, or indirectly via contact with polluted hands and objects.

Among wastewater-associated pathogens, *Klebsiella pneumoniae* is particularly significant as an opportunistic bacterium capable of colonizing both the gastrointestinal and respiratory tracts, leading to infections such as pneumonia, urinary tract infections, and septicemia (Martin and Bachman, 2018). The most vulnerable groups include children, the elderly, and immunocompromised individuals, who are more susceptible to infection. In resource-limited settings like Nigeria—where hostel drainage infrastructure is often substandard—inefficient wastewater management considerably increases the risk of outbreaks caused by enteric and respiratory pathogens, notably multidrug-resistant *Klebsiella* (Adegoke *et al*., 2020).

2.8.2. Potential Spread of Antimicrobial Resistance through Wastewater

One of the most pressing public health threats associated with wastewater-derived *Klebsiella* is its significant role in the propagation of antimicrobial resistance (AMR). Wastewater often harbors high concentrations of antibiotics, disinfectants, and resistant microorganisms originating from hospitals, households, and pharmaceutical sources, thereby creating a selective environment that fosters horizontal gene transfer through plasmids, integrons, and transposons (Rizzo *et al*., 2013). *Klebsiella* species are particularly notorious for carrying key resistance determinants such as extended-spectrum β-lactamases (ESBLs) and carbapenemases, which confer resistance to critical last-line antibiotics, including carbapenems (Pitout *et al*., 2015).

Following environmental discharge, *Klebsiella* strains from wastewater can disseminate resistance genes to commensal or environmental bacteria, establishing reservoirs of resistant pathogens with potential impacts on human and animal health. Studies have demonstrated that wastewater effluents released into rivers and drainage systems play a central role in the global dissemination of AMR across both industrialized and developing regions (Manaia, 2017). In African countries, where untreated or inadequately treated wastewater is frequently discharged into the environment, multidrug-resistant *Klebsiella* contaminates drinking water sources, agricultural soils, and food products (Olalekan *et al*., 2020). This not only heightens the risk of local disease outbreaks but also contributes to the worldwide escalation of AMR through interconnected environmental pathways.

2.8.3. Links to the One Health Concept (Human, Animal, Environment Interface)

The One Health framework, which emphasizes the interdependence of human, animal, and environmental health, offers a comprehensive perspective for understanding the public health implications of wastewater-associated *Klebsiella.* Wastewater serves as a critical interface where resistant *Klebsiella* strains and other pathogens can circulate among these interconnected domains. For instance, livestock and domestic animals exposed to contaminated environments may acquire resistant bacteria that can subsequently be transmitted to humans through direct contact or the consumption of animal-derived foods. Similarly, resistant *Klebsiella* strains can persist in aquatic ecosystems, contaminating fisheries and irrigation systems, and thereby introducing additional pathways for human exposure (Berendonk *et al.*, 2015).

Adopting a One Health approach underscores the importance of wastewater surveillance as an early-warning tool for detecting resistance patterns and emerging threats. Integrating such monitoring into national and regional public health frameworks can provide valuable insights into the dissemination of multidrug-resistant organisms and support evidence-based interventions to reduce environmental transmission. Addressing these challenges requires a multifaceted strategy encompassing advanced wastewater treatment technologies, prudent antimicrobial stewardship, and enhanced sanitation and hygiene practices. Ultimately, mitigating the risks associated with wastewater-borne *Klebsiella* demands coordinated, cross-sectoral collaboration among human health professionals, veterinarians, environmental scientists, and policymakers.

2.9 Control and Preventive Measures

The control and prevention of wastewater-associated *Klebsiella* infections—and the broader mitigation of antimicrobial resistance (AMR)—require a comprehensive, multi-pronged approach that combines environmental management, public health initiatives, and the One Health framework. Effective interventions should aim to minimize pathogen loads in wastewater, interrupt transmission pathways that facilitate human exposure, and strengthen surveillance systems to monitor resistance trends across human, animal, and environmental interfaces.

2.9.1. Wastewater Treatment and Disinfection Approaches

Effective wastewater treatment serves as a critical measure in minimizing the risks associated with *Klebsiella* contamination in effluents. Although conventional treatment processes—including sedimentation, filtration, and biological methods—significantly reduce microbial loads, multidrug-resistant (MDR) strains often persist through these systems (Rizzo *et al.*, 2013). To achieve higher efficiency, advanced treatment technologies such as membrane filtration, ozonation, and ultraviolet (UV) irradiation have shown superior effectiveness in eliminating resistant bacteria like *Klebsiella pneumoniae* (Zhang *et al.*, 2016).

Despite its widespread use and cost-effectiveness, chlorination poses challenges related to the formation of harmful disinfection by-products and its limited efficacy against resistant microorganisms, underscoring the need for integrated or complementary disinfection methods (Rizzo *et al.*, 2013). In resource-constrained regions such as Nigeria, the lack of adequate wastewater treatment infrastructure and poor maintenance of existing systems exacerbate environmental contamination and increase public health risks (Adegoke *et al.*, 2020). To mitigate these challenges, strengthening wastewater management capacity and adopting sustainable, low-cost alternatives—such as solar disinfection (SODIS) and constructed wetlands—offer practical and environmentally friendly solutions.

2.9.2. Good Sanitary and Hygienic Practices in Hostels and Institutions

Hostel and institutional settings are particularly vulnerable to wastewater-associated infections due to factors such as overcrowding, shared facilities, and inadequate sanitation infrastructure. Minimizing these risks necessitates the implementation of proper hygiene and waste management practices, including efficient waste disposal systems, well-maintained drainage networks, and regular cleaning of communal areas.

Hand hygiene remains one of the most effective preventive measures, as it disrupts both fecal–oral and contact transmission pathways for *Klebsiella* and other pathogenic microorganisms (Aiello *et al.*, 2008). Routine disinfection of toilets, restrooms, and drainage outlets also plays a crucial role in suppressing microbial proliferation within shared environments. In addition, health education and awareness programs targeted at students and institutional residents are vital for fostering responsible sanitation behaviors and discouraging habits that facilitate the spread of pathogens.

2.9.3. Surveillance and Monitoring of Antibiotic Resistance in Environmental Isolates

Monitoring antibiotic resistance in *Klebsiella* isolates from environmental sources provides essential information on emerging resistance trends and underpins evidence-based public health interventions. The use of wastewater-based epidemiology (WBE) has become increasingly recognized as an effective tool for tracking antimicrobial resistance (AMR) and monitoring pathogen circulation within communities (Hendriksen *et al.*, 2019). By analyzing wastewater effluents, health authorities can detect multidrug-resistant *Klebsiella* and other pathogens early, allowing for preventive measures before these strains cause widespread clinical infections.

In Nigeria and other low- and middle-income countries, integrating environmental surveillance into national AMR control frameworks would significantly strengthen efforts to identify resistance hotspots and implement timely interventions (Olalekan *et al*., 2020). Furthermore, the application of genomic surveillance techniques, such as whole-genome sequencing, offers detailed insights into resistance genes, plasmid architecture, and transmission pathways, thereby enhancing proactive strategies to mitigate the global AMR burden (Collignon and McEwen, 2019).

2.9.4. One Health Strategies in Combating Antimicrobial Resistance

Given the intrinsic links among human, animal, and environmental health, the One Health approach remains fundamental to addressing the public health challenges posed by wastewater-associated *Klebsiella.* This integrated framework emphasizes multisectoral collaboration among medical, veterinary, and environmental professionals to curb antimicrobial resistance (AMR) transmission. Core strategies include promoting the prudent use of antibiotics in healthcare and veterinary practices, strengthening infection prevention and control (IPC) programs in hospitals and agricultural settings, and enforcing stringent policies for safe wastewater management to prevent the release of untreated effluents into natural water bodies and farmlands (Berendonk *et al*., 2015).

Additionally, implementing environmental protection measures, alongside capacity-building initiatives, stakeholder participation, and international cooperation, is vital to ensuring that AMR containment efforts are effective, harmonized, and sustainable, particularly within resource-constrained regions.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study involved selected male hostel drains located within the University of Benin, Edo State, Nigeria. The University of Benin is situated in Benin City, the capital of Edo State. The city lies between latitude 6.50° North and longitude 5.80° East of the Greenwich Meridian. The male hostel drains serve as outlets for wastewater generated from bathing, laundry, cooking, and other domestic activities of students.

3.2 Sample Collection

A total of ten (10) wastewater samples were collected from drainage points within two selected male hostels on the university campus. The sampling locations comprised two (2) samples from the Bathroom Effluent Section (BES), two (2) samples from the Laundry Effluent Section (LES), and one (1) sample from the General Discharge Outlet (GDO) in each hostel. Sterile 500 mL screw-capped bottles were utilized for sample collection, with each bottle pre-rinsed using the respective wastewater prior to final sampling. All samples were properly labeled, placed in an ice-packed cooler, and immediately transported to the Microbiology Laboratory, Faculty of Life Sciences, University of Benin, for analysis within three (3) to four (4) hours of collection.

3.3 Enumeration of Total Heterotrophic Bacteria

3.3.1 Enumeration of Total Heterotrophic Bacteria

The total heterotrophic bacterial population was determined using nutrient agar (NA) medium. The medium (Lab M, Lancashire, United Kingdom) was prepared by dissolving 28 g of nutrient agar powder in 1000 mL of distilled water, followed by sterilization in an autoclave at 121 °C for 15 minutes.

Wastewater samples were subjected to serial ten-fold dilutions, in which 1 mL of the sample was transferred into 9 mL of sterile distilled water successively until a 10^{-3} dilution was achieved. Enumeration was performed using the spread plate technique, where 0.1µL of the 10^{-3} dilution was inoculated in duplicate onto sterile nutrient agar plates. The plates were then incubated at 37 °C for 18–24 hours.

Following incubation, colonies were counted, and results were expressed as colony-forming units per millilitre (CFU/mL). Distinct colonies were subsequently sub-cultured onto fresh nutrient agar plates to obtain pure isolates for further characterization.

3.4 Biochemical Characterization of *Klebsiella* Isolates

3.4.1 Characterization of Bacterial Isolates

Bacterial isolates suspected to belong to the *Klebsiella* genus were subjected to Gram staining and a range of biochemical assays for identification. These included the oxidase, indole, urease, citrate utilization, triple sugar iron (TSI), methyl red–Voges Proskauer (MR–VP), motility, and sugar fermentation tests.

3.4.2 Gram Staining

Gram staining was conducted to determine the Gram reaction and cell morphology of the bacterial isolates. Smears of each test isolate were heat-fixed onto clean glass slides and sequentially stained with crystal violet for 1 minute, followed by Gram's iodine for another minute. The slides were then decolorized with 95% ethanol for approximately 30 seconds and subsequently counterstained with safranin for 1 minute. After rinsing with distilled water and air-drying, the slides were examined under oil immersion (×100 objective lens). The observation of Gram-negative, rod-shaped cells was considered indicative of *Klebsiella* species.

3.4.3 Oxidase Test

The oxidase test was performed using the wet filter paper method with Kovac's oxidase reagent (1% tetramethyl-p-phenylenediamine dihydrochloride). A sterile inoculating loop was used to transfer a fresh bacterial colony onto a piece of filter paper previously soaked with the reagent. The appearance of a purple-blue coloration within 30 seconds was interpreted as a positive oxidase reaction, whereas the absence of any color change indicated a negative result.

3.4.4 Indole Test

The spot indole test was conducted using a 1% p-dimethylaminocinnamaldehyde reagent. A few drops of the reagent were dispensed onto a piece of filter paper, after which a loopful of bacterial culture was gently rubbed onto the reagent-saturated area. The development of a blue to blue-green coloration within 2–3 minutes signified a positive indole reaction, whereas the absence of color change indicated a negative result.

3.4.5 Urease Test

The urease test was performed using Christensen's urea agar slants. Each test isolate was streaked aseptically onto the slant surface and incubated at 37 °C for 24–48 hours. A color shift from yellow-orange to pink-red denoted a positive urease reaction, indicating the hydrolysis of urea to ammonia, whereas the absence of color change signified a negative result.

3.4.6 Citrate Utilization Test

The Simmons' citrate agar slants were inoculated with the test isolates and incubated at 37 °C for 24–48 hours. A color transition from green to blue signified a positive reaction, indicating the organism's ability to utilize citrate as the sole carbon source. Conversely, the absence of color change denoted a negative result.

3.4.7 Triple Sugar Iron (TSI) Agar Test

The TSI agar slants were inoculated by stabbing the butt and streaking the slant surface with each test isolate, followed by incubation at 37 °C for 24 hours. A yellow coloration of both the butt and slant indicated acid production resulting from the fermentation of glucose, lactose, and/or sucrose. The formation of black precipitate signified hydrogen sulfide (H_2S) production, while the presence of cracks or bubbles denoted gas formation during fermentation.

3.4.8 Motility Test

Motility was assessed using Sulphide-Indole-Motility (SIM) medium. The medium was inoculated by stabbing the center of the tube with a sterile needle containing the test culture and incubated at 37 °C for 24 hours. Diffused or hazy growth extending outward from the line of inoculation indicated a motile organism, whereas growth confined to the stab line signified a non-motile organism.

3.4.9 Methyl Red-Voges Proskauer (MRVP) Test

The MR–VP broth medium was inoculated with the test isolates and incubated at 37 °C for 48 hours.

For the Methyl Red (MR) test, 1 mL of the culture broth was transferred into a clean test tube, and 5 drops of methyl red indicator were added. The development of a red color indicated a positive reaction, while a yellow coloration indicated a negative result.

For the Voges–Proskauer (VP) test, 1 mL of the culture broth was mixed with 6 drops of 5% α-naphthol and 2 drops of 40% potassium hydroxide (KOH). The appearance of a pink to red coloration within 30 minutes indicated a positive VP reaction.

3.4.10 Sugar Fermentation Test

The ability of the isolates to ferment various carbohydrates—glucose, lactose, sucrose, and mannitol—was assessed using purple broth base containing 1% of each sugar and bromcresol purple as the pH indicator. Each tube was fitted with an inverted Durham tube to capture any gas produced during fermentation. The test tubes were inoculated with the isolates and incubated at 37 °C for 24–48 hours. A color change from purple to yellow indicated acid production, while the presence of gas bubbles in the Durham tube signified gas formation.

3.5 Data Analysis

Data obtained from the study were analyzed using Microsoft Excel 2013. Results of total bacterial counts were expressed as mean ± standard deviation (SD). Descriptive statistical methods were employed to summarize and present the microbial loads, frequency distributions, and biochemical characteristics of the bacterial isolates.

CHAPTER FOUR

4.0. RESULTS

Figure 4.1 presents the heterotrophic bacterial counts (Log_{10} cfu/ml) of wastewater samples collected from two male hostels (Hall 3 and Hall 4) in the University of Benin.

Figure 4.2 presents the total coliform bacterial counts of the wastewater samples from the two male hostels.

Table 4.1 presents the cultural, morphological, and biochemical characteristics of bacterial isolates recovered from the wastewater samples

Table 4.2 shows the distribution of bacterial isolates across the wastewater samples collected from Hall 3 and Hall 4 male hostels.

Figure 4.3 presents the frequency and percentage occurrence of bacterial isolates recovered from the wastewater samples.

Table 4.3 presents the antibiotic susceptibility profile of *Klebsiella* species isolated from the wastewater samples.

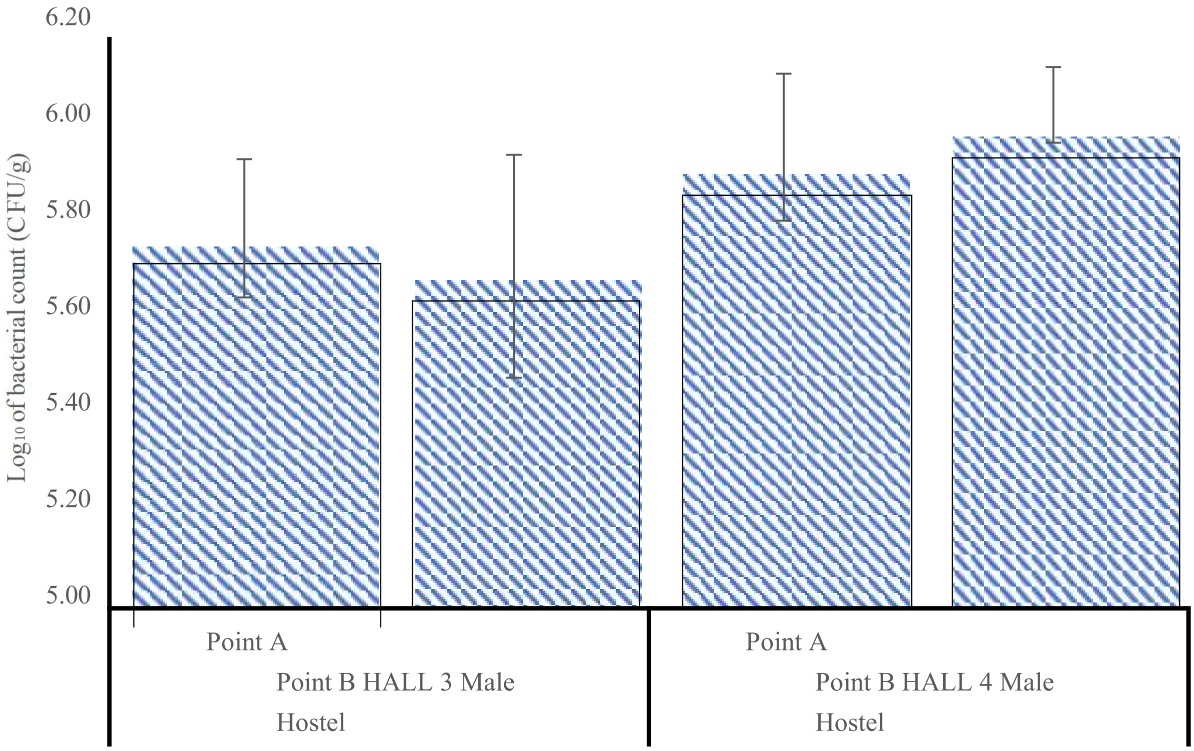

Figure 4.1. Heterotrophic bacterial counts of the wastewater samples from male hostels

KEYS:

Point A: Laundry waste water drainage

Point B: Bathroom waste water drainage

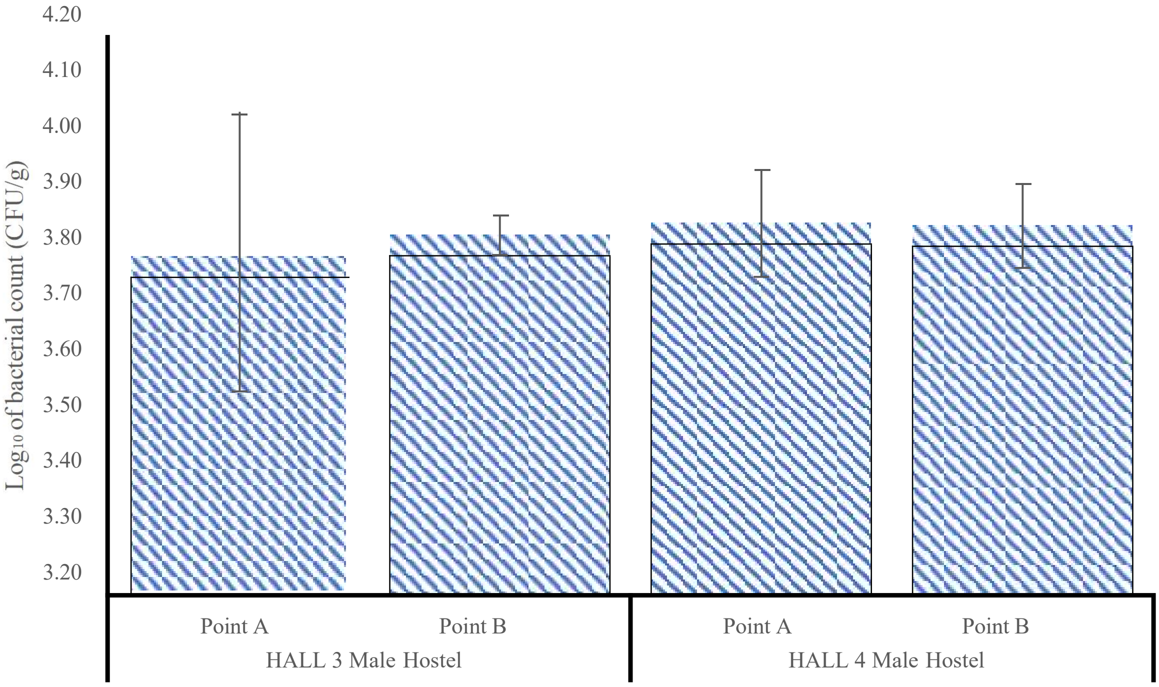

Figure 4.2. Total coliform bacterial counts of the wastewater samples from male hostels

KEYS:

Point A: Laundry waste water drainage

Point B: Bathroom waste water drainage

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

Morphological					
Elevation	Flat	Flat	Flat	Raised	Raised
Margin	Undulate	Entire	Undulate	Smooth	Entire
Color	Cream	Cream	Cream	Cream	Lemon
Shape	Irregular	Circular	Irregular	Irregular	Circular
Size	Large	Small	large	Small	Medium
Gr. diff. agar	EMB	EMB	BCA	MSA	PCA
Color	Green	Pink	Straw	Yellow	Green
Staining					
Gram stain	-	-	+	+	-
cell type	Rod	Rod	Rod	Cocci	Rod
Arrangement	Disperse	Rod disperse	disperse	Clusters	Disperse
Color	Pink	pink	purple	Purple	Pink
Spore staining	-	-	+	-	-
Biochemical					
KOH String Test	+	+	-	-	+
Catalase	+	+	+	+	+
Indole	+	-	-	-	-
Citrate	-	+	+	+	+
Oxidase	-	-	-	-	+
Motility	+	-	+	-	+
Urease	-	+	-	+	+
Glucose	+	+	+	+	-
Sucrose	-	+	+	+	-
Lactose	+	+	+	+	-
Mannitol	-	-	+	-	-
Gas formation	+	+	-	-	-
H2S formation	-	-	-	-	-
TSI (Slant/Butt) reaction	A/AG	A/AG	A/A	A/A*	K/K
Esculin Hydrolysis	-	+	-	-	-
Identity	*E. coli*	*Klebsiela oxytoca*	*Bacilus subtilis*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*

KEYS:

+ --- positive

- ---- negative

A/AG - Acid /Acid gas

A/A - Acid / Acid

K/K - Alkaline /Alkaline

Table 2. Distribution of bacterial isolates

	HALL 3 Male Hostel	**HALL 4 Male Hostel**	**HALL 4 Male Hostel**	**HALL 3 Male Hostel**
Isolates	**Point B**	**Point A**	**Point B**	**Point B**
E. coli	+	+	+	+
Klebsiela oxytoca	+	+	+	-
Bacilus subtilis	+	+	+	+
Pseudomonas aeruginosa	+	+	+	+
Staphylococcus aureus	+	+	-	+

Key: Present (+), Absent (-)

KEYS:

+ Positive

- negative

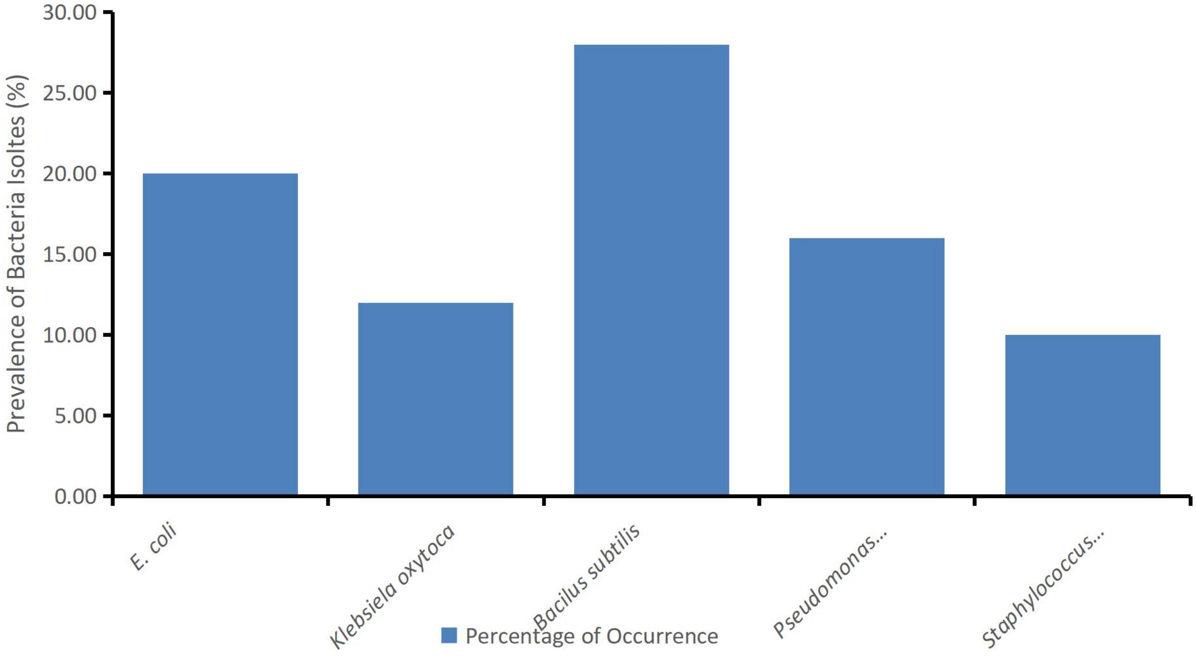

Figure 4.3. Frequency of bacterial occurrence obtained from the different sample

Table 4.3. Antibiotic susceptibility of *Klebsiella* species obtained from wastewater

Antibiotics	S (%)	I (%)	R (%)
NA	33	0	67
CRO	33	33	33
CKM	0	0	100
ACX	0	100	0
GX	0	67	33
IMP	0	100	0
OFX	0	33	67
ZEM	33	67	0
LBC	0	67	33
NF	0	33	67
AUG	0	67	33
GN	0	67	33

Key: (Based on CLSI/EUCAST example breakpoints for *Klebsiella pneumoniae*)
Sensitive (S): ≥21 mm (CRO, CKM); ≥17 mm (IMP); ≥18 mm (GN); ≥17 mm (OFX, GX, LBC); ≥17 mm (ZEM); ≥17 mm (NF); ≥20 mm (AUG); ≥13 mm (ACX); ≥19 mm (NA)
Intermediate (I): 15–20 mm (CRO, CKM); 14–16 mm (IMP, GN, and others)
Resistant (R): ≤14 mm (CRO, CKM); ≤13 mm (IMP); ≤14 mm (GN); variable for others

CHAPTER FIVE

5.0 DISCUSSION

Wastewater, especially from residential hostels, often harbors a complex mixture of human-derived microorganisms, including opportunistic pathogens that can persist and exchange resistance genes in the environment (Baquero *et al*., 2008; Rizzo *et al*., 2013). In university hostels where large populations coexist, the drainage systems become potential hotspots for microbial proliferation due to improper disposal of organic waste, detergents, and faecal matter. These conditions promote the survival and spread of antibiotic-resistant bacteria, which may later find their way into surface or groundwater sources (Manaia *et al*., 2016). *Klebsiella* species, a member of the Enterobacteriaceae family, is an opportunistic pathogen frequently isolated from such contaminated sites. Its growing resistance to commonly used antibiotics has made it an organism of great epidemiological concern (Podschun and Ullmann, 1998; Navon-Venezia *et al*., 2017; Wyres and Holt, 2018). This study seeks to investigate the Antimicrobial Resistance Profile of *Klebsiella* Species from Male Hostel Drains in the University of Benin.

The heterotrophic bacterial counts observed in this study ranged from 5.75 to 6.00 Log_{10} cfu/ml, indicating a considerable bacterial load across all sampling points. The highest count (6.00 Log_{10} cfu/ml) was recorded at Hall 4 Point B, while the lowest (5.75 Log_{10} cfu/ml) occurred at Hall 3 Point B. These elevated microbial loads suggest that the hostel drainage environments are highly conducive for bacterial proliferation, likely due to the continuous inflow of organic waste, detergents, and human-associated residues.

High bacterial counts in wastewater have been similarly reported by Eze and Okpokwasili (2010), who found heterotrophic counts ranging between 10^5 and 10^7 cfu/ml in domestic wastewater samples in Port Harcourt, Nigeria. Likewise, Aka and Nwachukwu (2017) reported comparable bacterial loads in hostel and domestic drainage systems in Owerri, attributing these high values to the accumulation of nutrient-rich organic matter. The results from this study therefore align with previous reports indicating that wastewater effluents from human settlements are significant carriers of diverse bacterial populations, including potential pathogens.

The total coliform counts ranged between 3.80 and 3.87 Log_{10} cfu/ml, suggesting the presence of fecal contamination in both hostels. Coliform bacteria, especially *Escherichia coli*, are key indicators of fecal pollution and poor environmental sanitation. The detection of such organisms in hostel drains implies that untreated or improperly managed waste may have infiltrated the drainage system, potentially posing health risks to residents.

This finding corroborates the work of Olowe *et al*. (2015), who reported high coliform counts in wastewater channels around residential areas in southwestern Nigeria. Similarly, Igbinosa and Okoh (2009) observed that municipal wastewater in South Africa contained coliform loads exceeding 10^3 cfu/ml, indicative of fecal contamination. The coliform counts obtained in this study thus reflect suboptimal sanitation and highlight the need for improved wastewater management practices in student residential areas.

The isolates identified include, *Escherichia coli*, *Klebsiella oxytoca*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* represent a mixture of both environmental and opportunistic pathogens. The isolation of *E. coli* and *Klebsiella* spp. is particularly significant because these enteric bacteria are commonly associated with fecal contamination and can act as opportunistic pathogens causing urinary tract infections, gastroenteritis, and wound infections (Brooks *et al*., 2022). The predominance of *Bacillus subtilis* (28%) may be attributed to its endospore-forming capability, allowing it to survive environmental stress and high organic loads typical of wastewater drains (Oyetibo *et al*., 2017; Igbinosa *et al*., 2017). Similarly, the presence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* reflects contamination from human activities such as bathing, handwashing, and discharge of body fluids, since these organisms are common skin and environmental commensals (Mena and Gerba, 2009; David and Daum 2010; Todar, 2019; Adesojiw *et al.,* 2023).

The detection of *Klebsiella oxytoca* in the wastewater is particularly significant. Members of the *Klebsiella* genus are opportunistic pathogens associated with pneumonia, urinary tract infections, and wound infections, and are often found in wastewater, hospital effluents, and domestic sewage (Sahoo *et al*., 2025). Its isolation from wastewater agrees with findings by Adefisoye and Okoh (2016)**,** who reported *Klebsiella* species in domestic effluents in the Eastern Cape, South Africa, highlighting their ability to survive in aquatic environments rich in organic nutrients. Their detection across multiple sampling points in both hostels implies continuous input of human-derived waste into the drainage system. *Klebsiella* species are known to survive for extended periods in moist environments, enabling them to colonize and persist in drainage pipes and biofilms (Bagley, 1985; Podschun and Ullmann, 1998).

The antibiotic susceptibility pattern of *Klebsiella oxytoca* revealed a concerning trend of multidrug resistance (MDR). The isolates were 100% resistant to Ceftazidime, and exhibited high resistance to Ofloxacin (67%), Nalidixic acid (67%), and Nitrofurantoin (67%). This pattern of multidrug resistance (MDR) is consistent with findings by Olowe *et al*. (2020) and Adeleke *et al*. (2019), who reported high resistance rates of *Klebsiella* species isolated from wastewater and hospital effluents in Nigeria. The observed resistance to β-lactam antibiotics (such as ceftazidime and amoxicillin-clavulanate) suggests possible production of extended-spectrum β-lactamases (ESBLs), enzymes known to hydrolyze and inactivate a broad range of β-lactam antibiotics **(**Paterson and Bonomo, 2005).

The moderate resistance to fluoroquinolones (Ofloxacin and Nalidixic acid) and the limited sensitivity to aminoglycosides (Gentamicin) suggest multiple mechanisms of resistance, possibly involving efflux pumps, plasmid-mediated quinolone resistance (PMQR), or alterations in DNA gyrase and topoisomerase IV (Jacoby *et al*., 2014). The isolates also demonstrated intermediate susceptibility to Imipenem and Amoxicillin-clavulanate, two antibiotics often considered as last-resort drugs for treating infections caused by Gram-negative bacteria. Although none of the isolates were completely resistant to Imipenem, the reduced susceptibility is worrisome, as it could indicate the emergence of carbapenemase-producing *Klebsiella* (Nordmann *et al*., 2011; Igbinosa *et al*., 2017; WHO, 2022).

Environmental dissemination of resistant *Klebsiella* strains can occur through horizontal gene transfer, particularly in wastewater systems where high bacterial densities and diverse microbial communities promote plasmid exchange (Van Hoek *et al*., 2011; Rizzo *et al*., 2013). This situation creates an ecological hotspot for AMR gene proliferation. The wastewater drains of the male hostels therefore represent not only a reservoir but also a potential conduit for the spread of resistant pathogens to surrounding environments, including soil and surface water.

The presence of multidrug-resistant *Klebsiella* species in hostel drainage systems poses a potential public health risk to students and nearby communities. Wastewater effluents can serve as vehicles for the dissemination of resistant genes into the wider environment, leading to contamination of surface water, groundwater, and food crops irrigated with contaminated water (Marti *et al*., 2014). The persistence of such pathogens in drainage systems increases the risk of human exposure, especially during flooding or poor waste disposal practices (Ngwa *et al*., 2021).

Furthermore, *Klebsiella* species have been implicated in hospital outbreaks and community-acquired infections (Wyres and Holt, 2018). The transition of resistant strains from environmental sources to human hosts is increasingly recognized as a component of the "One Health" framework, which emphasizes the interconnection between human, animal, and environmental health (Collignon *et al*., 2018). Therefore, monitoring wastewater systems for resistant bacteria provides early warning signals for emerging threats.

The detection of fecal indicator bacteria and multidrug-resistant *Klebsiella* species in hostel wastewater suggests that students and nearby communities could be exposed to potential pathogens through environmental contact, especially during flooding or leakage of drain contents. According to WHO (2023), wastewater is a critical but often overlooked pathway in the transmission of antimicrobial resistance. The improper disposal of wastewater can facilitate the dissemination of resistant genes into natural water bodies, soil, and food chains, ultimately compromising public health.

Several studies have corroborated these findings. For instance, Akpor *et al*. (2014) reported the isolation of *Klebsiella* species from hostel and domestic wastewater in Southwestern Nigeria, with high resistance to cephalosporins and quinolones. Similarly, Okoh *et al*. (2017) documented multidrug-resistant *Klebsiella pneumoniae* in hospital effluents, emphasizing the role of wastewater as a reservoir for resistance genes. Studies in other African universities have shown comparable trends, linking inadequate waste management systems to environmental dissemination of AMR (Tadesse *et al*., 2022). This present study thus aligns with global concerns that environmental sources—especially untreated wastewater—are major contributors to the evolution and spread of antimicrobial resistance.

5.1 CONCLUSION

This study demonstrates that wastewater drains in the male hostels of the University of Benin are heavily contaminated with diverse bacteria, including *Klebsiella* species exhibiting multidrug resistance. The high bacterial and coliform counts reflect poor sanitary and waste management practices, while the antibiotic susceptibility pattern highlights the environmental dissemination of resistant strains. The emergence of resistance to cephalosporins and quinolones among *Klebsiella* isolates suggests the potential presence of ESBL-producing strains, posing a significant threat to both environmental and public health. Effective monitoring and management of wastewater systems are therefore crucial to curb the spread of resistant bacteria in institutional settings.

5.2 RECOMMENDATION

- **Routine Surveillance:** Regular microbiological monitoring of hostel wastewater should be implemented to detect and track antimicrobial-resistant organisms.
- **Proper Waste Management:** The University should improve drainage infrastructure and establish proper wastewater treatment facilities to prevent environmental contamination.
- **Public Health Education:** Students and hostel residents should be educated on hygiene and waste disposal practices to minimize microbial contamination.
- **Antibiotic Stewardship:** Awareness programs should be promoted to discourage misuse and overuse of antibiotics among students and the general public.
- **Further Research:** Molecular studies should be conducted to identify specific resistance genes (e.g., *blaTEM*, *blaSHV*, *blaCTX-M*) in environmental *Klebsiella* isolates and to assess potential gene transfer mechanisms.

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