MICROBIAL AIR QUALITY OF MUNICIPAL BUSES IN BENIN CITY.

BY

Aisha Patricia MOMODU

LSC2003104

DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY.

JANUARY, 2025

MICROBIAL AIR QUALITY OF MUNICIPAL BUSES IN BENIN CITY.

BY

Aisha Patricia MOMODU

LSC2003104

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.

JANUARY, 2025

CERTIFICATION

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

________________________________ ________________________

DR. (MRS). R. ADAMS **DATE**

(Project Supervisor)

APPROVAL

This project work was carried out by **Aisha Patricia MOMODU** in partial fulfillment of the award of a Bachelor of Science, B.Sc (Hons) degree in the Department of Microbiology, University of Benin, Benin City.

______________________________ ______________________

PROF. (MRS.) F. I. AKINNIBOSUN **DATE**

(Head of Department)

DEDICATION

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

`

ACKNOWLEDGEMENTS

I would like to express my profound gratitude and appreciation to God Almighty and also to my supervisor **DR. (MRS). R. ADAMS** for her invaluable aid during my project. I am also grateful to **PROF. (MRS.) F. I. AKINNIBOSUN** the Head of Department, and all the staff members of the Department of Microbiology for their assistance.

I would also like to give a big thank you to my parents Mr. and Mrs. **MOMODU**, for their love, words of encouragement and support

I would like to appreciate Aduba Osaretin for his support and encouragement throughout my project work.

Finally, I would like to appreciate my friends Victor, Nana and Stephen Isunu for their encouragement and support throughout my academic journey and lastly my Brother **MOMODU OSHOKE** for also contributing totally towards the success of my academics. I love you all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Airborne microbial contamination poses a significant public health challenge, particularly in confined spaces such as public transport systems where ventilation may be inadequate and passenger density is high. This study investigates the microbial air quality of municipal buses in Benin City, Nigeria, focusing on bacterial contamination levels and the antimicrobial resistance (AMR) profiles of isolated organisms. Using the settle plate method for microbial isolation, bacterial samples were collected from four major bus routes in Benin City: New Benin, Ring Road, Mission Road, and Uselu. Identification of the isolates was based on cultural, morphological, and biochemical characteristics. The findings revealed varying levels of bacterial contamination across the bus routes, with the highest mean viable bacterial count ($2.34 \times 10^3 \pm 0.34$ CFU/m^3) recorded on the Ring Road bus and the lowest ($1.25 \times 10^3 \pm 0.08$ cfu/m^3) on the Mission Road bus. Bacterial species identified included *Escherichia coli, Bacillus* sp., *Proteus* sp., *Micrococcus* sp., *Pseudomonas aeruginosa, Staphylococcus* sp., and *Enterococcus faecalis*, with *Staphylococcus* sp. exhibiting the highest frequency of occurrence (60%) across all routes. Antibiotic susceptibility testing revealed alarming multidrug resistance (MDR) patterns, particularly in *Staphylococcus* sp., emphasizing the growing threat of AMR in public spaces. The bacterial counts observed in this study surpassed WHO-recommended indoor air quality limits, underscoring the necessity for improved sanitation and disinfection protocols within municipal abuses. The study provides critical data to inform public hygiene policies and supports initiatives aimed at enhancing microbial safety in public transport systems, particularly in resource-limited settings like Benin City.

Keywords: Microbial air quality, municipal buses, Benin City, bacterial contamination, antimicrobial resistance, multidrug resistance, settle plate method, indoor air quality, public transport hygiene, WHO standards.

CHAPTER ONE

INTRODUCTION

1.0 Background of study

Public transportation systems are vital to urban life, ensuring that large populations have access to affordable and efficient mobility. In cities like Benin City, Nigeria, where economic activities depend heavily on public buses, these vehicles play a critical role in maintaining the flow of goods and services. However, as essential as these systems are, they can also pose public health risks. One of the less visible but increasingly recognized hazards is the microbial air quality inside buses, which refers to the presence and concentration of various microorganisms, including bacteria, fungi, and viruses, in the air passengers breathe. Poor microbial air quality has potential implications for the spread of infectious diseases, particularly in densely populated environments (Hospodsky *et al.,* 2012). Human activities and the operation of equipment within indoor environments are considered the primary factors contributing to the accumulation and dissemination of airborne microbial contamination (Hospodsky *et al.,* 2012; Qian *et al.,* 2012; Taubel *et al.,* 2009).

As these buses operate continuously throughout the day, with numerous passengers boarding and alighting, the risk of bacterial contamination and the presence of airborne pathogens may increase significantly (Gao *et al.,* 2019). These buses operate in enclosed or semi-enclosed environments where air exchange with the outside is often limited, especially when windows are closed, or air conditioning is inadequate. These conditions create opportunities for airborne microorganisms to thrive and spread. Microbial contaminants can come from several sources, including passengers' skin, respiratory secretions, or clothing, as well as dust and dirt carried into the bus. Additionally, the bus's surfaces, such as seats, handles, and floors, can act as reservoirs for microbes, which can become aerosolized during normal bus activities like passenger movement and vibration from the bus itself (Almatawah *et al.,* 2022).

Benin City's tropical climate, characterized by high humidity and temperatures, can further exacerbate the microbial load within buses. Warm and humid environments have been shown to support microbial growth, particularly for fungi and bacteria. Additionally, high humidity increases the survival and dispersal of bacteria, making the microbial air quality a pressing concern, particularly in crowded and poorly ventilated buses (Pansanen *et al.,* 2000).

Inadequate ventilation further compounds the issue of poor air quality. Buses often lack the sophisticated air filtration systems found in modern buildings, and air exchange is limited when the windows are closed. As a result, airborne particles, including microorganisms, accumulate and circulate within the confined space. The limited air movement allows for a higher concentration of microbes in the air, increasing the risk of inhalation by passengers and bus operators. This issue is particularly concerning during rush hours when buses are overcrowded, and the chances of disease transmission are heightened (Van Ryswyk *et al.,* 2021).

The public health risks associated with poor microbial air quality in buses are significant, particularly in densely populated urban centers. Studies have demonstrated that pathogens commonly found in public transport systems, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, are capable of causing a variety of infections, ranging from respiratory illnesses to skin infections and gastrointestinal issues (Srikanth *et al.,* 2008, Mohammed, 2023).).

The prevalence of these organisms in public buses suggests that passengers, especially those with weakened immune systems, are at an increased risk of exposure to pathogens. Furthermore, the transmission of airborne microbes is facilitated by the close contact between passengers, prolonged occupancy, and limited airflow in buses.

Research has also highlighted the presence of antibiotic-resistant bacteria in public transportation, which presents an additional public health challenge. Bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and multi-drug resistant *Pseudomonas* species have been isolated from public transport systems in various cities worldwide, raising concerns about the role of these environments in the spread of antimicrobial resistance (O'Donnell *et al*., 2008) The presence of such organisms in buses in Benin City could have serious implications for the spread of resistant infections, particularly in a healthcare context where antibiotic resistance is already a growing concern.

The microbial air quality in buses cannot be examined in isolation from broader environmental and social factors. In many Nigerian cities, including Benin City, public health infrastructure is often inadequate to meet the demands of a growing population. Factors such as limited access to clean water, poor waste management, and overcrowded living conditions contribute to an environment in which microbes can proliferate and be easily transmitted (Tran, 2020). Public buses, which are used by a large segment of the population, become conduits for the spread of these microorganisms.

Moreover, economic constraints may limit the frequency and thoroughness of cleaning and maintenance practices within the transportation system. Without regular and effective cleaning of buses, microbial contamination is likely to persist and build up over time. A study by Kim *et al*. (2018) indicated that the frequency of cleaning significantly impacts microbial load in public transport vehicles, with buses that undergo routine cleaning showing lower microbial contamination levels compared to those that do not. In Benin City, where bus operators may lack the resources or awareness to implement adequate hygiene measures, the microbial air quality in public buses may be significantly compromised.

1.2. Research Aim and Objectives

The aim of this project is to assess the bacterial contamination of air quality in municipal in Benin City, Nigeria. Specifically, the project aims to determine the microbial load in municipal buses within different locations in Benin City, shedding light on the extent to which passengers contribute to microbial contamination.

To achieve this aim, several objectives have been outlined:

1. To quantify the microbial load in the air environment of municipal buses within different locations in Benin City
2. To determine the total bacterial count.
3. To identify potential microorganisms, present in the air environment of municipal buses.
4. To determine the antibiotic susceptibility of the bacteria isolated.

CHAPTER TWO

LITERATURE REVIEW

2.0 Air Quality

Air quality is defined as the state or quality of air in a certain location and is influenced by the surrounding atmospheric conditions. Volcanic eruptions, wind erosion, particulate matter (from dust), biogenic emissions from plants and trees (natural factors), fossil fuel combustion power stations, vehicular and traffic emissions, aerosols, and landfills (anthropogenic factors) all have an impact on air quality (n,). Air quality in urban environments can be influenced by factors such as building site and design, building renovations, occupant densities within the building, and maintenance of air-cleaning devices or systems (OSHA, 2011).

Indoor and outdoor air quality including that of a university environment are influenced by physical, chemical and biological factors (Badea *et al.*, 2015) such as vehicular and generator emissions, other power generating equipment and the burning of fossil fuels in the environment. As the quality of air worsens, the negative impact on individuals in the nearby or surrounding areas intensifies. Declining air quality signifies higher levels of harmful pollutants, leading to adverse health effects. Conversely, optimal air quality is defined by clean and unpolluted air, playing a vital role in ensuring the comfort, health, and overall wellness of people (Thomson *et al.*, 2003). Air quality can be ascertained by the presence of unpleasant odours or stuffy air. –Air Quality Index (AQI) is defined as a measure of the condition of air relative to the requirements of one or more biotic species or to any human need (Johnson *et al.,* 2012). The AQI is divided into ranges, which are numbered and labeled with color codes. It assigns a number ranging from a healthy standard level of zero to a very hazardous level of greater than 300 to indicate the level of health risk associated with air quality.

2.1 AIR POLLUTION

Air pollution refers to the presence of harmful substances or contaminants in the air that disrupt human health, well-being, and the environment negatively. The consequences of air pollution include health issues in humans, compromised unsafe conditons such as poor visibility in, dangerous driving circumstances, degradation of scenic locations, stained clothing, deterioration of buildings and metals, as well as the contamination of monuments and public structures (Sahu *et al*., 2014)

Air pollution is defined as all destructive effects of any sources which contribute to the pollution of the atmosphere and/or deterioration of the ecosystem. Air pollution is caused by both human interventions and/or natural phenomena. It is made up of many kinds of pollutants including materials in solid, liquid, and gas phases (vallero 2007). From an ecological standpoint, air pollution can lead to significant harm to the atmosphere, soil, and groundwater (Lovett *et al*., 2009; Mellouki *et al*., 2016). It is also a serious threat to the diversity of life. Studies on the relationship between air pollution and reducing species diversity clearly show the detrimental effects of environmental contaminants on the extinction of animals and plants species (Camargo *et al*., 2006). Harmful substances carried in the air can also result in reproductive issues in animals. Additionally, air pollution gives rise to ecological problems like acid rain, temperature inversions, and alterations in global climate patterns, which stem from the release of greenhouse gases into the atmosphere (Schneider, 1989).

2.2 AIR POLLUTANTS AND THEIR TOXICITIES

Air pollutants include any substances present in the atmosphere that can pose risks or cause harm to the human well-being and the environment. The World Health Organization (WHO) identifies six significant air pollutants responsible for detrimental effects on human health and ecosystems: particle pollution, ground-level ozone (O3), carbon monoxide (CO), sulfur oxides, nitrogen oxides, and lead (Pb). Suspended particulate matter like dust, fumes, smoke, mists, along with gaseous pollutants, hydrocarbons, volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), and halogen derivatives are among the various pollutants found in the air. Elevated concentrations of these pollutants are associated with increased susceptibility to various diseases, including various forms of cancer (Loomis *et al.,* 2014).

Airborne pollutants encompass substances existing in the atmosphere that can potentially harm human health and the environment. The World Health Organization (WHO) identifies six main air pollutants—particles, ground-level ozone (O3), carbon monoxide (CO), sulfur oxides, nitrogen oxides, and lead (Pb)—as agents that endanger human well-being and ecosystems. These pollutants include suspended particles like dust, smoke, and mists, as well as gaseous contaminants such as hydrocarbons, volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), and halogen derivatives. When present at high levels, these substances can lead to various diseases, including cancer, making individuals more vulnerable to health problems. The following section offers a brief overview of these primary air pollutants and their harmful effects on different organs in the human body, along with the diseases they are associated with.

Particle pollutants are major parts of air pollutants. In a simple definition, they are a mixture of particles found in the air. Particle pollution which is more known as PM is linked with most of pulmonary and cardiac-associated morbidity and mortality (Sadeghi *et al*., 2015). They have varied in size ranging mostly from 2.5 to 10 μm ($PM_{2.5}$ to PM_{10}).

The size of these particle pollutants plays a critical role in the development and progressionof the lungs and heart diseases. Smaller particles can reach the deeper parts of the respiratory system, increasing the potential for causing the lungs and heart diseases. Also, numerous scientific data have demonstrated that fine particulate pollutants are associated with premature death in individuals with heart and/or lung conditions, including problems like irregular heartbeats, nonfatal heart attacks, exacerbated asthma, and decreased lung function. The severity of illnesses stemming from exposure to particulate pollutants varies depending on the degree of exposure. Common clinical manifestations of respiratory issues due to air pollution include wheezing, coughing, dry throat, and reduced physical capacity due tobreathing difficulties (Bentayeb *et al.*, 2013; Guillam *et al.*, 2013).

Prolonged exposure to current levels of fine particulate matter (PM) in the air can significantly shorten one's lifespan. The increase of cardiopulmonary and lung cancer mortality are the main reasons for the reduction in life expectancy. Both adults and children experience decreased lung function, which can result in conditions like asthmatic bronchitis and chronic obstructive pulmonary disease (COPD). These illnesses lead to a lower quality of life and a reduced overall lifespan. Strong evidence on the effect of long-term exposure toPM on cardiovascular and cardiopulmonary mortality come from cohort studies (Zhou *et al.,* 2014).

2.2.1 Ground-level ozone

−Ozone, with the chemical formula O3, is a colourless gas that serves as a major component of the Earth’s atmosphere. This gas is present both at ground level and in the upper layers of the atmosphere known as the troposphere. Ground-level ozone (GLO) forms when oxides of nitrogen and volatile organic compounds (VOCs) from natural sources or human activities chemically react. GLO believed to have a potential link to an increased susceptibility to respiratory ailments, particularly conditions like asthma (Gorai *et al.,* 2014). Functioning as a potent oxidizing agent, ozone captures electrons from other molecules. The respiratory tract's surface lining and the underlying cell membranes contain a substantial amount of polyunsaturated fatty acids, which possess unstable double bonds. Ozone interacts with unpaired electrons, resulting in the creation of ozonides. These compounds then proceed through an unstable zwitterion or trioxolane formation, influenced by the presence of water. Ultimately, these structures recombine or decompose into lipohydroperoxides, aldehydes, and hydrogen peroxide. These pathways are believed to trigger the initiation of lipid radicals and the auto-oxidation process in cell membranes and larger molecules. Additionally, ozone exposure increases the likelihood of DNA damage in epidermal keratinocytes, leading to impaired cellular functioning (McCarthy *et al*., 2013).

Ozone (O_3) triggers a range of harmful effects in both humans and lab animals when present at levels found in many urban areas (Lippmann, 1989). These effects include changes in physical structure, functionality, immune responses, and biochemical processes. Due to its limited solubility in water, a significant proportion of inhaled O_3 reaches deep into the lungs, but its reactivity is filtered out by the nasopharynx in resting rats and humans, approximately 17% and 40% respectively (Hatch *et al*., 1994). On an ecological aspect, O_3 has the potential to decrease carbon assimilation in trees, potentially leading to deforestation. This long-term exposure could consequently impact global food security (Fares *et al*., 2013).

2.2.2 Carbon monoxide

Carbon monoxide (CO) is a colourless and odorless gas generated primarily from burning fossil fuels, especially in situations where combustion is incomplete, like when coal and wood are burned. CO has a strong attraction to haemoglobin, the molecule responsible for carrying oxygen in the bloodstream. Its affinity for haemoglobin is about 250 times higher than that of oxygen. Exposure to varying levels of CO, depending on concentration and duration, can lead to mild to severe poisoning. Common symptoms of CO poisoning include headaches, dizziness, weakness, nausea, vomiting, and even loss of consciousness. These symptoms are similar to those of other illnesses like food poisoning or viral infections.

It's been established that carboxyhemoglobin (COHb) levels below 2% don't pose evident risks to human health, while levels above 40% can be fatal. Hypoxia, apoptosis, and ischemiaare known mechanisms of underlying CO toxicity (Akyol *et al.,* 2014). CO exerts its toxic effects by binding competitively to haemoglobin's heme groups, which results in a reduced availability of oxygen (Hypoxia). Even CO exposures leading to COHb levels above 5% can induce cardiovascular changes. Studies conducted in the early 1990s by the Health Effects Institute investigated the association between CO exposure and cardiovascular disease, particularly angina pectoris, at COHb levels ranging from 2% to 6%. These results showed a potential for triggering premature angina under these circumstances, though the likelihood of ventricular arrhythmias remained uncertain. Consequently, decreasing ambient CO levels has the potential to decrease the risk myocardial infarction in predisposed individuals.

2.2.3 Sulphur dioxide

SO_2 is a colourless, highly reactive gas, which is considered as an important air pollutant. It is mostly emitted from fossil fuel consumption, natural volcanic activities, and industrial processes. SO_2 is very harmful for plant life, animal, and human health. People with lung disease, children, older people, and those who are more exposed to SO_2 are at higher risk of the skin and lung disease Exposure to elevated concentrations of SO_2 cause major health concerns, including respiratory irritation and dysfunction, along with the potential to worsen pre-existing cardiovascular conditions. SO_2 is mainly taken in by the upper respiratory passages.

Functioning as a sensory irritant, it can cause bronchospasms and stimulate mucus secretion in humans. Even lower concentrations of SO_2 (<1 ppm) in polluted urban air, as experienced by residents in industrialized areas, can lead to increased risk of bronchitis. The extent of SO_2 lung penetration is greater when breathing through the mouth compared to nose breathing. Increased airflow during deep, rapid breaths amplifies the gas's entry into the deeper lung regions. Consequently, individuals who exercise outdoors in polluted environments would inhale more SO_2, potentially leading to increased irritation. Upon depositing in airways, SO_2 dissolves into surface fluids as sulfite or bisulfite, distributing throughout the body. Evidence suggests that sulfite interacts with sensory receptors in the airways, causing both local and centrally mediated bronchoconstriction.

According to the Environmental Protection Agency (EPA) of the USA, the level of annual standard for SO_2 is 0.03 ppm. Due to its solubility in water, SO_2 contributes to acid rain formation and acidification of soils. SO_2 reduces the amount of oxygen in the water causing the death of marine species including both animals and plants. Exposure to SO_2 can cause damages to the eyes (lacrimation and corneal opacity), irritation mucous membranes, the skin problems (redness, and blisters), and respiratory tracts infections. Bronchospasm, pulmonary oedema, pneumonitis, and acute airway obstruction are the most common clinical findings associated with exposure to SO_2 (chen *et al.*, 2007)

2.2.4 Nitrogen oxide

Nitrogen oxides are important ambient air pollutants with the potential to increase the susceptibility to respiratory infections (chen *et al.*, 2007). These pollutants primarily originate from vehicle engines, making them closely associated with traffic-related air pollution. While they primarily affect the deeper parts of the lungs, they can lead to inhaled at high concentrations. In comparison to ozone (O3), nitrogen dioxide (NO2) is generally less harmful, but it still presents notable health risks. Notably, exposure to levels of 2.0–5.0 ppm has been observed to affect T-lymphocytes, especially CD8+ cells and natural killer cells that play a crucial role in defending the body against viruses. Although these concentrations might be considered high, epidemiologic studies demonstrate effects of NO_2 on respiratory infection rates in children.

The most common complications of nitrogen oxide toxicity include coughing and wheezing. Additionally, irritation of the eyes, nose, or throat, as well as headaches, difficulty breathing (dyspnea), chest pain, excessive sweating (diaphoresis), fever, bronchospasms, and even pulmonary edema can occur. Another study suggests that nitrogen oxide levels ranging from 0.2 to 0.6 ppm are not harmful to the general population (Hesterberg *et al*., 2009).

2.2.5 Other air pollutants

Other major air pollutants, categorized as substances with carcinogenic and mutagen**ic** compounds, are believed to contribute to the development and advancement of cancer in humans. These pollutants consist of volatile organic compounds (VOCs) like benzene, toluene, ethylbenzene, and xylene; polycyclic aromatic hydrocarbons (PAHs) such as acenaphthene, acenaphthylene, anthracene, and benzopyrene; and other organic pollutants like dioxins. Dioxins, which are unwanted chemical pollutants, are predominantly generated by industrial processes and human activities (Kansal, 2009).

2.4 AIR BORNE BIOAEROSOLS

Bioaerosols are tiny airborne particles, ranging in size from 0.001 to 100 µm, which originate biologically from plants or animals and may contain living organisms. Therefore, bioaerosols can host both pathogenic and non-pathogenic microorganisms such as viruses, bacteria, and fungi (Mandal and Brandl, 2011). Due to their small size and lightweight nature, these particles can be easily transported across different environments. In recent times, there has been increasing attention to the potential impact of bioaerosol exposure on human health, both in occupational and residential settings.

Occupational activities contribute to a wide array of bioaerosol sources, including tasks like waste sorting and composting, agriculture, food processing, and the livestock industry (Pearson *et al.*, 2015). Workers in such environments, susceptible to these exposures, have reported various respiratory symptoms and diseases, including allergic asthma, rhinitis, and airway inflammation (Beck *et al.*, 2012, Rohr *et al.*, 2015. Bioaerosols were estimated to be responsible for approximately 5 to 34% of indoor particulate matter air pollution. These indoor sources include, external elements infiltrating through windows, doors, and ventilationsystems, along with contributions from building materials, furnishings, occupants, pets, houseplants, and organic waste (Nazaroff, 2016). Everyday human activities like coughing, washing, toilet flushing, talking, walking, sneezing, and even floor sweeping can also generate bioaerosols. However, the prevailing environmental conditions, such as temperature and moisture levels, significantly influence the extent of bioaerosol formation and dispersion due to their role in microorganism growth (Dedesko *et al.*, 2015).

Consequently, the prevalence of bioaerosols can be associated with certain human diseases, such as pneumonia, influenza, measles, asthma, allergies, and gastrointestinal illness.

However, under certain circumstances, exposure to some microbes is beneficial for health in terms of developing a healthy immune system and protect children from developing allergies and asthma (Severson *et al*., 2010). Despite the recognized significance of bioaerosols and their influence on human health, it is yet difficult to accurately describe their role in the initiation or worsening of diverse symptoms and diseases.

2.2 Table 2: Microorganisms and some of the major resulting diseases.

Order	Species	Approximate size	Resulting disease	Infection/transmission
1	Legionella pneumophila	Length 2µm Width: 0.3-0.9 µm	Leginonnaires' disease	Inhalation of a water aeros containing the bacteria
2	*Mycobacterium tuberculosis*	Length: 2–4µm Width: 0.2–0.5 um	Tuberculosis	Person to person through the a
3	*Bordetella pertussis*	Length:40–100 nmDiameter: 2 nm	Whooping cough	Direct contact or inhalation ofairborne droplets
4	*Yersinia pestis*	Length: 1–3 µm Width:0.5–0.8 µm	Pneumonic plague	Being bitten by infected rodent flea or by handling infected animals
5	*Bacillus*	Length: 3–5 µm Width: 1.0–1.2 µm	Anthrax	Contact with infected animals, flies, and the breathing of aircontaining *anthrax spores*

(Kim *et al.*, 2018)

2.5. THE ATMOSPHERE AS A SOURCE OF PATHOGENIC MICROBES

Microorganisms carried by dust clouds in the air can directly impact human health by causing pathogenic reactions, exposing sensitive individuals to cellular components like pollen, fungal allergens, and lipopolysaccharides, and triggering sensitivities like asthma with prolonged exposure. Research has shown that airborne fungal and bacterial spores can lead to allergic responses. Many molecules derived from microorganisms, including endotoxins (lipopolysaccharides shed by Gram-negative bacteria membranes) and fungal mycotoxins, can also initiate respiratory issues. These microorganisms can travel vast distances, spanning continents, and dispersing to even the farthest corners of the world.

Microbes that form spores, such as Bacillus species from the Firmicutes phylum and other Gram-positive organisms like Actinobacteria, tend to dominate when examining the diversity of airborne microorganisms through culture-based surveys. Notably, the closest known associations between human disease of microbial origin and dust storms is the occurrence of meningitis outbreaks, particularly Neisseria meningitidis infections, within the meningitis belt of North Africa (Sultan *et al.*, 2005).

2.6 THE ATMOSPHERE AS A SOURCE OF BENEFICIAL MICROBES

Extremophilic microorganisms display the remarkable ability to thrive in environments thatappear inherently hostile, such as those with extreme acidity or alkalinity, high levels of salt,low oxygen levels, wide temperature ranges (from as low as -20°C to as high as 300°C),heavy metal concentrations, high pressure, intense solar irradiation (notably in theultraviolet-UV portion of the spectrum), and even desiccation (drying in the ambient air).Notably, even Earth's atmosphere, hosting strong chemical oxidants like ozone, hydroxyl,and nitrate radicals, can be considered an extreme environment where microorganisms canpersist.

However, while the atmosphere is indeed a challenging habitat, it isn't the most extreme microbial habitat. When measured against factors like pH, temperature, ultraviolet-UV radiation exposure, resource availability, and water presence, the atmosphere seems less extreme than many other environments that still permit microbial habitation and growth (Womack *et al*., 2010). The primary limiting factor for microbial activity in the air is generally low temperature, though research has shown that bacterial activity can occur even at subzero temperatures. Apart from temperature, other limiting factors that hinder microbial survival in the atmosphere include the influence of oxidants and the impact of solar radiation.

Microbial cells have mechanisms to counteract or trap radicals, thanks to specific enzymes like superoxide dismutase or peroxidases. They can also produce a variety of pigments like carotenoids, which absorb UV-B to red wavelengths, thereby preventing DNA damage. Dehydration and desiccation can also affect air microbiota, but their incorporation into cloud and fog droplets can safeguard them, preserving their viability and function. Cloud droplets, being liquid solutions containing carbon and nitrogen sources, offer an advantageous environment for microbial activity in the atmosphere (Delort *et al*., 2010).

Bio-aerosols found in the troposphere might effectively serve as cloud condensation nuclei or ice nuclei, influencing the formation and evolution of clouds. Airborne microorganisms are also thought to contribute to the atmospheric chemical composition by participating in the degradation of organic compounds. The first study demonstrating microbial degradation capabilities in the atmosphere involved bacteria from rainwater breaking down formic and acetic acids. In essence, microorganisms are believed to assume multiple roles in atmospheric chemistry and physics, although the complete extent of their influence remains largely unknown. Furthermore, since microorganisms can endure the rigorous conditions of the atmosphere, they might possess distinct genetic traits that targeted research could harness for societal benefits.

2.7. BACTERIA IN INDOOR AIR

Bacteria are tiny microscopic organisms, found everywhere in nature, air, water, objects and even in and on living things. They can be pathogenic or non-pathogenic. Air acts as a reservoir for bacteria as they cannot grow or multiply in the air but can survive long enough to find suitable medium for growth.

Bacteria in the air can originate from varied sources like humans, pets, walls, outdoor and different furniture. The human body is the home of trillions of microorganisms including viruses, bacteria, archaea, and protists (Xue *et al.,* 2020). These beneficial, neutral, and sometimes opportunistic microbes within the human body are known as normal microbial flora (Gupta *et al.,* 2022).

Indoor air never remains free from microorganisms/spores even clean rooms contain around 25spores/m^3 (Chirca, 2019). Common bacteria found in buildings are mostly saprophytic and they are a part of the normal microflora found in nose, mouth, ear (Moldoveanu, 2015). The most common building associated bacteria are saprophytic bacteria of the normal human skin, mouth and nose that are emitted into the indoor air and bacteria originating from outdoor air.

Other common indoor bacteria include heterotrophic bacteria that grow in water tanks or in damp areas of the building such as bathroom sinks, specially *Legionellae* and environmental non tuberculous mycobacteria thrive in biofilms in water pipes or in refrigeration system water reservoirs. *Actinobacteria, Bacillus* spp and others grow in moist building materials along with fungi. Elements of bacterial structures released into the air include cells fragment, spores, peptidoglycan, volatile organic compounds, exotoxins and other growth metabolites. Indoor airborne bacteria are associated with several infectious diseases and linked to the development and exacerbation of chronic respiratory illnesses including asthma (Fields *et al.,* 2002).

2.7.2. SOURCES OF BACTERIA IN THE INDOOR AIR

Humans are vital sources of bacteria in indoor spaces. The top layers of the skin are usually shed and renewed; the scales are shed into the environment. Bacteria can also be released by actions like talking, coughing, sneezing via aerosol droplets. Respiratory fluid are important sources of bacteria (Johnson and Morawska, 2009). The levels of contamination are dependent on the number of persons in a room and the efficiency of the ventilation system. Materials resuspended from surfaces as a result of human actions is an important source of indoor airborne particles (Ferro *et al.,* 2004)

Pets contribute to the presence of bacteria indoor. Animals like dogs, cats, vermin like mice can release bacteria from their fur into the air. Hygiene, proper disposal and storage of waste can influence the level of bacteria in the air. Accumulated waste can act as a breeding ground for many microbes/ bacteria. Proper disposal of waste, environmental clean up reduces not only the level of fermentation but also the number of bacteria pollutants in the air. In indoor environment, the key sources of microbial generations and transmission include air conditioners, fans, coolers and humidifiers.

2.7.2.1. *Staphylococcus* sp in indoor air

Staphylococcus is a genus of Gram-positive bacteria in the family Staphylococcaceae from the order Bacillales. Under the microscope it appears spherical or cocci and clustered or clumps. When grown on a petri dish with nutrient agar which is a general purpose medium it produces golden yellow colonies, round margin with shiny surface. They are facultative anaerobic microorganisms. It is known to be pathogens of humans and still remains one of the leading causes of nosocomial infections in hospitals with many strains being resistant.

Many species reside as normal flora of the human skin and mucous membrane and some are harmless while others are opportunistic pathogens. It can be released in the air through common human activities of talking, sneezing and coughing. A series of tests can be used to differentiate between Staphylococcus and other facultative anaerobes which are also Gram-positive cocci (Indra *et al.,* 2021). The production of coagulase is one of the most important features used to classify Staphylococci, coagulase is an enzyme that blocks blood clots. Majority of the strains of *S*taphylococcus aureus produce coagulase while others do not.

There are different species and strains of this genus, they include *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Staphylococcus hemolyticus* and so on. *Staphylococcus aureus* is both a normal flora and an opportunistic pathogen responsible for skin and soft tissue infections. *S aureus* possess certain features that enable it to invade and cause disease or aids in their pathogenicity (enzymes, toxins, surface proteins) (Gordon and Lowry, 2008). They can form endospores and biofilms (Conlon, 2014).

They are known to easily acquire resistance to antibiotics especially the penicillin family. This poses as a very serious problem especially in MRSA (Methicillin resistant *Staphylococcus aureus*), this notorious strain causes a lot of diseases from pneumonia to blood poisoning (Agostino *et al.,* 2017). Studies have shown that the most common means of transmission of this specie of staphylococcus of both sensitive and resistant strain is by human to human (Nadimpalli *et al.,* 2018). Some strains are capable of producing immune-modulatory toxins which possess stimulating and mitogenic effects towards T lymphocytes, which may induce shock (Sergelids and Angelidis, 2017). Other virulent factors include production of enzymes that can induce cytolytic effect, like proteases, lipases, evading immune systems, biofilm community (Gordon and Lowry, 2008). *Staphylococcus aureus* is able to form biofilm, defined as a collection of bacteria attached

on a polymeric substrate and communicate between themselves. It is a regulated ecosystem (White *et al.,* 2020). Infections of this bacterium is relating to skin and soft tissue infections.

2.7.2.2. *Pseudomonas* sp in indoor air

A Gram-negative rod, commonly found in the environment such as soil, water. It occurs in moist areas indoors such as sinks and baths. It grows well as 37 degrees and can survive 4-42 degrees. It can cause acute pneumonia with a high mortality rate. It can cause UTIs, bacteremia, bone and joint infection, skin and soft tissue infection, cystic fibrosis. *P aeruginosa* is the most common causative agent in cystic fibrosis, lung disease as other infection is established, survival, quality of lung function decline (Courtney *et al.,* 2007). *P aeruginosa* and other pseudomonads usually colonize moist surfaces. It is a type of bacteria commonly found in indoor air of healthcare facilities. HVAC systems in homes and hospitals are susceptible to this bacterium. It has been implicated with airborne transmission of cystic fibrosis (Jones *et al.,* 2003).

2.8. MICROBIAL COMPONENTS IN INDOOR AIR

2.8.1. Endotoxin

Endotoxins are identified as lipopolysaccharides (LPS) found in Gram-negative bacteria, possessing strong pro-inflammatory properties (Armstrong *et al*., 2013). These endotoxins comprise three main constituents: (1) a core polysaccharide chain, (2) O-specific polysaccharide side chains known as O-antigen, and (3) Lipid A, a lipid component responsible for its toxic effects. Humans are consistently exposed to endotoxins, which can easily attach to dust particles, making them easily inhalable. The body's response to these endotoxins varies based on factors like dosage, location, and exposure route. Exposure to endotoxins was suggested to a cause decrease in lung diffusion capacity along with different symptoms and diseases including fever, shivering, increased white blood cell count, inflammation of the airways with neutrophils, joint pain, difficulty breathing, chest tightness, and obstruction of the bronchial passages (Thilsing *et al*., 2015).

Endotoxins have been identified as a significant contributor to occupational lung diseases and organic dust toxic syndrome. Notably, various occupational groups have reported changes in lung function due to exposure to endotoxins, such as textile workers (average: 2160 EU/m3), dairy workers (average: 329 EU/m3), animal feed and grain workers (average: 662 EU/m3), and sewage treatment plant workers (average: 214 EU/m3) (Cyprowski *et al*., 2015).

2.8.2. β glucans

β-glucans are glucose polymers that occur naturally in the cell walls of various microorganisms such as bacteria, algae, lichens, fungi, yeasts, and certain plants like oats and barley (Kurek *et al*., 2016). In a previous study conducted in Ohio, USA, the indoor and outdoor concentrations of β-glucans were measured as 1.0 (range: 0.81–1.2) and 7.34 ng/m3 (range: 6.1–8.9) respectively. In Beijing, China, levels of airborne (1,3)-β-D-glucan were observed to vary from 0.02 to 1.2 ng/m3 across various sites including offices, hospitals, student dormitories, subway stations, and commercial streets (Dong and Yao, 2010). Another study conducted in 18 commercial concentrated animal feeding operations in Illinois, USA, found airborne (1,3)-β-D-glucan concentrations ranging from 2.4 to 538 ng/m3 (Yang *et al*., 2013). These β-glucans have been used to boost the immune system and in the treatment of conditions like high cholesterol, diabetes, and cancer. However, several studies have indicated that exposure to airborne β-glucans could trigger inflammatory responses and related respiratory symptoms (Richter *et al*., 2015). Therefore, the effect of inhaling β- glucans appears to be influenced by variables like the type of glucan and concurrent exposure.

2.8.3. Allergens

An allergen refers to a substance, often ingested or inhaled, which triggers an abnormal immune response leading to an allergic reaction. Typical symptoms associated with allergens include a runny or congested nose, scratchy throat, itchy eyes, and sneezing. Nevertheless, prolonged exposure to allergens can heighten the risk of allergic ailments such as asthma (Baxi and Phipatanakul, 2010). Allergens stem from various origins, including fungi (spores and hyphae), arthropods (mites and cockroaches), vascular plants (such as pollen, fern spores, and soy dust), pet dander, and even royal jelly (Jutel *et al*., 2016). Multiple factors, like mechanical disturbances, wind, rain, or active emission mechanisms, regulate the release of particles into the air. Indoor humidity and items affected by water damage (like carpets, ceilings, and walls) are notable sources of mite and mold allergens (Peden and Reed, 2010)."

2.9. AIR BORNE DISEASES

Airborne diseases are illnesses caused by microorganisms like bacteria, fungi, and viruses that can be transmitted through the air in the form of small dry particles or larger liquid droplets. These pathogens can be present in aerosols, dust, or liquids and can be inhaled or acquired by touching contaminated surfaces and then touching the eyes, nose, or mouth. Once inside the body, these pathogens establish themselves and multiply, leading to the development of the disease.

Particles that cause airborne diseases are small enough to cling to the air, attaching to dust particles, moisture droplets, or the breath until they are picked up. They can also be transferred through contact with bodily fluids such as mucus or phlegm. Sources of infection like bodily secretions from infected individuals or animals, as well as biological waste found in spaces like lofts, caves, and garbage, can generate infectious aerosols. These contaminated aerosols can remain in the air currents for extended periods, enabling them to travel significant distances. For instance, even a sneeze can propel infectious droplets across the entire length of a bus.

Airborne pathogens and allergens often lead to inflammation in the respiratory system, including the nose, throat, sinuses, and lungs. This inflammation can arise from touching contaminated surfaces and subsequently touching the eyes, nose, or mouth, as well as from inhaling these pathogens. Such inflammatory responses can affect both the respiratory system and other parts of the body. Symptoms like sinus congestion, coughing, and sore throats exemplify the inflammation occurring in the upper respiratory tract due to these airborne agents. Air pollution contributes significantly to airborne diseases, often associated with conditions like asthma. Pollutants are known to influence lung function by increasing airway inflammation.

Many common infections can spread through airborne transmission, at least in specific instances. These include diseases like Anthrax, Chickenpox, Influenza, Measles, Smallpox, Cryptococcosis, Tuberculosis, and the common cold. Airborne diseases also extend to non-human species. For instance, Newcastle disease affects various domestic poultry globally andis transmitted through airborne contamination (Mitchell *et al.*, 1994). Exposure to an individual or animal with an airborne disease does not guarantee contracting the disease. Factors such as changes in the host's immunity and the extent of exposure to airborne particles influence how the disease manifests in the body.

- **Symptoms**: Airborne diseases usually manifest in one or more of the followingsymptoms:

2.9.1. Inflammation of your nose throat, sinuses, or lungs, coughing, sneezing, congestion, runny nose, sore throat, swollen glands, headache, body aches, loss of appetite, fever, fatigue.

2.9.1.1. Prevention

Although it’s impossible to completely avoid airborne pathogens, there are somethings you can do to lower your chances of getting sick which include:

I. Wash your hands thoroughly with the appropriate hand disinfection (at least 20 seconds) and often, especially after sneezing or coughing.
II. Getting regular immunizations against diseases believed to be locally present.
III. Many public health specialists recommend social distancing to reduce the transmission of airborne infections.
IV. Avoid touching your face or other people with unwashed hands.
V. Wearing a respirator and limiting time spent in the presence of any patient likely to bea source of infection
VI. Antibiotics should be prescribed to patients with flu to control or prevent bacterial secondary infections but not to control viral infection and they may also be used in dealing with air-borne bacterial primary infections, such as pneumonic plague.
VII. Additionally, the Centres for Disease Control and Prevention (CDC) has told consumers about vaccination and following careful hygiene and sanitation protocols for airborne disease prevention.
VIII. Avoid close contact with people who have active symptoms of disease.
IX. Stay home when you’re sick. Don’t let vulnerable people come in close contact withyou.

X. If you must be around others, wear a face mask to prevent spreading or breathing ingerms.

XI. Cover your mouth when you cough or sneeze and use a tissue or your elbow to cutdown on the possibility of transmitting germs on your hands.

2.10. THE MICROBIAL ENVIRONMENT IN MUNICIPAL BUSES

Public buses are subject to frequent and varied human activity, which contributes significantly to the microbial load within these vehicles. The interior of buses, including seating, handrails, and ventilation systems, can harbor a diverse array of microorganisms, including bacteria, fungi, and viruses. The nature of these microorganisms and their prevalence can be influenced by several factors, including the frequency of cleaning, the age and condition of the bus, and environmental conditions.

Municipal buses have been identified as reservoirs for various bacterial species, some of which are pathogenic and pose health risks. Common bacterial isolates found in bus environments include *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus cereus*. *Escherichia coli*, often associated with fecal contamination, is a common finding in bus air samples. Its presence suggests potential hygiene issues and points to the need for regular disinfection practices (Kim *et al*., 2018). *Staphylococcus aureus*, which can cause skin infections, respiratory conditions, and more severe illnesses, is frequently found in the high-touch areas of buses. Its presence highlights the need for targeted cleaning protocols for commonly touched surfaces (Nokey and Wright, 2018). *Pseudomonas aeruginosa* is particularly concerning due to its resistance to multiple antibiotics. Its presence in bus air samples indicates a potential risk for the spread of antimicrobial-resistant strains (Ekpenyong and Udoh, 2020). *Bacillus cereus* is associated with foodborne illnesses and can also be present in the environment. Its detection in buses suggests possible cross-contamination from external sources (De Souza *et al*., 2017).

The warm and often humid conditions inside buses, especially in a city like Benin City with its high temperatures and humidity, create a favorable environment for fungal growth. Common fungal contaminants include *Aspergillus niger* and *Penicillium* species. *Aspergillus niger* is known for its ability to thrive in moist environments and can cause respiratory issues and allergies, particularly in sensitive individuals (Ekpenyong and Udoh, 2020). *Penicillium* species, while less pathogenic, can contribute to the overall fungal load and exacerbate allergic reactions and respiratory problems in passengers (Kim *et al*., 2018). Viruses, although less frequently studied in this context, can also be present in the air of municipal buses. Respiratory viruses such as influenza and rhinoviruses can be spread through airborne particles and are a concern in densely populated public transport systems.

Several factors contribute to the microbial contamination of municipal buses, Poor ventilation systems in buses can lead to the accumulation of microbial contaminants, as there is insufficient airflow to dilute or remove airborne particles. Inadequate ventilation exacerbates the spread of microorganisms, including bacteria and fungi (Kim *et al*., 2018). The frequency and effectiveness of cleaning practices play a critical role in controlling microbial contamination. Buses that are cleaned regularly and thoroughly exhibit lower levels of microbial contamination compared to those that are infrequently cleaned (Nokey and Wright, 2018). Cleaning protocols should include disinfection of high-touch surfaces and periodic cleaning of air filters to reduce microbial loads. High passenger turnover increases the likelihood of microbial contamination. Each passenger can introduce new microorganisms into the bus environment, and close proximity facilitates the spread of pathogens (Ekpenyong and Udoh, 2020). The climate of Benin City, characterized by high temperatures and humidity, creates an ideal environment for microbial growth. High humidity levels, in particular, promote the proliferation of fungi and exacerbate the persistence of microbial contaminants in the bus environment (De Souza *et al*., 2017).

The presence of microbial contaminants in municipal buses poses significant public health risks. Passengers are exposed to potential pathogens that can cause a range of health issues, from mild respiratory infections to more severe illnesses. The spread of antimicrobial-resistant bacteria in public transportation systems is of particular concern, as it contributes to the broader issue of antibiotic resistance (Kim *et al*., 2018; Nokey and Wright, 2018).

Effective strategies to mitigate microbial contamination include improving cleaning practices, enhancing ventilation systems, and implementing public health interventions to raise awareness about hygiene and infection prevention. Regular monitoring and maintenance are essential to ensuring a safe and healthy environment for bus passengers and transit workers.

CHAPTER THREE

MATERIALS AND METHODS

3.0. Study Design and Sample Collection

Samples were collected from municipal buses in New Benin, Ring road, Mission road and Ugbowo area of Benin City, Edo state, Nigeria. The collection strategy involved taking samples 15 mins prior to boarding, 15 mins after boarding for the initial interval, and during the final mins before the end of the journey, which naturally varied.

3.1. Sampling Procedure

The settle plate method was used, employing 90mm diameter Petri dishes to conduct the sampling. The sampling height was carefully chosen to align with the human breathing zone, which is approximately 1 meter above the floor. Samples were collected at various positions within the bus, including the front, middle, and back seats. To assess microbial contaminationover time, sampling intervals of 15 mins before passengers boarded, 15 mins after boarding during the initial interval, and periodically until the journey's conclusion were established. Following sample collection, all specimens were transported to the laboratory and subsequently incubated at a temperature of 37°C for a duration of 24 hrs.

3.2. Preparation of Culture Media

All media were prepared according to manufacturer's instruction. The media used in this study include nutrient agar and MacConkey agar.

3.2.1. 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 was placed in an autoclave to sterilize it for 15 mins at 121 °C. After sterilization, the flask was allowed to cool.

3.3. MICROBIAL COUNT

The plates were brought to the microbiology lab after exposure and collection, where they were incubated at 37 °C for 24 hrs to isolate bacteria. The total number of bacteria found in the air samples taken from various shuttle buses were counted. The number of colony forming unit per milliliter (cfu/ml) was calculated using the formula below:

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

Where a= number of colonies on plates

P= Surface area =^{2}

t= time of exposure (15 mins).

3.4. 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. Biochemical tests were also carried out to further identify the bacterial isolates.

3.4.1 Gram staining

Smars 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 .

3.4.2. Potassium Hydroxide (KOH) test

The potassium hydroxide (KOH) test is a useful supplement to the Gram stain and antibiotic disk susceptibility testing for the initial classification of anaerobic bacteria. This test is based on differences in the chemistry of the bacterial cell wall as the cell wall of gram-negative bacteria is easily disrupted when exposed to dilute alkali solutions, which gives rise to the viscosity of the suspension in KOH due to the release of relatively unfragmented threads of deoxyribonucleic acid. Weak alkali has no detectable effect on the cell wall of gram-positive bacteria. Two drops of 3% solution of KOH were applied on a clean glass slide and a loopful of pure bacterial growth was stirred in a circular motion in the slide. The loop was occasionally raised and observed for the presence of a string of the mixture. The solution was observed to be of a viscous and mucoid consistency indicating a Gram-negative bacterium. No reaction (absence of stringing) indicates a Gram-positive bacterium (Roberts and Sandle, 2008).

3.4.3. BIOCHEMICAL TEST

3.4.3.1. 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.

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

3.4.3.2. 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.

3.4.3.3. Urease Test

The urease test is used to determine the ability of an organism to split urea in the presence of the enzyme urease. The bacterial isolates were inoculated into slants of urea medium and incubated at 37°C for 24-48 hr. Urease positive cultures produced a red-pink color due to changes in the color of the indicator.

$$NH_2CO.NH_2 + H_2O \rightarrow 2NH_3 + CO_2$$

3.4.3.4. 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.

3.4.3.5. 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.

3.4.3.6. Triple sugar iron

This test was done to test the fermentation of glucose, sucrose and lactose with or without gas production it also test for the production of $H_2S$from amino acid. A gram of distilled water was dissolved in 150ml of distilled water and mixed thoroughly it was sterilized and slanted. The sample organism was inoculated and stabbed at the bottom of the tubes it was incubated for 24- 48hrs at 37℃. Color change at the slant indicates the fermentation of lactose and/or sucrose. Bottom color change indicates the fermentation of glucose.H_2S formation is done by blackening of the medium.

3.5. 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). The antibiotic discs used in the study with their corresponding codes and concentrations include

3.6. 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.

3.7. Statistical Analysis

Data obtained in this study were collected and analysed using Microsoft excel and by statistical package for social scientist (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA). Normal distributed data was expressed as mean ± standard deviation and means were compared by analysis of variance (Ogbeibu, 2014).

CHAPTER FOUR

4.0 RESULTS

Table 4.1 presents the mean total viable bacterial counts (in colony-forming units per cubic meter, CFU/m³) obtained from air samples collected in municipal buses across different routes in Benin City. The highest count was recorded, with Ring Road bus showing the highest mean count of $2.34 \times 10^3 \pm 0.34$ and the Mission road bus showing the lowest of $1.25 \times 10^3 \pm 0.08$.

Table 4.2. Shows the result of the cultural, morphological and biochemical identification of the bacterial isolates. The characteristics were used for morphological identification of the isolates and these includes; shape, size, arrangement, cell type, colour and surface appearance of each isolates. The biochemical test conducted were Gram stain, Urease test, Citrate test, Indole test, Catalase, Lactose and gas formation test etc. Possible organism identified include , *Escherichia coli, Bacillus sp, Proteus* sp*., Micrococcus* sp., *Pseudomonas aeruginosa*, *Staphylococus* sp. and *Enterococcus faecalis*

Figure 4.1: shows the frequency of occurrence of the bacterial isolates with *Staphylocococcus* sp. having the highest occurring frequency.

Table 4.3 shows the distribution of the bacterial isolates across the four different sampling locations, *Staphylocococcus* sp. having the highest distribution pattern.

Table 4.4: shows the resistance and susceptibility of the various isolates to the specific antibiotics used which include: Cefotaxime, Ampicillin, Ofloxacin ,Cefixime,Gentamicin, Levofloxacin, Cefuroxime, Imipenem, Nitrofurantoin and Nalidixic acid.

Figure 4.2: shows the multiple antibiotic resistant (MAR) index of the isolated organisms indicating the isolates with potential health implications.

Table 4.1: Mean Total Viable Bacterial Counts (CFU/m³) from Air Samples in Municipal Buses in Benin City, Nigeria

SAMPLE CODE	MEAN TOTAL VIABLE COUNT (CFU/m³)
NBB	1.59 x 10^3 ± 0.42
RRB	2.34 x 10^3 ±0.34
MRB	1.25 x 10^3 ± 0.08
UB	1.92 x 10^3 ± 0.25

Key:

NEW BENIN BUSS - NBB

RIGN ROAD BUSS - RBB

MISSION ROAD BUS - MRB

USELU BUS - UB

Table 4.2: Cultural and morphological, biochemical tests of the bacterial isolates

Shape	Irregular	Circular	Irregular	Circular	Irregular	Circular	Circular
Size	Medium	small	Medium	Large	Medium	Medium	Medium
Colour	Golden yellow	Cream	Milky	Red	Milky	Cream	Cream
Cell type	Rod	Rod	Cocci	Rod	Rod	Cocci	Rod
Cell arrangement	Disperse	Clusters	Disperse	Cluster	Disperse	Disperse	Disperse
Gram	-	+	+	-	-	+	-
KOH	+	-	-	+	+	-	+
Gas formation	+	-	+	+	-	-	-
Indole	+	-	-	-	-	-	-
Citrate	-	+	+	-	-	-	+
Oxidase	-	-	+	-	-	-	+
Catalase	+	+	+	+	+	+	+

H_2S formation	-	+	+	+	-	-	-
Sucrose	+	+	-	-	+	+	-
Glucose	+	+	+	+	+	+	-
Lactose	+	-	-	-	+	+	-
TSI reaction (slant/butt)	A/A	(K/A)	K/K	K/A	A/A	A/A	K/K
Identity	*Escherichia coli*	*Bacillus cereus*	*Micrococcus* sp	*Proteus* sp	*Enterococcus faecalis*	*Staphylocococcus* sp	*Pseudomonas aeruginosa*

KEY:

+: Positive to test, -: Negative to test . A)acid; (K) alkaline; (G) gas production (bubble); (H_2S) hydrogen sulphide (black precipitate); (KOH) Potassium hydroxide test; (TSI) Triple sugar iron test;

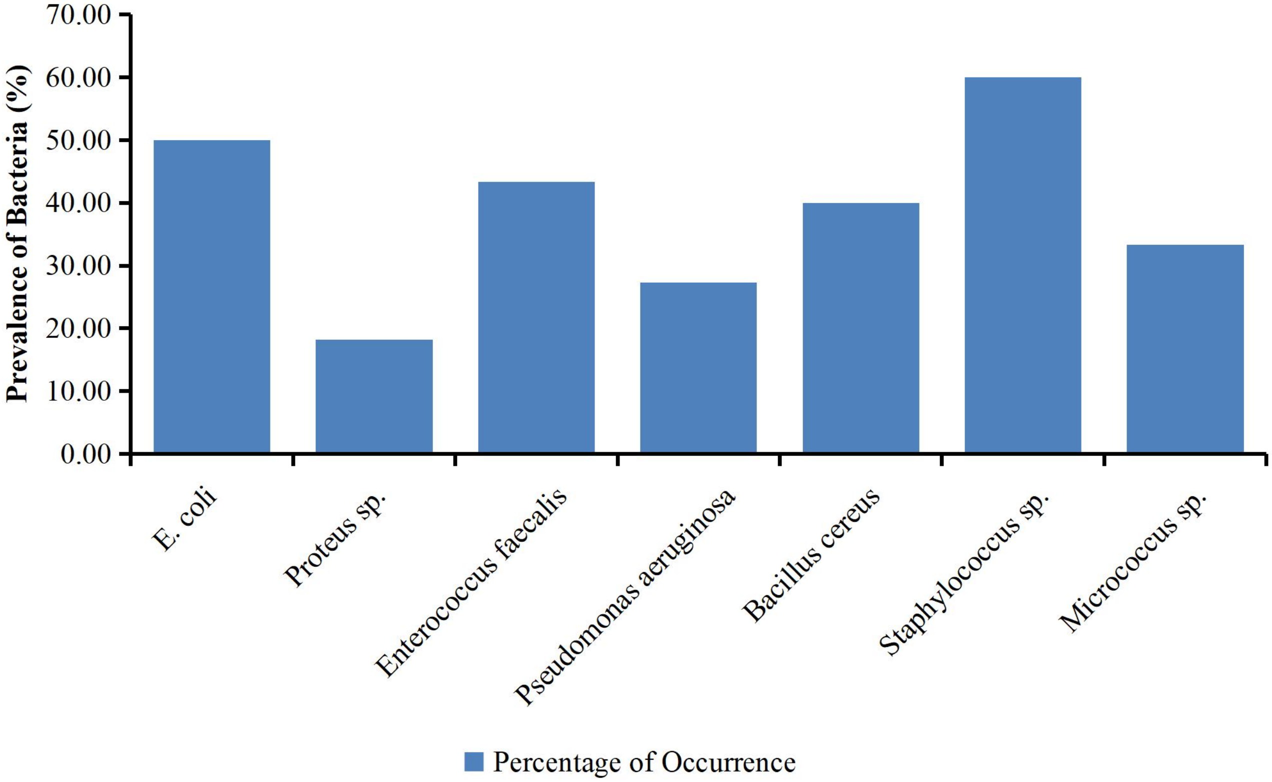

Figure 4.1. Prevalence of bacterial isolates (%)

Table 4.3: Distribution of Bacterial Isolates within the sampling location

Distribution of Bacterial Isolates

Isolates	New Benin	Ring road	Mission road	Uselu
E. coli	+	+	-	+
Proteus sp.	-	+	-	+
Enterococcus faecalis	+	-	+	+
Bacillus cereus	+	+	-	+
Staphylococcus sp	+	+	+	+
Bacillus cereus	-	+	-	+
Pseudomonas aeruginosa	+	-	+	+

Key: Present (+), Absent (-)

Table 4.3: Antibiotic sensitivity test on the bacterial isolates represented as zones of inhibiton measured in millimeters

ISOLATES	CTX	OFX	GEN	CFX	AMP	NIT	CFM	LEV	IPM	NAI
Escherichia coli	0(R)	20(S)	18(S)	10(R)	12(I)	0(R)	9(R)	12(I)	4(R)	16(I)
Staphylococcus sp	3(R)	17(S)	20(S)	14(I)	0(R)	7(R)	11(I)	14(I)	16(I)	14(I)
Proteus sp	10(R)	18(S)	16(S)	0(R)	8(R)	0(R)	10(R)	17(S)	0(R)	9(R)
Micrococcus sp	8(R)	14(S)	22(S)	8(R)	0(R)	10(R)	4(R)	22(S)	24(S)	19(S)
Enterococcus faecalis	14(I)	20(S)	16(I)	12(I)	0(R)	8(R)	4(R)	22(S)	24(S)	20(S)
Pseudomonas aeruginosa	8(R)	14(I)	22(S)	9(R)	14(I)	6(R)	0(R)	15(I)	0(R)	11(I)
Bacillus cereus	6(R)	14(S)	18(S)	8(R)	0(R)	12(I)	4(R)	22(S)	24(S)	19(S)

KEY:

Resistant (R) = 0 – 10mm

Intermediate (I) = 11 – 16mm

Susceptible (S) = 17mm and above.

CTX=Cefotaxime AMP=Ampicillin
OFX=Ofloxacin CFM=Cefixime
GEN=Gentamicin LEV=Levofloxacin
CFX=Cefuroxime IPM=Imipenem
NIT=Nitrofurantoin NAL=Nalidixic acid

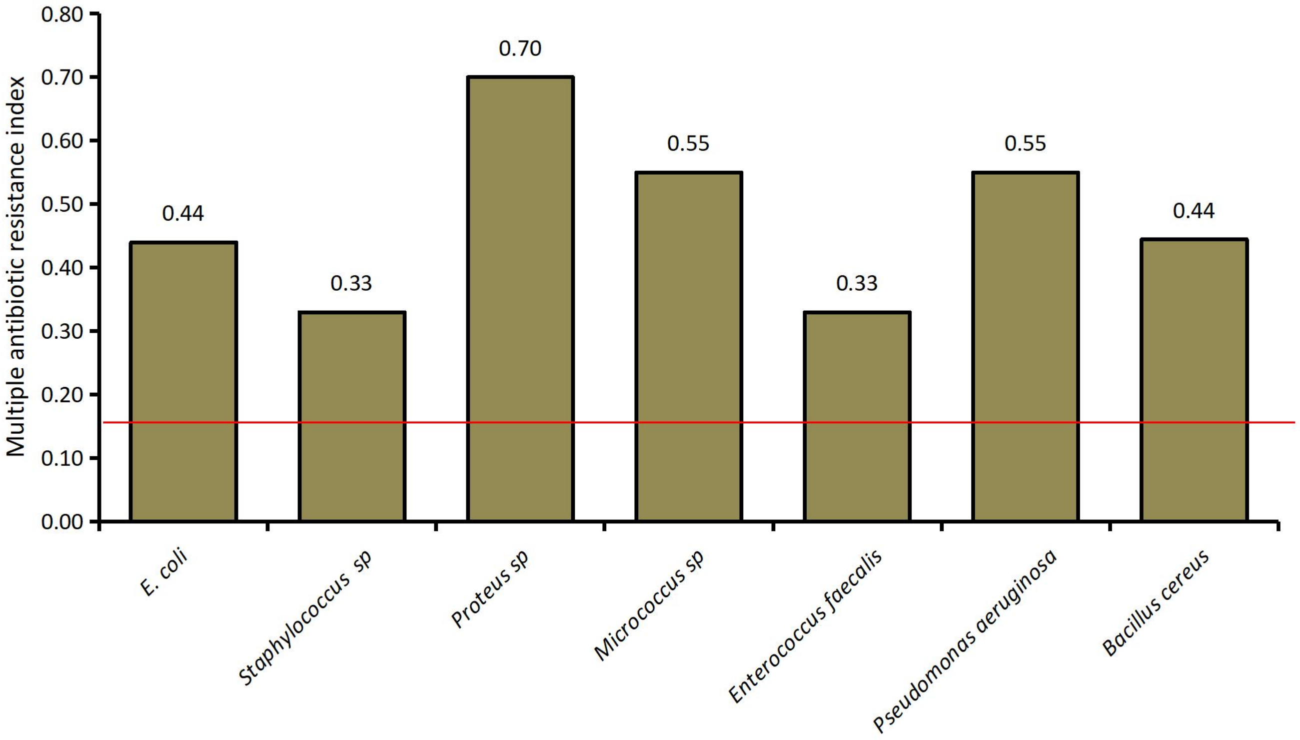

Figure 4.2.: Multiple antibiotic resistance index of the bacterial isolates

CHAPTER FIVE

DISCUSSION

The assessment of indoor air quality through microbiological analysis is a crucial investigation to identify and address microbial pollution within enclosed spaces. It provides essential insights into both potential health risks and the development of indoor air quality standards. This study specifically focuses on evaluating the presence of airborne bacteria in municipal bus within selected location in Benin City, Nigeria, shedding light on the extent to which individuals contribute to microbial contamination.

The microbial air quality assessment of indoor air in municipal buses in Benin City provided detailed insights into bacterial contamination levels, as demonstrated by the total viable counts (TVC) reported in Table 4.1. The study revealed a significant variation in bacterial contamination across different bus routes. Specifically, the Ring Road bus exhibited the highest mean bacterial count of $2.34 \times 10^3 \pm 0.34$ CFU/m^3, indicating a higher level of microbial presence in the air compared to the Mission Road bus, which had the lowest count of $1.25 \times 10^3 \pm 0.08$ CFU/m^3. This discrepancy in bacterial counts can be attributed to multiple contributing factors. One key factor is the differences in bus routes and their respective characteristics (Frankel *et al*., 2012). The Ring Road bus route, being a major thoroughfare, likely experiences higher passenger turnover and greater bus traffic compared to the Mission Road bus. This increased passenger movement can introduce a larger quantity of microorganisms into the bus environment. High passenger density not only brings more people who may carry various microorganisms but also increases the frequency of entry and exit, further contributing to microbial load (Andualem *et al*., 2019). Similar findings were reported by Hayleeyesus *et al*.

(2015), who observed higher microbial loads in indoor dormitories with increased humidity and occupancy.

Another factor influencing the higher bacterial count in the Ring Road bus could be related to maintenance and cleaning practices. Buses operating on high-traffic routes may face challenges in maintaining cleanliness due to continuous use and frequent boarding and alighting of passengers. Inadequate or infrequent cleaning of these buses can lead to the accumulation of microorganisms on surfaces and in the air, exacerbating microbial contamination (Wamedo *et al*., 2012). The Ring Road bus, with its higher microbial load, might be subjected to less rigorous cleaning protocols or might not be cleaned as frequently as needed to manage high passenger traffic effectively. Conversely, the Mission Road bus, which recorded a lower bacterial count, may benefit from factors such as a lower volume of passengers or more stringent cleaning practices. A less crowded bus environment could result in fewer opportunities for microorganisms to be introduced or spread within the vehicle. Additionally, if the Mission Road bus adheres to more effective cleaning routines, this would contribute to a reduction in microbial load. The combination of fewer passengers and better maintenance practices can significantly impact the overall microbial quality of the indoor air (Wamedo *et al*., 2012).

The cultural, morphological, and biochemical identification of bacterial isolates, as detailed in Table 4.2, revealed the presence of various bacterial species, including *Escherichia coli*, *Bacillus sp.*, *Proteus sp.*, *Micrococcus sp.*, *Pseudomonas aeruginosa*, *Staphylococcus sp.*, and *Enterococcus faecalis*. Each of these bacteria was identified based on distinct morphological characteristics such as shape, size, and arrangement, as well as biochemical tests including Gram stain, urease test, citrate test, indole test, catalase test, and lactose fermentation (Cowan, 2004;

Cheesbrough, 2006). This result align with Kumar *et al.*'s (2021) findings of *Staphylococcus* sp., *Escherichia. coli*, *Proteus* sp. and *Micrococcus* in residential indoor air. Among the identified bacteria, *Staphylococcus sp.* showed the highest frequency of occurrence (Figure 4.1). This finding is significant as *Staphylococcus* species are known to be resilient and commonly found in various environments, including those frequently contacted by humans. Their high occurrence in the air samples may reflect their adaptability and survival in indoor settings with frequent human interactions (Stephens *et al.*, 2019).

Table 4.3 illustrates the distribution of bacterial isolates across different sampling locations, with *Staphylococcus sp.* again demonstrating the highest distribution pattern. This widespread presence across sampling sites highlights the potential for cross-contamination and the need for regular cleaning and disinfection protocols in municipal buses. The prevalence of *Staphylococcus sp.* could be linked to its ability to thrive in various environmental conditions and its association with human skin, which may lead to its transfer into the bus environment through direct or indirect contact (Zieliński *et al.*, 2020).

The antibiotic resistance profiles, as shown in Table 4.4, provide insights into the susceptibility and resistance patterns of the isolated bacterial strains. The antibiotics tested include Cefotaxime, Ampicillin, Ofloxacin, Cefixime, Gentamicin, Levofloxacin, Cefuroxime, Imipenem, Nitrofurantoin, and Nalidixic acid. The results indicate varying levels of resistance among the isolates, with some strains demonstrating multi-drug resistance (MDR) as evidenced by the MAR index in Figure 4.2 (Afshan *et al.*, 2021). The presence of antibiotic resistant bacteria in the air of municipal buses is of significant concern. Multi-drug-resistant strains pose a potential public health risk as they can lead to difficult-to-treat infections. The MAR index highlights the extent of resistance and suggests that some isolates have developed resistance to multiple antibiotics, reflecting the broader issue of antimicrobial resistance (AMR) in bacterial populations (WHO, 2021). The spread of resistant bacteria in public transportation settings underscores the importance of stringent hygiene measures and the need for surveillance to manage and mitigate the risk of AMR.

The findings from this study emphasize the need for improved sanitation practices in municipal buses to reduce bacterial contamination. Regular cleaning and disinfection of bus interiors, particularly high-touch surfaces, can help minimize microbial load (Briñas *et al*., 2008). There is no universally accepted limit for microbial load in indoor air; some studies suggest a threshold of less than 1000 cfu/m^3 according to WHO standards, while others suggest 750 cfu/m^3 for contamination (Capitelli and Sorlini, 2010). The European Commission recommends 300-500 cfu/m^3 for fungi and bacteria, respectively. The results of this study exceeded these limits, indicating a need for improved environmental hygiene, sanitary practices, monitoring of outdoor sources, ventilation regulation, and general management of factors that promote microbial growth.

5.1 CONCLUSION

These findings highlight the critical role of bus maintenance and passenger management in controlling microbial contamination. Enhanced cleaning protocols and monitoring of passenger density could mitigate bacterial levels and improve indoor air quality. Addressing these factors is essential for reducing the risk of microbial exposure and promoting better public health within municipal transportation systems. Future efforts should focus on implementing regular disinfection practices and evaluating the impact of these interventions on microbial contamination in public transportation.

REFERENCES

Afshan, N., Fatima, R., and Khan, S. (2021, January). Multiple Antibiotic Resistance Indexing of Coliforms. In *2021 International Bhurban Conference on Applied Sciences and Technologies (IBCAST)* (pp. 1-10). IEEE.

Afunwa, R. A., Ezeanyinka, J., Afunwa, E. C., Udeh, A. S., Oli, N. A. and Unachukwu, M. (2020). Multiple antibiotic resistant index of Gram-negative bacteria from bird droppings in two commercial poultries in Enugu, Nigeria. *Open Journal of Medical Microbiology*. **10**: 171 – 181.

Akyol, S., Erdogan, S., Idiz, N., Celik, S., Kaya, M. and Ucar, F. (2014). The role of reactive oxygen species and oxidative stress in carbon monoxide toxicity: An in-depthanalysis. *Redox Report* **19**(5): 180-189.

Akyol, S., Erdogan, S., Idiz, N., Celik, S., Kaya, M. and Ucar, F. (2014). The role of reactive oxygen species and oxidative stress in carbon monoxide toxicity: An in-depthanalysis. *Redox Report* **19**(5): 180-189.

Al-Joda Mulamattathil, S. G., Bezuidenhout, C. and Mbewe, M. (2015). Analysis of physico- chemical and bacteriological quality of drinking water in Mafikeng, South Africa. *Journal of Water and Health* **41**: 1143-1152.

Allred, E. N., Bleecker, E. R., Chaitman, B. R., Dahms, T. E., Gottlieb, S. O., Hackney, J. D., Pagano M., Selvester R.H. and Walden S.M. (1989). Acute effects of carbon monoxide exposure on individuals with coronary artery disease. *Research Report (Health Effects Institute)* **10**(25): 1-79.

Almatawah, Q. A., Al-Rashidi, M. S., Yassin, M. F. and Varghese, J. S. (2022). Microbiological contamination of indoor and outdoor environments in a desert climate. *Environmental Monitoring and Assessment* **194**(5), 355.

American Academy of Pediatrics Committee on Environmental Health. (2005). Lead exposure in children: Prevention, detection, and management. *Pediatrics* **116**(4): 1036-1046.

Andualem, Z., Gizaw, Z., Bogale, L. and Dagne, H. (2019). Indoor bacterial load and its correlation to physical indoor air quality parameters in public primary schools. *Multidisciplinary Respiratory Medicine* **14**: 2.

Bentayeb, M., Simoni, M., Norback, D., Baldacci, S., Maio, S., Viegi, G. and Annesi-Maesano, I. (2013). Indoor air pollution and respiratory health in the elderly. *Journal of Environmental Science and Health*, Part A: **48**(14), 1783-1789.

Bouillard, L., Michel, O., Dramaix, M. and Devleeschouwer, M. (2005). Bacterial contamination of indoor air, surfaces, and settled dust, and related dust endotoxin concentrations in healthy office buildings. *Annals of Agricultural and Environmental Medicine* **12**:187–192.

Cabral, J.P.S. (2010) Water Microbiology. Bacterial Pathogens and Water.*International Journal of Environmental Research and Public Health* **7**(10):3657-3703.

Camargo, J.A. and Alonso, Á. (2006). Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International* **32**(6) : 831-849.

Chen, Q. and Hildemann, L. M. (2009). The effects of human activities on exposure to particulate matter and bioaerosols in residential homes. *Environmental Science & Technology* **43**(13): 4641–4646.

Chen, T. M., Kuschner, W. G., Gokhale, J. and Shofer, S. (2007). Comprehensive Review: Outdoor Air Pollution. *The American Journal of the Medical Sciences* **333:**(4) 249- 256.

D. L. and McKee, J. (1994). Ozone dose and effect in humans and rats: A comparison using oxygen-18 labeling and bronchoalveolar lavage. *American Journal of Respiratory and Critical Care Medicine* **150**(3): 109-162.

Dedesko, S., Stephens, B., Gilbert, J. A. and Siegel, J. A. (2015). Methods to assess human occupancy and occupant activity in hospital patient rooms. *Building and Environment* **90**: 136–145.

Delort, A.-M., Vaïtilingom, M., Amato, P., Sancelme, M., Parazols, M., Mailhot, G., Laj, P., Deguillaume, L., 2010. A short overview of the microbial population in clouds: potential roles in atmospheric chemistry and nucleation processes. *Atmospheric Research* **98**:2-4

Fares, S., Vargas, R., Detto, M., Goldstein, A. H., Karlik, J., Paoletti, E. and Vitale, M. (2013). Tropospheric ozone reduces carbon assimilation in trees: estimates from analysis of continuous flux measurements. *Global Change Biology* **19**(8): 2427-2443.

Farhat, A. S., Parizadeh, S. M., Balali, M., Balali, M. and Khademi, G. R. (2005). Comparison of blood lead levels in 1-7 year old children with and without seizure. *Neurosciences Journal* 1**0**(3): 210-212.

Foarde, K. K., Dulaney, P., Cole, E., Van Osdell, D., Ensor, D. and Chang, J. (1993). Assessment of fungal growth on ceiling tiles under environmentally characterized conditions. *Indoor Air* **4**: 357-362

Foysal, M. J**.** and Lisa, A. K. (2018). Isolation and characterization of Bacillus sp. strain BC01 from soil displaying potent antagonistic activity against plant and fish pathogenic fungi and bacteria. *Journal of Genetic Engineering and Biotechnology*. **15**:387 – 392.

Frankel, M., Bekö, G., Timm, M., Gustavsen, S., Hansen, E. W., and Madsen, A. M. (2012). Seasonal variations of indoor microbial exposures and their relation to temperature, relative humidity, and air exchange rate. *Applied and environmental microbiology* **78**(23):8289-8297.

Gao, M., Cao, J., Seto, E. and Zhang, Q. (2019). Personal exposure to air pollutants in a Bus Rapid Transit System: Impact of fleet age and emission standard. *Environment International* **126**: 346-354.

Georgakopoulos, D. G., Despres, V., Frohlich-Nowoisky, J., Psenner, R., Ariya, P. A., Posfai,

Gerceker, D., Karasartova, D., Elyürek, E., Barkar, S., Kıyan, M., Özsan, T. M., Calgin,

M. K., and Sahin, F. (2009). A new, simple, rapid test for detection of DNase activityof microorganisms: DNase Tube test. *The Journal of General and Applied Microbiology* **55**: 291-294.

Gerrity, T. R., Weaver, R. A., Berntsen, J., House, D. E. and O'Neil, J. J. (1988). Extrathoracic and intrathoracic removal of O3 in tidal-breathing humans. *Journal ofApplied Physiology* **65**(1), 393-399.

Gorai, A. K., Tuluri, F. and Tchounwou, P. B. (2014). A GIS based approach for assessing the association between air pollution and asthma in New York State, USA. *International Journal of Environmental Research and Public Health* **11**(5): 4845- 4869.

Guillam, M. T., Pédrono, G., Le Bouquin, S., Huneau, A., Gaudon, J., Leborgne, R., Dewitte, J. D. and Ségala, C. (2013). Chronic respiratory symptoms of poultry farmers and model-based estimates of long-term dust exposure. *Annals of Agricultural andEnvironmental Medicine* **20**(2): 307–311.

Harwood, V. J., Delahoya, N. C., Ulrich, R. M., Kramer, M. F., Whitlock, J. E., Garey, J. R.and Lim, D. V. (2004). Molecular confirmation of *Enterococcus faecalis* and *E. faecium* from clinical, faecal and environmental sources. *Letters in Applied Microbiology*. **38**: 476 – 482.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Stanley, J. T. and William, S. T. (1994). Bergey's Manual of Determinative Bacteriology. Williams and Wilikins, Baltimore, 786- 788pp.

Hospodsky, D., Qian, J., Nazaroff, W. W., Yamamoto, N., Bibby, K., Rismani-Yazdi, H. and Peccia, J. (2012). Human occupancy as a source of indoor airborne bacteria. *PLoS ONE* **7**(4): 16-26

Igbinosa, E. O., Beshiru, A., Akporehe, L. U., Oviasogie, F. E. and Igbinosa, O. O. (2016). Prevalence of methicillin-resistant *Staphylococcus* aureus and other*Staphylococcus* species in raw meat samples intended for human consumption in Benin City, Nigeria: Implications for public health. *International Journal of Environmental Research and Public Health*. **13**: 949.

J. D. and Ségala, C. (2013). Chronic respiratory symptoms of poultry farmers and model-based estimates of long-term dust exposure. *Annals of Agricultural and Environmental Medicine* **20**(2): 307–311.

Jerrett, M., Finkelstein, M. M., Brook, J. R., Arain, M. A., Kanaroglou, P., Stieb, D. M., Gilbert, N. L., Verma, D., Finkelstein, N., Chapman, K. R. and Sears, M. R. (2009). A cohort study of traffic-related air pollution and mortality in Toronto, Ontario, Canada. *Environmental Health Perspectives* **117**(5): 772–777.

Kalogerakis, N., Paschali, D., Lekaditis, V., Pantidou, A., Eleftheriadis, K. and Lazaridis, M. (2005). Indoor air quality-bioaerosol measurements in domestic and office premises. *Journal of Aerosol Science* **36**: 751-761.

Kianoush, S., Balali-Mood, M., Mousavi, S. R., Moradi, V., Sadeghi, M., Dadpour, B., Rajabi, O. and Shakeri, M. T. (2011). Comparison of therapeutic effects of garlic and d-Penicillamine in patients with chronic occupational lead poisoning. *Basic andClinical Pharmacology and Toxicology* **110**(5): 476-481.

Kumar, D., Tyagi, N. and Gupta, A. B. (2012). Sensitivity analysis of field test kits for rapid assessment of bacteriological quality of water. *Journal of Water Supply: Research and Technology-AQUA* **23**: 283-290.

Kunwar, A., Tamrakar, S., Poudel, S., Sharma, S. and Parajuli, P. (2019). Bacteriological assessment of the indoor air of different hospitals of Kathmandu District. *International Journal of Microbiology* **2**: 1-9.

Lidsky, T. I. and Schneider, J. S. (2006). Adverse effects of childhood lead poisoning: The clinical neuropsychological perspective. *Environmental Research* **100**(2), 284-293.

Lippmann, M. (1989). Health effects of ozone: A critical review. *JAPCA* **39**(5): 672-695.

Loomis, D., Huang, W. and Chen, G. (2014). The International Agency for Research on Cancer (IARC) evaluation of the carcinogenicity of outdoor air pollution. *Chinese*

Journal of Cancer **33**: 189–196.

Loomis, D., Huang, W. and Chen, G. (2014). The International Agency for Research on Cancer (IARC) evaluation of the carcinogenicity of outdoor air pollution. *Chinese Journal of Cancer* **33**: 189–196.

Lovett, G. M., Tear, T. H., Evers, D. C., Findlay, S. E. G., Cosby, B. J., Dunscomb, J. K., Driscoll, C. T. and Weathers, K. C. (2009). Effects of air pollution on ecosystems and biological diversity in the eastern United States. Annals of the New York Academy of Sciences 1162: 143-169.

M. and Ahern, H. E. (2009). Microbiology and atmospheric processes: biological, physical and chemical characterization of aerosol particles. *Biogeosciences* **6**: 721–737.

Mandal, J. and Brandl, H. (2011). Bioaerosols in the indoor environment - a review with special reference to residential and occupational locations. *Open Environmental Biology Monitoring Journal* **4**: 83–96.

McCarthy, J. T., Pelle, E., Dong, K., Brahmbhatt, K., Yarosh, D. and Pernodet, N. (2013). Effects of ozone in normal human epidermal keratinocytes. *Experimental Dermatology* **22**(5): 360-361.

McCarthy, J. T., Pelle, E., Dong, K., Brahmbhatt, K., Yarosh, D. and Pernodet, N. (2013). Effects of ozone in normal human epidermal keratinocytes. *Experimental Dermatology* **22**(5): 360-361.

Meadow, J., Altrichter, A., Kembel, S., Kline, J., Mhuireach, G. and Moriyama, M., (2014). Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source. *Indoor Air* **24**: 41–48.

Mellouki, A., George, C., Chai, F., Mu, Y., Chen, J. and Li, H. (2015). Sources, chemistry, impacts, and regulations of complex air pollution. *Journal of Environmental Sciences* **40**: 1-2.

Nazaroff, W. W. (2016). Teaching indoor environmental quality. *Indoor Air* **26**: 515–516.

Niazi, S., Hassanvand, M. S., Mahvi, A. H., Nabizadeh, R., Alimohammadi, M., Nabavi, S., Faridi, S., Dehghani, A., Hoseini, M., Moradi-Joo, M., Mokamel, A., Kashani, H., Yarali, N., and Yunesian, M. (2015). Assessment of bioaerosol contamination (bacteria and fungi) in the largest urban wastewater treatment plant in the Middle East. *Environmental Science and Pollution Research* **22**(22): 16014-16021.

O'Donnell, S., Humphreys, H., and Hughes, D. (2008). Distribution of virulence genes among colonising and invasive isolates of methicillin-resistant Staphylococcus aureus. *Clinical Microbiology and Infection* **14**(6):625-626.

Ozcan, N. S. and Cubukcu, K. M. (2018). The relationship between urban air pollution and urban planning decisions. *Asian Journal of Quality of Life* **3**(11): 181.

P. A. (2009). Critical review of the human data on short-term nitrogen dioxide (NO2) exposures: Evidence for NO2 no-effect levels. *Inhalation Toxicology* **39**(10): 743-781.

Pearson, C., Littlewood, E., Douglas, P., Robertson, S., Gant, T. W. and Hansell, A. L. (2015). Exposures and health outcomes in relation to bioaerosol emissions from composting facilities: A systematic review of occupational and community studies.

Pelucchi, C., Negri, E., Gallus, S., Boffetta, P., Tramacere, I. and La Vecchia, C. (2009). Long-term particulate matter exposure and mortality: a review of European epidemiological studies. *BMC Public Health*:**9**(453).

Pepper, I. I., and Dowd, S. E. (2009). *Pseudomonas* in hospitals. *Environmental Microbiology* **1**: 6-25.

Qian, J. and Ferro, A. R. (2008). Resuspension of dust particles in a chamber and associated environmental factors. *Aerosol Science and Technology* **42**:566–578.

Qian, J., Hospodsky, D., Yamamoto, N., Nazaroff, W. and Peccia, J. (2012). Size-resolved emission rates of airborne bacteria and fungi in an occupied classroom. *International Journal of Indoor Environment and Health* **22**(4), 339-351.

Qian, J., Hospodsky, D., Yamamoto, N., Nazaroff, W. W., Peccia, J. (2012). Size resolved emission rates of airborne bacteria and fungi in an occupied classroom. *Indoor Air* **22**:339–351.

Roberts, J. and Sandle, T. (2008). Assessment of the potassium hydroxide test as a complementary test for the Gram-stain. *Pharmig Excellence in Microbiology* **31**: 1- 12.

Rohr, A. C., Campleman, S. L., Long, C. M., Peterson, M. K., Weatherstone, S., Quick, W. and Lewis, A. (2015). Potential occupational exposures and health risks associated with biomass-based power generation. *International Journal of Environmental Research and Public Health* **12**(7): 8542-8605.

Sadeghi, M., Ahmadi, A., Baradaran, A., Masoudipoor, N. and Frouzandeh, S. (2015). Modeling of the relationship between environmental air pollution, clinical risk factors, and hospital mortality due to myocardial infarction in Isfahan, Iran. *Journal of Research in Medical Sciences* **20**(8): 757-762

Sadeghi, M., Ahmadi, A., Baradaran, A., Masoudipoor, N. and Frouzandeh, S. (2015). Modeling of the relationship between environmental air pollution, clinical risk factors, and hospital mortality due to myocardial infarction in Isfahan, Iran. *Journal of Research in Medical Sciences* **20**(8): 757-762

Sahu, D., Kannan, G. M. and Vijayaraghavan, R. (2014). Carbon Black Particle Exhibits Size Dependent Toxicity in Human Monocytes. *International Journal of Inflammation* **6**:1-10

Schneider, S.H. (1989). The Greenhouse Effect: Science and Policy. *Science* **243**(4892): 771-781

Severson, K. M., Mallozzi, M., Driks, A. and Knight, K. L. (2010). B cell development in galt: Role of bacterial superantigen-like molecules. *Journal of Immunology* **184**(12): 6782–6789.

Soto T, Lozano M, Vicente-Soler J, Cansado J, Gacto M.(2009) Microbiological survey of the aerial contamination in urban areas of the city of Murcia, Spain. *Analytical Biology* **31**: 7-13.

Soto T, Murcia RM, Franco A, Vicente-Soler J, Cansado J, Gacto M. (2009) Indoor airborne microbial load in a Spanish university (University of Murcia, Spain). *Analytical Biology* **31**: 109-115.

Srikanth, P., Sudharsanam, S. and Steinberg, R. (2008). Bio-aerosols in indoor environment: composition, health effects, and analysis. *Indian Journal of Medical Research* **26**: 302–312.

Stephens, B.; Azimi, P.; Thoemmes, M.S.; Heidarinejad, M.; Allen, J.G.; Gilbert, J.A. (2019) Microbial exchange via fomites and implications for human health. *Current. Pollution. Report* **5**:198–213

Majumder, M. A. A., Rahman, S., Cohall, D., Bharatha, A., Singh, K., Haque, M., and Gittens-St Hilaire, M. (2020). Antimicrobial stewardship: fighting antimicrobial resistance and protecting global public health. *Infection and drug resistance*, 4713-4738.

Sundell, J. (2004). On the history of air quality and health. *Indoor air* (2004); **14**: 51-58

Sykes, P., Jones, K. and Wildsmith, J. D. (2007). Managing the potential public health risks from bioaerosol liberation at commercial composting sites in the UK: An analysis of the evidence base. *Resources, Conservation and Recycling* **52**(2): 410-424.

Tagesu, A. (2018). General Characteristics and Miscellaneous Examination of Microorganism. International Journal of Veterinary Science and Research, 105-108.

Täubel, M., Rintala, H., Pitkäranta, M., Paulin, L., Laitinen, S., Pekkanen, J., Hyvärinen, A. and Nevalainen, A. (2009). The occupant as a source of house dust bacteria. *Journal of Allergy and Clinical Immunology* **124**(4): 834-840

Van Leuken, J. P. G., Swart, A. N., Havelaar, A. H., Van Pul, A., Van der Hoek, W. and Heederik, D. (2016). Atmospheric dispersion modelling of bioaerosols that are pathogenic to humans and livestock—a review to inform risk assessment studies. *Microbial Risk Analysis* **1**: 19–39.

Wilkinson, S., Mills, G., Illidge, R. and Davies, W. J. (2012). How is ozone pollution reducing our food supply? *Journal of Experimental Botany* **63**(2): 527-536.

Yao, W., Liu, K., Liu, H., Jiang, Y., Wang, R., Wang, W. and Wang, T. (2021). A valuable product of microbial cell factories: Microbial Lipase. *Frontiers in Microbiology* **12**: 1-16.

Zhao, Y., Gu, C. and Song, X. (2023). Evaluation of indoor environmental quality, personal cumulative exposure dose, and aerosol transmission risk levels inside urban buses in Dalian, China. *Environmental Science and Pollution Research* **30**: 55278-55297.

Zhou, M., Liu, Y., Wang, L., Kuang, X., Xu, X. and Kan, H. (2014). Particulate air pollution and mortality in a cohort of Chinese men. *Environmental Pollution* **186**: 1-6.

Zhou, M., Liu, Y., Wang, L., Kuang, X., Xu, X. and Kan, H. (2014). Particulate air pollution and mortality in a cohort of Chinese men. *Environmental Pollution* **186**: 1-6.

Zieliński, W., Korzeniewska, E., Harnisz, M., Hubeny, J., Buta, M. and Rolbiecki, D., 2020. The prevalence of drug-resistant and virulent Staphylococcus spp. in a municipal wastewater treatment plant and their spread in the environment. *Environment International* **143**: 105914.