ISOLATION AND IDENTIFICATION OF AIRBORNE BACTERIA FROM HALL 2 TOILETS, UNIVERSITY OF BENIN

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CERTIFICATION

This is to certify that this research work was carried out by **Peace Ojemenwan ISIWELE** LSC1806742 in the Department of Microbiology, University of Benin, Benin City, Edo State, Nigeria. In partial fulfillment of the requirements for the award of B.Sc. in Microbiology of the University of Benin.

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DEDICATION

I would like to dedicate this work to God Almighty, the giver of life and to my family. I am thankful and blessed to have the ultimate support and motivation from my family. My Loving mom Mrs. ANGELINA ISIWELE and my sister Mrs Jane Iyare, whose words of encouragement and push for tenacity ring in my ears and my other r siblings who never left my side.

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ABSTRACT

Public toilets are dynamic environments affected by both microbial load and indoor air quality from the occupants' activities. Management at University of Benin faces a challenge when it comes to maintaining satisfactory air quality in the hostel toilets. Regular monitoring is therefore, necessary to evaluate air control effectiveness and to detect the irregular introduction of airborne microorganisms via occupants. The principal aim of this study is to assess the microbial quality of the air in some selected toilets at hall two female hostel, University of Benin by measuring indoor bacterial loads. Samples were collected from Female Student Toilets in Hall 2 Hostel, University of Benin by setting exposed plates for 15minutes. Bacteria were incubated at 37°C for 24hrs. The identified bacterial isolates were *Escherichia coli, Staphylococcus* sp*., Proteus* spp*., Citrobacter* sp*., Micrococcus* sp. and *Bacillus cereus.* There were high levels of bacterial contamination in the toilet. These findings underscore the importance of thorough cleaning and disinfection protocols in public toilets to minimize the risk of bacterial contamination and subsequent infections.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Indoor and outdoor environments are home to microorganisms, with the majority of individuals residing there. In various locations such as homes, businesses and offices, educational institutions, and healthcare facilities, where they are exposed to various bioaerosols (airborne biological contaminants such as bacteria, viruses, mushrooms or their by-products). Today, poor ventilation, crowded conditions, and increased indoor air conditions can favor the spread and survival of airborne particles, and can also increases the risk of airborne infections. Among the dust particles present in the indoor environment, fungi reproduce by forming spores, some bacteria including gram-positive bacteria, and some viruses can survive for a long time in the air (Sheik and al., 2015).

Indoor air quality is the specific constituent of the air that fills and surrounds buildings and structures, and its significance lies in the health and comfort of occupants. The quality of air that an individual breathes in the environment in which they find themselves largely determines that individual's health (Leech et al., 2002). The indoor air quality has become a significant issue in recent times due to the fact that most individuals spend time indoors, whether at home, at work, or at schools or other public places, and are exposed to multiple microorganisms. affect their health. and physical condition (Stryjakowska-Sekulska et al., 2007).

A building that has one or more toilets that can be easily used by everyone or the general public (to urinate, defecate or both) is called a public toilet and they are mainly found in facilities, schools, airports, cinemas, parking lots, etc. A restroom is a small room or building containing one or more

toilets and is available to the public (Yassin and Almouqatea, 2010).

Microorganisms are found in both indoor and outdoor environments, most of the people spend their lives indoors: in houses, industries, offices, colleges, schools, hospitals etc., where they are exposed to many bioaerosols (biological air borne contaminants such as bacteria, viruses, fungi or their byproducts). Poor ventilation, crowded conditions and increase in number of air conditions inside building nowadays can facilitate the spreading and the survival rates of airborne particles and also can increase the chance of people at risk of airborne infections. Among dust particles present in the indoor environment, fungus which reproduce by forming spores, some bacteria especially Gram positive bacteria and some viruses can survive for a long time in the air (Sheik *et al*., 2015).

Indoor air quality is defined as the quality of air within and around buildings and structures, and its significance especially in relation to health and comforts of occupants. The quality of air inhaled by an individual within an environment in which the individual finds himself determines to a great extent, the well-being of that individuals (Leech *et al*, 2002). In recent years, indoor air quality has become a topic of serious concern, since most people spend their indoors, either in their house, office, school or other public places, where they are exposed to some indoor microorganisms which have much effects on their health and physical condition (Stryjakowska-Sekulska *et al*., 2007).

A building containing one or more toilets that is easily accessible for use (for urination, defecation or both) to everyone or the public is referred to as public toilet and they are mainly found in different establishments, schools, airports, cinemas, motor parks, etc. The toilet is a room or small building housing one or more toilets and is made available for public usage (Yassin and Almouqatea, 2010) The public toilets (away from home toilet) comprise of traditional ‘street toilets’ and toilets that are no longer used which is accessed by the public. In some public toilets, people only access

them after paying while some are accessed without payment of an amount of money.

A toilet assessed by different persons could be a reservoir for infections especially if the toilets are not properly managed. (Sheik *et al,* 2015), the public toilet was referred to as "paying to get infections. Infection via the use of public toilets is increasing and there is a need to identify the microorganisms that may be responsible for these infections so as to speed up treatment. Toilets are one of the public facilities used by people and are located indoor. Therefore, maintaining good air quality in toilets is essential in order to keep it hygienic and sanitarily conducive for usage. In order to create a healthier and safer indoor environment, the first step is to maintain a good indoor air in toilets and washrooms (Mirbahar *et al*, 2005). Air is the easiest means by which agents of pathogenic microbes are disseminated, which can cause significant problems in the environment; especially, in public rooms such as toilets (Stryjakowska-Sekulska *et al*., 2007)

Insufficient ventilation, high influx of people and improper management of public toilets, are main sources of indoor air contamination in public toilets. Transmission of pathogenic organisms can be direct or indirect. In direct transmission, droplets containing microbial agents from individuals can be released into the air, which when inhaled by another can lead to an infection. Droplets are generated mainly through coughing and sneezing. Dissemination can be either by airborne droplets containing the organisms that remain suspended in the air for a long period of time or dust particles containing the infectious agents. Indirect transmission can occur through contact with contaminated surfaces and objects or through insect vectors.

Hall 2 is a Female Hostel in the University of Benin, consisting of many series, common room and reading room, so it is a place with influx of people and the toilets becomes a point of call and a place of relief for these people. This usually leaves the toilets untidy, making it a den of airborne

pathogens. The results revealed that the wetness of toilets, lack of litter bins and refuse lids and lack of ownership of some public toilets are the most contributing factors to the problem of hygiene and dirtiness of public toilets. These automatically impacts on the indoor air quality of these toilets. Microorganisms are ubiquitous and propagate rapidly wherever water is available. The dust and dirt's normally present in most public toilets provide sufficient nutrients to support extensive microbial growth. Mold specifically grows on all materials, including the dirty toilet bins and under the toilet seats; following the damp nature of most toilets, leading to mold growth. Microbial growth may result in greater numbers of spores, cell fragments, allergens, mycotoxins, endotoxins, β-glucans and volatile organic compounds in indoor air (Mera-ul-Haque *et al.*, 2016). The causative agents of adverse health effects have not been identified conclusively, but an excess level of any of these agents in the indoor environment is a potential health hazard. Microbial interactions and moisture-related physical and chemical emissions from toilet materials may also play a role in dampness-related health effects.

1.2 AIM AND OBJECTIVES

The aim of this study is to isolate and identify bacteria from Hall 2 Female Hostel Toilets, University of Benin.

The specific objectives were to;

i. enumerate and identify the airborne bacterial isolates.

ii. determine the antibacterial sensitivity pattern of the bacterial isolates against commonly used antibacterial agents.

iii. determine the public health significance of isolated pathogens using their multiple antibiotic resistance index (MARI).

CHAPTER TWO

LITERATURE REVIEW

2.1 Air Quality

Clean air is one of the fundamental needs and conditions for human life. Poor air quality has a detrimental effect on human health, and it is caused by anthropogenic activities and natural disasters (such as volcanoes), both of which have become more frequent as a result of industrialization and the ambition for human growth. The activities of human releases some gaseous emissions (SO_2, NO_2, CO, H_2S, VOCs and hydrocarbons) as well as particle emissions (smoke, soot, air pollutants, dust, fumes and aerosols) that, in excessive concentrations, could harm the environment and people's health. According to epidemiological studies, poor air quality significantly increases the risk of death and a variety of cardiovascular and respiratory diseases (Heft-Neal *et al*., 2018).

2.2 Airborne Diseases

Any disease that can spread through the air in the form of small, dry particles or larger liquid droplets is considered to be airborne. The pathogens that cause these diseases can be any type of microbe, including bacteria, fungi, or viruses, and they can spread through aerosols, dust, or liquids. Inhaled airborne pathogenic germs establish a home inside the host. By contacting a contaminated surface, the host can also pick up germs Small enough to attach to the air, disease causing particles can hang out on dust, moisture droplets, or even human breath until they are taken up. They can also be acquired by contact with bodily secretions like phlegm or mucus (Man *et al*., 2017). Once inside the body, the viruses multiply until a person becomes ill. The bodily secretions of an ill animal or person, as well as biological wastes that build up in lofts, caves, and rubbish, can produce aerosols. Such contaminated aerosols may float in air currents long enough to cover great distances

Inflammation in the sinuses, lungs, nose, throat, and pharynx is frequently brought on by airborne microorganisms or allergens. This is brought on by inhaling germs that harm a person's respiratory system or even the rest of their body after contacting a contaminated surface and touching your own eyes, nose, or mouth. These airborne chemicals can cause the upper respiratory tract to inflame, as seen by symptoms including sore throats, coughing, and sinus congestion. Asthma-related airborne disorders are heavily influenced by air pollution. According to some theories, pollutants affect lung function via escalating airway inflammation. Many common illnesses, including Otitis media, Pneumonia, Anthrax, Chickenpox, Influenza, Measles, Smallpox, Cryptococcosis, and Tuberculosis, can spread via airborne transmission, at least under some circumstances (Schaal *et al.,* 1991)

2.2.1 TUBERCULOSIS (*Mycobacterium tuberculosis*)

Tuberculosis is an infectious disease usually caused by *Mycobacterium tuberculosis* (MTB) bacteria which generally affects the lungs, but can also affect other parts of the body. Tuberculosis is spread through the air when people who have active TB in their lungs cough, spit, speak or sneeze. Most infections do not have symptoms in which case it is known latent tuberculosis. About ten percent of latent infections progress to active disease which, if left untreated, kills about half of those affected. People with latent TB do not spread the disease and active infection occurs more often in people with HIV/AIDS and those who smoke. The symptoms include; chronic cough with blood containing mucus, fever, night sweat and weight loss. The diagnosis of latent TB relies on the tuberculin skin test (TST) or blood tests. It can also be diagnosed by considering those with signs of lung disease or constitutional symptoms lasting longer than two weeks, carrying out chest X-ray and multiple sputum cultures for acid-fast bacilli. A definitive diagnosis of TB is made by identifying M. tuberculosis in a clinical sample (e.g., sputum, pus, or a tissue biopsy). However, the difficult culture process for this slow-growing organism can take two to six weeks for blood or

sputum culture. Thus, treatment is often begun before cultures are confirmed and Mantoux tuberculin skin test is often used to screen people at high risk for TB. Nucleic acid amplification tests and adenosine deaminase testing may allow rapid diagnosis of TB. These tests, however, are not routinely recommended, as they rarely alter how a person is treated. It can be prevented and controlled by vaccination of infants as well as its detection and appropriate treatment of active cases with the right antibiotics *(Nishiuchi et al.,* 2017)

2.2.2 WHOOPING COUGH

Whooping cough, also known as pertussis or 100-day cough, is a highly contagious bacterial disease and respiratory tract infection (Carbonetti, 2007). In many people, it's marked by a severe hacking cough followed by a high-pitched intake of breath that sounds like "whoop". Pertussis is caused by the bacterium *Bordetella pertussis*, it is an airborne disease (through droplets) that spreads easily through the coughs and sneezes of an infected person. People are infectious from the start of symptoms until about three weeks into the coughing fits while those treated with antibiotics are no longer infectious after five days. Before the vaccine was developed, whooping cough was considered a childhood disease.Now whooping cough primarily affects children too young to have completed the full course of vaccinations and teenagers and adults whose immunity has faded. Deaths associated with whooping cough are rare but most commonly occur in infants. That's why it's so important for pregnant women and other people who will have close contact with an infant to be vaccinated against whooping cough. Once infected with whooping cough, it takes about 7 to 10 days for signs and symptoms to appear, though it can sometimes take longer. They're usually mild at first and resemble those of a common cold which includes runny nose, nasal congestion, red watery eyes, fever and cough. After a week or two, signs and symptoms worsen. Thick mucus accumulates inside your airways, causing uncontrollable coughing. Severe and prolonged coughing attacks may

lead to provoke vomiting, result in a red or blue face, extreme fatigue, End with a high- pitched "whoop" sound during the next breath of air.

It can be diagnosed based on the symptoms, by running lab test which involves culturing of nasopharyngeal swabs on a nutrient medium and serology may be used for adults and adolescents who have already been infected for several weeks to determine whether antibody against pertussis toxin or another virulence factor of *B. pertussis* is present at high levels in the blood of the person. It can be prevented by vaccination and can also be treated by the use of antibiotics such as erythromycin, clarithromycin, or azithromycin.

2.2.3 PNEUMONIA

Pneumonia is an infection that affects the lungs, causing inflammation and potentially severe respiratory symptoms. It is caused by a variety of bacteria, including S*treptococcus pneumonia,* Kl*ebsiella pneumoniae* and P*seudomonas aeruginosa.* The symptoms of pneumonia can vary in severity, depending on the individual and the type of organism involved. Common symptoms include cough (with or without mucus production), fever, chills, difficulty breathing or shortness of breath, chest pain that worsens with deep breaths, fatigue, and sometimes confusion, especially in older adults. Additionally, some individuals may experience headache, muscle aches, and sore throat. To diagnose pneumonia, a healthcare professional will typically perform a physical examination and may order various tests. These can include chest X-rays to look for signs of infection in the lungs, blood tests to assess the presence of infection, and cultures of respiratory secretions to identify the specific organism causing the infection. In some cases, more specialized tests like a sputum culture or a bronchoscopy may be needed. To prevent pneumonia, it's important to practice good hygiene, such as washing hands regularly and covering your mouth when coughing

or sneezing. Vaccination against common pathogens like *Streptococcus pneumoniae* can also help prevent pneumonia. Treatment options may include antibiotics, rest, staying hydrated, and taking over-the-counter pain relievers to manage symptoms (Hoge *et al*., 1994)

2.3 MICROBES IN AIR

In addition to nitrogen, oxygen, and carbon dioxide, the air we breathe also contains minute amounts of other gases, inorganic particles, and biologically derived particles. The latter are known as bio-aerosols, and a large portion of them are made up of microorganisms that colonize soil, water bodies, plant surfaces, rocks, and structures and are easily discharged into the atmosphere by wind erosion and water splashing. Microorganisms can travel great distances in the air by attaching to background particles or forming water droplets in clouds, fog, and precipitation (rain, snow, and hail) (Bauer *et al*., 2002). Air microbiota can then be returned to the surfaces of the earth through dry and wet deposition processes, where they may have an impact on the diversity and efficiency of aquatic and terrestrial ecosystems or impose risks to human health through the spread of microbial infections. Viruses, bacteria, fungi and their spores, fragments of lichen, protists (including protozoa, algae, and diatoms), plant spores and fragments, pollen, small seeds, invertebrates (such worms, mites, spiders, and insects) and faecal waste are all examples of bio-aerosol particles.

Additionally to their effects on the environment and public health, there are significant air microorganisms appear to be metabolically active and well adapted to the challenging atmospheric circumstances (Rothschild *et al*., 2001). Numerous microbes found in outdoor air are related to or identical to isolates previously characterized from aquatic habitats, certain soil bacteria, or fungus. It's interesting to note that fungi and bacteria have been found in a variety of atmospheric layers, including the boundary layer (up to 1.5 km above sea level), the upper troposphere (up to 12 km

above sea level), and even the stratosphere at heights of 20 and 41 km (Griffin et al, 2004). Additionally, isolated cultures of the bacteria *Micrococcus albus* and *Mycobacterium luteum* as well as the common mould *Penicillium notatum* have been collected at altitudes of 77 km and height, respectively. Due to their small size, microorganisms can travel/deposit to the farthest reaches of the globe via upper air current over large distances within or between continents. According to (Womack *et al*. 2010), spore-forming organisms like Bacillus species (phylum of Firmicutes) and other Gram-positives like Actinobacteria typically predominate in culture-dependent surveys of airborne microbial diversity.

2.4. THE ATMOSPHERE AS A SOURCE OF PATHOGENIC MICROBE

By way of pathogenesis, exposing susceptible people to cellular components (such as pollen, fungal allergens, and lipopolysaccharide), and the development of sensitivities (such as asthma) through prolonged exposure, airborne microorganisms carried by dust clouds can also have a direct impact on human health (Griffin *et al*., 2007). It has been proven that being exposed to bacterial and fungal spores in the air can result in a number of allergic reactions. Numerous microorganism-derived substances can also cause respiratory issues, including endotoxins (membrane lipopolysaccharides released by Gram-negative bacteria) and fungi's mycotoxins. They can travel/deposit to the farthest reaches of the globe over lengthy distances within or between continents. Organisms that produce spores, like the phylum Bacillus species

2.5. INDOOR AIR QUALITY

Indoor air quality is an important public health concern, as people spend a significant amount of time indoors (Mandal and Brandl, 2011). Bacteria are one of the most common types of microorganisms found in indoor air, and the identification and characterization of these bacteria is important for understanding their pathogenic potential and for developing effective control strategies. The most common bacterial genera isolated from indoor air samples include *Staphylococcus, Bacillus* and *Clostridium*. These bacteria can cause a range of health problems, including respiratory infections, allergies, and asthma. To identify these bacteria, researchers use a variety of techniques, including culture-based methods, molecular methods, and microscopy (Hayleeyesus *et al.,* 2014).

2.6. FACTORS AFFECTING THE DISTRIBUTION OF BACTERIA IN INDOOR ENVIRONMENTS

The characteristic warm, moist climate found in a toilet makes it ideal for microbe survival. For weeks or even months, many pathogens can live on environmental surfaces. Environmental factors such as temperature, humidity, and air flow can influence the distribution and abundance of airborne bacteria in indoor environments. For example, high humidity levels can promote the growth of bacteria, while low humidity levels can lead to the release of endotoxins from bacterial cells. In addition, the composition of bacterial communities in indoor environments can vary depending on factors such as occupancy, ventilation, and building materials. Overall, the isolation and identification of bacteria isolates from indoor air is an important area of research that has implications for human health and environmental quality. The use of advanced molecular techniques can provide a more comprehensive view of bacterial diversity and can help to identify

potential sources of indoor air pollution (McKinney *et al.*, 2006).

2.7. TOILET AS AN INDOOR ENVIRONMENT

A public toilet is a building with urinals and sinks for use by general public. Public toilets are typically found in many different places: inner-city locations, offices, factories, schools, universities and other places of work and study. Toilets are essential sanitation facilities, yet they also serve as reservoirs for potentially harmful bacteria. Toilets can also play a role in transmission both through direct contact and through the generation of aerosols during flushing (Johnson *et al.,* 2013; Seun *et al.,* 2019). Previous studies have shown that flushing toilets produces a plum that can last up to 8 minutes and seed the toilet seat with bacteria (Johnson *et al*., 2013). Understanding the diversity and prevalence of bacteria found in toilets is crucial for maintaining hygiene, preventing infections, and developing effective sanitation strategies. Public toilets serve as important facilities for maintaining public health and hygiene. However, due to their high footfall and shared nature, they are susceptible to being hotspots for various microorganisms, including bacteria. Understanding the types of bacteria present in public toilets is crucial for public health measures, sanitation protocols, and the prevention of contagious diseases. When it comes to bacteria in public toilets, it's important to understand that they can come from various sources such as fecal matter, urine, or even from people's hands.

2.8 COMMON BACTERIA IN PUBLIC TOILETS

Some common bacteria found in public toilets include *Escherichia coli, Staphylococcus aureus, Salmonella enterica* and *Pseudomonas aeruginosa*

2 8.1 *Escherichia coli*

Echerichia coli is Gram negative, rod shaped, non sporulating, facultative anaerobe, that commonly inhabits the Gastrointestinal tract of humans and other warm blooded animals, food and the environment (Campbell *et al.,* 2002). *Escherichia coli*, a member of the *Enterobacteriaceae* family, grows optimally at 37°C under aerobic conditions, although it is a facultative anaerobe and can therefore grow under anaerobic conditions. It has also been previously reported that some strains of *E. coli* have been known to grow at temperatures of up to 53°C (Fotadar *et al*., 2005). Cells are typically rod-shaped and range in size from 1 to 3 micro metres (m) by 0.4 to 0.7 m (m), with a volume of 0.6 to 0.7 m. Due to the peritrichous flagellar configuration, it is motile; very few strains are not. *E. coli* is recognized to be a component of healthy intestinal flora but can also cause intestinal and extra intestinal disease in people. Numerous different *E. coli* strains have been found, causing illnesses ranging from mild, self-limiting gastroenteritis to renal failure and septic shock. *E. coli*'s virulence makes it easier for it to overcome host defenses and acquire antibiotic resistance. These bacteria can potentially cause gastrointestinal infections if proper hygiene practices are not followed. The presence of *E. coli* in public toilets can lead to the spread of infections and illnesses, which can have negative economic consequences. For instance, individuals who contract *E. coli* may require medical attention, resulting in healthcare expenses. Additionally, if an outbreak occurs due to unsanitary conditions, it could impact public trust and confidence in the facilities, potentially leading to decreased patronage. The most likely cause of infection with resistant *E. coli* is poor toilet hygiene, therefore it is important to put in the practice the habit of good toilet hygiene (Alteri and Mobley, 2012)

2.8.2 *Staphylococcus aureus*

As a non-motile member of the *Micrococcaceae* family, *Staphylococcus aureus* is also Gram-positive, coccus, facultative anaerobe, non-capsulated, catalase-positive, oxidase-negative, facultative anaerobic, and ferments glucose and mannitol both anaerobically and mesophilically. The temperature range for growth of S. aureus is 7–48°C, with an optimum of 37°C. *Staphylococcus aureus* is resistant to freezing and survives well in food stored below -20°C; however, viability is reduced at temperatures of -10 to 0°C. *Staphylococcus aureus* is readily killed during pasteurization or cooking. It's about 0.5 – 1.0 μm in diameter. They grow in clusters, pairs and occasionally in short chains. Growth of occurs over the pH range of 4.0–10.0, with an optimum of 6–7 (Stewart, 2003).

Staphylococcus aureus colonizes the nasal mucosa and skin of healthy individuals. This organism can cause a wide range of diseases from skin or soft tissue infections to systemic and fatal diseases (Wertheim *et al.*, 2005; Tong *et al*., 2015). *Staphylococcus aureus* produces an astounding array of virulence factors. These include a plethora of toxins and immune evasion factors, and a vast array of protein and non-protein factors that enable host colonization during infection. Staphylococcus aureus is a bacterium that can cause various infections, including skin infections, respiratory infections, and food poisoning. It is worth mentioning that any presence of harmful bacteria like *Staphylococcus aureus* in public toilets is not associated with economic benefits but rather potential negative consequences for public health and the economy. If public toilets are not maintained and cleaned properly, *Staphylococcus aureus* can flourish, leading to increased risks of infections and illnesses for individuals (Małecka-Adamowicz, 2019).

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2.8.3 *Salmonella enterica*

One of the two *Salmonella* species and a member of the *Enterobacteriaceae* family is *Salmonella enterica*. It is a Gram-negative facultative anaerobe with rods that are motile and non-spore forming and range in size from 0.7 to 1.5 by 2.0 to 5.0 m. Salmonella grows best in temperatures ranging from 37 to 42 °C. Any *Salmonella* species can infect people. Salmonella's natural habitat is the intestinal tracts of both humans and animals, and because they are excreted by both, they are frequently detected in environmental samples. *Salmonella enterica* is a zoonotic pathogen of substantial concern to global human and animal health. It can also be found in the environment (Leigh *et al, 2019*). *Salmonella enterica* infections typically are fever, diarrhea and abdominal pain.

Enteric Salmonella (TS) possesses specific virulence factors including typhoid toxin and virulence capsular polysaccharide (Vi antigen) that are involved in the development of symptoms and immune evasion. Since the intestinal tract is the habitat of *Salmonella enterica* getting infected by the bacteria is common in public toilets (Hagjoo *et al*., 2004; Liston *et al.,* 2016).

2.8.4 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative rod measuring 0.5 to 0.8 μm by 1.5 to 3.0 μm. It is heterotrophic and a facultative anaerobe. Almost all strains are motile by means of a single polar flagellum. *Pseudomonas aeruginosa* grows well at 37 °C, but it can survive in broad temperatures ranging from 4–42 °C. *Pseudomonas aeruginosa* infections can occur in various parts of the body, including the respiratory tract, urinary tract, gastrointestinal tract, wounds, or bloodstream. It is often associated with healthcare-associated infections, especially among individuals in hospitals or long-term care facilities. *Pseudomonas aeruginosa* has a natural ability to acquire resistance to multiple classes of antibiotics. This, coupled with its ability to form biofilms (a complex matrix that

protects bacteria), can make infections challenging to treat and potentially life-threatening.

The presence of *Pseudomonas aeruginosa* in toilets can lead to a range of complications depending on the site of infection and the individual's health. These can include pneumonia, urinary tract infections, skin and soft tissue infections, sepsis, and even more severe conditions when left untreated (Yagoub and Elagbashi, 2010).`

2.9. BACTERIA PRESENCE IN PUBLIC TOILETS: SIGNIFICANT FACTORS

1. High Traffic:

Public toilets experience a large number of users throughout the day, increasing potential for bacterial transmission from person to person.

2. **Insufficient Cleaning:**

Inadequate cleaning practices or lack of regular cleaning can result in the accumulation of bacteria on various surfaces, such as toilet seats, and door handles and sinks

3. Moisture:

Public toilets often have warm and humid conditions providing an ideal breeding ground for bacteria to thrive and multiply

4. Inadequate Ventilation:

Poor ventilation can lead to stagnant air, trapping bacteria and preventing proper airflow, further contributing to bacterial growth and persistence (Osman and Ibrahim, 2018)

2.10. EFFECTS OF BACTERIA IN PUBLIC TOILETS

Bacteria in public toilets can have a significant effects. These tiny organisms, which are part of the microbial community, can both positively and negatively affect the environment of these facilities.

One positive impact of bacteria in public toilets is their role in the natural decomposition process. Some bacteria are involved in breaking down organic waste, helping to prevent the buildup of waste and reducing unpleasant odors. These bacteria contribute to the overall cleanliness and hygiene of the toilets.

However, not all bacteria in public toilets are beneficial. Some harmful bacteria, such as *Escherichia coli (E. coli), Salmonella*, and *Staphylococcus aureus*, can be present in these facilities. These bacteria can cause various infections, including gastrointestinal illnesses, urinary tract infections, and skin infections. They can be transmitted through contact with contaminated surfaces or through improper hand hygiene practices. To minimize the negative impact of bacteria in public toilets, proper hygiene practices are crucial. The presence of bacteria in public toilets highlights the importance of maintaining proper sanitation practices.

2.11. HEALTH RISKS ASSOCIATED WITH BACTERIAL CONTAMINATION

The presence of bacteria can pose several health risks to individuals

1. Gastrointestinal Infections:

Bacteria such as *Escherichia coli* and *Salmonella enterica*, commonly found in public toilets can cause gastrointestinal infections if ingested through contaminated hands or food

2. Skin Infections:

Staphylococcus aureus which can be present in surfaces in public toilets can lead to skin infections and even more severe conditions like cellulitis.

3. Urinary Tract Infections:

Escherichia coli can cause Urinary Tract Infections, often associated with poor hygiene practices or improper cleaning of toilet seats.

2.12 MITIGATING BACTERIAL CONTAMINATION IN PUBLIC HEALTH

Efforts must be made to minimize bacterial contamination in public toilets and promote a safe and hygienic environment

1. Regular Cleaning:

Public toilets should undergo frequent and thorough cleaning using appropriate sanitizing agents to remove bacteria from surfaces and minimize biofilm formation

2. Good Hand Hygiene:

Promoting proper hand washing practices among users is crucial in preventing the spread of bacteria. Providing hand sanitizers in public toilets can also encourage hand hygiene

3. Adequate Ventilation:

Ensuring sufficient ventilation is pulic toilets aids in reducing moisture levels and improves airflow, inhibiting bacterial growth.

4. Education and Awareness:

Public health campaigns and clear signage promoting good hygiene practices, such as proper hand washing techniques and the importance of flushing toilets can help raise awareness among users and encourage responsible behavior.

5. Maintenance of Plumbing Systems:

Regular inspection and maintenance of plumbing systems in public toilets are essential to prevent leaks, water stagnation and the growth of bacteria in hidden areas.

The relationship between bacteria and public toilets highlights the importance of maintaining cleanliness and implementing effective measures to minimize bacterial contamination. By understanding the factors contributing to bacterial presence, potential health risks and implementing preventive strategies such as regular cleaning, promoting good hand hygiene, proper ventilation, education and maintenance, we can help ensure that public toilets remain safe and hygienic

environments for all users.

2.13. IMPACT ON PUBLIC HEALTH:

Understanding the diversity and prevalence of bacteria in public toilets is crucial in minimizing the potential health risks. By identifying pathogenic and opportunistic we understand the types of bacteria present in these facilities, we can take necessary actions to prevent the spread of infections and protect the well-being of the public.

Firstly, by conducting regular bacterial analysis, we can identify harmful pathogens that may be lurking in public toilets. Bacteria such as E. coli, Salmonella, and Staphylococcus aureus are commonly found in these environments and can cause gastrointestinal infections. Knowing which bacteria are present allows public health authorities to develop targeted strategies to minimize the risk of transmission. This could include implementing more frequent cleaning and disinfection practices or providing educational materials on proper hygiene practices.

Secondly, isolating bacteria from public toilets helps us understand their antibiotic resistance patterns. Antibiotic resistance is a significant global concern, and identifying resistant strains in public toilets provides valuable insights into the prevalence and spread of these bacteria. This knowledge can guide healthcare providers in prescribing appropriate antibiotics and developing effective infection control measures. It also highlights the importance of responsible antibiotic use to prevent the further emergence and spread of antibiotic-resistant bacteria.

Moreover, identifying and isolating bacteria from public toilets contributes to the development of improved cleaning and sanitation protocols. By understanding the specific types of bacteria present, cleaning products and techniques can be tailored to effectively target and eliminate these pathogens. This helps maintain a cleaner and safer environment for the public, reducing the risk of infections. In addition to immediate health impacts, this information can also inform long-term public health strategies. By studying the bacterial composition of public toilets over time, researchers can identify trends and patterns in bacterial prevalence and resistance. This data can guide the development of

policies and interventions to improve public toilet hygiene standards and overall public health.

Furthermore, the identification and isolation of bacteria from public toilets can help raise awareness among the general public about the importance of proper hygiene practices. When people are informed about the presence of potentially harmful bacteria in these facilities, they are more likely to take necessary precautions, such as washing their hands thoroughly and using toilet seat covers. This increased awareness can contribute to a healthier and safer community.

Overall, the impact of identifying and isolating bacteria from public toilets on public health is significant. It allows for targeted interventions, improved infection control measures, and the development of more effective cleaning practices.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Preparation and Sterilization of Culture Media

All culture media was prepared according to the manufacturer's instructions. Sterilization was at 121ºC at 15psi for 15 min unless otherwise stated by manufacturer.

3.2 Agar Preparation

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

3.3 Collection of Samples

Air samples were collected from Hall 2 Female Hostel Toilets, University of Benin. The samples were transported aseptically to Microbiology Laboratory, University of Benin, Benin City for microbiological analysis.

3.3.1 Phenotypic identification of Bacteria from samples

Several tests such as Gram reaction, catalase, urease, indole, oxidase, sugar fermentation, respective reaction on triple sugar iron agar tests were carried out to presumptively identify bacterial isolates (Okafor *et al*., 2020).

3.4 Morphological Identification

3.4.1 Gram staining:

This test was carried out to verify the sort of bacteria cell that would be utilized. In order to distinguish between Gram-positive and Gram-negative bacteria, Gram staining techniques were

applied. Gram-positive organisms are those that, after being decoloured, maintain the primary stain, whereas Gram-negative organisms do not. The cell composition is the cause of the stain's non-retention. Crystal violet (the primary stain), iodine (as a mordant), alcohol (a decolorizer), and safranin (the counter stain) are some of the reagents used in Gram staining.

The procedure for Gram staining is as follows:

A smear of the organism to be Gram stained was made on a sterile slide using an inoculating wire loop .The slide was air dried and heat fixed by passing it gently over the flame. The smear was stained with 1% crystal violet for one minute and washed with distilled water. Gram iodine was added as a mordant for one minute, after which alcohol was added for 30 seconds. The slide was then rinsed with distilled water. The slide was finally flooded with counter stain, safranin for one minute and then washed off with distilled water and air dried. The preparation was observed under the microscope with an oil immersion and their morphology (shape), arrangement and Gram result was obtained.

3.5. Biochemical Identification

Biochemical test was carried out so as to help in the identification of the bacteria isolates as phenotypic (cultural) characteristics is not sufficient. The various biochemical test carried out are shown below;

3.5.1 Oxidase Test

This is mainly used to differentiate between *Pseudomonas* from other gram-negative rod bacteria. Oxidase test was carried out to identify bacteria species that will produce cytochrome oxidase enzyme. *Staphylococcus aureus* and *Escherichia coli* which are Gram-positive and Gram-negative respectively were employed as control. 2-3 drops of freshly prepared oxidase reagent (1% aqueous tetramethyl-3-phenyl nediamine dichloride) was added to a sterilized piece of filter paper.A smear

of the isolates were dropped on the filter paper using a sterile wire loop. A positive oxidase test is indicated by purple colouration within 10 seconds.

3.5.2 Urease Test.

This is used to test organisms that have the abilities to produce the enzyme urease which catalyzes the breakdown of urea to produce ammonia. The test is usually used to differentiate organisms like *Proteus mirabilis* from other non-urease positive organism. The procedure for the test is as follows: 5ml of sterilized medium was dispensed into test tubes aseptically and kept in a slant position for it to solidify. The test bacteria isolated were inoculated into the medium and incubated at 37 ^{o}C for 24 hours. A change in colour from yellow to red-pink confirmed the presence of urease.

3.5.3 Indole Production Test

Tryptophan is hydrolyzed by tryptophanase to produce three possible end products; indole, pyruvate, and ammonia. Indole production is detected by Kovac's or Ehrlich's reagent. Indole, if present, combines with the aldehyde in the reagent to produce a pink to red-violet quinoidal compound (if benzaldehyde reagent is used) or a blue to green color (if cinnamaldehyde reagent is used). The absence of enzyme results in no color production (i.e. indole negative). Spot indole test is performed by using one of the three methods mentioned below .Saturate a piece of filter paper with the reagent. Use a wooden stick or bacteriologic loop to remove a small portion of a bacterial colony from the agar surface and rub the sample on the filter paper. The development of a brown-red to purple-red color (benzaldehyde reagents) or blue color (cinnamaldehyde reagent) within 20 seconds indicates the presence of indole. A negative test is colorless.

3.5.4 Catalase Test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdowns 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.

3.5.5 Sugar fermentation and production of gases using Triple sugar iron agar (TSI)

TSI was prepared following manufacturer's instruction and the5ml of prepared media was dispensed into a test tube aseptically and kept in a slant position for it to solidify. The slant and butt of the medium was inoculated with the test bacterium using a sterile loop and it was incubated for 18-24 hr. The results were read on the basis of acid or alkaline production in the slant or butt region of the tube and gas production was confirmed by the presence of air bubbles in the slant or butt region. More so, production of hydrogen sulphide was confirmed by the blackening of the medium. A prepared laboratory chat was used for result interpretation in line with microbiological standard protocol as well as other biochemical tests carried out on the isolates to confirm or ascertain their identity.

3.6 Antibiotic Sensitivity Test

Antimicrobial susceptibility testing was done according to the CLSI protocol. Isolates were taken out of the fridge and streaked onto set plates of Nutrient Agar, and the petri dishes were fastened together with a masking tape, labeled and incubated by inverting them in the 37 ^{o}C incubator for 24 hours. Plates of Muller Hinton Agar were also poured, allowed to dry and bundled together with masking tape, and also put into the 37 ^{o}C incubator for 24hours. Plates of Muller Hinton Agar that showed no contamination after the 24hours were used in the procedure. Test tubes containing 5ml of saline solution were prepared and sterilized by autoclaving. An inoculating loop was used to take growth that appeared on the streak lines on the nutrient agar plate and inoculated into a test tube containing saline solution. The test tube was beat with the finger in order for the inoculated colony

to mix well in the solution, causing it to become cloudy around 0.5 McFarland. A cotton stick was dipped into the suspension and streaked unto to a labeled pre incubated plate of Mueller Hinton Agar, and allowed to dry. A multidisc of antibiotics was placed on the dried Mueller Hinton Agar plate, aseptically, and lightly pressed unto the agar surface with a sterile forceps. The plate was subsequently incubated by inverting it in the 37 ^{o}C incubator. This procedure was repeated for all plates of Nutrient Agar that showed growth after 24hours. Zones of inhibition were later measured after 24hours, using a ruler and the results recorded, and interpreted according to the CLSI breakpoint (2013).

CHAPTER FOUR

RESULTS

The study was aimed at isolating and identifying bacteria isolate present in air samples from students hall of residence(HALL 2) of the University of Benin, Benin City.

Figure 4.1. Shows the total heterotrophic bacteria count obtained form the air samples. The figure shows the difference in the number of heterotrophic bacteria count between both weeks of sampling

Table 4.3.Shows the morphological and biochemical characteristics of the bacteria isolate from both locations, the biochemical reactions carried out further reveal vital information necessary for accurate identification of the bacteria isolate from air sample from the tiolet

Table 4.4 Shows the resistance and susceptibility of the different isolate to specific antibiotics Cefotaxime, Ofloxacin, Gentamicin, Cefuroxime, Ampicillin, Nitrofurantoin, Cefixime Levofloxacin, Imipenem and Nalidixic acid

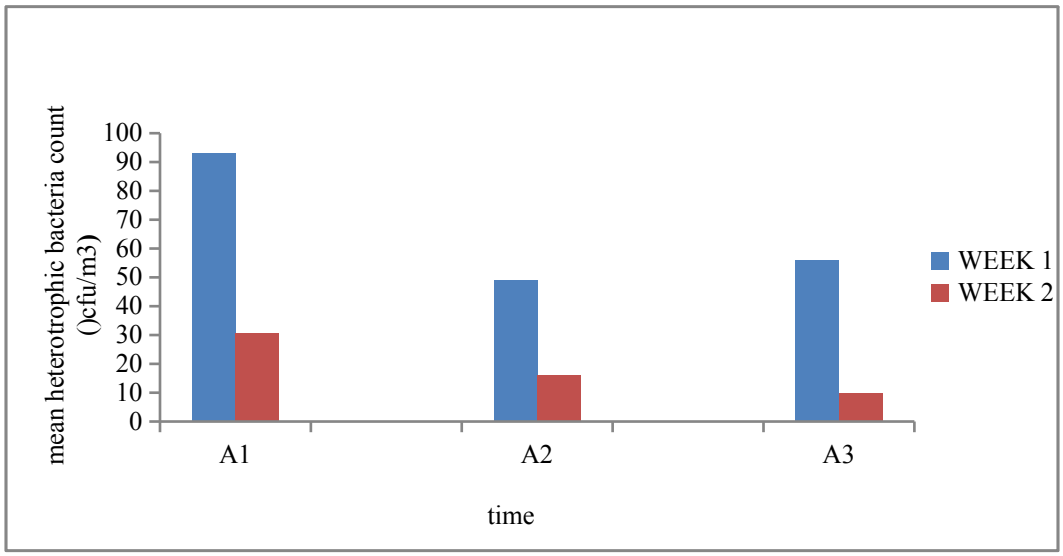

Figure 4.1: Mean heterotrophic bacteria counts (cfu/m^3)

Key:

A1: Sample 1

A2: Sample 2

A3: Sample 3

Table. 4.1 Cfu/m3 for both sampling period

First week	4.07X10^3
Second week	1.01X10^3

Table 4.2: Cultural characteristics and Biochemical test on the Bacteria isolate.

Cultural						
Shape	irregular	Circular	Circular	irregular	Circular	Irregular
Size	Large	Medium	Medium	large	Medium	Medium
Colour	Green	Straw	Black	Red	Pink	Golden yellow
Morphological						
Gram stain	-	+	+	-	-	+
cell type	Rod	Rod	Cocci	Rod	Rod	Cocci
Arrangement	Disperse	Disperse	Cluster	Disperse	Disperse	Cluster
Color	Pink	purple	Purple	purple	Pink	Purple
Biochemical						
KOH test	+	-	-	+	+	-
Indole	+	-	-	-	-	-
Oxidase	-	-	+	-	-	-
Urease	-	+	-	+	+	+
Glucose	+	+	+	+	+	+
Sucrose	+	+	-	-	+	+
Lactose	+	-	-	-	+	+
Gas formation	+	-	+	+	+	-
H2S formation	-	-	+	+	+	-
Catalase	+	+	+	+	+	+
Probable Identity	*E. coli*	*Bacillus cereus*	*Micrococcus* spp.	*Proteus* sp.	*Citrobacter* sp.	*Staphylococcus* sp.

Key: + = positive; - = negative;

Table 4.3 Antibiotic sensitivity results

ISOLATES	CTX	OFX	GEN	CFX	AMP	NIT	CFM	LEV	IPM	NAI
E.coli	0(R)	16(S)	14(S)	0(R)	0(R)	0(R)	9(R)	12(I)	4(R)	16(S)
Staphylococcus sp	0(R)	14(S)	15(S)	8(R)	0(R)	7(R)	11(I)	14(S)	16(S)	14(S)
Bacillus sp	0(R)	22(S)	15(S)	10(I)	17(S)	7(R)	0(R)	9(R)	0(R)	17(S)
Proteus sp	0(R)	16(S)	16(S)	0(R)	0(R)	0(R)	0(R)	14(S)	0(R)	9(R)
Micrococcus spp	4(R)	14(S)	17(S)	2(R)	0(R)	0(R)	4(R)	17(S)	0(R)	14(S)
Citrobacter sp	0(R)	14(S)	15(S)	5(R)	9(R)	0(R)	0(R)	15(R)	0(R)	11(I)

Key:

Sensitive=14-22, Resistance=0-9, Intermediate=10-13.

S = sensitive, R=Resistance, GEN=Gentamicin, CFX=Cefuroxime, AMP=Ampicillin

NIT=Nitrofurantoin, CFM=Cefixime, LEV=Levofloxacin, IPM=Imipenem

NAL=Nalidixic acid.

CHAPTER FIVE

DISCUSSION

The study of the microbiological quality assessment of indoor air is one of the most vital investigations to determine the microbial indoor air pollution. The information on the indoor microbial concentrations of airborne bacteria is necessary both to estimate the health hazard and to create standards for indoor air quality control. The concentrations of bacterial and aerosols in the indoor environment of Hall 2 hostel toilets, estimated with the use of the settle plate method, ranged between 4.07 X10^3 -1.01x10^3 cfu/m3 for both sampling periods.

The concentrations of bacteria measured in hall 2 hostel toilets were significantly different to each other (P=0.000). These can be mainly explained by the variation of density of occupant during sampling time as well as the variation of ventilation conditions (Frankel *et al.,* 2012). The highest cfu/m^3 recorded during the first sampling might be due to the number of occupants during assessment which is 4 times higher than the second sampling. These situations increase the shedding of bacteria and agitation of air. Similar studies revealed that, the presence of aerial bacteria was associated to the presence of personnel into the air of the partially closed premises (Adam *et al.,* 2015). Hence, an obvious practice to improve a more healthy quality of indoor air in the building would be to avoid overcrowding and to design good ventilation systems.

Thus, the microbial loads of the buildings were favored by the environmental conditions which enhance their development. And also it was stated by World Health Organization (2011) that dampness situation has to be considered as the risk indicator for health risks of biological contaminants of indoor air. A quantitative interpretation of the results describing the air quality in

hall 2 hostel toilets is difficult due to the lack of widely accepted normative and reference values. Universally applicable standards defining an acceptable level of indoor air contamination with microorganisms have not yet been established. Evaluation of the air quality in the designated areas on the premises of the toilets in hall 2 hostel was based on the sanitary standards for non-industrial premises formulated by the European Commission in 1993. According to this classification, the air in the all toilets was in the range of highly or very highly contaminated with bacteria.

The bacteria isolate identified during the first sampling were similar to the isolate identified during the second sampling. This remark, coupled to the fact that more than 14 m3 of air inhaled daily by a human adult (Bogaert *et al.,* 2017) leads to the conclusion that the airborne microbial intake per day of the users of the analyzed building might likely exceed by at least 14-fold the average number of microorganisms expressed above. The control of the microbial load of the surrounding air is thus important to establish the quality and health conditions of the services rendered by any public institution.

In this study, the identified bacterial isolates were *Micrococcus* spp., *Staphylococcus aureus, Escherichia coli*, *Proteus* sp., *Bacillus* sp. and *Citrobacter* sp. It has been reported that Gram-positive bacteria seem to predominate in dusts of animal and human origin, whereas Gram-negative bacteria predominate in dusts of plant origin (Swan *et al*., 2002). This study supports the aforementioned assumption that the detected species and levels of bacteria are mainly due to human presence and its activities and density. Similarly, certain studies in Europe have demonstrated that Gram-positive bacteria are the most commonly found bacteria in indoor air environment (Gorny and Dutkiewicz, 2002). In addition, other study on US office buildings revealed that Gram-positive cocci are the most prevalent in both indoor and outdoor air (Tsai and Macher, 2005).

5.2. CONCLUSION

This study reveals the bacterial isolates present in toilet environments and highlights the importance of maintaining proper hygiene practices. The study revealed that public toilets serve as a reservoir for various bacteria, including pathogenic ones, which can pose a potential risk to public health. As the findings of this study contribute to the existing knowledge on bacterial ecology within public toilets, it serves as a basis for further research and the development of improved strategies to mitigate the spread of infectious diseases. Continued efforts in monitoring and maintaining hygienic conditions in public restrooms will have a positive impact on public health and overall well-being.

Overall, the research on isolation and identification of bacteria from public toilets emphasizes the need for ongoing vigilance and adequate hygiene practices to minimize bacterial contamination and enhance public safety. By implementing effective preventive measures, we can collectively contribute to creating healthier environments for everyone.

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