MICROBIAL ASSESSMENT OF INDOOR AIR OF MICROBIOLOGY LABORATORY IN UNIVERSITY OF BENIN, BENIN CITY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY,

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IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.SC.) DEGREE IN MICROBIOLOGY.

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CERTIFICATION

We certify that this project work was carried out by **IDEHEN REBECCA OGHOGHO** in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc.) Degree in Microbiology.

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PROF. E. I. ATUANYA **DATE**

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(Head of Department)

DEDICATION

This project is dedicated to the Almighty God, my helper and my source, and also to my lovely parents

ACKOWLEDGMENTS

I would like to express my gratitude and appreciation to all those who gave me the possibility to come thus far. First of all I want to thank God for His grace, His mercy and love which has brought me this far . And to my parents Mr and Mrs Idehen, I want to say may God continue to bless you for me for your support towards me since the beginning of my academic journey till now, I'm entirely grateful. My sincere gratitude to my supervisor, **PROF. E. I. ATUANYA** supported by **DR. O. N. IGIEHON** for their generosity, kindness and excellent supervision throughout my project work. To my big sister Mrs Susan Benson I love you and I say a big thank you for your encouragement, help, support and love. To my big brother Harry Idehen, I say thank you for all you do, to my little miss perfect Idehen Naomi I love you. To the best cousins anyone could ask for, I say a big thank you for everything you've done for me, I love you Osariemen Imasuen and Judith Imasuen . To my wonderful lecturers, especially my Course Adviser Mrs. S. I. Ogbebor, I say thank you ma'am. To my wonderful aunty Mrs Mabel, Imasuen , I appreciate you ma for your assistance and love. To my teachers in church and my pastor Mrs Helen Ikpeama, I say thank you for your prayers and mentorship. To my friends Flourish Ogbemudia, Enabulele Obosa, Enabulele Emmanuel, Ubah Prosper, Osifo Praise, Enoch Uduak, Osayamen Samuel, Nosakhare Wilfred, Precious popoola, Mrs, Blessing Abu, Igbinosa Kingdave, Desmond osazuwa, Acholo success, Osaze Donald, Micah Idowu , Eromosele Philip, Ogenemaro precious, I love you guys and thank you all for your love and support. And to my favorite people, my girls, Ogbomo Esohe Grace, Ogagavwiare, Precious Oghenefejiro, Omotehinshe Fumilayo Victoria. I love you guys and can't wait to see us at the top. And to Mr Aduba and my boss MR, I. T. DANIEL for their moral support.

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ABSTRACT

Microbiological quality assessment of indoor air study is one of the most vital investigations to determine the microbial indoor air contaminant. The information on the indoor microbial concentrations of airborne bacteria and fungi is necessary both to estimate the health hazard and to create standards for indoor air quality control. Standard bacteriological methods were used to determine the total bacterial count of the air samples. The bacterial isolates were characterized and identified using morphological and biochemical methods. The percentage distribution and frequency of the isolates were evaluated using statistical method. The results obtained from this study showed that laboratory one had the microbial load of $8.04x10^4 \pm 6.0$ Cfu/m^3 while laboratory two had the microbial load of $6.85x10^4 \pm 0.5$ Cfu/m^3. Using standard morphological and cultural characterization, the isolates obtained in this study were *Staphylococcus aureus*, *Micrococcus* sp, *Bacillus subtilis, Streptococcus* and *Escherichia coli*. The Gram positive bacteria were all susceptible to gentamycin and augmentin while the Gram negative showed resistance to these antibiotics. However the isolates were all susceptible to ofloxacin. The selected sampling laboratories of Faculty of Life Sciences, University of Benin were contaminated with bacteria. Thus, attention must be given to control those environmental factors which favor the growth and multiplication of microorganisms in indoor environment of the school to safeguard health of users and workers, and it is vital to control visitors and students in and out the laboratories.

CHAPTER ONE

INTRODUCTION

1.1. Background to the study

Indoor air quality holds greater importance for human health due to the extended exposure time people spend indoors compared to outdoors. On average, a person breathes in approximately 6-10 liters per minute and requires around 15 cubic meters of air daily (Dang *et al*., 2020). Since most human activities occur indoors—in places like offices, dormitories, laboratories, and homes—indoor air quality significantly impacts health (Enitan *et al*., 2017). Currently, individuals spend over 80% of their time indoors, including at work and in their living spaces. Everyday activities such as talking, sneezing, coughing, walking, and washing can introduce biological dust into the air. Additionally, items such as food, houseplants, dust, clothing, carpets, wood materials, and furniture can intermittently release various microorganisms into the indoor environment. Greater attention is required for indoor air quality in laboratories because of the extensive microbial and pathogenic activities that occur there, posing risks to humans, especially laboratory workers (Dang *et al*., 2020).The concentration of fungi in indoor environments fluctuates based on variables like temperature, humidity, materials, the number and movement of people, building characteristics, geographical and climatological conditions, and the heating, cooling, and ventilation systems in place (Özkan, 2020). Therefore, it is crucial to study and monitor indoor air quality to safeguard health. Various genera of microorganisms, including *Staphylococcus, Bacillus, Micrococcus*, *Cladosporium, Aspergillus, and Penicillium*, can be identified in indoor air (Degois *et al*., 2021b).Previous research has shown that the microbial

profile of indoor air varies significantly with season, time, ventilation, and location, particularly in laboratory settings (Fujiyoshi *et al*., 2017). Studies have reported that indoor air temperatures above 25°C can promote fungal growth (Zender-Świercz *et al*., 2019), and there is a suggested correlation between the amount of fungi and indoor air temperature (Stojanović Bjelić *et al*., 2020). Molds produce millions of spores that can become airborne with minimal air disturbance. These spores, typically measuring between 1-20 micrometers, can remain airborne and be inhaled deeply into the respiratory system. They are highly resistant to dryness, temperature changes, UV light, and some chemicals (Giri, 2020).Bacteria such as Streptococcus, Mycoplasma, and Staphylococcus can cause skin diseases, respiratory issues, and allergies, leading to increased infection rates among exposed individuals. Ideally, indoor air should contain fewer microorganisms than outdoor air (Salihu *et al*., 2018). Another study identified *Corynebacterium* and *Staphylococcus* as prevalent bacterial genera indoors, accounting for 45% and 88% of bacterial reads, respectively (Degois *et al*., 2021a).High humidity and poor room cleanliness promote robust fungal growth. Additionally, environmental factors like vegetation, urbanization, and airborne particulate matter influence mold and bacterial proliferation in indoor air (Dang *et al*., 2020). The US Environmental Protection Agency (EPA) and the World Health Organization (WHO) recognize indoor air quality (IAQ) as a multidisciplinary issue and have categorized pollutants accordingly. The presence of fungi and bacteria indoors can cause severe health problems, including respiratory symptoms like asthma, coughing, reduced lung function, wheezing, allergies, allergic bronchopulmonary aspergillosis (ABPA), and allergic fungal sinusitis (AFS) (Mannan & Al-Ghamdi, 2021).Mechanical ventilation systems can also adversely affect health, increasing the risk of asthma, dry eyes, and sick building syndrome symptoms (Lim *et al*., 2021). A report by the Royal College of Physicians estimated that nearly 3

billion people globally are exposed to poor indoor air quality daily due to the use of solid fuels for cooking, heating, and lighting. This report concluded that household air pollution significantly contributes to global morbidity and mortality, particularly affecting the respiratory system. Fungal spores are a well-known cause of respiratory allergies in both the upper and lower respiratory tracts. Allergic reactions typically occur at the site where allergens are deposited. Inhaling a high number of fungal spores can deposit them in the nasopharynx, causing nasal and/or ocular symptoms commonly known as hay fever (rhinitis). Spores smaller than 5 micrometers can reach the lower airways, triggering allergic reactions and asthma (Giri, 2020). A study found that increased indoor carbon dioxide (CO2) levels, which correlate with ventilation rates, are linked to higher risks of eye fatigue, allergic rhinitis, and atopic dermatitis symptoms. A 100 ppm rise in CO2 was associated with an increased risk of eye fatigue and coughing in adults. Additionally, high indoor relative humidity was linked to lower risks of skin dryness in adults but higher risks of coughing and rhinitis in children (Lim *et al*., 2021).Human health is closely linked to the balance of microbial communities (Blaser, 2014). The human microbiome helps protect against skin pathogens (Grice & Segre, 2011), aids in digestion, supplies nutrients, and activates the immune system (Eckburg *et al*., 2005; Walia *et al*., 2014; Adar *et al*., 2016). A balanced microbial community is more resilient and better able to defend against pathogen invasion. Dysbiosis can destabilize this community, making the host susceptible to infection and inflammation, with some studies linking dysbiosis to immune disorders (Honda & Littman, 2012). In larger ecosystems, like coral reefs, greater biodiversity improves the efficiency of resource utilization by ecological communities. The term "holobiont," coined in 1992, describes host-microbe symbioses (Mindell, 1992). Corals, for example, form symbiotic relationships with specific zooxanthellae, gaining various nutrients from them. Corals also utilize

metabolites from microorganisms and cyanobacteria (Thompson *et al*., 2014; Cardini *et al*., 2016). These symbiotic microorganisms support their host by providing nutrients, aiding development, and offering pathogen resistance (Thompson *et al*., 2014). Understanding holobiotic systems is crucial for managing human health and disease, as the human microbiome is linked to health outcomes (Postler & Ghosh, 2017). Bacterial composition changes readily due to dietary shifts, antibiotic use, infections, and environmental factors (Eckburg *et al*., 2005; Walia *et al*., 2014; Adar *et al*., 2016).Indoor environments, where people spend up to 90% of their time, significantly influence health (Klepeis *et al*., 2001). Daily, humans inhale air containing about 10^6 airborne microorganisms (Mandal & Brandl, 2011). Some of these microorganisms cause pneumonia (e.g., nontuberculous mycobacteria, Legionella, Mycoplasma species), asthma, or allergies (Dannemiller *et al*., 2016; Montagna *et al*., 2016; Nishiuchi *et al*., 2017). Reduced microbial diversity exposure during childhood in developed countries has been linked to rising allergies and autoimmune disorders (Fujimura *et al*., 2014; Fang *et al*., 2016; Man *et al*., 2017). High-density indoor environments, such as correctional facilities, military training centers, and dormitories, facilitate human-to-human transmission (Hoge *et al*., 1994; Brundage *et al*., 1988). Airborne bacteria in healthcare settings can lead to nosocomial infections (Schaal, 1991). The relationship between mental health and the indoor microbiome is explored by Hoisington *et al*. (2015). Therefore, identifying bacteria in indoor environments is essential for human health, particularly in laboratories where various research activities are conducted.

AIM:

The aim of this study was to assess indoor air of Microbiology laboratories in University of Benin

Objectives

The various objectives are:

I. evaluation of bacterial population in laboratory indoor air

II. phenotypic identification of bacterial.

III. antibiotic profiling of bacterial isolates

CHAPTER TWO

LITERATURE REVIEW

2.1. BIOAEROSOLS IN LABORATORY

A bioaerosol refers to airborne biological material, which can include bacterial cells, fragments of cells, fungal spores, fungal hyphae, viruses, and by-products of microbial metabolism. Other biological entities, like pollen grains, can also be part of bioaerosols. These microbial aerosols are produced in both outdoor and indoor environments due to various natural and human activities. For example, wind, rain, wave splashes, spray irrigation, wastewater treatment activities, cooling towers, air handling water spray systems, and agricultural processes such as harvesting and tilling contribute to the generation of outdoor bioaerosols.Inside buildings, bioaerosols are produced and spread through mechanical and human activities. Industrial operations, manufacturing processes, and bio-fermentation practices can lead to high concentrations of microbial aerosols. Heating, ventilation, and air conditioning (HVAC) systems, water spray devices like showerheads and humidifiers, and cleaning activities such as dusting, sweeping, vacuuming, and mopping facilitate the movement of microbial materials in the air. Individuals talking and coughing can also generate bioaerosols, some of which may be infectious. Medical, dental, and animal care facilities can produce infectious microbial aerosols. The particle

size of bioaerosols typically ranges from 0.3 to 100 micrometers in diameter; larger particles settle quickly and are less likely to be transported through the air. Virus particles are nanometers in size, bacterial cells are around 1 micrometer in diameter, and fungal spores are larger than 1 micrometer. These microorganisms can be airborne as single units or in aggregates, with larger aggregates exhibiting different aerodynamic properties compared to single-cell units. Aggregates provide protection from environmental stresses such as desiccation, UV radiation, ozone, and other atmospheric pollutants.Bacterial cells and virus particles are often associated with skin cells, dust, and other organic or inorganic materials. During agricultural practices, such as harvesting and tilling, fungal spores are released from plant surfaces and soil, hitching a ride on other particulate matter. This "rafting" alters their aerodynamic properties and survival in the bioaerosol. When biological material disperses from water sources (e.g., splash, rainfall, cooling towers, fountains), it is typically surrounded by a thin water layer, enhancing aerodynamic properties and the microorganisms' survival while airborne. Airborne particles remain suspended until they settle or are inhaled. Larger particles tend to lodge in the upper respiratory tract (e.g., nose, nasopharynx) upon inhalation.Indoor Air QualityIndoor air quality significantly impacts health and well-being, as people spend 80–90% of their time indoors (Reynolds *et al*., 2001). An average person inhales 10 cubic meters of air daily, populated with various microorganisms in the form of a colloidal suspension known as bioaerosol (Srikanth *et al*., 2008). Airborne microorganisms are ubiquitous and can even be found in highly controlled environments like operating theaters (Ayoudgu, 2010; Ghanizadeh *et al*., 2012). Fungi are notable sources of allergic reactions and general health issues (Khan & Karuppayil, 2012). Indoors, these microorganisms can originate from people, organic dust, stored products, and air circulated through natural and artificial ventilation systems (Bragoszewska *et al*., 2018). Approximately

30% of office workers experience health issues due to poor indoor air quality (Szymczak *et al*., 2010). Exposure to these contaminants can cause allergic reactions, infections, and intoxication, leading to various molecular reactions (Husman, 1996; Fung *et al*., 2001; Reijula *et al*., 2004; Khan *et al*., 2012; Baxi *et al*., 2016). Health problems related to indoor air quality in office environments are referred to as Sick Building Syndrome (SBS) (WHO, 1988). The World Health Organization (WHO) prioritized studies on airborne microorganisms and other air pollutants in 2009 (WHO, 2009).Microorganisms can adapt to different conditions, resulting in significant variations in their concentration within different areas of the same indoor environment (Fernström & Goldblatt, 2013). Seasonal variations in temperature, relative humidity, and air exchange rates influence the composition and abundance of airborne microorganisms indoors (Frankel *et al*., 2012; Kumari *et al*., 2016; Park *et al*., 2016). In pre-nursery schools, the presence of airborne microorganisms can affect children's respiratory health and well-being (Madamarandawala *et al*., 2019). Common fungal genera found in indoor areas of Kolkata, India, include Aspergillus, Curvularia, Penicillium, and Rhizopus (Karmakar *et al*., 2020). Mycotoxin-producing micromycetes in indoor environments play a crucial role in causing SBS in occupants (Švajlenka *et al*., 2017).Hospitals are critical indoor environments for the spread and propagation of aero-microflora (Chirca, 2019). An air sampling study in a hematology hospital found Aspergillus and Penicillium to be the dominant fungal genera both indoors and outdoors (Cho *et al*., 2019). Surveys are an effective method for analyzing the health effects of indoor air quality (Wu *et al*., 2018). A significant cross-sectional survey conducted by Herr *et al*. (2003) found that waking up due to coughing, coughing, and bronchitis were major problems linked to microbial contamination of outdoor air. Another cross-sectional study in Australia suggested that non-

biological factors such as overcrowding, dust, and water supply are associated with health issues like respiratory problems, asthma, and skin conditions (Melody *et al*., 2016).

2.2.2. Airborne microorganisms in laboratory

Microorganisms constitute the majority of the Earth's biomass and are ubiquitous, thriving in environments with extreme radiation, heat, pressure, salinity, cold, and darkness (Rothschild *et al*., 2001). Air contains nitrogen, oxygen, carbon dioxide, trace gases, inorganic particles, and particles of biological origin. Bioaerosol particles encompass bacteria, fungi, viruses, spores, lichen fragments, protists (such as protozoa, algae, and diatoms), plant fragments, pollen, small seeds, and fecal material (Lacey, 2006). Airborne microorganisms transported by dust clouds can directly affect human health through pathogenesis, exposure to cellular components, and the development of sensitivities such as asthma from prolonged exposure (Griffin *et al*., 2007). The Earth's lower atmosphere contains a wide variety of microbes, from algae to viruses. In addition to gases, dust particles, and water vapor, air also harbors microorganisms. Exposure to sunlight, higher temperatures, and lower moisture levels in the air causes many of these microbial forms to perish. Air currents play a crucial role in dispersing microorganisms over long distances. The presence of microorganisms in the atmosphere was first demonstrated by Spallanzani's experiments in the mid-18th century (Capanna, 1999) and later by Pasteur in the 19th century (Pasteur, 1890). Despite these early discoveries, the atmosphere remains a significant area of study for microbiologists. Beyond classical aerobiology pursuits (describing the abundance and diversity of atmospheric microorganisms, their responses to atmospheric conditions, and their dispersal), microbes are typically found within 300-10,000 feet above the ground.Fungal spores such as *Alternaria, Cladosporium, Penicillium*, and Aspergillus have been detected above 4,000 feet in both polar and non-polar air masses. Organisms found below 500 feet, particularly in

densely populated areas, include spores of Bacillus and Clostridium, yeast ascospores, mycelium fragments, molds, Streptomycetaceae, pollen, protozoan cysts, algae, M*icrococcus*, and Corynebacterium. Air in schools, hospitals, or the living spaces of individuals with infectious diseases has also been associated with pathogens like tubercle bacilli, streptococci, and pneumococci (Polymenakou, 2012). Air, the simplest environment comprising a single gas phase, does not naturally support the growth and reproduction of microorganisms due to insufficient moisture and nutrients. Despite this, air still contains gases, dust particles, water vapor, and microorganisms. Microorganisms such as bacteria, fungi, viruses, and their spores are almost always present in the air. The quality of indoor environments is difficult to control, which can pose risks to laboratory technicians (Jaffal *et al*., 1997). Microorganisms are primary sources of indoor air contamination because enclosed spaces can trap aerosols, allowing them to reach infectious levels (Jaffal *et al*., 1997). Laboratory air can harbor large amounts of potentially harmful microorganisms due to various factors, including human normal flora and activities. Sources of laboratory air microflora include staff normal flora, visitors, students, and materials within the laboratory. Human activities like coughing, sneezing, talking, and yawning also contribute to laboratory infections (Ekhaise *et al*., 2008). Materials such as files can also be viable sources of microorganisms in the laboratory (Burge *et al*., 2000). Seasonal variations can influence indoor microbial analysis results. Reponen *et al*. (1992) found that indoor fungal counts were significantly lower in winter compared to other seasons, while airborne bacterial counts did not exhibit a clear seasonal pattern. Common microorganisms isolated from indoor air include *Staphylococcus* spp., *Micrococcu*s spp., *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., and *Rhizopu*s spp.

2.3 Factors influencing the composition of airborne bacterial communities

Despite the challenges of spatial and temporal variability and the lack of standardized methods for air collection and sample processing, which complicate comparisons across studies, general trends have been observed that correlate bacterial community composition with environmental factors. These factors, including seasonality, meteorological conditions, human influences, and variability in bacterial sources, significantly shape the abundance and composition of airborne bacterial communities over time and space. However, the extent and manner of these effects are highly context-dependent. For instance, in Milan, Italy, bacterial communities sampled during the summer showed less variation compared to those sampled in other seasons, potentially due to the stability of air and particulate matter levels during the summer (Bertolini *et al*., 2013). Additionally, summer stressors like ozone, drought, and solar radiation may consistently apply selective pressure, favoring the survival of adapted species.Two general theories explain the correlation between meteorological factors and community composition: first, atmospheric stressors select for bacteria with certain adaptations (such as spore formation or pigmentation); second, wind, temperature, precipitation, and season affect the contributions of different source environments to the airborne bacteria at a specific location (e.g., an increase in leaf-associated bacteria during summer) (Jones and Harrison, 2004; Bowers *et al*., 2013; Huffman *et al*., 2013).In the latter case, the primary factor influencing bacterial abundance is the contribution from different sources, suggesting that airborne bacteria are merely a collection of organisms dispersed from various sources, rather than integral parts of an atmospheric ecosystem. However, airborne bacterial communities are often distinct from their source environments, possibly because many bacteria do not survive the atmospheric conditions (Bowers *et al*., 2011a). Other ecological factors, such as the availability of specific substrates, may also exert selection pressures in the atmosphere. This aligns with the "atmosphere biome hypothesis" proposed by

Morris *et al*. (2011), which suggests that airborne microbes actively interact with each other and their environment. Studies on the adaptations and metabolic activity of airborne bacteria support this hypothesis (as discussed in section 2.6). It is likely that both explanations coexist, as suggested by Womack *et al*. (2010), indicating that only a certain fraction of airborne bacteria are metabolically active.

2.4. **Airborne bacteria in laboratory atmosphere**

2.4.1. Sources

Bacteria enter the near-surface atmosphere through aerosolization from various surfaces affected by air currents. Jones and Harrison (2004) propose that bacteria are released from soil and plant surfaces into the atmosphere through particle resuspension processes, a theory supported by multiple observations. Firstly, several studies have found correlations between land cover and near-surface atmospheric bacterial concentrations, including those by Bertolini *et al*. (2013), Shaffer and Lighthart (1997), and Tong and Lighthart (2000). Secondly, 'source-tracking studies' estimate the relative contributions of different sources to airborne bacteria at specific locations. These studies use taxonomic identifications of airborne organisms to determine the likely sources where these taxonomic units are typically found (Bowers *et al*., 2011a, 2011b; Cao *et al*., 2014). Thirdly, upward bacterial fluxes from soil and vegetation can be quantified. For example, Lighthart and Shaffer (1994) recorded a maximum flux of 17,000 CFU/m²/h above desert-like

scrubland, and they found that the upward flux was correlated with the intensity of sensible heat due to its role in driving convective air movements. Additionally, oceans and seas also contribute to the atmospheric bacterial content through the ejection of aerosol droplets into the air (Aller *et al*., 2005).

2.4.2. Dispersal

Bacteria can exist in the atmosphere as individual cells or attached to other particles like soil dust, leaf fragments, spores, or other microorganisms (Lighthart, 1997; Maki *et al*., 2008; Maron *et al*., 2005; Tong and Lighthart, 2000). Once aerosolized, bacteria can be lifted by convective air movements and, due to their small size, can remain airborne for extended periods. Intercontinental transport of bacteria has been observed, particularly with dust plumes from deserts and drought-affected areas (Barberan *et al*., 2014; Hara and Zhang, 2012; Kellogg and Griffin, 2006; Lim *et al*., 2011; Polymenakou *et al*., 2008). These events significantly alter the atmospheric bacterial community downwind (Maki *et al*., 2013). For example, desert dust-associated bacteria can outnumber local atmospheric bacteria tenfold at locations over 1000 km from the source (Jeon *et al*., 2011). Dust plumes may also pick up marine bacteria while traveling over oceans or seas (Kellogg and Griffin, 2006; Maki *et al*., 2013). Besides dust plumes, tropical storms or transport into the higher troposphere can facilitate the long-range movement of airborne bacteria (Burrows *et al*., 2009a; DeLeon-Rodriguez *et al*., 2013; Stres *et al*., 2013). Increasingly, studies on airborne microbes use backward trajectory modeling to better understand their origins, as seen in research by Bottos *et al*. (2014), Fahlgren *et al*. (2010), Lee *et al*. (2007), and Murata and Zhang (2014).

2.4.3. Deposition

Bacteria are eventually removed from the atmosphere by either "dry" deposition or "wet" deposition (Jones *et al*., 2008). Dry deposition is explained by adherence to buildings, plants, water surfaces, the ground and other surfaces in contact with the air (Jones and Harrison, 2004; Jones *et al*., 2008). The wet deposition of bacteria is caused by the precipitation of rain, snow or hail that has collected atmospheric particles (Christner *et al*., 2008a; Jones *et al*., 2008; Monteil *et al*., 2014; Peter *et al*., 2014). In some cases, wet deposition can be actively induced by the bacteria themselves.

2.5. Survival of airborne bacterial isolates

Despite the common notion that the atmosphere is a harsh environment for bacterial survival, diverse bacterial communities have been identified in other extreme settings such as the deep sea, hot springs, and deserts (Rothschild and Mancinelli, 2001; Womack *et al*., 2010; An *et al*., 2013; Puspitasari *et al*., 2015). Atmospheric conditions are marked by nutrient scarcity, UV radiation, desiccation, temperature and pH fluctuations, and reactive oxygen species. Airborne microbes likely survive due to DNA repair mechanisms, pigmentation, aggregation mechanisms, and metabolic adaptations like cytochrome biosynthesis to cope with iron deficiency (Tringe *et al*., 2008; Womack *et al*., 2010). Bowers *et al*. (2013) found greater diversity on coarser particles compared to finer ones, suggesting that bacteria attached to substrates have higher survival rates. Similarly, Stres *et al*. (2013) reported a strong link between dust particle abundance and bacterial cell count. Amato *et al*. (2015) observed that bacterial aggregation in a cloud simulation chamber promoted survival. These aggregates likely facilitate the activities and interactions characteristic of an atmospheric biome. Another bacterial survival strategy is entering a nondividing (dormant) state, transforming into spores or modifying their cell walls, thereby slowing or halting metabolic activity (Bär *et al*., 2002; Delort *et al*., 2010). These changes enhance resistance to physical

stresses like UV radiation (Kobayashi *et al*., 2015), increasing atmospheric survival chances. For example, during Asian dust events, the viable airborne bacteria often include mainly spore-forming *Bacillus* species (Maki *et al*., 2013; Yamaguchi *et al*., 2014).

2.6. Effect of airborne bacteria in Human health

The atmosphere is often recognized as a significant carrier of bacterial pathogens, including *Streptococcus pneumoniae, Streptococcus pyogenes, Mycoplasma pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Mycobacterium tuberculosis*. Understanding the source, survival, dispersal, and environmental relationships of outdoor pathogens is crucial for their management. Dust events play a significant role in aerosolizing and transporting bacteria, with potential implications for disease spread (Griffin, 2007). An illustrative example is the occurrence of meningococcal meningitis outbreaks across sub-Saharan Africa, closely associated with dry seasons marked by dust storms, and subsiding with the onset of the wet season (Molesworth *et al*., 2002).Dust particles, especially in low absolute humidity conditions, are believed to enhance infection by causing abrasions in the nasopharyngeal mucosa, facilitating the entry of Neisseria meningitidis. Besides pathogens, airborne microbes and their components (e.g., endotoxins, mycotoxins, glucans) can significantly impact health in specific contexts. Airborne biological agents have emerged as notable safety and health concerns in agriculture, biotechnology, and industrial settings (Eduard *et al*., 2012; Martinez *et al*., 2004).Endotoxins, also known as lipopolysaccharides (LPS), are extensively studied in the air. Present in the outer membrane of Gram-negative bacteria, they can evoke a robust immune response irrespective of bacterial viability. Moreover, endotoxins are remarkably resilient and commonly enter our respiratory tracts due to their widespread presence. While high concentrations of endotoxins can cause acute and chronic health effects (Rylander, 2006), the

absence of an occupational exposure limit is primarily attributed to inter-laboratory variability and the lack of a standardized international protocol for sampling and analyzing airborne endotoxins.Factors influencing the nature and intensity of our immune response to endotoxins are multifaceted and may result in either beneficial or adverse outcomes. These factors encompass an individual's immune susceptibility (prior exposures and genetic predisposition), duration and level of exposure, and synergistic contaminants (Liu, 2002). For instance, endotoxins are integral components of airborne particulate matter and are believed to enhance the immune response of particulate matter co-pollutants such as transition metals (Degobbi *et al*., 2011). Such synergistic effects have been predominantly demonstrated in cell models (Imrich *et al*., 1999; Smets *et al*., 2016; Long *et al*., 2001) and corroborated by the Childhood Allergy and Air Pollution high-risk birth cohort study, indicating that exposure to traffic-related particles and endotoxin during infancy is associated with wheezing at age 3 years (Ryan *et al*., 2009).Moreover, airborne endotoxins do not always exert detrimental effects. The most extensively studied beneficial role involves immune stimulation and maturation. Some research suggests that early childhood exposure to microbes and their components, including endotoxins, is pivotal for immune system development and mitigating allergies and atopic asthma onset (Mutius, 2000; Liebers *et al*., 2008; Schuijs *et al*., 2015). This aligns with epidemiological evidence indicating lower allergy and asthma prevalence in children raised in rural and farming communities compared to urban populations (Braun-Fahrlander *et al*., 1999; Ernst and Cormier, 2000).

2.7. Factors influencing bacteria survival in air

Despite the variability in space and time and the absence of standardized methods for air collection and sample processing, which complicates comparisons across studies, there is a

noticeable trend in various studies linking the composition of bacterial communities with environmental factors. Among these factors, seasonality, meteorological conditions, human activities, and variations in bacterial sources play significant roles in shaping the abundance and composition of airborne bacterial communities across different locations and time periods. However, the extent to which these factors influence bacterial communities is highly dependent on the specific context.For instance, in Milan (Italy), communities during the summer exhibited less variation compared to those sampled in other seasons, possibly due to the stability of air and particulate matter levels during the summer months (Bertolini *et al*., 2013). It is conceivable that stressors prevalent in summer, such as ozone, drought, and solar radiation, collectively exert a consistent selective pressure, leading to the survival of adapted species. Regarding the correlation of meteorological factors with community composition, there are two general speculations: atmospheric stressors may drive the selection of different bacterial adaptations (e.g., spore formation, pigmentation), or variations in wind, temperature, precipitation, and seasonal changes may influence the contribution of different source environments to airborne bacteria at specific locations (Jones and Harrison, 2004; Bowers *et al*., 2013; Huffman *et al*., 2013).In the latter case, the contribution of sources plays a predominant role in determining the abundance of various bacteria in the atmosphere, suggesting that airborne bacteria primarily consist of organisms dispersed from diverse sources, rather than forming an integral part of an atmospheric ecosystem. However, airborne bacterial communities exhibit distinct differences from their source environments, potentially due to the inability of many bacteria to survive in the atmospheric conditions (Bowers *et al*., 2011). Moreover, the specific selection pressure in the atmosphere may be influenced by ecological factors such as the availability of certain substrates. This ecological perspective aligns with the "atmosphere biome" hypothesis proposed by Morris

et al. (2011), suggesting that airborne microbes actively interact with each other and the environment.It is likely that both explanations coexist, indicating that only a fraction of bacteria in the atmosphere are metabolically active, as noted by Womack *et al*. (2010).

Prevention of airborne bacteria in laboratories

Laboratories serve as hubs for scientific exploration, innovation, and advancement. Preserving the integrity of research findings and safeguarding the well-being of laboratory staff are paramount concerns in upholding the sanctity of these environments (Bowers *et al*., 2011). Airborne bacterial contamination poses a persistent threat to laboratories, potentially compromising experimental accuracy, endangering personnel health, and undermining the credibility of research institutions. This article explores the multifaceted approaches employed to mitigate airborne bacterial contamination in laboratories, encompassing regulatory frameworks, facility design, staff training, and state-of-the-art technologies (Bowers *et al*., 2011).Airborne bacteria infiltrate laboratories through diverse pathways, including human activities, contaminated equipment, and environmental factors. The consequences of such contamination are extensive, ranging from compromised research integrity to potential health hazards for laboratory personnel (Bertolini *et al*., 2013). It is imperative to identify the sources and comprehend the health risks associated with airborne bacteria to develop effective prevention strategies. Existing regulatory guidelines, such as those established by the Occupational Safety and Health Administration (OSHA) and the Centers for Disease Control and Prevention (CDC), provide standards for laboratory safety. Adhering to these protocols entails the formulation and implementation of rigorous standard operating procedures (SOPs), routine inspections, and accreditation processes. Compliance with these regulations is fundamental for effective prevention (Polymenakou, 2012).Critical to prevention efforts is the design of laboratory

facilities. Ventilation systems play a crucial role in preserving air quality, with tailored systems meeting specific laboratory requirements. Utilization of High-Efficiency Particulate Air (HEPA) filters and Ultraviolet (UV) germicidal irradiation constitutes essential components of air purification technologies. Furthermore, physical containment measures like negative pressure chambers aid in mitigating airborne contamination. Ensuring proper utilization of Personal Protective Equipment (PPE) is indispensable for preventing airborne bacterial infiltration. Lab coats, gloves, goggles, and respirators are indispensable elements of PPE (Polymenakou, 2012). Implementation and enforcement of stringent PPE protocols, alongside comprehensive staff training initiatives, bolster defenses against bacterial intrusion (Bertolini *et al*., 2013). Regular hygiene practices, encompassing cleaning, disinfection, and waste management strategies, serve as the foundation of prevention efforts. Fostering a culture of hygiene and accountability among laboratory personnel, coupled with continuous training endeavors, ensures sustained adherence to best practices (Bertolini *et al*., 2013).

CHAPTER THREE

Materials and Methods

3.1 Study site

The research work was carried out in the Microbiology laboratory of the University of Benin, Benin City.

3.2 Sample collection

The air sampling was done in Microbiology laboratories, University of Benin, Benin City. The samples was done using different time frame and was later transported aseptically to the Microbiology laboratory, University of Benin, Benin City for microbial analysis.

3.3. Sterilization of Materials.

Glass wares (conical flasks, round bottom flasks, bottles.) were washed, drained and dried. They were wrapped in aluminium foil and sterilized in a hot-air oven at 160 °C for an hour. They were allowed to cool at about 40 °C after sterilization. An antiseptic working environment was achieved with the use of a spirit lamp and ethanol for swabbing the working desk.

3.4 Preparation of bacteria media

3.4.1 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 minutes at 121 °C. After sterilization, the flask was allowed to cool.

3.5 Heterotrophic (Standard) Plate Count

Heterotrophic plate count was carried out using the pour-plate method as described by America Public Health Association, APHA (1998). The standard plate count method consists of diluting a sample with sterile saline or phosphate buffer diluent until the bacteria are dilute enough to count accurately. Thus, the number of colonies should give the number of bacteria that can grow under the incubation conditions employed.

3.7.1. Gram staining

The Gram staining technique is used to differentiate Gram positive from Gram negative bacterial stains according to (Hemraj *et al*., 2013). A drop of sterile distilled water was placed on a neat and clean glass slide containing a single isolated colony of 24hours. The smear was made by spreading the culture. This smear was air dried and fixed by rapidly passing the slide three times over the flame. It was then flooded with crystal violet for 1minute and then washed off with

distilled water. Then Grams iodine solution was added to the smear and the glass slide was left for one minute and rinsed with distilled water. Alcohol was added and safaranin was added as counter stain and viewed Cheesbrough (2006).

Biochemical Tests

3.7.2 Catalase test

This test was carried out to differentiate between a catalase enzyme-producing bacterium such as *Staphylococcus aureus* and non-catalase enzyme producing bacteria such as *Streptococcus* sp.. Two milliliters (2 ml) of 3% hydrogen peroxide solution was measured and transferred into test tube. Using a sterile glass rod, several colonies of the test organisms were removed and immersed in the hydrogen peroxide solution. Immediate bubbling in the tube shows a positive catalase test.

3.7.3. Coagulase test

This test was used to differentiate coagulase-producing *Staphylococcus aureus* from the nonproducing ones. A drop of distilled water was placed on each end of a clean slide. Colonies of the test organisms were emulsified in each of the drops to make two thick suspensions. A loopful fresh human plasma was added to one of the suspension, and mixed gently. Clumping of the organisms within 10 minutes indicates a positive coagulase test.

3.74. Oxidase test

Oxidase test strip (Oxoid, England) was used. This test was used to differentiate between oxidase-positive and oxidase-negative bacteria. Several colonies of the test organisms were robbed on the strip using sterile glass rod. Formation of purple colouration within 5 seconds indicates a positive oxidase test.

3.7.5. Urease test

The bacterial isolates were inoculated into slants of urea medium and incubated at 37^0C for 24 to 48 hours. Urease positive cultures produced a red-pink colour due to changes in the colour of the indicator (Cheesbrough, 2005).

$NH_2CO.NH_2 + H_2O \rightarrow 2NH_3 + CO_2$ - Equation I

3.7.6. Indole Test

This test was used to determine which of the isolates has the ability to split indole from tryptophan present in buffered peptone water. The test is usually used as an aid in the differentiation of gram-negative bacilli especially those of the enterobacteriaceae. Peptone water was prepared and about 3ml of it was dispensed in test tubes using a sterile pipette. Then, fresh sterile loops were used to pick a well-isolated colony of bacteria and inoculated into the test tubes, thereafter, the tubes were incubated at 37ºC for 48hours.After incubation, 0.5ml of Kovac's indole reagent was added to the inoculated bijou tubes. The tubes were subjected to gentle shaking and examined for red colour in the surface layer within 10 minutes. A red ring on top of the tube indicates indole positive reaction.

3.7.7. Triple Sugar Iron Agar (TSIA) Test

The Triple Sugar Iron agar (TSIA) test is designed to differentiate among the different groups or genera of the Enterobacteriaceae, which are all Gram negative bacilli capable of fermenting glucose with the production of acid, and to distinguish them from other Gram negative intestinal bacilli. The differentiation is based on fermentation of glucose and lactose or sucrose and hydrogen sulfide (H_2S) production. With a straight inoculation needle, touch the top of a well-isolated colony. Inoculate TSI by first stabbing through the center of the medium to the bottom

of the tube and then streaking the surface of the agar slant. Leave the cap on loosely and incubate the tube at 35 °-37 °C in ambient air for 18 to 24 hours. An alkaline/acid (red slant/yellow butt) reaction: It is indicative of dextrose fermentation only. An acid/acid (yellow slant/yellow butt) reaction: It indicates the fermentation of dextrose, lactose and/or sucrose. An alkaline/alkaline (red slant, red butt) reaction: Absence of carbohydrate fermentation results. Blackening of the medium: Occurs in the presence of H_2 Gas production: Bubbles or cracks in the agar indicate the production of gas (formation of CO_2 and H_2)

3.8. ANTIBIOTICS SENSITIVITY TESTING

The antibiotic susceptibility of the bacterial isolates was determined by the Kirby-Bauer agar disc diffusion technique (Bauer *et al.,* 1966). Antimicrobial susceptibility testing was carried out on all the bacteria isolates using the disk diffusion method. The isolates were spread on the already prepared agar plates containing Mueller-Hinton agar, after which the antibiotic disks were pressed firmly on the agar plates containing the isolates, and was incubated at 37 °C for 24 hr. After incubation, the Petri dishes were examined for zones of inhibition. The zones of inhibition were measured, and susceptibility was measured as Susceptible (S), Intermediate (I), or Resistant (R).

3.9 Data Analysis

The data were analysed using the SPSS package version 21.0. All data are mean of three replicates. The mean, range and standard deviation of each parameter was determined. The means were separated using Duncan's Multiple Range test (SPSS, 2010).

CHAPTER FOUR

RESULT

4.1

This study was aimed on isolation and characterization of bacterial isolates from laboratory indoor air in University of Benin, Benin City. The result of the cfu/m^3 is presented in table 1 while table 2 represents the cultural, morphological, biochemical and sugar fermentation test. Table three represents the abiotic test conducted in this study. The highest bacterial count was

obtained in lab 1 with bacterial population of $8.04x10^4 \pm 6.0$ while the lowest was obtained in lab 2 with value of $6.85x10^4 \pm 0.5$. The isolates obtained were mostly Gram positive which include*; Staphylococcus aureus*, *Streptococcus pyogenes*, *Micrococcus* sp, *Bacillus subtilis* and the Gram negative isolate was *Escherichia coli*.

Table 4.1: Mean bacterial count (cfu/m^3) for respective duration

SAMPLE CODE	CFU/m^3	P-VALUES

LAB 1	$8.04x10^4 \pm 6.0$	0.09
LAB 2	$6.85x10^4 \pm 0.5$	0.105

Note: values are mean ± standard error. Mean followed by different p-values are significantly different ($p<0.05$)

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

CULTURAL					
Shape	Round	Rhizoid	Round	Circular	Round
Elevation	Convex	Flat	Convex	Flat	Flat
Colour	Golden yellow	Cream	yellowish	Greyish White	Green metallic sheen

MORPHOLOGICAL					
Gram stain	Positive	Positive	positive	Positive	Negative
Cell type	Cocci	Rod	Cocci	Cocci	Rod
Cell arrangement	Cluster	Single	Single	Single	Single
BIOCHEMICAL					
Urease	+	-	-	-	-
Indole	-	-	-	-	+
Citrate	+	+	-	+	-
Catalase	+	+	+	-	+
Coagulase	+	-	+	-	-
Oxidase	-	+	-	-	-
H2S	-	-	-	-	-
Glucose	+	+	-	+	+
Sucrose	+	+	-	-	+
Lactose	+	-	-	-	+
Bacteria	*Staph. aureus*	*Bacillus subtilis*	*Micrococcus* sp	*Streptococcus pyogenes*	*Escherichia coli*

Table 4.3 Antibiotic sensitivity profile of isolated bacteria

ISOLATES	GEN	CTR	ERY	CXC	OFL	AUG	CAZ	CRX
Saphylococcus aureus	S	R	S	R	S	S	R	R
Bacillus subtilis	S	R	R	R	S	S	R	R

Micrococcus sp	S	R	S	R	S	S	R	R
Eschericia coli	R	R	R	R	S	R	R	R
Streptococcus	S	R	S	R	S	S	R	R

Key: GEN=Gentamycin, ERY=Erythromycin, CTR=Ceftriaxone, CXC=Cloxacillin, OFL=Ofloxacin, AUG=Augmentin, CAZ=Ceftazidime, CRX=Cefuroxime

CHAPTER FIVE

DISCUSSION

Microbiological quality assessment of indoor air study is one of the most vital investigations to determine the microbial indoor air contaminant. The information on the indoor microbial concentrations of airborne bacteria and fungi is necessary both to estimate the health hazard and to create standards for indoor air quality control. The concentrations of bacteria measured in all laboratories were significantly different to each other (P=0.001). These can be mainly explained by the variation of density of occupant during sampling time as well as the variation of ventilation conditions (Wamedo *et al.,* 2012). The results obtained from this study showed that laboratory one had the microbial load of $8.04x10^4$ ± 6.0 Cfu/m^3 while laboratory two had the microbial load of $6.85x10^4$± 0.5 Cfu/m^3 This result is in agreement with study conducted by Amoah *et al.* (2020) on indoor air pollution

Human activities like speaking, sneezing, coughing, walking, and cleaning can generate biological particles in the air. Additionally, various sources such as food, houseplants, dust, clothing, carpets, wood materials, and furniture may intermittently release different types of microorganisms indoors (Dang *et al*., 2020). In this study, standard morphological and biochemical tests identified isolates including *Staphylococcus aureus, Micrococcus* sp, *Bacillus subtilis, Streptococcus*, and *Escherichia coli*, which are consistent with findings by Hassan *et al*. (2021).Laboratory one exhibited the highest bacterial count per cubic meter (CFU/m3), possibly due to a higher number of occupants relative to its size, while the Department of Microbiology at the University of Benin had the lowest concentration. Similar studies have associated airborne bacteria with the presence of individuals in enclosed spaces (Meadow *et al*., 2014). Furthermore, the bacterial concentrations measured were significantly different (p-value = 0.001), likely influenced by variations in occupancy density and environmental factors. Environmental conditions conducive to microbial growth may have contributed to higher microbial loads,

consistent with WHO's assertion that dampness is a risk factor for indoor air quality (WHO, 2009).Gram-positive bacteria were susceptible to gentamycin and augmentin, while Gram-negative bacteria showed resistance to these antibiotics but were susceptible to ofloxacin. Predominantly, the isolated bacteria were Gram-positive cocci associated with human skin and mucosa, suggesting that human presence was the primary source of suspended bacterial contamination in indoor air. Longer sampling durations corresponded to higher CFU/m3 counts, as observed by Soto *et al*. (2009), with similar findings reported by others (Soto *et al*., 2017).Airborne microorganisms can lead to infectious diseases when inhaled, posing a contamination risk among humans, as emphasized by Yusup *et al*. (2014). Microbial air pollution adversely affects health, particularly when pathogenic species are present, potentially causing inflammation, allergies, and infections (Wei *et al*., 2017).

CONCLUSION

The selected sampling laboratories of Faculty of Life Sciences, University of Benin were contaminated with bacteria. Thus, attention must be given to control those environmental factors which favor the growth and multiplication of microorganisms in indoor environment of the school to safeguard health of users and workers, and it is vital to control visitors and students in and out the laboratories. Additionally, it is necessary to adopt the guidelines for the design and construction of new facilities and for renovation of existing facilities in order to control indoor air-quality. Moreover, it is advisable that strict measures be put in place to check the increasing microbial load in the laboratories.

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