PUBLIC HEALTH SIGNIFICANCE OF AIRBORNE BACTERIAL ISOLATES FROM PRIVATES EATRIES IN UNIVERSITY OF BENIN.

BY

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LSC2104008

DEPARTMENT OF MICROBIOLOGY

UNIVERSITY OF BENIN

BENIN CITY.

OCTOBER, 2025.

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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.

OCTOBER, 2025.

CERTIFICATION

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

PROF. F.O. EKHAISE **DATE**

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

(Head of Department)

DEDICATION

This project work is dedicated to God Almighty, for bringing me this far in life. I am truly grateful.

ACKNOWLEDGEMENT

I wish to begin by expressing my profound gratitude to God Almighty**,** whose endless love, grace, and mercy have been my constant source of strength, wisdom, and guidance throughout this academic journey. His divine favor has been instrumental in the successful completion of this project.

I wish to extend my profound gratitude to the Head of Department, Prof. E. O. Igbinosa, for his leadership, academic excellence, and commitment to creating an enabling environment for learning and research within the Department.

My heartfelt appreciation goes to my beloved parents, Rev. and Pastor (Mrs.) Osamede Jeremiah, for their unwavering love, prayers, encouragement, and financial support. Their sacrifices and steadfast belief in my potential have been the foundation of my academic accomplishments.

I am deeply grateful to my supervisor, Prof. F. O. Ekhaise, for his invaluable guidance, mentorship, and constructive criticism throughout the course of this research. His patience, insight, and dedication have greatly contributed to the success and quality of this work.

My sincere appreciation also goes to my co-supervisor, Dr. (Mrs.) I. B. Idemudia, for her continuous support, encouragement, and useful suggestions that helped in refining this study.

Finally, I acknowledge all my lecturers, colleagues, and friends who, in diverse ways, contributed to the successful completion of this project. Your support and encouragement are deeply appreciated. May God bless you all abundantly.

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ABSTRACT

Indoor air quality is an important determinant of public health, particularly in food-handling environments where airborne microorganisms can contaminate food and surfaces, leading to potential disease transmission. This study was conducted to isolate and identify airborne bacterial species from private eateries within the University of Benin (UNIBEN), Edo State, Nigeria. The study was carried out in two selected eateries, University Buka and Home and Away. A cross-sectional study design was adopted, and airborne bacterial samples were collected over a three-week period using the settle plate method. Sterile nutrient agar plates were exposed for 15 minutes at a height of approximately 1 meter above the ground to allow the natural settling of airborne bacteria. The plates were then incubated and analyzed for total bacterial load, followed by morphological, Gram staining, and biochemical characterization to identify the bacterial species present. The results showed that the mean bacterial load ranged from $0.7 \pm 0.04 \times 10^2$ CFU/m^3 at Home and Away in Week 3 (the lowest) to $3.22 \pm 0.5 \times 10^2$ CFU/m^3 at University Buka in Week 1 (the highest). Six bacterial species were isolated and identified: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella spp.*, *Bacillus spp.* and *Pseudomonas aeruginosa*. Among these, *S. aureus* was the most frequently occurring species (26.8%), followed by *Bacillus spp.* (21.4%) and *S. epidermidis* (17.9%), while *E. coli*, *Klebsiella spp.*, and *P. aeruginosa* had lower frequencies. Antibiotic susceptibility testing revealed widespread multidrug resistance among the isolates. *Klebsiella spp.* demonstrated the highest resistance (88.9%) with a Multiple Antibiotic Resistance (MAR) index of 0.89, followed by *E. coli* (0.78), *S. epidermidis* and *P. aeruginosa* (0.67 each), *S. aureus* (0.56), and *Bacillus spp.* (0.44). Ofloxacin (OFL) and gentamicin (GEN) were the most effective antibiotics, while ampicillin (AMP), ampiclox (AMPX), and clarithromycin (CLT) were the least effective across most isolates. The public health assessment based on bacterial load classification revealed that University Buka consistently recorded high contamination levels (H) in all three weeks, while Home and Away maintained low levels (L). The presence of antibiotic-resistant pathogens such as *S. aureus*, *Klebsiella spp.*, and *P. aeruginosa* in indoor air poses significant health risks, particularly to food handlers and consumers. This study highlights the presence of potentially pathogenic and multidrug-resistant airborne bacteria in private eateries within UNIBEN, emphasizing the need for improved ventilation, regular disinfection, and strict adherence to hygiene practices to safeguard public health.

CHAPTER ONE

INTRODUCTION

1.1. Background to the Study

The microbiological quality of the air in food preparation and service environments is a critical determinant of food safety, yet it is often overlooked compared to direct food and surface contamination (Møretrø and Langsrud, 2017). The presence of airborne microorganisms especially bacteria in eateries poses a significant risk to public health because such organisms can settle on exposed food, utensils, work surfaces, and packaging materials, thereby acting as indirect vehicles of foodborne disease transmission (Li and Chen, 2021; Aldosary *et al*., 2025). This form of contamination is particularly insidious because it may occur without any visible sign of spoilage, making it difficult for consumers and food handlers to detect.

Airborne bacteria originate from multiple sources, including human occupants, raw food materials, waste disposal sites, dust particles, and poorly maintained ventilation systems (Prussin and Marr, 2015). In indoor food service environments, bioaerosols can be generated through human activities such as talking, coughing, sneezing, food handling, and cooking processes, as well as through the movement of air that re-suspends settled dust particles (Cox and Wathes, 1995; Shale and Lues. 2007). Cooking activities such as frying, grilling, or boiling release heat and water vapor that can create upward air currents, aiding the dispersal of microorganisms into the surrounding space. Likewise, inadequate cleaning schedules and the absence of high-efficiency air filtration systems can lead to the accumulation of airborne particles that harbor bacteria, fungi, and other potential pathogens (Masotti *et al*.,2019).

In densely populated environments like university campuses, private eateries are essential for meeting students' nutritional needs, but they are also potential hotspots for airborne bacterial contamination. The University of Benin, located in Edo State, Nigeria, is home to a large student population drawn from diverse geographic and socio-economic backgrounds. Many of these students depend daily on privately owned food outlets and restaurants within and around the campus. These establishments often operate under conditions of high customer turnover, limited preparation space, and inconsistent hygiene practices, creating an environment conducive to the accumulation and dissemination of airborne microorganisms. Where ventilation is inadequate and cleaning practices are inconsistent, the microbial load in the air can increase significantly, raising the likelihood of food contamination (Madigan *et al*., 2018).

Numerous studies in Nigeria and other developing countries have documented that the indoor air establishments often harbor potentially pathogenic bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and members of the *Bacillus* genus (Ekhaise *et al*., 2010; Gulumbe and Kawo, 2018; Chimbekujwo *et al*., 2022; Ologbosere and Ekhaise, 2023). These organisms are of concern not only because they are capable of surviving in airborne particles but also because they can cause a range of diseases in humans (Agbonrofo *et al*., 2022). *Staphylococcus aureus* can produce heat-stable enterotoxins that remain active even after cooking, resulting in rapid-onset food poisoning (Mairi *et al*., 2025). *Echerichia coli*, especially enterohaemorrhagic strains like *E. coli* O157:H7, can cause severe gastrointestinal disease and, in extreme cases, haemolytic uremic syndrome (Ibama *et al*., 2019). *Klebsiella pneumoniae* and *P. aeruginosa* are associated with opportunistic infections and can persist in moist or water-rich environments such as sinks and drains, allowing them to re-enter the air during cleaning or cooking activities (WHO, 2017).

Beyond acute foodborne diseases, airborne bacterial contaminants have emerged as potential contributors to the spread of antimicrobial resistance (AMR). Antibiotic-resistant bacteria in the air of eateries can be ingested through contaminated food or inhaled directly, creating both immediate and long-term health risks (Gulumbe and Kawo, 2018). The World Health Organization has identified AMR as one of the top ten global public health threats, emphasizing the role of food environments as key reservoirs and transmission points for resistant pathogens (WHO, 2015). In Nigeria, research has shown that bacteria from environmental sources including food service environments often display resistance to multiple antibiotics such as ampicillin, tetracycline, gentamicin, and ciprofloxacin (Nwankwo *et al*., 2014). This is particularly concerning in community settings because infections with resistant bacteria can lead to treatment failures, longer hospital stays, and increased healthcare costs.

The risk of airborne contamination is amplified in university contexts where large groups of students dine in close proximity, often in poorly ventilated rooms. This facilitates not only the spread of foodborne pathogens but also the possible airborne transmission of respiratory pathogens, thereby compounding public health risks. Environmental factors such as overcrowding, inadequate waste disposal systems, poor ventilation, and limited regulatory oversight of private food outlets exacerbate these problems (Gulumbe and Kawo, 2018). Eateries located near open drains, refuse dumps, or unpaved roads are particularly vulnerable to high microbial loads, as airborne dust and debris from these areas can easily infiltrate food preparation and serving spaces (Jay *et al*., 2005). Furthermore, poor structural designs that fail to physically separate cooking areas from dining spaces can allow microorganisms to travel freely between zones.

The public health threat posed by airborne bacterial contamination in these settings is twofold. First, deposition of airborne bacteria on ready-to-eat foods and food-contact surfaces increases the risk of foodborne disease outbreaks, which can impair students' academic performance, increase absenteeism, and place additional pressure on healthcare facilities. Second, the presence of antibiotic-resistant strains among airborne isolates represents an added layer of risk, as these organisms may cause infections that are difficult to treat and contribute to the wider problem of antimicrobial resistance (WHO, 2015).

Despite the plausibility and documented occurrence of airborne bacterial contamination in eateries, there is a paucity of data specifically addressing the microbial air quality of private eateries in the University of Benin. Without such evidence, it is difficult to design and implement effective interventions to mitigate airborne contamination and protect public health. This knowledge gap is particularly concerning given the high reliance of the university community on these eateries and the potential for rapid spread of pathogens within a densely populated academic environment.

The absence of systematic surveillance and locally relevant data on the types, concentrations, and antibiotic resistance patterns of airborne bacteria in these food service environments limits the capacity of the university and public health authorities to respond proactively. Consequently, there is a need to investigate the airborne bacterial profile of private eateries within the University of Benin to inform targeted hygiene interventions, strengthen food safety policies, and safeguard the health of the campus community.

1.2 Aim and Objectives of the Study

The aim of this study was to isolate and identify airborne bacterial isolates from private eateries within the University of Benin, Edo State, Nigeria, and to assess their potential public health significance.

The specific objectives of this study were to;

1. enumerate the bacterial isolates of the air samples collected from selected private eateries in the University of Benin.
2. isolate and identify bacterial isolates present in the eateries.
3. assess the antibiotic susceptibility pattern of the bacterial isolates.
4. evaluate the potential public health implications of the identified airborne bacterial isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1. Overview of airborne microorganisms in indoor environments.

Indoor environments such as offices, homes, classrooms, hospitals, and food-service establishments are recognized as reservoirs for a wide variety of airborne microorganisms, including bacteria, fungi, viruses, and microbial fragments. These microorganisms originate from numerous sources, such as human occupants through activities like breathing, talking, coughing, and shedding of skin cells, as well as from pets, plants, building materials, heating, ventilation and air-conditioning (HVAC) systems, and outdoor air that infiltrates the indoor space (Lindsley and Nazaroff, 2019). Airborne bacteria in particular may exist in several forms: as free-floating cells, attached to dust particles, or encapsulated within liquid droplets or droplet nuclei produced by respiratory or mechanical actions (Jones and Harrison, 2004). Their abundance and diversity within indoor environments are influenced by a combination of environmental factors, including temperature, humidity, ventilation efficiency, occupant density, and the nature of activities carried out in the space (Peitzsch *et al*., 2012).

Food-service areas, including restaurants, cafeterias, commercial kitchens, and small-scale eateries, represent a unique category of indoor environments where airborne bacterial contamination poses significant public health concerns. These spaces combine high human traffic with food preparation and handling, creating ideal conditions for the dissemination and deposition of airborne microorganisms. Airborne bacteria can settle on food contact surfaces, utensils, packaging materials, and even directly onto exposed food, potentially leading to spoilage or foodborne illnesses (Mujumdar and Vinodhkumar, 2021). Furthermore, employees in food-service environments are at risk of occupational exposure to airborne pathogens, which may result in respiratory tract infections or other health complications (Peitzsch *et al.*, 2012). The presence of pathogenic bacteria in such settings also jeopardizes consumer health and may negatively impact the reputation and economic viability of food businesses.

The study of airborne bacteria in food-service environments is therefore critical for several reasons. First, it provides insight into the potential routes of microbial contamination in ready-to-eat foods, which is important for hazard analysis and the development of preventive measures (WHO, 2010). Second, it aids in identifying risk factors and environmental conditions that favor the survival and spread of airborne bacteria, thereby informing the design of effective ventilation systems, air-filtration devices, and cleaning protocols (Lindsley and Nazaroff, 2019). Third, monitoring airborne bacteria aligns with regulatory and public health priorities, as many food safety agencies require strict control of environmental hygiene in food production and retail spaces (Mujumdar and Vinodhkumar, 2021).

Indoor air quality (IAQ) is increasingly recognized as a crucial determinant of public health, given that people spend a significant proportion of their daily lives indoors. Poor IAQ has been linked to a wide spectrum of health effects, ranging from acute symptoms such as headaches, nasal congestion, and eye irritation, to more severe consequences like asthma exacerbations, allergic responses, and increased susceptibility to infections (WHO, 2010). The microbial component of IAQ is particularly important in food-service contexts, where both the health of occupants and the safety of food products can be compromised by high levels of airborne bacteria (Jones and Harrison, 2004). Prolonged exposure to microbial-laden air can also contribute to chronic respiratory conditions and impose additional health burdens on vulnerable populations such as children, the elderly, and immunocompromised individuals.

Airborne bacteria in indoor environments, and particularly in food-service establishments, pose both direct and indirect risks to public health. These microorganisms can compromise food safety, threaten occupational health, and degrade indoor air quality. Therefore, a comprehensive understanding of their sources, prevalence, and control measures is essential for safeguarding consumer well-being and ensuring compliance with food safety and occupational health regulations.

2.2 Concept of Aeromicrobiology

Aeromicrobiology is a specialized branch of microbiology that focuses on the study of airborne microorganisms and their interactions with the surrounding environment. It involves identifying, quantifying, and understanding the behavior, transport mechanisms, and survival of microorganisms in the atmosphere (Cox and Wathes, 2020). This includes both bioaerosols particles of biological origin that are suspended in the air and particulate matter that carries microorganisms. Bioaerosols may range in size from nanometers to several hundred micrometers, influencing how far they travel, how long they remain suspended, and the type of health or environmental effects they cause (Després *et al*., 2012).

The scope of aeromicrobiology extends across multiple disciplines, including public health, occupational safety, atmospheric science, agriculture, and environmental monitoring. In public health, it is essential for understanding the airborne transmission of infectious agents such as *Mycobacterium tuberculosis*, influenza virus, and *SARS-CoV-2* (Tellier *et al*., 2019). In agriculture, aeromicrobiology helps track the dispersal of plant pathogens like *Puccinia graminis* (wheat stem rust) or *Phytophthora infestans* (potato late blight), which can cause devastating crop losses (Bowers *et al*., 2013). Industrially, the discipline informs guidelines for controlling bioaerosol levels in food processing, pharmaceutical production, and biotechnology facilities (Macher, 1999).

Air acts as both a carrier and a habitat albeit a temporary one for microorganisms, hence Aeromicrobiology also examines how environmental factors such as humidity, temperature, Ultra Violet radiation, and wind speed influence microbial survival and dissemination (Tang, 2009). The discipline's ultimate goal is to provide scientific insights for mitigating airborne microbial risks, improving air quality, and protecting human, animal, and plant health.

2.2.1. Classification of Airborne Microorganisms

Airborne microorganisms are diverse, encompassing bacteria, fungi, and viruses. They can exist in the air as single cells, spores, pollen grains, or as aggregates attached to dust particles or other organic matter (Prussin and Marr, 2015).

2.2.1.1.Bacteria

Airborne bacteria can originate from soil, water, plants, animals, humans, and various anthropogenic sources. They are released into the atmosphere through wind erosion, water splashes, coughing, sneezing, talking, or disturbance of contaminated surfaces. Many airborne bacteria are non-pathogenic environmental species such as *Micrococcus* and *Bacillus*, but pathogenic species like *Mycobacterium tuberculosis*, *Legionella pneumophila*, and *Streptococcus pneumoniae* are of major public health concern (Cox and Wathes, 2020). Some bacteria produce

endospores (e.g., *Bacillus anthracis* and *Clostridium* spp.), allowing them to survive harsh atmospheric conditions for prolonged periods (Setlow, 2014).

2.2.1.2.Fungi

Fungal spores are among the most abundant biological particles in the air and are a major component of outdoor and indoor bioaerosols. Common airborne fungi include *Aspergillus*, *Penicillium*, *Cladosporium* and *Alternaria* species (Eduard, 2009). These spores are released from soil, plants, decaying organic matter, and damp building materials. Airborne fungi are important in allergy and asthma epidemiology and can cause opportunistic infections in immunocompromised individuals. Certain species produce mycotoxins, which can be harmful when inhaled over time (Macher, 1999).

2.2.1.3.Viruses

Viruses are obligate intracellular parasites that rely on host cells for replication, but many can remain infectious in the air for varying periods. Airborne viruses are typically transmitted via respiratory droplets (>5 µm) or droplet nuclei (<5 µm) generated by coughing, sneezing, speaking, or breathing (Tellier *et al*., 2019). Examples include influenza virus, measles virus, varicella-zoster virus, and *SARS-CoV-2*. Some viruses are highly infectious via the airborne route, capable of spreading over considerable distances in indoor spaces with poor ventilation (Li *et al*., 2021).

2.2.2. Pathways of Microbial Dissemination in Air

Microorganisms enter and travel through the air via several distinct pathways, influenced by both natural processes and human activity. These can be broadly categorized as mechanical dispersal**,** biological dispersal, and environmental/atmospheric transport**.**

2.2.2.1.Mechanical Dispersal

Physical activities such as sweeping, vacuuming, construction, industrial operations, and tillage disturb contaminated surfaces or soil, releasing microorganisms into the air. In food service environments, activities like chopping, washing, or the movement of staff can re-suspend settled dust particles carrying bacteria and fungi (Macher, 1999). Aerosolization from wastewater treatment plants, compost facilities, or animal housing units also falls under this category (Douwes *et al*., 2003).

2.2.2.2.Biological Dispersal

Humans, animals, and plants naturally emit microorganisms into the air. Humans release skin flakes, hair, respiratory droplets, and even fecal bioaerosols (Prussin and Marr, 2015). Animals shed microorganisms through fur, saliva, and excreta, while plants release pollen grains and fungal spores into the atmosphere. Some plant pathogens also produce microscopic infectious propagules that can travel with the wind and infect crops at considerable distances (Bowers *et al*., 2013).

2.2.2.3.Environmental and Atmospheric Transport

After microorganisms are released into the air, they are subject to transport by atmospheric currents. Wind speed, direction, turbulence, and convection currents influence the distance they travel. Rain splash, wave action, and soil erosion can generate microbial aerosols that become part of atmospheric circulation (Burrows *et al*., 2009). Some airborne microorganisms can travel across continents via dust storms or jet streams, contributing to the global dispersal of pathogens and allergens (Griffin, 2007). Environmental survival during transport depends on multiple factors such as temperature, humidity, solar radiation, and particulate association. Microbes attached to dust or organic particles generally survive longer due to protection from UV light and desiccation (Tang, 2009).

2.3. Sources of Airborne Bacteria in Eateries

The presence and persistence of airborne bacteria in eateries are largely influenced by multiple interconnected factors, including human activities, food preparation processes, ventilation systems, environmental surfaces, and the potential for cross-contamination from raw food items. Understanding these sources is essential for designing effective control measures to safeguard food quality, protect public health, and maintain compliance with hygiene standards.

2.3.1. Human Activities (Talking, Coughing, Sneezing)

Human activities are among the most significant contributors to airborne bacterial load in eateries. Activities such as talking, coughing, and sneezing can release large quantities of bioaerosols containing pathogenic or opportunistic microorganisms, which remain suspended in the air and may subsequently settle on food contact surfaces (Tang *et al*., 2013). A single sneeze can release up to 40,000 droplets, many of which are small enough (<5 μm) to remain airborne for extended periods (Han *et al*., 2013). These droplets can contain bacteria such as *Staphylococcus aureus*, *Streptococcus pneumonia* and other respiratory commensals or pathogens that may contaminate exposed food items (Božiková *et al*., 2019). In poorly ventilated food service environments, airborne bacteria originating from human activities can accumulate, increasing the risk of contamination events.

The role of food handlers as both a direct and indirect source of bacterial contamination has been widely documented. Food handlers carrying asymptomatic infections or with poor personal hygiene practices can shed bacteria into the environment through respiratory emissions, hair shedding, and skin flakes (World Health Organization [WHO], 2020). Therefore, employee hygiene, mask usage, and adherence to cough etiquette are critical components of airborne contamination control in eateries.

2.3.2. Food Preparation and Handling Processes

Food preparation and handling processes also play a substantial role in generating airborne bacterial particles. Cutting, chopping, blending, grinding, and frying can release microscopic food particles and associated microorganisms into the air (Stilo and Parisi, 2018). Heat and moisture from cooking may facilitate microbial aerosolization, especially when contaminated raw materials are involved. Meat processing activities can release *Listeria monocytogenes*, *Escherichia coli* and *Salmonella* into the air, while flour handling in bakeries can aerosolize *Bacillus* spores (Wang *et al*., 2015).

Furthermore, improper food handling techniques—such as leaving prepared foods uncovered—provide an opportunity for airborne bacteria to settle on ready-to-eat items, posing a direct public health risk. The microbial composition of the indoor air in food preparation areas has been found to closely match the microbial flora of the food products handled within those spaces (Mandal and Brandl, 2011). This correlation underscores the necessity of integrating airborne hygiene into food safety protocols, particularly in open kitchen or buffet settings where food is exposed to the ambient air for extended periods.

2.3.3. Ventilation Systems and Environmental Surfaces

Ventilation systems, while designed to improve indoor air quality, can paradoxically serve as reservoirs and dissemination pathways for airborne bacteria if not adequately maintained. Accumulation of dust, moisture, and organic matter within air ducts, filters, and vents can provide a nutrient-rich environment for bacterial growth, including species such as *Legionella pneumophila* and *Pseudomonas aeruginosa* (Li *et al*., 2007). When airflow is initiated, these microorganisms can be dispersed into food preparation and dining areas, leading to widespread contamination.

Environmental surfaces such as countertops, chairs, tables, and door handles can also act as secondary sources of airborne bacteria through mechanical disturbance. Sweeping floors or moving chairs can resuspend settled bacterial particles back into the air (Qian *et al*., 2014). In eateries with high customer turnover, surface cleaning practices that involve dry dusting rather than wet wiping can exacerbate airborne microbial loads. Proper ventilation design, high-efficiency particulate air (HEPA) filtration, and scheduled maintenance are therefore crucial for reducing airborne bacterial concentrations.

2.3.4. Cross-Contamination from Raw Food Items

Cross-contamination from raw food items represents another important source of airborne bacteria in eateries. Raw meat, poultry, seafood, and unwashed produce can harbor high loads of pathogenic microorganisms such as *Salmonella* spp., *Campylobacter* spp. and *Listeria monocytogenes* (Schmidt, 2011). Handling or processing these raw materials—particularly chopping, grinding, or washing—can generate aerosolized droplets containing these bacteria, which may travel short distances and contaminate nearby surfaces or ready-to-eat foods (Gibson *et al*., 2012).

Studies have demonstrated that airborne dissemination from raw food handling areas is a potential route of cross-contamination within kitchens, particularly when food preparation zones are not adequately segregated (Flores *et al*., 2012). In buffet or open-display settings, raw and cooked foods in close proximity without proper physical barriers are especially vulnerable. This highlights the importance of implementing separation between raw and ready-to-eat food handling zones, maintaining strict cleaning schedules, and using covered containers or sneeze guards to prevent microbial deposition.

2.4. Types of Airborne Bacteria Commonly Found in Eateries

Airborne bacteria represent a diverse group of microorganisms that can remain suspended in the air for varying periods, depending on their size, density, and environmental conditions. In eateries, the composition of airborne bacterial populations is influenced by factors such as human activities, food preparation processes, ventilation systems, and the presence of contaminated raw food materials. The types of airborne bacteria commonly found in such settings vary, but they often include Gram-positive cocci**,** Gram-negative bacilli**,** spore-forming bacteria and a variety of opportunistic and pathogenicspecies that have significant implications for food safety and public health.

2.4.1. Gram-Positive Cocci

Gram-positive cocci are among the most prevalent airborne bacteria in indoor environments, including eateries. They are characterized by their spherical shape and thick peptidoglycan cell wall, which provides resistance to desiccation, enabling them to survive in air for extended periods (Madigan *et al*., 2021). A prime example is *Staphylococcusaureus*, a facultative anaerobe that is well known for its role in foodborne illnesses through the production of heat-stable enterotoxins. *S. aureus* is frequently shed from the skin, nasal passages, and hair of food handlers, particularly during talking, coughing, or sneezing (Le Loir *et al*., 2003). This organism can contaminate ready-to-eat foods such as pastries, salads, and cooked meats, causing staphylococcal food poisoning.

Another common Gram-positive coccus is *Micrococcus luteus***,** which is generally considered non-pathogenic but is a reliable indicator of human skin contamination. It is frequently isolated from the air of kitchens and dining spaces, reflecting poor hygiene practices or inadequate ventilation (Wilson *et al*., 2020). Although it rarely causes disease in healthy individuals, M. luteus can act as an opportunistic pathogen in immunocompromised persons.

2.4.2. Gram-Negative Bacilli

Gram-negative bacilli are less tolerant to desiccation than Gram-positive bacteria due to their thinner peptidoglycan layer; however, some species survive in aerosols long enough to pose a health risk in eateries. They are often associated with wet surfaces, drains, raw vegetables, and improperly handled meat products.

Pseudomonas aeruginosa is a notable Gram-negative bacillus isolated from food preparation environments. It is an opportunistic pathogen capable of surviving in moist conditions such as sinks, dishwashers, and cutting boards. While it is not a major cause of foodborne illness, its presence in air samples may indicate poor sanitation and water handling practices (Morita *et al*., 2014).

Escherichia coli, particularly pathogenic strains like E. coli O157:H7, is of greater food safety concern. Although primarily transmitted through contaminated food and water, E. coli can be aerosolized through splashing during food washing or handling raw meat. Its airborne presence in eateries underscores cross-contamination risks and inadequate hygiene measures (Holah*et al*., 2004).

2.4.3. Spore-Forming Bacteria

Spore-forming bacteria are highly resilient microorganisms capable of surviving extreme environmental conditions, including heat, desiccation, and disinfectants, due to their ability to form dormant spores. In eateries, the most frequently encountered spore-formers in air samples are species of the genus *Bacillus*.

Bacillus cereus is a well-known foodborne pathogen that produces toxins responsible for two types of food poisoning: the emetic type (caused by cereulide) and the diarrheal type (caused by enterotoxins). Its spores can remain airborne for prolonged periods and subsequently settle on food preparation surfaces, utensils, or food items (Ehling-Schulz *et al*., 2019). Other Bacillus species, while non-pathogenic, may contribute to food spoilage through enzymatic degradation of proteins, lipids, and carbohydrates.

2.4.4. Opportunistic and Pathogenic Species Relevant to Food Safety

In addition to the well-defined groups above, several opportunistic and pathogenic bacteria may be present in the air of eateries. These organisms are of particular concern in environments where high-risk foods—such as dairy products, salads, and meat dishes—are handled.

For instance, *Listeria monocytogenes*, though not as commonly airborne as Bacillus species, has been detected in aerosols generated during food processing, particularly in meat and dairy facilities (Wilks *et al*., 2006). Its presence in eateries, even in low concentrations, poses a severe risk to vulnerable populations, including pregnant women, neonates, and immunocompromised individuals.

Other bacteria, such as *Acinetobacter* spp.**,** *Enterobacter* spp. and *Klebsiella pneumoniae***,** may not always cause immediate illness in healthy individuals but can lead to serious infections in immunocompromised persons. Their airborne occurrence often reflects contamination from raw food materials or water systems. The detection of these bacterial groups in eateries highlights the potential for airborne transmission of foodborne pathogens and cross-contamination during food preparation and serving. Airborne microorganisms can settle on exposed food, utensils, and contact surfaces, contributing to outbreaks of foodborne illnesses (Duan *et al*., 2020). Consequently, understanding the types of airborne bacteria present in eateries is crucial for implementing targeted hygiene measures, improving ventilation, and ensuring safe food handling practices.

2.5 Factors Affecting Airborne Bacterial Load in Indoor Environments

The concentration and diversity of airborne bacteria in indoor environments are influenced by multiple interrelated environmental, human, and operational factors. In food service settings such as eateries, these factors can significantly impact indoor air quality and, consequently, food safety and public health. Understanding these determinants is essential for designing effective control measures to reduce bacterial contamination risks.

2.5.1. Temperature, Humidity and Ventilation

Temperature and relative humidity play critical roles in the survival and proliferation of airborne microorganisms. Most bacteria thrive within a mesophilic temperature range (20–40 °C), which is typical for indoor environments, especially in tropical climates such as Nigeria's. Elevated temperatures can promote bacterial metabolic activity, while extreme cold or heat may reduce viability (Tang, 2009). Similarly, relative humidity affects the ability of bacteria to remain suspended in the air; many airborne bacteria survive longer at moderate humidity levels (40–60%), while very low humidity can cause desiccation and cell death, and very high humidity can promote settling and surface contamination (Hospodsky *et al*., 2012).

Ventilation also plays a pivotal role by influencing the rate of air exchange and the dilution of airborne particles. Poor ventilation allows bacterial aerosols to accumulate, increasing exposure risk, whereas adequate ventilation can significantly lower airborne bacterial load by introducing fresh air and exhausting contaminated air (Qian *et al*., 2014). Mechanical ventilation systems, if not properly maintained, can also act as reservoirs and dispersal routes for bacterial contaminants.

2.5.2. Occupant Density and Movement

The number of people present in a confined space directly affects the bacterial load of the air. Humans are significant sources of airborne bacteria, releasing microorganisms through activities such as talking, coughing, sneezing, and even normal respiration (Meadow *et al*., 2014). Higher occupant density increases the probability of bacterial shedding, while movement — such as walking or serving food — can resuspend bacteria from floor surfaces and other fomites into the air. This is particularly relevant in busy eateries where staff and customers are in constant motion. Studies have shown that airborne bacterial concentrations in indoor environments can spike during peak occupancy hours (Qian *et al*., 2012).

2.5.3. Cleaning Frequency and Sanitation Practices

Effective cleaning and sanitation are essential in reducing both surface and airborne bacterial load. Inadequate cleaning practices allow bacteria to accumulate on surfaces, which can later become aerosolized through human activity or airflow. Regular cleaning with appropriate disinfectants reduces the microbial reservoir and limits the risk of resuspension (Zhao *et al*., 2014). The method of cleaning also matters; dry sweeping can increase airborne bacteria through dust disturbance, whereas wet mopping can reduce aerosolization (Foarde *et al*., 1999). In eateries, cleaning frequency should be increased during high customer turnover to minimize bacterial contamination risk.

2.5.4. Type of Food Served and Method of Preparation

The nature of food handled in an eatery can also influence airborne bacterial concentrations. Raw meat, poultry, and seafood are known to harbor higher bacterial loads compared to cooked or processed foods, and their handling can release bacteria into the air via splashes, aerosols, and contaminated utensils (Holah, 2000). Cooking methods such as grilling, frying, or boiling may produce aerosols containing microbial particles, particularly when contaminated ingredients are used (Chowdhury *et al*., 2017). Conversely, foods that require minimal handling or are pre-packaged typically pose a lower risk of contributing to airborne bacterial load. The degree of adherence to hygienic food preparation protocols can therefore significantly influence airborne contamination levels in food service environments.

Airborne bacterial load in indoor environments such as eateries is determined by a complex interplay of environmental conditions, human factors, cleaning regimes, and food-related activities. Control measures should therefore be multifaceted, incorporating environmental monitoring, improved ventilation, optimal cleaning practices, and strict adherence to food safety standards.

2.6 Public Health Implications of Airborne Bacteria in Eateries

The presence of airborne bacterial isolates in eateries has significant public health implications, particularly in relation to food safety, antimicrobial resistance, and the protection of vulnerable population groups. Indoor environments where food is prepared and served are often characterized by high human activity, diverse microbial sources, and varying hygiene practices. These factors collectively contribute to an increased risk of microbial contamination of food and subsequent foodborne disease transmission. Understanding these implications is crucial for formulating preventive measures and enhancing public health surveillance.

2.6.1 Potential for Foodborne Illnesses

Airborne bacterial isolates can serve as an important source of contamination in the food service environment. Pathogenic species such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes* may become airborne through coughing, sneezing, talking, or through mechanical activities such as sweeping, cleaning, and food preparation (Nyarubega*et al.*, 2020). Once airborne, these microorganisms can settle on exposed ready-to-eat foods, utensils, or food contact surfaces, creating a pathway for ingestion and subsequent infection.

Foodborne illnesses associated with airborne contamination often present with gastrointestinal symptoms such as diarrhea, vomiting, abdominal cramps, and fever, which may be severe in susceptible individuals (Lues*et al.*, 2006). In poorly ventilated eateries, airborne bacterial concentrations can be significantly higher, increasing the likelihood of cross-contamination and facilitating localized outbreaks. Thus, effective control of indoor air quality is an important component of comprehensive food safety management systems.

2.6.2 Antimicrobial Resistance in Airborne Pathogens

An emerging concern in public health is the detection of antibiotic-resistant bacteria in the air of food service establishments. Studies have reported airborne isolates of *S. aureus*, *Pseudomonas aeruginosa* and *Enterobacter* spp. showing resistance to multiple antibiotic classes, including beta-lactams, tetracyclines and macrolides (Li *et al.*, 2018). The presence of antimicrobial-resistant bacteria in eatery environments not only poses a direct infection risk but also contributes to the spread of resistance genes via horizontal gene transfer.

From a One Health perspective, airborne dissemination of antimicrobial resistance bridges environmental, human, and food safety sectors. This makes eateries potential hubs for resistance transmission, especially in densely populated urban areas where these facilities are frequented daily by large numbers of people (Jin *et al.,* 2022)

2.6.3 Risk to Vulnerable Populations

Certain groups, including young children, the elderly, pregnant women, and immunocompromised individuals, face a heightened risk from airborne bacterial exposure in eateries (FAO/WHO, 2008). Their immune systems are either underdeveloped, weakened by age, or compromised by illness, making them more susceptible to infection and severe disease outcomes. *Listeria monocytogenes* infections, though relatively rare, can be life-threatening for pregnant women and neonates. Similarly, elderly individuals may experience prolonged recovery periods and increased hospitalization rates following foodborne infections.

The frequent patronage of eateries by these vulnerable populations such as school canteens, hospital cafeterias, and nursing home dining areas necessitates stringent air quality and hygiene standards in such settings.

Documented cases highlight the link between poor air hygiene and disease outbreaks in eateries. For instance, norovirus outbreaks in restaurant settings have been attributed to aerosolization of viral particles during vomiting events, which subsequently contaminated food and surfaces (Marks *et al*., 2000). Although norovirus is a virus, similar airborne dispersal mechanisms apply to bacterial pathogens, particularly those capable of surviving in desiccated states, such as *Bacillus cereus*.

In some outbreak investigations, inadequate ventilation, overcrowding, and poor cleaning practices were identified as contributing factors (Lues*et al*., 2006). These examples underscore that airborne contamination in eateries is not merely theoretical but a documented route of transmission with tangible health consequences.

2.6.5 Public Health Significance

From a public health standpoint, the implications of airborne bacterial contamination in eateries include increased incidence of foodborne illness, greater difficulty in managing infections due to antimicrobial resistance, and disproportionate impact on vulnerable populations. Addressing this issue requires an integrated approach involving:

- Implementation of adequate ventilation and air filtration systems.
- Regular monitoring of airborne microbial loads in high-risk settings.
- Strict enforcement of hygiene protocols for food handlers.
- Public health education emphasizing respiratory and hand hygiene in food service contexts.

By incorporating airborne contamination control into food safety management systems, it is possible to reduce the risk of outbreaks, slow the spread of antimicrobial resistance, and safeguard public health in the community.

2.7 Airborne Bacteria as Indicators of Hygiene Standards in Food Establishments

The assessment of airborne bacteria in food establishments serves as a critical indicator of hygiene standards and the overall microbiological safety of the indoor environment. Microbial air quality monitoring provides valuable insights into the extent of microbial contamination, the effectiveness of cleaning and sanitation protocols, and the potential risks of cross-contamination between the environment, food handlers, and food products (Duan *et al.*, 2020). In food service areas such as restaurants, cafeterias, and eateries, where food is prepared, handled, and served, airborne microorganisms can originate from multiple sources, including human activities, food preparation processes, and ventilation systems. As such, quantifying airborne bacteria is not only a measure of environmental cleanliness but also an indirect evaluation of operational hygiene practices (Kalwasinska *et al.*, 2012).

Microbial air quality assessment is often integrated into Hazard Analysis and Critical Control Points (HACCP)systems and Good Hygiene Practices (GHP) as a monitoring tool to ensure compliance with food safety standards. By periodically measuring the bacterial load in the air, food establishments can detect potential hygiene lapses early and implement corrective measures to prevent foodborne illness outbreaks (Kusumaningrum *et al.*, 2003). In this context, airborne bacteria serve as “sentinels” of environmental hygiene, reflecting not only current cleanliness but also the potential for pathogenic contamination.

2.7.1. Acceptable Microbial Load Limits in Indoor Air

Several regulatory and advisory bodies, including the World Health Organization (WHO), HACCP guidelines, and International Organization for Standardization (ISO), provide benchmarks for acceptable microbial air quality in food handling environments. While specific numerical limits can vary depending on the type of establishment and the regulatory framework, general recommendations suggest that the total aerobic bacterial count in food preparation areas should not exceed 200–500 cfu/m³ for high-risk zones, and should remain below 1,000 CFU/m³ for less critical areas (WHO, 2002).

The WHO (2002) emphasizes that maintaining microbial loads within these thresholds is essential to reduce the likelihood of airborne transmission of pathogenic microorganisms, such as *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus*, which have been implicated in foodborne outbreaks linked to poor air hygiene. HACCP-based protocols reinforce this requirement by recommending environmental microbiological monitoring at defined intervals, especially in sensitive areas where ready-to-eat (RTE) foods are handled.

Similarly, ISO 14698-1 specifies that microbial air sampling should be part of a comprehensive contamination control program, particularly in environments where airborne transmission poses a risk to product safety. This involves using both passive (settle plates) and active (air samplers) monitoring techniques to capture quantitative and qualitative data on airborne bacteria. These results are then benchmarked against pre-established alert and action limits, allowing for timely intervention when contamination levels exceed acceptable thresholds (Kalwasinska*et al.*, 2012).

2.7.2. Hygiene Indicator Role of Airborne Bacteria

Airborne bacterial counts in food establishments can serve as a proxy measure of surface cleanliness, ventilation efficiency, and staff hygiene practices. Elevated microbial loads often indicate inadequate cleaning frequencies, poor air filtration, overcrowding, or improper handling of raw and cooked foods (Whyte *et al.*, 2004). Furthermore, the presence of certain bacterial species, such as *S. aureus* or coliform bacteria, can specifically point to lapses in personal hygiene among food handlers or cross-contamination from raw ingredients.

In addition, long-term microbial air quality data can be used to evaluate the effectiveness of intervention strategies. For instance, improvements in ventilation systems, increased frequency of surface disinfection, and the introduction of high-efficiency particulate air (HEPA) filtration have been shown to significantly reduce airborne bacterial counts in food environments (Sowiak*et al*., 2020).

Ultimately, the consistent monitoring of airborne bacteria provides both a preventive and corrective mechanism for ensuring food safety. When integrated into food safety management systems, it reinforces compliance with local and international standards, safeguards consumer health, and enhances public trust in food establishments.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was carried out in selected private eateries located within the University of Benin (UNIBEN), Benin City, Edo State, Nigeria. Benin City, the capital of Edo State, experiences a tropical climate with distinct wet and dry seasons, which can influence airborne microbial load. The selected eateries (University Buka and Home and Away) were situated in different areas of the campus and were representative of typical food service environments within the University. These eateries were characterized by moderate to high customer traffic, basic or mixed ventilation systems, and variable levels of environmental hygiene.

3.2 Study Design

A cross-sectional study design was employed, and airborne bacterial samples were collected from indoor environments of the selected eateries. Sampling was conducted during peak business hours when customer activities were at their highest, as this period was associated with increased microbial load due to human movement, talking, and food handling. The sampling focused on areas where food preparation, serving, and customer interactions were most frequent.

3.3 Sampling Sites

Airborne bacterial samples were collected from two eateries within the University of Benin. Within each eatery, three specific sampling points were identified:

1. **Food Preparation Area** – where meals were cooked or prepared before serving.
2. **Serving Area** – where food was displayed or served to customers.
3. **Customer Seating Area** – where customers consumed their meals

The selection criteria included high customer traffic, indoor cooking or food-serving operations, and varied ventilation conditions (natural or mechanical).

3.4 Sample Collection Method

The settle plate method, as described by the American Public Health Association (APHA, 2017), was used for sampling airborne bacteria. Sterile Petri dishes containing nutrient agar (for total heterotrophic bacteria) were prepared and labeled. The plates were exposed at a height of approximately 1 meter above the ground (representing the average breathing zone) and 1 meter away from walls or obstructions. Each plate was exposed for 15 minutes to allow airborne bacteria to settle on the medium. Control plates were left unopened to ensure media sterility. Following exposure, the plates were immediately covered, sealed with parafilm, and transported in sterile containers to the Microbiology Laboratory, Faculty of Life Sciences, University of Benin, for analysis within 1 hour of collection.

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°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

Twenty-eight grams (28 g) of nutrient agar was dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium 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 ofCitrate agar

A 24.28grams 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 dispensing 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/m^3**)** using a standard formula for settle plate enumeration:

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

Where:

- **a** = number of colonies counted on the agar plate
- **p** = surface area of the plate (in cm^2); for a 90 mm Petri dish, p = 63.6 cm^2
- **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/m^3.

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. These parameters were evaluated by physical examination. Microscopy was also carried out using n-lactophenol cotton blue staining and a bright field microscope.

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

The Triple Sugar Iron (TSI) test is an ability to test an organism's capability to ferment sugars and to produce hydrogen sulphide (H_2S) or gas (O_2), or both. The test was used primarily to differentiate members of the *Enterobacteriaceae* family based on their sugar fermentation patterns and from other Gram-negative rods. 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 1.5×10^8 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.

$$MARindex = \frac{y}{nx}$$

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

$$n = number of isolates and$$

$$x = total number of antibioitics$$

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

The mean bacterial load of air samples collected from selected eateries within the University of Benin (UNIBEN) over a three-week period is presented in Table 4.1. Among the eateries, University Buka consistently exhibited higher bacterial loads compared to Home and Away throughout the study. The lowest bacterial load was recorded at Home and Away in Week 3 ($0.7 \pm 0.04 \times 10^2$ CFU/m^3), while the highest was observed at University Buka in Week 1 ($3.22 \pm 0.5 \times 10^2$ CFU/m^3).

The cultural, morphological, and biochemical characteristics of bacterial isolates are presented in Table 4.2. Six distinct bacterial species were identified: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella* spp., *Bacillus* spp.and *Pseudomonas aeruginosa*. The identification was based on colony elevation, margin, color, shape, size, Gram staining, cell arrangement, and biochemical tests such as catalase, indole, citrate utilization, oxidase, sugar fermentation, and triple sugar iron (TSI) reaction

The distribution of airborne bacterial isolates over the three weeks is presented in Table 4.3. *Staphylococcus aureus* was the most frequently isolated organism, accounting for 26.8% of the total isolates, followed by *Bacillus* spp.(21.4%) and *Staphylococcus epidermidis* (17.9%). *Escherichia coli* contributed 12.5%, while *Klebsiella* spp. and *Pseudomonas aeruginosa* each accounted for 7.1% of the isolates. The distribution pattern showed that *S. aureus* and *B. subtilis* were consistently present throughout the study period, while *K. pneumoniae* and *P. aeruginosa* were absent in Week 3.

Table 4.1: Mean Bacterial Load (x10^2 CFU/m³) of Air Samples from Selected Eateries in UNIBEN Over Three Weeks

Week	University Buka (CFU/m³)	Home and Away (CFU/m³)
Week 1	3.22 ± 0.5	0.8 ± 0.05
Week 2	2.33 ± 0.8	0.9 ± 0.06
Week 3	3.04 ± 0.6	0.7 ± 0.04

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

Characteristics						
Elevation	Raised	Raised	Flat	Flat	Raised	Flat
Margin	Entire	Entire	Undulate	Entire	Undulate	Irregular
Color (Colony)	Golden yellow	White/cream	Cream	Cream/mucoid	Cream	Greenish (pigmented)
Shape (Colony)	Circular	Circular	Irregular	Circular	Circular	Irregular
Size	Medium	Small	Large	Large	Medium	Medium
Gram Stain	+	+	-	-	+	-
Cell Type	Cocci	Cocci	Rod	Rod	Rod	Rod
Arrangement	Clusters	Clusters	Disperse	Disperse	Chains	Disperse
Color (Gram Reaction)	Purple	Purple	Pink	Pink	Purple	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/A	K/K
Identity	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Escherichia coli*	*Klebsiella* spp.	*Bacillus* spp.	*Pseudomonas aeruginosa*

Key: (-) negative test; (+) positive test; (A) Acid; (K) Alkaline; (G) Gas production (bubbles); (H_2S) Hydrogen sulphide (black precipitate); (KOH) Potassium hydroxide test; (TSI) Triple sugar iron test.

Table 4.4 shows the frequency of occurrence of isolates from both eateries. *S. aureus* was the most frequently occurring bacterium, representing 27.8% of isolates, with slightly higher presence at Home and Away (29.2%) compared to University Buka (26.7%). *Bacillus* spp.followed with 22.2%, occurring more in University Buka (23.3%) than in Home and Away (20.8%). *S. epidermidis* occurred at 18.5% overall, while *E. coli* accounted for 13.0%. *Klebsiella* spp. and *P. aeruginosa* were only isolated from University Buka (5.6% each) and were absent in Home and Away.

The antibiotic susceptibility profile of the bacterial isolates is presented on Table 4.5. Most isolates showed high resistance to multiple antibiotics. *Klebsiella* spp. exhibited the highest resistance, with 88.9% of tested antibiotics being ineffective. *E. coli* also showed a high resistance rate (77.8%), while *S. epidermidis* and *P. aeruginosa* recorded 66.7% and 55.6% resistance, respectively. On the other hand, *Bacillus* spp. displayed the least resistance (44.4%). Among the antibiotics tested, ofloxacin (OFL) and gentamicin (GEN) were the most effective across several isolates, while ampiclox (AMPX), ampicillin (AMP), and clarithromycin (CLT) were largely ineffective.

The MAR index values of bacterial isolates are shown in Table 4.6. *K. pneumoniae* had the highest MAR index (0.89), followed by *E. coli* (0.78), *S. epidermidis* and *P. aeruginosa* (0.67 each), and *S. aureus* (0.56). *Bacillus* spp.exhibited the lowest MAR index (0.44).

Table 4.7 presents the public health significance of bacterial loads in air samples from selected eateries within the University of Benin over a three-week period. The contamination levels were categorized based on established codes, ranging from very low (VL) to very high (VH) bacterial presence. University Buka consistently exhibited high (H) bacterial contamination across all three weeks, indicating a substantial microbial presence in the indoor air. In contrast, Home and Away maintained low (L) contamination levels throughout the same period

Table 4.3: Distribution of Airborne Bacterial Isolates from Selected Eateries in UNIBEN Over Three Weeks

Bacterial Isolates	**Week 1 (n, %)**	**Week 2 (n, %)**	**Week 3 (n, %)**	**Total (n, %)**
Staphylococcus aureus	4 (25.0%)	6 (27.3%)	5 (27.8%)	15 (26.8%)
Staphylococcus epidermidis	3 (18.8%)	4 (18.2%)	3 (16.7%)	10 (17.9%)
Bacillus spp.	3 (18.8%)	5 (22.7%)	4 (22.2%)	12 (21.4%)
Escherichia coli	2 (12.5%)	3 (13.6%)	2 (11.1%)	7 (12.5%)
Klebsiella spp.	2 (12.5%)	2 (9.1%)	–	4 (7.1%)
Pseudomonas aeruginosa	2 (12.5%)	2 (9.1%)	–	4 (7.1%)
Total Isolates	16 (100%)	22 (100%)	18 (100%)	56 (100%)

Table 4.4: Frequency of Occurrence of Airborne Bacterial Isolates from University Buka and Home and Away over Three Weeks

Bacterial Isolates	**University Buka (n, %)**	**Home and Away (n, %)**	**Total (n, %)**
Staphylococcus aureus	8 (26.7%)	7 (29.2%)	15 (27.8%)
Staphylococcus epidermidis	5 (16.7%)	5 (20.8%)	10 (18.5%)
Bacillus spp.	7 (23.3%)	5 (20.8%)	12 (22.2%)
Escherichia coli	4 (13.3%)	3 (12.5%)	7 (13.0%)
Klebsiella spp.	3 (10.0%)	–	3 (5.6%)
Pseudomonas aeruginosa	3 (10.0%)	–	3 (5.6%)
Total Isolates	30 (100%)	20 (100%)	50 (100%)

Table 4.5: Antibiotic Susceptibility Profile of Bacterial Isolates

Antibiotics	***S. aureus***	***S. epidermidis***	***E. coli***	*Klebsiella* spp.	*Bacillus* spp.	***P. aeruginosa***
AUG	R	S	R	R	S	R
PEF	R	R	R	R	S	R
CLT	R	R	S	R	R	R
CHL	S	R	R	R	S	R
AMP	R	R	R	R	I	R
OFL	S	S	R	R	S	S
AMPX	R	R	R	R	R	R
GEN	S	S	R	R	R	S
CIP	S	I	R	R	R	S
NR (%)	5 (55.6%)	6 (66.7%)	7 (77.8%)	8 (88.9%)	4 (44.4%)	5 (55.6%)
NS (%)	4 (44.4%)	2 (22.2%)	2 (22.2%)	1 (11.1%)	3 (33.3%)	4 (44.4%)
NI (%)	Nil	1 (11.1%)	Nil	Nil	1 (11.1%)	Nil

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

NR % = Number of resistance in percentage
NS = Number of susceptible in percentage NI% = Number of intermediate in percentage

Table 4.6: Multiple Antibiotic Resistance (MAR) Index of Bacterial Isolates

Bacterial Isolates	**No. of Antibiotics Tested**	**No. of Antibiotics Resistant**	**MAR Index**
Staphylococcus aureus	9	5	0.56
Staphylococcus epidermidis	9	6	0.67
Escherichia coli	9	7	0.78
Klebsiella spp.	9	8	0.89
Bacillus spp	9	4	0.44
Pseudomonas aeruginosa	9	6	0.67

Table 4.7: Public Health Significance of Bacterial Load from Selected Eateries Over Three Weeks (×10^2 CFU/m^3)

Contamination Level (Code):

Week	**University Buka**	**Home and Away**
Week 1	H	L
Week 2	H	L
Week 3	H	L

Key:

Values	**Degree of Contamination**	**Code**
<50	Very low	VL
50–100	Low	L
100–500	Intermediate	I
500–2000	High	H
>2000	Very high	VH

CHAPTER FIVE

DISCUSSION

Airborne bacteria constitute a significant aspect of environmental microbiology and have important implications for public health, particularly in settings where food is prepared and consumed. Eateries, especially those located in densely populated institutions such as universities, serve as potential reservoirs and transmission points for microbial contaminants. Exposure to pathogenic and opportunistic bacteria through inhalation or contact with contaminated surfaces can lead to foodborne illnesses, respiratory infections, and other health complications (Qian *et al*., 2012; Li *et al*., 2016). This study presents the bacteriological assessment of air samples collected from selected private eateries within the University of Benin (UNIBEN), highlighting the public health significance, patterns of antibiotic resistance, and implications for food safety.

The results from this study indicate significant differences in the mean bacterial load between University Buka and Home and Away over a three-week period. University Buka consistently exhibited higher bacterial loads, with the highest recorded at $3.22 \pm 0.5 \times 10^2$ CFU/m^3 in Week 1, while Home and Away maintained lower levels, with the lowest at $0.7 \pm 0.04 \times 10^2$ CFU/m^3 in Week 3. These findings align with studies by Osimani*et al*. (2016), who reported that environmental factors such as poor ventilation, high human traffic, and inadequate sanitation contribute to elevated airborne bacterial loads in food establishments. University Buka's consistently high contamination levels suggest environmental conditions conducive to microbial proliferation, such as crowded settings, inadequate air circulation, or suboptimal cleaning practices. In contrast, Home and Away's lower bacterial loads (categorized as "Low") may reflect better hygiene practices, improved ventilation, or lower customer density.

The variation in bacterial loads between the two eateries underscores the importance of environmental management in food service settings. According to the World Health Organization (WHO, 2019), high microbial loads in indoor air can increase the risk of respiratory infections and food contamination, particularly in settings where food is prepared and consumed. The high bacterial loads at University Buka suggest a potential public health concern, as airborne bacteria can settle on food surfaces, utensils, or food handlers, increasing the risk of foodborne illnesses. Similar observations have been made in studies assessing airborne contamination in food processing environments, where microbial aerosols from human activity and poor sanitation were identified as key contributors to elevated bacterial counts (Denyerand Baird, 2007). Furthermore, research on air quality in school dining services has shown that bacterial loads in indoor air can directly correlate with surface contamination, amplifying cross-contamination risks in eateries (2015). In a study evaluating kitchen air in restaurants, bacterial densities were found to vary seasonally, with higher loads in warmer periods potentially exacerbating contamination in tropical climates like Nigeria's (Asgharzadeh*et al*., 2019).

In this study, six bacterial isolates (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*) were identified based on cultural, morphological, and biochemical characteristics. The predominance of *S. aureus* (26.8%) and *B. subtilis* (21.4%) across the study period is consistent with their ubiquitous nature in indoor environments. *S. aureus*, a common human commensal, is often shed from skin and respiratory secretions, as noted by Dancer (2008), and its high prevalence in both eateries suggests contamination from human sources, such as food handlers or customers (Kadariya *et al*., 2014). *B. subtilis*, a spore-forming bacterium, is commonly found in dust and soil, and its presence may indicate environmental contamination from external sources, as reported by Shaffer and Lighthart (1997).

The presence of *E. coli* (12.5%) and *K. pneumoniae* (7.1%) is particularly concerning due to their association with fecal contamination and opportunistic infections. *E. coli* is a well-documented indicator of poor hygiene, often linked to inadequate handwashing or contaminated water sources (WHO, 2017; Dokuta *et al*., 2025). Its detection in both eateries, albeit at a lower frequency in Home and Away, suggests potential lapses in sanitation practices. *K. pneumoniae* and *P. aeruginosa*, both absent in Week 3and only isolated from University Buka, are known opportunistic pathogens associated with respiratory and wound infections (Podschunand Ullmann, 1998; Li *et al*., 2023). Their exclusive presence in University Buka further highlights the eatery's poor environmental hygiene compared to Home and Away.

The consistent isolation of *S. aureus* and *B. subtilis* across all weeks suggests their resilience in indoor environments, likely due to their ability to survive on surfaces and in aerosols (Kowalski, 2011). In contrast, the absence of *K. pneumoniae* and *P. aeruginosa* in Week 3 may reflect temporal variations in environmental conditions, such as improved cleaning or reduced human activity, although further investigation is needed to confirm this. Additionally, concentrations of *Staphylococcus* species in indoor air have been associated with human occupancy, reinforcing the role of crowd density in eateries like University Buka (Madsen *et al*., 2018).

The antibiotic susceptibility profiles reveal alarming levels of resistance among the bacterial isolates, with *K. pneumoniae* exhibiting the highest resistance (88.9%) and *B. subtilis* the lowest (44.4%). The high multiple antibiotic resistance (MAR) indices, particularly for *K. pneumoniae* (0.89) and *E. coli* (0.78) (Table 4.6), indicate significant exposure to selective pressures, likely from overuse or misuse of antibiotics in the environment or community. These findings are consistent with global trends reported by the WHO (2020), which highlight the growing threat of antimicrobial resistance (AMR) in community settings, including food establishments (Larsson and Flach, 2022).

The effectiveness of ofloxacin (OFL) and gentamicin (GEN) against most isolates suggests that these antibiotics remain viable treatment options for infections caused by these bacteria. However, the widespread resistance to ampiclox (AMPX), ampicillin (AMP), and clarithromycin (CLT) is concerning, as these are commonly used antibiotics. The high resistance rates of *K. pneumoniae* and *E. coli* align with studies by Messi *et al*., (2015), who noted that Gram-negative bacteria in indoor environments often exhibit multidrug resistance due to their ability to acquire resistance genes. The relatively lower resistance of *B. subtilis* may be attributed to its environmental origin and lower exposure to clinical antibiotics, as suggested by Logan and De Vos (2009).

The MAR index values provide a quantitative measure of resistance severity, with values above 0.2 indicating significant environmental antibiotic exposure (Krumperman, 1983). All isolates in this study exceeded this threshold, with *K. pneumoniae* approaching near-total resistance (0.89). This suggests that the eateries' environments may serve as reservoirs for resistant bacteria, posing a risk of transmission to food handlers, customers, and the broader community. Research on airborne antibiotic resistomes has shown that human-impacted environments, such as food processing areas, act as hotspots for the dissemination of resistance genes through aerosols (Gwenzi *et al*., 2022). Moreover, antibiotic-resistant airborne bacteria in restaurant environments have been detected, illustrating the potential for kitchens to become sources of multidrug-resistant pathogens. Studies linking air pollution to rising antibiotic resistance further emphasize how environmental factors in densely populated areas like university campuses could exacerbate AMR in airborne microbes.

The bacterial loads in University Buka as "High" (H) across all three weeks, while Home and Away maintained "Low" (L) contamination levels. The high bacterial loads in University Buka, coupled with the presence of pathogenic and multidrug-resistant bacteria, indicate a significant public health risk. Airborne bacteria can contribute to the spread of respiratory infections, skin infections, and foodborne illnesses, particularly in settings with high human traffic (Hoseinzadeh*et al*., 2013). The presence of *S. aureus*, a known cause of food poisoning and skin infections, and *E. coli*, an indicator of fecal contamination, underscores the need for stringent hygiene measures in University Buka.

The lower contamination levels in Home and Away suggest better adherence to hygiene protocols, such as regular cleaning, proper ventilation, or food handler training. However, the presence of *S. aureus* and *E. coli* in Home and Away, albeit at lower frequencies, indicates that even well-maintained eateries are not immune to microbial contamination. This is consistent with findings by Stellato*et al*. (2015), who noted that airborne bacteria in food establishments are influenced by multiple factors, including human activity and environmental conditions, regardless of hygiene practices. Airborne contamination in the food industry has been reviewed as a microbiologist's perspective, emphasizing its variable public health significance depending on the setting.

The high prevalence of antibiotic-resistant bacteria, particularly *K. pneumoniae* and *E. coli*, raises concerns about the potential for treatment failures in infections acquired from these environments. The WHO (2021) emphasizes that AMR in community settings can exacerbate the burden of infectious diseases, particularly in low-resource settings like university campuses, where access to advanced medical care may be limited. The findings suggest that University Buka, in particular, could serve as a hotspot for the dissemination of resistant bacteria, necessitating targeted interventions. Pathogenic airborne fungi and bacteria have been reviewed for their health impacts, noting increased risks under polluted conditions. Additionally, food safety risks from unsafe foods containing harmful bacteria contribute to over 200 diseases, aligning with the observed microbial profiles.

Thisresult highlights the need for improved environmental hygiene and infection control measures in University Buka. Strategies such as enhanced ventilation systems, regular air quality monitoring, and strict adherence to food safety protocols could reduce bacterial loads and mitigate public health risks. Training food handlers on proper hygiene practices, including handwashing and surface disinfection, is critical, as human activities are a primary source of airborne bacteria (Dancer, 2008). Additionally, the high levels of antibiotic resistance underscore the importance of antimicrobial stewardship programs to curb the misuse of antibiotics in the community.Home and Away's lower bacterial loads and absence of certain pathogens (*K. pneumoniae* and *P. aeruginosa*) suggest that it serves as a model for better hygiene practices. Comparative studies of the two eateries' operational practices could provide insights into effective interventions for reducing airborne bacterial contamination. Monitoring airborne pathogen transmission in food industries has been proposed to enhance safety, particularly for aerosolized bacteria in meat processing, which shares similarities with eatery environments. Person-to-person transfer of microorganisms through food further emphasizes the role of airborne routes in contamination chains.

5.2 Conclusion

The study revealed that indoor air in selected eateries within UNIBEN harbors diverse bacterial populations, including opportunistic and pathogenic species. University Buka exhibited higher bacterial loads and contamination levels compared to Home and Away, suggesting potential health risks for patrons. The high antibiotic resistance observed among isolates, particularly *K. pneumoniae* and *E. coli*, highlights the threat of multidrug-resistant bacteria in indoor air, which could complicate treatment of infections. Overall, the findings highlight the public health significance of airborne bacteria in food establishments and the need for effective control measures to protect consumers.

5.3. Recommendations

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

1. **Implementation of hygiene protocols:** Eateries should adhere to strict cleaning and sanitization standards to minimize microbial contamination.
2. **Regular monitoring of indoor air quality:** Routine microbial assessments should be conducted to identify potential health risks and implement corrective measures.
3. **Antibiotic stewardship:** Proper use of antibiotics in the community should be encouraged to reduce the spread of multidrug-resistant organisms.
4. **Education of food handlers:** Staff should be trained in personal hygiene, safe food handling practices, and contamination prevention strategies.
5. **Improvement of ventilation systems:** Enhanced airflow and proper ventilation can reduce microbial accumulation in indoor air.

6. **Further research:** Additional studies should investigate the seasonal variations of airborne bacteria, their virulence factors, and potential links to foodborne illnesses

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