ISOLATION AND IDENTIFICATION OF AIRBORNE BACTERIAL ISOLATES FROM LIFE SCIENCE, HALL 3 AND HALL 4 DUMPSITES. UNIBEN, NIGERIA

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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 FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc. Hons) IN MICROBIOLOGY

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CERTIFICATION

This is to certify that this project work was carried out by **Marcel Oshiokpekhai ISAAC** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

…… ……..

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(Project Supervisor)

…… ……..

PROF. E.O IGBINOSA **DATE**

(Head of Department)

DEDICATION

This project work is dedicated to God and my family members for their unwavering support.

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I express my wholehearted gratitude to God whose endless love, grace and mercy have been a source of inspiration, strength, wisdom and guidance through this academic journey and for the completion of this work.

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ABSTRACT

Airborne bacteria from refuse dumpsites represent a significant environmental and public health concern, as these microorganisms can disperse widely, contaminate the surrounding air, and serve as potential reservoirs of pathogenic and antibiotic-resistant species. This study investigated the isolation and identification of airborne bacterial isolates from selected refuse dumpsites within the University of Benin, Ugbowo Campus, Benin City, Nigeria. A cross-sectional study design was employed, and air samples were collected from three major dumpsites—Hall 3, Hall 4, and the Faculty of Life Sciences, using the settle plate method. Results showed that the highest bacterial load recorded at the Hall 3 dumpsite in Week 2 ($7.6 \pm 0.42 \times 10^3$ cfu/m³) and the lowest at the Faculty of Life Sciences in Week 1 ($3.9 \pm 0.22 \times 10^3$ cfu/m³). Six bacterial species were identified: *Staphylococcus aureus*, *Bacillus* spp. *Escherichia coli*, *Klebsiella* spp. *Pseudomonas* spp. and *Proteus* sp. Among these, *Staphylococcus aureus* had the highest frequency of occurrence (26.5%), while *Pseudomonas* spp. and *Proteus* sp. were the least frequent (10.3% each). The antibiotic susceptibility tests revealed that *S. aureus* and *Proteus sp.* exhibited the highest susceptibility (77.8%) and the lowest resistance (22.2%), whereas *E. coli* displayed the lowest susceptibility (33.3%) to tested antibiotics. Ofloxacin, ciprofloxacin, and gentamicin were the most effective antibiotics, while ampicillin and ampiclox showed the least efficacy.The Multiple Antibiotic Resistance (MAR) indices ranged from 0.44 to 0.89, with *Klebsiella spp.* exhibiting the highest MAR index (0.89), suggesting exposure to environments with high antibiotic selective pressure. The study underscores the need for improved waste management practices, regular sanitation, and environmental monitoring to minimize microbial air pollution and prevent the spread of resistant bacteria.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Airborne microorganisms form a significant component of the atmospheric environment and include bacteria, fungi, spores and viruses that are suspended in the air (Chen *et al*., 2020). They originate from natural sources such as soil, water, plants and animals, as well as from anthropogenic activities like agriculture, industrial processes, waste disposal (Bowers *et al*., 2013). Among these sources, refuse dumpsites are recognized as major contributors to airborne bacterial loads because they serve as reservoirs of organic matter and moisture that favor microbial growth and proliferation. Through the processes of waste decomposition, volatilization and disturbance by wind or human activity, these microorganisms are released into the surrounding air as bioaerosols (Bamotra *et al*., 2025).

In many parts of the world, particularly in developing countries, waste disposal is poorly managed. In Nigeria, open dumping remains the most prevalent method of municipal solid waste disposal due to inadequate infrastructure for sanitary landfills, incineration and recycling (Nnaji, 2015; Aluko *et al*., 2022). These open dumpsites are often located near residential areas, markets, schools and even within academic institutions, where they pose significant health and environmental risks. The University of Benin, a large institution with thousands of students and staff, generates substantial quantities of waste daily from hostels, cafeterias, classrooms, offices, and laboratories. Most of this waste is disposed of in open dumpsites within or close to the

campus. These dumpsites provide an ideal environment for the multiplication and dispersal of bacteria into the atmosphere (Ekhaise and Oni, 2012).

Several studies have reported that airborne bacteria commonly isolated from dumpsites include *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus* species, some of which are known pathogens (Oviasogie and Agbonlahor, 2013; Omusi *et al*., 2017; Obumneme *et al*., 2019; Okwuanaso *et al*., 2023). These bacteria are capable of causing respiratory tract infections, gastrointestinal diseases and skin disorders when inhaled or deposited onto food and water sources consumed by humans. Additionally, many of these bacterial isolates have been shown to develop resistance to multiple antibiotics, thereby complicating treatment options and contributing to the global threat of antimicrobial resistance (Oviasogie and Agbonlahor, 2013).

In the University of Benin, the presence of refuse dumpsites within or near student residential areas, lecture halls and food vendor locations increases the risk of exposure to airborne bacterial pathogens. Constant human activity and vehicular movement, combined with wind action, enhance the dispersion of bioaerosols beyond the immediate vicinity of the dumpsites. This raises concern for the health of students, staff, and visitors who may inhale contaminated air or come into contact with surfaces onto which these bacterial aerosols settle.

Globally, the World Health Organization (WHO, 2018) recognizes improper waste management and bioaerosol exposure as serious environmental health concerns. Yet, despite the clear risks, few studies have focused on the aeromicrobiology of dumpsites within Nigerian universities. Most research has concentrated on the bacteriological analysis of soil, leachates and water

sources contaminated by dumpsites, with limited attention to airborne bacteria, which represent one of the most direct pathways of exposure.

Therefore, there is a pressing need to investigate the bacterial composition of air around refuse dumpsites within the University of Benin. Identifying and characterizing these airborne bacterial isolates will provide essential data for assessing health risks, strengthening waste management policies on campus and protecting the university community from preventable infectious diseases.

1.2 Statement of the Problem

The problem of solid waste management remains a critical environmental and health challenge in Nigeria. Open refuse dumping, the most common waste disposal method, creates a fertile environment for microbial proliferation and facilitates the release of bacteria into the atmosphere (Siddiqua *et al*., 2022). Within the University of Benin, the daily generation of waste from hostels, lecture halls, offices and cafeterias has resulted in open dumpsites situated close to residential and academic areas. These dumpsites serve as constant sources of bacterial aerosols, which are dispersed by wind, vehicular activity, and human movement into the surrounding environment.

Exposure to airborne bacterial pathogens from dumpsites poses serious health threats to the university community. Students, staff, and visitors are at risk of inhaling contaminated air, leading to respiratory tract infections, allergic reactions, and other opportunistic diseases. The situation is worsened by the fact that some bacteria commonly isolated from dumpsites such as *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* are not only pathogenic but

also increasingly resistant to multiple antibiotics (Oviasogie and Agbonlahor, 2013). This presents a double threat: the risk of infection and the difficulty of treatment.

Despite these potential health hazards, little attention has been given to studying airborne bacteria in Nigerian universities, particularly within the University of Benin. Most available studies on dumpsites focus on soil and leachates, leaving a gap in knowledge regarding the microbial quality of air in such environments. Without such information, effective risk assessment and preventive measures cannot be adequately developed.

This lack of data represents a serious oversight, given the high population density of the University of Benin community and the daily exposure of students and staff to waste-associated aerosols. It is, therefore, necessary to isolate and identify the airborne bacterial species present in refuse dumpsites within the university to provide scientific evidence that can guide health and environmental management strategies.

1.3 Aim and Objectives

The aim of this study was to isolate and identify airborne bacterial isolates from selected refuse dumpsites within the University of Benin.

The specific objectives of this study were to:

1. Determine the bacterial load (total viable count) of airborne bacterial isolates from selected refuse dumpsites within the University of Benin.
2. Isolate and identify the bacterial species present in airborne samples collected from the dumpsites using standard bacteriological techniques.

3. Determine the antibiotic susceptibility patterns of the bacterial isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Airborne Bacteria

Airborne bacteria, also referred to as bioaerosols, are microscopic bacterial cells or spores suspended in the air, capable of being transported over varying distances by air currents (Smets *et al*., 2016) . These microorganisms, typically ranging in size from 0.5 to 5 micrometers, can exist as single cells, aggregates, or attached to particulate matter such as dust or organic debris (Lighthart, 1997). Their ability to remain viable in the atmosphere depends on environmental factors such as humidity, temperature, ultraviolet (UV) radiation, and the presence of nutrients (Després *et al*., 2012).

Airborne bacteria exhibit diverse morphological and physiological characteristics, including Gram-positive and Gram-negative species, with some forming resilient spores that enhance their survival in harsh atmospheric conditions (Griffin, 2007). Common genera include *Bacillus*, *Staphylococcus*, *Pseudomonas* and *Actinomyces*, each with unique adaptations that enable persistence in the air. For instance, *Bacillus* species form endospores that resist desiccation and UV exposure, while *Pseudomonas* species are metabolically versatile, thriving in varied environmental conditions (Madigan *et al*., 2015). The physical properties of these bacteria, such as cell wall composition and hydrophobicity, influence their aerosolization potential and deposition patterns in the environment (Jones and Harrison, 2004).

Airborne bacteria can exist in both viable and non-viable states. Viable bacteria retain the ability to grow and reproduce under favorable conditions, while non-viable bacteria may still pose

health risks due to the presence of endotoxins or allergens (Douwes *et al*., 2003). Their concentration in the air is typically measured in colony-forming units per cubic meter (cfu/m^3), with levels varying significantly based on location, human activity, and environmental conditions (Shaffer and Lighthart, 1997).

The study of airborne bacteria is critical for both public health and environmental science due to their implications for human health, ecosystem dynamics, and environmental quality. From a public health perspective, airborne bacteria are associated with a range of respiratory and infectious diseases. Pathogenic species, such as *Mycobacterium tuberculosis*, *Legionella pneumophila* and *Staphylococcus aureus*, can be transmitted through the air, posing risks to vulnerable populations (Peccia and Hernandez, 2006). Inhalation of bioaerosols containing endotoxins, produced by Gram-negative bacteria, can trigger inflammatory responses, leading to conditions like asthma or chronic obstructive pulmonary disease (COPD) (Thorne *et al*., 2005). Additionally, antibiotic-resistant bacteria, increasingly prevalent in environments like dumpsites, pose a growing threat to global health security (Li *et al*., 2018).

In environmental science, airborne bacteria play a significant role in atmospheric processes and ecosystem interactions. They contribute to the formation of bioaerosols that act as cloud condensation nuclei, influencing precipitation patterns and climate dynamics (Möhler *et al*., 2007). Bacteria also participate in nutrient cycling by depositing onto terrestrial and aquatic ecosystems, affecting soil and water microbial communities (Burrows *et al*., 2009). In the context of refuse dumpsites, the release of airborne bacteria can alter local microbial ecosystems and contribute to air pollution, with potential downstream effects on nearby agricultural or residential areas (Adeleke and Olaitan, 2016).

Studying airborne bacteria also informs waste management practices and environmental policy. Understanding the types and concentrations of bacteria emitted from dumpsites can guide strategies to mitigate their spread, such as improved waste covering or controlled composting (Pankhurst *et al*., 2011). Furthermore, monitoring airborne bacteria provides insights into microbial ecology and the resilience of bacterial communities in extreme environments, contributing to broader microbiological research (Després *et al*., 2012).

2.2. Sources of Airborne Bacteria in the Environment

Airborne bacteria originate from a wide range of natural and anthropogenic sources. Natural sources include soil, water bodies, vegetation, and animal-associated microbiomes. Soil, for instance, is a significant reservoir, with disturbance from wind or human activity releasing bacteria such as *Bacillus* and *Actinomyces* into the air (Bowers *et al*., 2011). Aquatic environments, including oceans and freshwater systems, contribute to bioaerosol formation through wave action and bubble bursting, which aerosolize bacteria like *Vibrio* and *Pseudomonas* (Aller *et al*., 2005). Vegetation, particularly plant surfaces, harbors diverse bacterial communities that can become airborne during processes like transpiration or mechanical disruption (Lindow and Brandl, 2003).

Anthropogenic sources significantly amplify airborne bacterial concentrations, particularly in urban and industrial settings. Refuse dumpsites, wastewater treatment plants, agricultural activities, and composting facilities are notable contributors (Pankhurst *et al*., 2011). Dumpsites, the focus of this study, release bacteria due to the decomposition of organic waste, which provides a nutrient-rich environment for microbial growth. Physical disturbance from waste handling or wind can aerosolize these bacteria, increasing their atmospheric presence (Adeleke

and Olaitan, 2016). Indoor environments, such as hospitals, schools, and homes, also serve as sources, with human activities like coughing, sneezing, or shedding skin cells releasing bacteria such as *Staphylococcus* and *Streptococcus* (Qian *et al*., 2012).

The diversity and concentration of airborne bacteria from these sources are influenced by environmental factors. For example, high humidity enhances bacterial survival, while arid conditions may reduce viability but increase spore dispersal (Tong and Lighthart, 2000). Seasonal variations also play a role, with higher bacterial loads often observed during warmer, wetter months due to increased microbial activity (Bowers *et al*., 2013).

2.3 Refuse Dumpsites as Sources of Airborne Bacteria

Refuse dumpsites whether formally engineered landfills, open municipal dumps, or informal community piles are dynamic biogeochemical microenvironments that act as continual, spatially heterogeneous sources of microbial life into the surrounding atmosphere. Because they concentrate organic substrates, moisture, and heat while remaining exposed to wind, traffic and human activity, dumpsites produce complex **bioaerosols**: airborne particles that carry bacteria (viable or non-viable), bacterial fragments, endotoxins, and associated particulate matter. These emissions are of direct relevance to occupational health (waste workers and scavengers), to communities living nearby, and to the broader environment through downwind transport and deposition (Nair, 2021)

2.3.1 Composition and characteristics of refuse dumpsites

Refuse dumpsites are heterogeneous mixtures of organic and inorganic wastes. Typical components include putrescible food waste, yard and garden refuse, paper and cardboard,

textiles, plastics, construction debris, sewage-contaminated material, animal remains and—at many uncontrolled sites—medical and industrial wastes. The physical arrangement is usually uneven: fresh waste layers interleave with older, decomposed strata, with pockets that are aerobic at the surface and largely anaerobic deeper in the mass. This vertical and horizontal heterogeneity creates distinct microhabitats (thermophilic cores, moist anaerobic zones, drying crusts) each supporting different microbial assemblages and metabolic processes (aerobic decomposition, anaerobic fermentation, methanogenesis) (Bassey *et al*., 2021; Nair, 2021).

Microbial communities in dumpsites are therefore diverse and dynamic. On exposed surfaces and in leachate-wet zones, bacterial genera associated with the gastrointestinal tracts of humans and animals (e.g., *Escherichia, Klebsiella, Enterococcus*), environmental Gram-positives (e.g., *Bacillus, Staphylococcus*), and numerous opportunistic and soil-associated taxa have been repeatedly identified in both bulk waste and air samples collected around landfill/dump sites (Cyprowski *et al*., 2019). The combination of high nutrient availability, alternating oxic/anoxic conditions and physical disturbance selects for organisms able to grow rapidly, form biofilms, sporulate, or otherwise persist in changing conditions — characteristics that influence which organisms become airborne (Ghanbarian *et al*., 2020).

2.4. Factors contributing to bacterial proliferation in dumpsites

Multiple, often interacting environmental and anthropogenic factors determine bacterial growth, survival, and therefore the potential pool of organisms available for aerosolization:

Organic substrate quality and quantity. High loads of degradable carbon (food scraps, vegetable waste, animal tissue) and nitrogenous material provide abundant energy and nutrient

sources that favor rapid bacterial proliferation and high biomass (Palaniveloo *et al*., 2020). Decomposition releases soluble organic matter that supports both planktonic bacteria and biofilm formation on waste surfaces — a key precursor to aerosol release (Rastogi *et al*., 2020)

2.4.1. Moisture content.

Moisture is a critical driver. Moderately wet conditions enhance microbial metabolism and biomass accumulation; very dry conditions limit growth but increase dust and particulate resuspension (potentially mobilizing desiccated cells) (Liang *et al*., 2003) . During wetting events (rainfall, leachate movement), bacteria can be mobilized into liquids and later aerosolized by splash, bubble bursting, or evaporation. Several composting and landfill studies have identified moisture as a dominant control on microbial activity and on emissions (Rastogi *et al*., 2020)

2.4.2. Temperature regimes

Temperature greatly affects both the rate of organic matter breakdown and the composition of the microbial community (Lin *et al* 2022). Surface layers subject to solar heating or active biological decomposition can reach mesophilic to thermophilic temperatures, selecting for thermotolerant taxa; meanwhile cooler deeper layers support anaerobes. Temperature also influences bioaerosol survival after release: elevated temperatures combined with UV exposure can inactivate many bacteria unless they are shielded within organic particles (Fernandez *et al*., 2019)

2.4.3. Oxygen availability and redox microenvironments

The presence or absence of oxygen shapes community composition (aerobes vs anaerobes) and metabolic by-products (e.g., ammonia, hydrogen sulfide) that can indirectly affect survival and aerosol formation. Aerobic decomposition at the waste surface tends to produce more particulate material and spores/vegetative cells that are readily entrained (Nair, 2021).

2.4.4. Vectors and biological interactions

Flies, rodents, scavenger birds and humans act as mechanical vectors that both concentrate microbes and promote dispersal. Insects visiting waste for feeding or egg-laying can pick up high bacterial loads and shed these organisms into the air or onto surrounding surfaces; similarly, scavengers' movement and feeding increase mechanical disturbance and resuspension (Ghanbarian *et al*., 2020).

2.4.5. Anthropogenic disturbance and operational factors

Vehicle traffic, bulldozing, open burning, compaction, sorting and manual handling all generate mechanical energy that resuspends dust and particulate-associated bacteria. Seasonal patterns of waste generation, episodic burning of waste piles, and the practice of open dumping versus controlled covering further modulate emission rates. Studies at operational waste facilities show clear increases in airborne bacterial counts during active disturbance (Wikuats *et al*., 2020; Zhang *et al*., 2023).

2.5 Common Airborne Bacterial Isolates from Refuse Dumpsites

Refuse dumpsites act as ecological niches where diverse bacterial populations thrive, proliferate, and are eventually aerosolized into the surrounding atmosphere. These sites contain decomposing organic matter, sewage residues, animal remains, medical wastes, and other biodegradable materials that provide a nutrient-rich environment for microbial growth. Airborne transmission from these sites occurs through wind action, scavenger activities, vehicular movement, and microbial metabolic processes, leading to the dissemination of both environmental and pathogenic bacteria (Nwankwo *et al*., 2021; Adeyemi and Onilude, 2022). The bacteria frequently isolated from refuse dumpsites are heterogeneous and include both spore-forming genera and non-spore-forming opportunistic pathogens. Notably, *Bacillus, Staphylococcus, Pseudomonas, Klebsiella, Escherichia, Micrococcus, Enterobacter, Proteus,* and *Acinetobacter* are among the most commonly reported airborne isolates from such environments (Odonkor and Mahami, 2020; Igbinosa and Aighewi, 2017).

2.5.1. *Bacillus* spp.

Members of the genus *Bacillus* are aerobic or facultatively anaerobic, Gram-positive, rod-shaped, spore-forming bacteria that are ubiquitous in nature. Their ability to produce heat- and desiccation-resistant endospores gives them a distinct ecological advantage in harsh environments such as refuse dumpsites, where fluctuations in temperature, humidity, and nutrient availability are common (Logan and De Vos, 2015). The resilience of *Bacillus* spores allows them to survive for extended periods in aerosols, making them one of the most frequently isolated bacterial genera in airborne samples from waste environments.

Several species within this genus have clinical and environmental relevance. *Bacillus cereus* is of particular concern due to its pathogenic potential. It is widely recognized as a cause of foodborne illnesses, particularly emetic and diarrheal syndromes resulting from the production of toxins such as cereulide and enterotoxins (Ehling-Schulz *et al*., 2019). In addition to gastrointestinal disease, *B. cereus* can cause opportunistic systemic infections, including bacteremia, endocarditis, meningitis, and ocular infections, particularly following traumatic injury to the eye (Bottone, 2010). The detection of *B. cereus* in dumpsite aerosols raises concerns not only about inhalation risks but also about the potential contamination of food products exposed to such environments.

Other members of the genus, such as *Bacillus anthracis*, though less frequently isolated from dumpsites, represent a potential hazard due to their pathogenicity. *B. anthracis*, the causative agent of anthrax, forms durable spores capable of long-term survival in soil and aerosols. While environmental isolation from refuse dumpsites is not routinely reported, the possibility of contamination via discarded animal products or waste material remains a public health concern (Turnbull, 2008).

Non-pathogenic species like *Bacillus subtilis* and *Bacillus licheniformis* are often considered harmless and even beneficial in biotechnology and agriculture; however, under certain conditions, they can act as opportunists in immunocompromised individuals (Hong *et al*., 2005). Their detection in dumpsite aerosols is significant because their high spore counts may act as indicators of environmental microbial load and potential allergenic exposure.

In terms of antimicrobial resistance**,** *Bacillus* species from environmental sources have demonstrated varying levels of resistance to antibiotics such as β-lactams, tetracycline, and

erythromycin (Agata *et al*., 2002). The waste environment may facilitate the acquisition and dissemination of resistance genes via horizontal gene transfer, particularly when antibiotics are improperly disposed into dumpsites. Consequently, the persistence of *Bacillus* spores in airborne particles poses dual threats: (i) allergenic or infectious risk through inhalation, and (ii) contribution to the environmental resistome that fuels the antimicrobial resistance crisis.

Overall, the prevalence of *Bacillus spp.* in dumpsite aerosols underscores their ecological resilience and clinical importance. Their ability to survive, persist, and disperse through the air highlights the significance of dumpsites as sources of airborne microbial contamination with both direct and indirect impacts on human health.

2.5.2. *Staphylococcus* spp.

Staphylococcus species, particularly *Staphylococcus aureus*, are frequently detected in aerosols from dumpsites due to their association with human and animal waste, food residues, and discarded medical materials. *S. aureus* is a Gram-positive, non-spore-forming coccus that occurs in clusters and has remarkable environmental persistence. Its ability to withstand desiccation and survive for long periods on surfaces enables its persistence in the dumpsite environment and subsequent aerosolization (Tong *et al*., 2015).

Clinically, *S. aureus* is one of the most important opportunistic pathogens, responsible for a wide range of infections including skin abscesses, cellulitis, pneumonia, endocarditis, osteomyelitis, and septicemia. Its pathogenicity is mediated by a variety of virulence factors, such as surface proteins that promote adherence, toxins like α-hemolysin, and superantigens that trigger exaggerated immune responses (Lowy, 1998). Inhalation of *S. aureus*-laden aerosols from

dumpsites could predispose individuals, especially those with weakened immunity, to respiratory and systemic infections.

Of particular concern is the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in environmental samples, including waste sites (Nwankwo *et al*., 2021). MRSA strains possess the mecA gene, which confers resistance to β-lactam antibiotics, making infections difficult to treat. Additional resistance to macrolides, tetracyclines, and aminoglycosides has been documented, raising alarms about the potential dissemination of multidrug-resistant strains from waste dumps into surrounding communities (Otter and French, 2010).

Thus, dumpsites represent important reservoirs for *S. aureus* and MRSA, with implications for public health given the proximity of many waste dumps to residential areas in developing countries.

2.5.3. *Pseudomonas* spp.

The genus *Pseudomonas*, particularly *Pseudomonas aeruginosa*, is a prominent group of Gram-negative, rod-shaped bacteria frequently isolated from refuse dumpsite aerosols. Their metabolic versatility allows them to thrive in moist, nutrient-rich environments typical of waste decomposition. *P. aeruginosa* is well-adapted to survive in hostile environments due to its ability to produce pigments (pyocyanin, pyoverdine), form biofilms, and utilize diverse organic compounds (Lister *et al*., 2009). Clinically, *P. aeruginosa* is an opportunistic pathogen of major concern. It causes severe infections in immunocompromised individuals, including pneumonia, urinary tract infections, wound infections, and septicemia. Inhalation of *Pseudomonas*-contaminated aerosols from dumpsites may contribute to respiratory tract colonization,

particularly in children, elderly persons, and individuals with underlying conditions such as cystic fibrosis (Gellatly and Hancock, 2013).

P. aeruginosa also demonstrates intrinsic resistance to multiple antibiotics due to efflux pumps, low membrane permeability, and the production of β-lactamases. Environmental isolates often show resistance to cephalosporins, aminoglycosides, and carbapenems, making treatment options extremely limited (Lister *et al*., 2009). The detection of multidrug-resistant *Pseudomonas* in dumpsite aerosols underscores the risk of environmental transmission of hard-to-treat infections.

2.5.4. *Klebsiella* spp.

Klebsiella pneumoniae is a Gram-negative, encapsulated bacterium that is frequently detected in dumpsite aerosols, largely due to fecal contamination from human and animal waste. The thick polysaccharide capsule of *Klebsiella* enhances environmental survival and increases virulence by protecting the bacterium from phagocytosis and complement-mediated killing (Podschun and Ullmann, 1998). This organism is clinically important as a causative agent of community-acquired and hospital-acquired pneumonia, urinary tract infections, wound infections, and septicemia. Airborne exposure to *K. pneumoniae* from dumpsites is of particular concern for nearby populations, especially those living in crowded or unhygienic environments.

Resistance among *Klebsiella* isolates is a growing challenge. Extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae* strains are frequently isolated from waste environments (Igbinosa and Aighewi, 2017). Even more alarming is the emergence of carbapenem-resistant *Klebsiella pneumoniae* (CRKP), which is listed by the World Health Organization as a critical-

priority pathogen due to its limited treatment options (WHO, 2021). Dumpsites, therefore, act as environmental reservoirs and potential dissemination points of resistant *Klebsiella* strains.

2.5.5. *Escherichia coli*

Escherichia coli is a facultatively anaerobic, Gram-negative bacterium that serves as a fecal indicator organism and is commonly isolated from dumpsite aerosols. While many strains of *E. coli* are harmless commensals, pathogenic strains such as enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), and enteropathogenic *E. coli* (EPEC) are responsible for severe diarrheal diseases, hemolytic uremic syndrome, and extraintestinal infections (Croxen *et al*., 2013).

The presence of aerosolized *E. coli* in dumpsites is a major public health concern since these bacteria may be inhaled or deposited onto food and water sources in nearby communities. Outbreaks of diarrheal illness linked to poor waste management have been reported in several regions of Africa (Igbinosa and Aighewi, 2017). Resistance among *E. coli* strains is widespread, particularly against fluoroquinolones, sulfonamides, and β-lactams. ESBL-producing *E. coli* are increasingly reported from environmental and clinical settings, and their presence in airborne particles suggests the possibility of airborne dissemination of resistant enteric pathogens (Fair and Tor, 2014).

2.5.6. *Micrococcus* spp.

Micrococcus species, such as *Micrococcus luteus*, are Gram-positive, spherical bacteria often considered harmless environmental saprophytes. They are widely distributed in soil, dust, and human skin, explaining their frequent detection in dumpsite aerosols (Kandi *et al*., 2016).

Although traditionally regarded as non-pathogenic, *Micrococcus* can cause opportunistic infections in immunocompromised hosts, including respiratory tract colonization, septicemia, and skin infections. Their ability to withstand environmental stress such as desiccation and UV radiation contributes to their persistence in dumpsite aerosols. While less virulent compared to other genera, their presence in large numbers may indicate microbial pollution and contribute to allergenic responses in sensitive individuals.

2.5.7. *Enterobacter* spp.

Enterobacter cloacae and related species are opportunistic pathogens increasingly associated with environmental and clinical infections. They are commonly isolated from dumpsite aerosols due to their association with decaying organic matter and fecal waste. *Enterobacter* species are implicated in urinary tract infections, lower respiratory tract infections, and bloodstream infections, particularly in hospitalized patients (Mezzatesta *et al*., 2012).

Environmental isolates of *Enterobacter* often display multidrug resistance, including resistance to cephalosporins, fluoroquinolones, and aminoglycosides. Their survival in dumpsite aerosols not only facilitates dissemination but also enhances the risk of horizontal gene transfer of resistance determinants to other bacteria in the environment.

2.5.8. *Proteus* spp.

Proteus mirabilis and *Proteus vulgaris* are Gram-negative enteric bacteria that thrive in decaying organic matter and are frequently detected in dumpsite aerosols. These bacteria are well-known for their swarming motility, which aids in colonization of diverse habitats including waste environments (O'Hara *et al*., 2000).

Clinically, *Proteus* species are opportunistic pathogens responsible for urinary tract infections, wound infections, and septicemia. They also produce urease, which can lead to kidney stone formation in urinary tract infections. Isolates from waste environments have demonstrated resistance to ampicillin, tetracycline, and sulfonamides, highlighting their contribution to the environmental resistome.

2.5.9. *Acinetobacter* spp.

Acinetobacter baumannii is one of the most notorious pathogens found in dumpsite aerosols. These Gram-negative, non-fermentative coccobacilli are capable of surviving on dry surfaces for extended periods, making them well-suited for persistence in waste environments (Peleg *et al*., 2008). *A. baumannii* is strongly associated with hospital-acquired infections, including ventilator-associated pneumonia, bloodstream infections, and wound infections. Its detection in airborne particles from dumpsites is concerning because of its extensive antimicrobial resistance profile, including resistance to carbapenems, aminoglycosides, and even colistin in some strains. These multidrug-resistant isolates are sometimes referred to as “superbugs” due to the paucity of treatment options.

The dumpsite environment, enriched with discarded pharmaceuticals, facilitates the selection and spread of resistant *Acinetobacter* strains, which may be disseminated through aerosols to surrounding communities.

2.6. Mechanisms of Bacterial Aerosolization from Dumpsites

Aerosolization refers to the process by which bacteria or bacteria-laden particles detach from a surface or liquid medium and become suspended in the air. Within dumpsites, this process occurs

through several overlapping physical, chemical, and biological mechanisms, each contributing to the release and dispersal of microbial particles into the surrounding atmosphere.

One of the most common pathways is the resuspension of dust and dry particles**.** As surface waste dries out, organic matter, biofilm residues, soil particles, and dust become easily entrained into the air by environmental forces such as wind shear, vehicular traffic, or human activity. These airborne particles vary in size and influence microbial transport. Larger particles, typically greater than 2.5 μm in aerodynamic diameter, settle quickly and contaminate nearby surfaces, while finer particles, less than 2.5 μm, can remain suspended for longer periods and be transported over greater distances (Fernandez *et al*., 2019; Wikuats *et al*., 2020).

Another important mechanism is mechanical agitation and handling of waste**.** The activities of bulldozers, compactors, trucks, and human scavengers generate localized turbulence that dislodges bacteria from surfaces and biofilms. These actions create dense plumes containing both particulate matter and microbial cells, a phenomenon frequently documented in occupational exposure studies of landfill workers and recycling facility operators (Ghanbarian *et al*., 2020; Zhang *et al*., 2023).

In addition, splashing and bubble-bursting from leachate or water-logged waste surfaces contribute to aerosol formation. Rainfall striking dumpsite surfaces, the splashing of contaminated pools, and the bursting of microbial-laden bubbles all generate fine droplets. These droplets may either remain airborne or evaporate rapidly to form droplet nuclei that can carry viable bacteria, a process well documented in wastewater aerosol studies (Alsved *et al*., 2020; Liu *et al*., 2023).

Thermal and convective forces also play a role. Heat generated by microbial decomposition, chemical oxidation, or solar radiation produces rising air currents capable of lifting fine particles and microbes from waste surfaces. These convective plumes aid the vertical transport of bioaerosols, potentially facilitating their long-distance dispersal under suitable meteorological conditions (Nair, 2021).

A further mechanism is the fragmentation of biofilms and microbial cells. Biofilms adhering to organic substrates may break apart due to desiccation, enzymatic activity, or mechanical disruption. Such fragments, often containing viable clusters of bacterial cells or spores, are readily aerosolized. Spore-forming organisms such as Bacillus species are particularly resilient, as their desiccation resistance enhances survival following aerosolization and subsequent atmospheric stress (Fernandez *et al*., 2019; Shen *et al*., 2025).

Finally, bacteria may become airborne through attachment to other particulate matter. Microbes frequently associate with soil particles, soot, or organic detritus, which provides physical shielding against environmental stressors such as desiccation and ultraviolet radiation. These associations not only improve bacterial survival but also alter the aerodynamic properties of the particles, enabling longer suspension times and wider dispersal. Studies consistently show that viable bacteria in dumpsite and waste facility environments are more often associated with composite particles rather than as free, isolated cells (Wikuats *et al*., 2020; Shen *et al*., 2025).

2.7 Environmental and Health Impacts of Airborne Bacteria

Airborne bacteria represent an important environmental and public health concern due to their potential to disseminate widely through aerosols and dust particles. These microorganisms, once

suspended in the atmosphere, can remain viable over extended periods and be transported across long distances, thereby facilitating both direct and indirect impacts on human health, ecosystems, and environmental quality (Franzetti *et al*., 2011; Nazaroff, 2014). The environmental and health impacts of airborne bacteria are particularly pronounced in urban centers with poorly managed refuse dumpsites, where microbial load in the air tends to be significantly elevated (Adhikari *et al*., 2004; Osagbemi *et al*., 2021).

2.7.1. Role of Airborne Bacteria in Disease Transmission

Airborne bacteria play a crucial role in the transmission of infectious diseases, especially those caused by pathogenic and opportunistic species. Aerosolized bacteria can be inhaled directly into the respiratory tract, leading to infections such as pneumonia, tuberculosis, and staphylococcal infections (Eduard, 2009; Tang *et al*., 2011). Some bacterial species, including *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*, have been frequently reported in aerosols and are associated with respiratory tract infections, septicemia, and other opportunistic conditions (Kembel *et al*., 2012; Fang *et al*., 2016). Vulnerable populations such as children, the elderly, and immunocompromised individuals are at greater risk of infection. Moreover, antibiotic-resistant strains have also been detected in airborne environments, raising serious concerns about the spread of antimicrobial resistance through inhalation routes (Li *et al*., 2018).

2.7.2. Impact of Dumpsite-Derived Bacteria on Nearby Communities

Refuse dumpsites act as reservoirs and amplifiers of bacterial communities, including pathogenic strains, which can be aerosolized by wind, vehicular movement, and waste scavenging activities

(Aluko *et al*., 2018; Adeyemi *et al*., 2020). Communities living in proximity to dumpsites are frequently exposed to elevated levels of bioaerosols, increasing their risk of respiratory infections, skin irritations, and allergic responses (Ohimain, 2016). Studies in Nigeria and other parts of sub-Saharan Africa have shown that dumpsite-derived airborne bacteria contribute significantly to the burden of respiratory diseases such as bronchitis, asthma, and chronic obstructive pulmonary disease (COPD) among nearby residents (Bello *et al*., 2020; Akinbile *et al*., 2021). The presence of enteric bacteria like *E. coli* and *Enterococcus* in aerosols around dumpsites further indicates the possible transmission of fecal-oral pathogens through inhalation or deposition on food surfaces (Nwankwoala and Ememu, 2018). Dumpsite workers, scavengers, and waste pickers are particularly at high risk due to prolonged and repeated exposure to bacterial aerosols.

2.7.3. Environmental Implications: Contribution to Air Pollution and Ecosystem Disturbance

Beyond human health, airborne bacteria derived from dumpsites also contribute to broader environmental degradation. High microbial loads in the air contribute to bioaerosol pollution, which is increasingly being recognized as a critical component of air quality assessment (Després *et al*., 2012). Airborne bacteria can interact with dust, particulate matter, and chemical pollutants, thereby intensifying air pollution and its negative effects on climate and visibility (Smets *et al*., 2016). Additionally, bacteria that are deposited onto soil and water bodies from the atmosphere may disrupt natural microbial ecosystems, alter nutrient cycling, and introduce antibiotic-resistant genes into environmental reservoirs (Zhu *et al*., 2017). This ecological disturbance may reduce biodiversity and compromise ecosystem services such as soil fertility

and water quality. Furthermore, airborne bacteria can act as cloud condensation and ice-nucleating particles, influencing atmospheric processes and potentially contributing to climate variability (Morris *et al*., 2014).

2.8. Mitigation Strategies for Airborne Bacteria from Dumpsites

The environmental and health risks posed by airborne bacteria in and around dumpsites necessitate the adoption of effective mitigation strategies. Such measures must be multidimensional, combining engineering controls, public health interventions, regulatory frameworks, and community-based approaches**.** Proper implementation not only reduces bacterial load in the atmosphere but also improves environmental quality and safeguards vulnerable populations.

2.8.1 Improved Waste Management Practices

The most effective long-term strategy for mitigating airborne bacteria is the adoption of sustainable solid waste management practices. Open dumping and uncontrolled burning of refuse—common practices in many Nigerian cities—should be replaced with **sanitary landfills, controlled composting, and waste-to-energy conversion systems** (Wilson *et al*., 2012). Properly engineered landfills with daily soil cover minimize exposure of waste to wind and reduce the release of bioaerosols. Segregation of waste at source into biodegradable and non-biodegradable fractions also limits bacterial proliferation in organic-rich refuse (Guerrero *et al*., 2013).

2.8.3 Air Quality Monitoring and Surveillance

Routine monitoring of bioaerosols around dumpsites is critical for assessing exposure risks. Air sampling and microbial analysis should be incorporated into municipal air quality programs to identify pathogenic species and antibiotic-resistant bacteria (Franzetti *et al*., 2011). Data from such monitoring can guide public health interventions, including issuing health advisories to at-risk communities and regulating waste management practices.

Mitigating health risks requires both preventive and protective strategies. Residents and waste handlers should be educated on the health implications of airborne bacteria and encouraged to adopt protective measures, such as wearing face masks, gloves, and protective clothing during waste handling. Regular medical check-ups and vaccination campaigns, especially against respiratory pathogens, are also recommended for scavengers and workers frequently exposed to dumpsites (Adeyemi *et al*., 2020). Community health awareness campaigns can reduce risky behaviors such as children playing near refuse dumps.

2.8.5 Regulatory and Policy Measures

Governmental policies play a pivotal role in ensuring the sustainability of mitigation measures. Enforcement of environmental sanitation laws, restriction of residential building near dumpsites, and implementation of Nigeria's National Environmental (Sanitation and Waste Control) Regulations are essential (NESREA, 2009). Incentivizing recycling and private-sector participation in waste management can also reduce the volume of waste deposited in open dumpsites. Modern technologies such as biofilters, biocovers, and microbial-based deodorization systems can reduce microbial aerosols from dumpsites (Han *et al*., 2016).

Additionally, the use of geotextile covers, anaerobic digestion, and incineration with emission controls offers advanced approaches for minimizing microbial spread. In the long term, integrating smart waste management systems powered by artificial intelligence (AI) and sensor technologies can help in predicting microbial risks and optimizing waste disposal practices in urban centers (Gupta *et al*., 2019).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was carried out within the University of Benin, Ugbowo Campus, located in Benin City, Edo State, Nigeria. The university is situated at approximately latitude 6°24′N and longitude 5°37′E, within the humid tropical rainforest zone of Southern Nigeria. The region experiences high rainfall, high humidity, and average daily temperatures between 24°C and 34°C, which provide conditions conducive for microbial growth and dispersal.

Three refuse dumpsites within the university were selected for the study: the dumpsite close to Hall 3 Hostel, the dumpsite close to Hall 4 Hostel**,** and the dumpsite behind the Faculty of Life Sciences. These sites were chosen due to their high waste generation rates and their proximity to areas with significant human activity, including student hostels, lecture halls, and food vendors.

3.2 Study Design

The research was designed as a cross-sectional study aimed at isolating and identifying airborne bacterial isolates from refuse dumpsites. Air samples were collected from the selected dumpsites using standard microbiological air sampling techniques and analyzed in the laboratory using culture-based methods.

3.3 Sample Collection Method

Air sampling was carried out at the three designated dumpsites (Hall 3, Hall 4, and Faculty of Life Sciences) using the settle plate method as described by Mounyr *et al*. (2016). In this

method, sterile Petri dishes containing different prepared culture media (nutrient agar, MacConkey agar, mannitol salt agar, and blood agar) were exposed to the atmosphere at each dumpsite.

At each site, plates were exposed at a height of approximately 1.5 meters above ground level (representing the human breathing zone) for 15 minutes during peak human and waste disposal activity (between 9:00 am and 12:00 pm). Three replicates were collected per site to ensure reproducibility. After exposure, the plates were immediately covered, sealed with masking tape, labeled, and transported to the microbiology laboratory of the Faculty of Life Sciences for analysis.

3.5.Sterilization of Materials

Materials such as Petri-dishes, pipette, glass containers (conical flask, round bottom flask) and bottles were washed, drained and dried. They were wrapped with aluminum foil and sterilized in a hot-air oven at 160^{o}C for an hour. They were allowed to cool after sterilization before usage. An aseptic working environment was achieved with the use of Bunsen burner flame and disinfection of work surfaces with alcohol.

3.5.1 Preparation and Sterilization of media

Materials used include; Glass wares such as test tubes, beakers, conical flasks, Petri-dishes, McCartney bottles, Sterile cotton swabs, Sterile gloves, Normal saline, Sterile sampling containers, stirring glass rod and measuring cylinder. Media and Biochemical test reagents and Gram's staining kit . All glassware which include MacCartney bottles, Petri dishes, test tubes,conical flasks, measuring cylinders and pipettes, were sterilized at 160 ^{o}C for 1 hr in a hot-air-oven before use. The media used in this study were sterilized at 121 ^{o}C for 15 min in an

autoclave. Agar media, agar slant and biochemical reagents were prepared freshly and refrigerated at 3-4 ^{o}C. Aseptic conditions were ensured during inoculation and subculturing.

3.5.1.1 Preparation of Nutrient agar

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

3.5.1.2. Preparation of Citrate agar

A (24.28)grams of agar was dissolved in 1000 ml distilled water and heated to boiling, to dissolve the medium completely. It was then mixed properly and distributed in conical flasks. The medium was sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 mins and then left to cool before dispension on sterile petri dishes.

3.5.1.3 Preparation of Triple Sugar Iron agar

A (64.6) g of powder was dissolved in 1L of distilled water and then heated to properly dissolve the mixture. The mixture was autoclaved to sterilize the agar before it is dispensed into tubes and sterilized again at 121 °C for 15 mins. The agar was then left to solidify with short slant and good butts.

3.6 Bacterial Enumeration

Following the air sampling exercise, all exposed nutrient agar plates were transported aseptically to the microbiology laboratory for incubation. The plates were incubated at a temperature of 37°C for 24 to 48 hours to allow for optimal bacterial growth. After incubation, distinct bacterial

colonies on each plate were visually enumerated. The number of airborne bacteria was then expressed as colony-forming units per cubic meter of air (cfu/m³) using a standard formula for settle plate enumeration:

$$\text{cfu/ml} = \frac{a\ x\ 10{,}000}{\text{p x t x } 0.2}$$

Where:

a = number of colonies counted on the agar plate

p = surface area of the plate (in cm²); for a 90 mm Petri dish, p = 63.6 cm²

t = time of exposure in minutes **(**15 minutes**)**

0.2 = sedimentation constant for passive air sampling

This calculation provided a standardized estimate of bacterial load in the bathroom air of the hostels, expressed in CFU/m³.

3.6.1 Subculturing of Pure Isolates

After colony counting, well-isolated colonies with distinct morphologies were selected and subcultured onto fresh Nutrient Agar plates to obtain pure cultures. These pure cultures were then subjected to further identification tests, such as biochemical and morphological characterization.

3.7.BACTERIAL IDENTIFICATION

The bacterial isolates were characterized based on colonial morphological characteristics such as colony shape, size, elevation, optical activity, margination and pigmentation on nutrient agar and MacConkey agar. Biochemical tests were also carried out to further identify the bacterial

isolates. The fungal isolates were identified using colonial morphological characteristics such as size, texture colour and reverse colour.

3.7.1 Gram staining

Smears of the bacterial isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with distilled water. The slides were flooded with dilute Grams' iodine solution for one minute. This was washed off with distilled water and the smears were decolorized with 95% alcohol for 30 seconds and rinsed off with distilled water. The smears were then counter stained with safranin solution for one minute. Finally, the slides were washed off with distilled water, air dried and observed under oil immersion objective (Cheesbrough, 2005).

3.7.2. Potassium Hydroxide (KOH) test

Two drops of a 3% potassium hydroxide (KOH) solution were placed on a clean glass slide, after which a loopful of pure bacterial growth was emulsified in the solution by stirring in a circular motion. During mixing, the loop was occasionally lifted to observe the formation of a string in the mixture. The development of a viscous and mucoid consistency indicated a Gram-negative bacterium, whereas no reaction (absence of string formation) was interpreted as indicative of a Gram-positive bacterium (Roberts and Sandle, 2008).

3.8. BIOCHEMICAL TEST

3.8.1 Catalase Test

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

smeared on a slide. The production of gas bubble indicated catalase enzyme positive (Cheesbrough, 2005).

3.8.2 Oxidase Test

A piece of filter paper was wet with a few drops of the dilute (1%) solution of oxidase reagent (tetramethyl-pphenylenediamine-dihydrochloride) which was prepared by standard procedure. A bit of growth from the nutrient agar slant was obtained using sterilized platinum wire loop and smeared on the wet piece of paper. Development of an intense purple color by the cells within 30 seconds indicates a positive oxidase test (Cheesbrough, 2005).

3.8.3 Citrate Utilization Test

This test is based on the ability of some organisms to utilize citrate as a sole source of carbon. This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was incubated at 37°C for 24 - 48 hr. The development of deep blue colour after incubation indicates a positive result (Cheesbrough, 2005).

3.8.4 Indole Test

Indole test is performed to determine the ability of the organism to split tryptophan molecule into indole. This test is performed to help differentiate species of the family enterobacteriaceae. Kovac's reagent which contains hydrochloric acid, dimethyl-aminobenzaldehyde and amyl alcohol is used. The broth was inoculated with the test organism and incubated for 18 hours at 37°C. 5ml of Kovac's reagent was then added down the inner wall of the tube. Development of bright red colour at the interface of the reagent and the broth within seconds after adding the reagent was indicative of the presence of indole and a positive result.

3.8.5. Triple sugar iron (TSI) agar test

An agar slant prepared of a TSI agar was used in carrying out this test in a sterile test tube at a slanted angle. The slanted medium was inoculated with TSA pure culture using a straight inoculation needle by stabbing first through the center to the bottom of the tube and streaking the agar slant's surface. After inoculations, the test tubes were covered with foil paper and left at an ambient temperature of 36°C to incubate for 24 hours. Reactions on test tubes were examined, and sugar fermentations were indicated by the production of H_2S, gas and a change in colours from red (alkaline) to yellow (acid). When an alkaline/acid (red top/yellow bottom) slant reaction appeared, it only indicated dextrose (glucose) fermentation. When an acid/acid (yellow top/yellow bottom) slant reaction appeared, it showed the fermentation of dextrose, lactose and/or sucrose. The appearance of an alkaline/alkaline (red top/red bottom) slant reaction represented the absence of sugar fermentation. The blackening of the medium in the slant indicated H_2S production. Bubbles, cracks, or bottom-raised space in the slanted agar indicated gas production (formation of CO_2 and H_2) (Fawole and Oso, 2007).

3.9. Antibiotic susceptibility test

The identified colonies of bacteria were used to determine the susceptibility and resistance of bacterial isolates, which were subjected to standard antibacterial susceptibility testing (AST) to decipher their resistance or susceptibility to common antibiotics used for treatment within the locality. The standard discs were produced by Oxoid, UK, which was used to execute the disc diffusion method employed in this study. For this assay, a fully grown bacterial culture (from 18-24 hours) was cultured on MHA. The inoculum corresponding to 1.5 x 10^8 cells/ml McFarland standard was streaked using a sterile loop onto the MHA plates before the introduction of antibiotic discs and were added with extreme care to the plates with the aid of sterile forceps.

The susceptibility results were recorded after incubation for 24 hours at 37 ºC. Following the standard or rules of AST established in 2017 by CLSI (Clinical Laboratory Standards Institute). The inhibition zone around each disc (measured using a meter rule in diameter) was assessed and interpreted based on the 2020 CLSI standard as Resistant (R), Intermediate resistant (I) and Sensitive (S) (Odonkor and Addo, 2011)

3.10. Multiple Antibiotic Resistance (MAR) Index

This index is obviously a good tool which identifies the region where the isolates were obtained. Whether they are from places of high or low risks or from arears where antibiotics are abused. This tool becomes necessary for health risk assessment. According to Davis and Brown (2016), an index of ≥ 0.2 and above is indicative of a 'high-risk' contamination source. In this study the MAR index was determined by employing the methods delineated by Chitanand*et al.* (2010). The formula below was used to decipher MAR index of bacterial isolates.

$$MAR\ index = \frac{y}{nx}$$

$$where\ y = number\ of\ resistance\ scored,$$

$$n = number\ of\ isolates\ and$$

$$x = total\ number\ of\ antibioitics$$

It is a general established rule that MAR index greater than 0.2 is indicative of the fact that the bacterium originates from areas where antibiotics have been abused (or regularly used) or worse still from areas of high-risk source of contamination.

CHAPTER FOUR

RESULTS AND DISCUSSION

The mean bacterial load of air samples from selected dumpsites in UNIBEN (Hall 3, Hall 4, and Faculty of Life Sciences) across three weeks is presented in Table 4.1. In Week 1, the highest bacterial load was recorded at Hall 3 ($5.2 \pm 0.31 \times 10^3$ cfu/m³), while the lowest occurred at the Faculty of Life Sciences ($3.9 \pm 0.22 \times 10^3$ cfu/m³). Week 2 showed a general increase across all locations, with Hall 3 reaching the peak value of the study ($7.6 \pm 0.42 \times 10^3$ cfu/m³) and the Faculty of Life Sciences recording $6.1 \pm 0.35 \times 10^3$ cfu/m³. In Week 3, there was a slight decline, although Hall 3 ($6.2 \pm 0.33 \times 10^3$ cfu/m³) remained the highest, and the Faculty of Life Sciences ($4.6 \pm 0.27 \times 10^3$ cfu/m³) remained the lowest. Overall, the lowest bacterial load was observed at the Faculty of Life Sciences in Week 1 ($3.9 \pm 0.22 \times 10^3$ cfu/m³), and the highest load at Hall 3 in Week 2 ($7.6 \pm 0.42 \times 10^3$ cfu/m³).

Table 4.2 presents the cultural, morphological, and biochemical characteristics of bacterial isolates. Six distinct species were identified: *Staphylococcus aureus, Bacillus* spp., *Escherichia coli, Klebsiella* spp., *Pseudomonas* spp. and *Proteus* sp. The identification was based on colony morphology, Gram reaction, cell arrangement, and biochemical reactions.

Figure 4.3 shows the frequency of occurrence of airborne bacterial isolates across the three dumpsites. Hall 3 recorded the highest occurrence (30 isolates, 44.1%), followed by Hall 4 (26 isolates, 38.2%) and the Faculty of Life Sciences dumpsite (22 isolates, 32.4%). In terms of species, *Staphylococcus aureus* was the most frequently recovered bacterium (26.5%), while *Pseudomonas* spp. and *Proteus* sp. were the least frequent (10.3% each).

The antibiotic susceptibility profile of bacterial isolates is presented in Table 4.4. *Staphylococcus aureus* and *Proteus* sp. showed the highest susceptibility (77.8%) with the lowest resistance (22.2%). The lowest susceptibility was observed in *Escherichia coli* (33.3%). Among the antibiotics, ofloxacin (OFL), gentamicin (GEN), and ciprofloxacin (CIP) were the most effective, while ampicillin (AMP) and ampiclox (AMPX) were the least effective.

Figure 4.3 shows the Multiple Antibiotic Resistance (MAR) index of bacterial isolates. The highest MAR index was recorded in *Klebsiella* spp. (0.89), followed by *Escherichia coli* (0.78). *Pseudomonas* spp. and Proteus sp. each had MAR values of 0.67, while *Staphylococcus aureus* (0.56) and *Bacillus* spp. (0.44) showed the lowest indices

Table 4.1: Mean Bacterial Load (CFU/m³) of Air Samples from Selected Refuse Dumpsites in UNIBEN Over Three Weeks

Week	Hall 3 Dumpsite	Hall 4 Dumpsite	Faculty of Life Sciences Dumpsite
Week 1	$5.2 \pm 0.31 \times 10^3$	$4.7 \pm 0.28 \times 10^3$	$3.9 \pm 0.22 \times 10^3$
Week 2	$7.6 \pm 0.42 \times 10^3$	$6.9 \pm 0.39 \times 10^3$	$6.1 \pm 0.35 \times 10^3$
Week 3	$6.2 \pm 0.33 \times 10^3$	$5.4 \pm 0.30 \times 10^3$	$4.6 \pm 0.27 \times 10^3$

Table 4.2: Cultural, Morphological and Biochemical Characteristics of Bacterial Isolates

Characteristics	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
Elevation	Raised	Flat	Flat	Raised	Flat	Flat
Margin	Entire	Undulate	Undulate	Entire	Irregular	Entire
Color (Colony)	Golden yellow	Cream	Cream	Cream/mucoid	Greenish (pigmented)	Pale
Shape (Colony)	Circular	Irregular	Irregular	Circular	Irregular	Circular
Size	Medium	Large	Large	Medium	Medium	Small
Gram Stain	+	+	-	-	-	-
Cell Type	Cocci	Rod	Rod	Rod	Rod	Rod
Arrangement	Clusters	Chains	Disperse	Disperse	Disperse	Disperse
Color (Gram Reaction)	Purple	Purple	Pink	Pink	Pink	Pink
KOH String Test	-	-	+	+	+	+
Catalase	+	+	+	+	+	+
Indole	-	-	+	-	-	+
Citrate	-	+	-	+	+	+
Oxidase	-	-	-	-	+	-
Glucose	+	+	+	+	+	+
Sucrose	-	-	-	+	-	-
Lactose	-	-	+	+	-	-
Gas Formation	-	-	+	+	-	+
H_2S Formation	-	-	-	-	-	+
TSI (Slant/Butt)	K/A	K/A	A/AG	A/AG	K/K	A/AG
Identity	*Staphylococcus aureus*	*Bacillus* spp.	*Escherichia coli*	*Klebsiella* spp.	*Pseudomonas* spp.	*Proteus* sp.

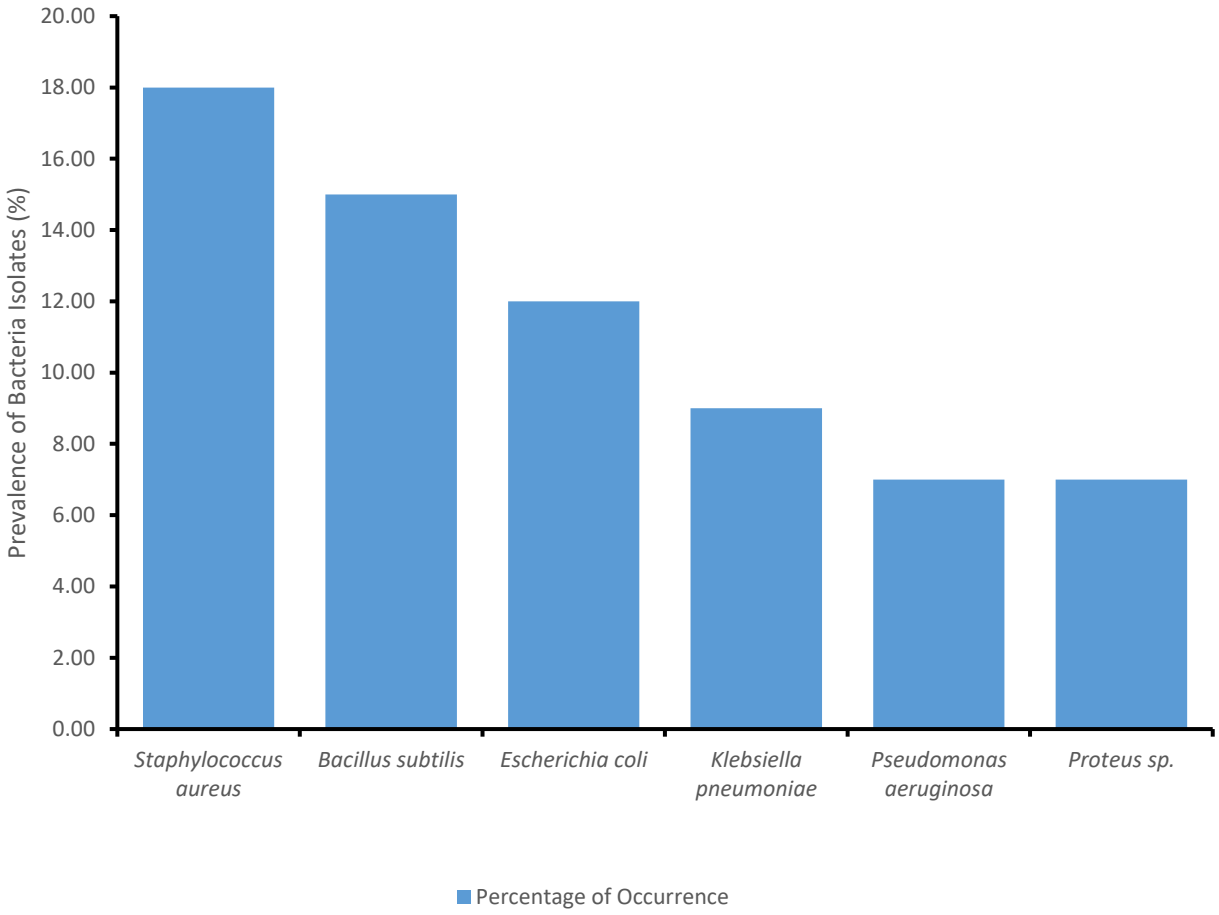

Figure 4.1. Percentage Frequency of Occurrence of Airborne Bacterial Isolates

Table 4.4: Antibiotic Susceptibility Profile of Bacterial Isolates

Antibiotics	*Staphylococcus aureus*	*Bacillus* spp	*Escherichia coli*	*Klebsiella* spp.	*Pseudomonas* spp.	*Proteus sp.*
AUG	S	S	R	S	R	S
PEF	S	S	R	S	S	S
CLT	S	S	S	R	S	S
CHL	S	S	S	S	R	S
AMP	R	I	R	R	R	R
OFL	S	S	S	S	S	S
AMPX	R	R	R	R	R	R
GEN	S	S	S	R	S	S
CIP	S	S	S	S	S	S
NR (%)	2 (22.2)	2 (22.2)	5 (55.6)	4 (44.4)	3 (33.3)	2 (22.2)
NS (%)	7 (77.8)	6 (66.7)	3 (33.3)	4 (44.4)	5 (55.6)	7 (77.8)
NI (%)	0 (0.0)	1 (11.1)	0 (0.0)	1 (11.1)	0 (0.0)	0 (0.0)

Keys:

Resistant (R)=0-10mm

Intermediate (I) = 11-16mm

Sensitive (S) =17mm and above

Aug = Augumentin , PEF = Pefloxacin , CLT = Clarithomycin , CHL = Chlorophenicol, AMP = Ampicillin , OFL = Ofloxacin , AMPX = Amplicox , GEN = Gentamicin, CIP = Ciprofloxacin

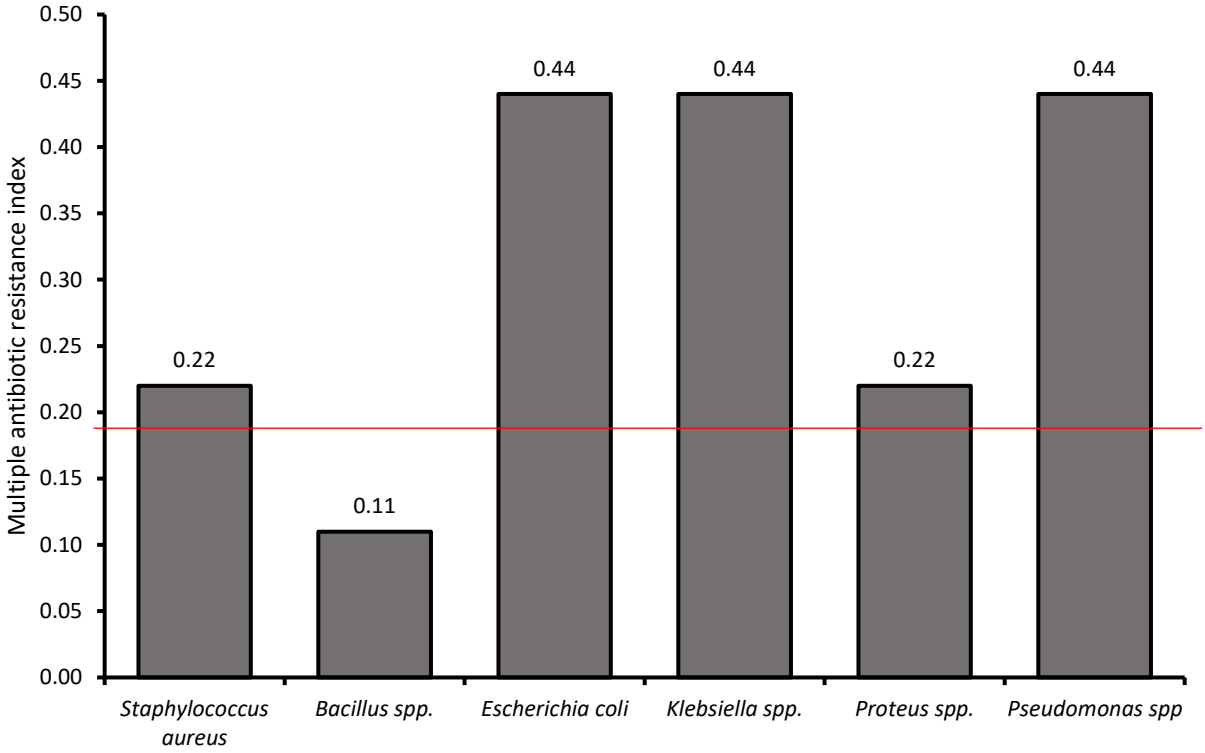

Figure 4.2: Multiple Antibiotic Resistance (MAR) Index of Bacterial Isolates

CHAPTER FIVE

DISCUSSION

Open refuse dumpsites are widely recognized as significant sources of environmental pollution and public health hazards (Siddiqua *et al*., 2022). In many developing countries, including Nigeria, indiscriminate waste disposal into open dumps remains a common practice due to inadequate waste management infrastructure (Oguntoyinbo, 2012). These dumpsites serve as reservoirs for diverse microorganisms, which thrive on decomposing organic matter and are subsequently dispersed into the atmosphere by wind, human activity, and other environmental factors (Choudhary *et al*., 2024). Previous studies have shown that dumpsites are hotspots for airborne bacteria, fungi, and other bioaerosols that can cause respiratory, gastrointestinal, and systemic infections, particularly in densely populated communities and institutional environments (Akpeimeh *et al*., 2019; Aleruchi *et al*., 2024). The microbial contamination associated with open dumpsites is further compounded by the presence of antimicrobial residues from domestic and industrial waste, creating environments conducive to the emergence and spread of antibiotic-resistant microorganisms.

This study investigated the airborne bacterial load and diversity of isolates from refuse dumpsites located within the University of Benin, specifically Hall 3, Hall 4, and the Faculty of Life Sciences. The results revealed varying bacterial concentrations across weeks and dumpsites, with Hall 3 recording the highest bacterial load ($7.6 \pm 0.42 \times 10^3$ CFU/m^3) in Week 2, while the Faculty of Life Sciences consistently showed the lowest counts. This variation may be attributed to differences in refuse volume, the nature of waste deposited, human traffic, and environmental

conditions such as wind speed, humidity, and temperature, which have been shown to influence airborne microbial dynamics (Obumneme *et al*., 2019; Aleruchi *et al*., 2024).

When compared with studies conducted in other institutions, the results obtained here fall within the lower to moderate category of contamination. For instance, Aleruchi *et al*. (2024) reported airborne bacterial loads of 1.43×10^3 to 5.8×10^4 CFU/m³ at Rivers State University, while Anele *et al*. (2025) found much higher loads in Madonna University, Elele, with values ranging from 1.1×10^5 to 3.0×10^5 CFU/m³ at dumpsites, compared to just 5×10^3 CFU/m³ at control sites located ten meters away. These differences highlight the influence of waste type, quantity, sanitation practices, and proximity of the dumpsites to human activities. The bacterial genera isolated in this study, which included *Staphylococcus aureus, Bacillus* spp., *Escherichia coli, Klebsiella* spp., *Pseudomonas* spp. and *Proteus* sp., are also consistent with those reported by Aleruchi *et al*. (2024) in similar dumpsite environments, underscoring the widespread nature of these organisms in waste-polluted air.

The diversity of bacterial isolates further confirmed the impact of waste composition and human activity on airborne microbial profiles. *Staphylococcus aureus* (26.5%) was the most predominant isolate, which agrees with its ubiquity in the environment and its frequent dispersal from human skin and nasal passages through aerosols (Otter and French, 2020). The high prevalence of *Bacillus* spp. (22.1%) can be explained by their spore-forming capacity, which allows them to withstand harsh environmental conditions and remain airborne for long periods (Checinska *et al*., 2015). The detection of enteric bacteria such as *Escherichia coli* (17.6%), *Klebsiella* spp. (13.2%), and *Proteus* sp. (10.3%) clearly indicates fecal contamination of refuse, which may have originated from decomposing food waste and human or animal excreta. This

finding is in line with Adegunloye (2020), who reported that airborne coliforms around dumpsites in Ekiti State were strongly linked to poor sanitation and decaying waste. The occurrence of *Pseudomonas* spp. (10.3%) also has significant implications since it is an opportunistic pathogen known for its persistence in environmental reservoirs and association with hospital-acquired infections (Karnwal *et al*., 2025).

Temporal and spatial variations were also evident in this study. Week 2 recorded the highest bacterial load and the greatest number of isolates, which may be attributed to increased accumulation of refuse and heightened human activity during this period. Hall 3 consistently showed the highest isolate frequency (44.1%), underscoring the role of localized factors such as the volume of waste deposited and population density in determining microbial dispersal patterns. These findings support earlier reports by Zhao *et al*. (2022), who demonstrated that areas of high human interaction and intense anthropogenic activity tend to exhibit elevated airborne microbial concentrations.

The antibiotic susceptibility profiles of the isolates revealed a worrisome trend. *Staphylococcus aureus* and *Proteus* sp. were the most susceptible, with susceptibility rates of about 77.8%, while *Escherichia coli* had the lowest susceptibility of approximately 33.3%. The high resistance observed in *E. coli* and *Klebsiella* spp. is consistent with previous findings linking enteric bacteria in polluted environments to multidrug resistance (Okoh *et al*., 2021). The Multiple Antibiotic Resistance (MAR) index further demonstrated the extent of resistance, with *Klebsiella spp.* recording a MAR index of 0.89 and *E. coli* 0.78. According to Krumperman (1983), MAR indices above 0.2 are indicative of contamination sources where antibiotics are frequently used. The extremely high MAR values obtained in this study therefore suggest that the airborne

isolates likely originated from anthropogenic sources, including pharmaceutical waste, indiscriminate disposal of antibiotics, and untreated human or animal excreta. This aligns with the findings of Nyandjou *et al*. (2019), who reported that 57.2% of isolates from Zaria dumpsites were multidrug-resistant and that 76.2% had MAR indices of 0.2 or higher.

The implications of these findings are profound. The detection of multidrug-resistant airborne bacteria within a university environment is a critical public health concern. The presence of pathogens such as *S. aureus, E. coli,* and *Klebsiella spp.* in the air around refuse dumpsites increases the likelihood of respiratory and gastrointestinal infections among students, staff, and neighboring communities. The risk is further exacerbated by the fact that airborne bacterial particles smaller than 10 µm can penetrate deep into the lungs, potentially causing serious infections (Nair, 2021). Akpeimeh *et al*. (2019) reported that between 41% and 76% of airborne bacteria, fungi, and *Aspergillus fumigatus* in dumpsites fall within this respirable size range, while workers at the Olusosun dumpsite in Lagos were found to suffer from chronic respiratory symptoms due to prolonged exposure to high bioaerosol concentrations (Akpeimeh *et al*., 2019). In a university setting characterized by dense human traffic and close building proximity, the circulation of such pathogens—especially those with multidrug resistance—poses a heightened risk of outbreaks and infections that may be difficult to treat.

From a public health standpoint, the presence of both pathogenic and opportunistic bacteria in dumpsite air represents a serious threat. Infections caused by these organisms may range from minor respiratory illnesses to severe systemic infections, particularly in immunocompromised individuals. The problem is compounded by the antibiotic resistance exhibited by many of the isolates, which makes treatment more challenging and costly (Ayandiran *et al*., 2019). This

situation underscores the importance of the One Health perspective, which stresses the interconnectedness of human, animal, and environmental health and the need for integrated approaches to waste management and antimicrobial stewardship (Danasekaran, 2024).

5.2 Recommendations

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

1. **Improved Waste Management:** The University of Benin should adopt modern waste management strategies, including frequent removal of refuse, provision of covered bins, and establishment of centralized waste collection points away from residential areas.
2. **Air Quality Monitoring:** Regular monitoring of airborne microbial load around dumpsites should be carried out to provide early warnings of potential health hazards.
3. **Public Health Education:** Awareness campaigns should be organized for students and staff on the risks of exposure to dumpsites and the importance of personal hygiene.
4. **Strengthening Antimicrobial Stewardship:** Policies should be implemented to reduce indiscriminate antibiotic use, as resistance genes in environmental bacteria may eventually be transmitted to human pathogens.
5. **Adoption of the One Health Approach:** Environmental health, human health, and veterinary sectors should collaborate in addressing the risks associated with microbial air pollution and antibiotic resistance from waste sources.
6. **Further Research:** More studies should be conducted to investigate seasonal variations in airborne bacterial loads, molecular characterization of resistance genes, and the role of dumpsites in broader urban health risks.

5.3 Conclusion

This study revealed that refuse dumpsites within the University of Benin harbor a high bacterial load in the surrounding air, with isolates including both environmental organisms and potential human pathogens such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Some of these isolates demonstrated resistance to commonly used antibiotics, underscoring the role of dumpsites as reservoirs of multidrug-resistant bacteria. The findings highlight the public health risks associated with poor waste management practices and emphasize the need for improved hygiene, proper refuse disposal, and periodic environmental monitoring. Dumpsites, if left unmanaged, may contribute significantly to the spread of airborne pathogens and antimicrobial resistance within institutional and urban environments.

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