ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA ON DOOR HANDLES OF BANKS WITHIN THE UNIVERSITY OF BENIN

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UNIVERSITY OF BENIN

BENIN CITY

FEBRUARY 2025.

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FUFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY.

FEBRUARY 2025.

CERTIFICATION

This is to certify that this project work was successfully carried out by **FAVOUR CHINONSO LOUIS** with matriculation number **LSC2003103,** of the department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria, under my supervision.

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(Project Supervisor)

APPROVAL

This project work was carried out by **FAVOUR CHINONSO LOUIS** with matriculation number **LSC2003103,** in partial fulfillment of the award of a Bachelor of Science, B.Sc. (Hons) degree in the Department of Microbiology, University of Benin, Benin City.

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Prof. Mrs. F.I AKINNIBOSUN **Date**

(Head of Department)

DEDICATION

This report is primarily dedicated to the Divine Providence for His abundant blessings, mercy, and guidance throughout my university journey.

I am also dedicating this project to my parents for their constant love and support.

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First and foremost, I wish to give my profound gratitude to God Almighty for His faithfulness, goodness and grace throughout my life and academic journey.

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ABSTRACT

Door handles in public spaces serve as potential reservoirs for microbial contamination, posing risks for the transmission of pathogenic bacteria. This study aimed to isolate, identify, and assess the antibiotic susceptibility of bacterial contaminants on door handles of selected banks within the University of Benin, Benin City, Edo State, Nigeria. The research was conducted across five banks within the university campus, selected due to their high patronage, which increases the likelihood of microbial contamination. Sterile swab samples were collected from both entrance and interior door handles of each bank over a two-week period, during peak business hours (10:00 AM – 12:00 PM). The collected swabs were transferred into sterile peptone water and transported to the microbiology laboratory within one hour for bacterial analysis. The total heterotrophic bacterial count (THBC) was determined using standard culture techniques, with counts ranging from $2.03 \pm 0.03 \times 10^4$ CFU/ml (Bank D) to $4.72 \pm 0.31 \times 10^4$ CFU/ml (Bank E). Biochemical and morphological characterization of isolates identified seven bacterial species, including *Staphylococcus* sp., *Bacillus* sp., *Streptococcus* sp., *Pseudomonas* sp., *Enterobacter* sp., Escherichia coli, and *Klebsiella* sp. The frequency of occurrence varied, with *Escherichia coli* having the highest prevalence (28%), followed by *Staphylococcus* sp. (20%)**,** while *Pseudomonas* sp. had the lowest occurrence (12%). Antibiotic susceptibility testing against ten commonly used antibiotics revealed varying resistance patterns among isolates. The multiple antibiotic resistance (MAR) index ranged from 0.3 to 0.5, with the highest recorded for *Escherichia coli, Pseudomonas* sp*.,* and *Micrococcus* sp*.* The results highlight significant bacterial contamination on frequently touched surfaces, emphasizing the need for regular disinfection and improved hygiene practices in high-contact public spaces.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

In the context of public health and environmental microbiology, frequently touched surfaces in public spaces such as door handles have emerged as critical reservoirs for microbial contamination. Door handles, particularly in high-traffic environments like banks, are subjected to constant human contact and, as a result, can become unwitting vehicles for the transmission of harmful microorganisms. These surfaces, often neglected in routine cleaning procedures, pose significant health risks, especially in settings where individuals interact without strict hygiene practices. Given the continuous flow of people through banks, with varied socio-economic and hygienic backgrounds, the potential for the accumulation of bacteria on door handles is greatly amplified (Boyce, 2007; Choi *et al*., 2018).

The contamination of door handles is not just an inconvenience; it represents a public health concern with broad implications for disease transmission. High-contact surfaces like door handles can harbor numerous microorganisms, including opportunistic and pathogenic bacteria. Several studies have documented the presence of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and other microbial agents on door handles in public spaces (Otter and French, 2009; Russotto *et al*., 2015). These bacteria are linked to a variety of infections, including respiratory and urinary tract infections, skin conditions, and gastrointestinal diseases. *S. aureus*, for instance, is a known cause of both hospital-acquired and community-acquired infections, and the presence of *E. coli* on surfaces poses a significant risk for gastrointestinal infections (Kramer *et al*., 2006).

Microorganisms such as *Pseudomonas aeruginosa* and *Klebsiella* spp. have also been shown to persist on environmental surfaces, adding to the complexity of microbial transmission in areas with high foot traffic (Mukherjee *et al*., 2014). These pathogens are often resistant to environmental stressors, allowing them to survive on surfaces for extended periods. Such persistence not only increases the chances of transmission between individuals but also heightens the risk of outbreaks in densely populated areas like banks, where numerous individuals come into contact with shared surfaces throughout the day.

The situation in Nigeria is further complicated by factors such as inadequate sanitation practices, limited awareness of surface hygiene, and lack of resources for routine cleaning and disinfection (Onuoha and Fatokun, 2014). Public spaces, particularly in urban centers like Benin City, may not be cleaned or sanitized frequently enough, which leaves door handles and other high-contact surfaces vulnerable to bacterial accumulation. This is particularly concerning for financial institutions like banks, which are constantly frequented by a diverse range of individuals. The poor sanitation infrastructure in many public spaces exacerbates the risk of bacterial contamination, with potential consequences for public health (Alves *et al*., 2020).

In developed countries, regular cleaning and disinfection of frequently touched surfaces in public places have been recognized as effective preventive measures for reducing microbial loads (Aiello *et al*., 2008). However, in low-resource settings like Nigeria, such measures are often not fully implemented due to a lack of infrastructure and awareness. The challenge of inadequate surface hygiene is particularly pressing in urban Nigerian settings, where population density and rapid urbanization increase the risk of cross-contamination in public spaces (Zhu *et al*., 2014). As such, there is a critical need for research into the microbial

contamination of surfaces in public spaces, particularly in banks, to assess the scale of the problem and identify practical solutions.

The University of Benin, located in Benin City, Edo State, Nigeria, is home to a number of financial institutions, with banks being key components of the daily operations of the university's staff and students. Banks in this area experience high foot traffic, with individuals constantly entering and exiting the premises. The door handles, therefore, become focal points for potential bacterial contamination. The contamination of high-contact surfaces in public spaces remains an often overlooked public health challenge, particularly in the context of developing countries like Nigeria. Surfaces such as door handles, which are regularly touched by multiple individuals, represent a major vector for the spread of infectious diseases. In the absence of rigorous cleaning protocols, door handles can serve as a repository for bacteria, increasing the likelihood of transmission between individuals in public settings (Boyce, 2007; Mukherjee *et al*., 2014).

While the importance of hand hygiene has been widely promoted as a preventive measure against disease transmission, the significance of sanitizing environmental surfaces like door handles is less emphasized. This gap in surface hygiene practices, especially in high-traffic areas such as banks, has implications for the spread of pathogenic bacteria. The continuous use of door handles by individuals, combined with inadequate cleaning and disinfection practices, exacerbates the potential for bacterial contamination. In densely populated settings like banks, the transmission of bacteria through contaminated door handles can occur rapidly, leading to potential outbreaks and contributing to the overall burden of infectious diseases (Russotto *et al*., 2017).

Despite the growing global recognition of the importance of environmental hygiene, particularly in high-contact public spaces, there is a paucity of research on microbial

contamination in Nigerian public institutions. While hospitals and healthcare settings have received considerable attention in studies of microbial contamination, financial institutions such as banks—where individuals frequently come into contact with shared surfaces—have largely been ignored. In Benin City, Edo State, the microbial contamination of door handles in banks has not been systematically investigated, creating a significant knowledge gap. This lack of data on the types of bacteria present, their resistance profiles, and the potential health risks associated with contaminated surfaces hinders the development of effective sanitation strategies and public health policies (Zhu *et al*., 2014).

In addition, the increasing prevalence of antibiotic-resistant bacteria presents an added layer of complexity. The global rise in antimicrobial resistance (AMR) has made it more difficult to control infections, particularly in environments where bacteria can rapidly evolve resistance. High-touch surfaces like door handles not only harbor common pathogens but may also serve as reservoirs for multidrug-resistant organisms. This complicates infection control efforts and makes it imperative to understand the diversity and resistance profiles of bacteria present on these surfaces (Boyce, 2007; Aiello *et al*., 2008).

This study seeks to address the critical knowledge gap surrounding the bacterial contamination of door handles in banks within the University of Benin. It aims to identify the microbial species present, assess their pathogenic potential, and evaluate their antibiotic resistance profiles. By doing so, this research will contribute to the understanding of the risks posed by contaminated door handles in public spaces and provide recommendations for improving sanitation practices in Nigerian financial institutions.

1.2 AIM AND OBJECTIVES

To investigate the bacterial contamination of door handles in selected banks within the University of Benin, Benin City, Edo State, Nigeria.

The specific objectives of the study were to:

1. Enumerate and identify the bacterial isolates from door handles in the selected banks.
2. To determine the antibiotic resistance pattern of the bacterial isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1. Microbial Contamination on High-Touch Surfaces

Public spaces, particularly those with high human traffic, have long been recognized as potential hotspots for the accumulation and transmission of harmful microorganisms. These spaces—such as shopping malls, airports, schools, and healthcare facilities—serve as points of convergence for individuals from varied backgrounds and health conditions. The sheer volume of human activity in these environments increases the likelihood of microbial contamination. Frequent contact with surfaces such as door handles, elevator buttons, handrails, and public seating creates an ideal environment for the transmission of bacteria, viruses, and other pathogens. This chapter focuses on the microbial contamination of high-touch surfaces, which are commonly overlooked in regular cleaning protocols despite their significant role in disease transmission (Boyce, 2007; Aiello *et al*., 2008).

Public spaces, especially those that accommodate large crowds, provide a unique challenge in terms of sanitation. The constant flow of individuals, coupled with the frequent touching of shared surfaces, escalates the risk of pathogen transmission. Studies have shown that high-touch surfaces harbor a variety of microorganisms, ranging from transient bacteria to more persistent and potentially dangerous pathogens. These include both opportunistic pathogens—such as *Staphylococcus aureus* and *Escherichia coli*—and more resilient disease-causing organisms like *Clostridium difficile* and *Norovirus* (Fletcher *et al*., 2009; Otter and French, 2009). These pathogens can pose significant public health threats, especially in environments where individuals with compromised immune systems, such as the elderly or the sick, may be present.

Surfaces that are frequently touched by individuals—such as door handles, elevator buttons, and handrails—are particularly susceptible to microbial contamination. These high-touch surfaces act as a bridge between individuals and the environment, facilitating the transfer of bacteria, viruses, and fungi. For example, a study by Aiello *et al*. (2008) revealed that high-touch surfaces in hospitals and schools often contained a wide variety of microorganisms, including those capable of causing infectious diseases. The ease with which people transfer pathogens between surfaces and hands exacerbates the problem, as microorganisms can travel from one person to another in a matter of seconds. The nature of these surfaces, along with their continuous exposure to human hands, allows microbes to thrive, making the transmission of infections particularly efficient.

Several studies have highlighted the importance of these surfaces in the spread of nosocomial infections in healthcare environments. Otter and French (2009) emphasized that in hospitals, the spread of infection can be directly linked to contaminated surfaces such as bed rails, door handles, and light switches. In other public spaces, such as airports or shopping centers, the variety of microbial species found on these surfaces includes not only bacteria but also viruses, fungi, and parasitic organisms that can be inadvertently spread to people (Fletcher *et al*., 2009). Inadequate sanitation and inconsistent cleaning protocols increase the microbial load on these surfaces, heightening the risk of infection transmission. For instance, studies on hospital environments have shown that door handles are often among the most heavily contaminated surfaces, serving as key points for the transfer of multidrug-resistant organisms (MDROs) (Zhu *et al*., 2014).

2.2 Microbial Contamination of Door Handles: A Focus on Financial Institutions

Door handles in public spaces are among the most frequently touched surfaces, and as such, they are often exposed to a high volume of microbial contamination. In settings such as financial institutions, particularly banks, the risk of microbial contamination is even more pronounced due to the constant flow of people interacting with these surfacs6es. Financial institutions are often characterized by moderate to high exposure to microbial contamination, with customers and employees interacting with door handles throughout the day. Many of these individuals may not consistently practice hand hygiene, further exacerbating the potential for pathogen transmission (Otter and French, 2009).

Financial institutions serve as hubs for a wide range of people, including those with varying hygiene practices and health statuses. Banks are visited by individuals who may have touched contaminated surfaces before entering the facility, or who may carry bacteria or viruses on their skin. As such, door handles in these settings act as a potential conduit for pathogens, including those capable of causing serious infections. Several studies have explored the microbial contamination of door handles in various public spaces, including financial institutions, and have found a significant prevalence of harmful microorganisms on these surfaces. This section will delve into the factors contributing to microbial contamination on door handles, the common pathogens identified in these environments, and the potential public health implications, particularly in developing countries such as Nigeria.

2.3. Fomites and the Role of Door Handles in Disease Transmission

Fomites are inanimate objects that play a significant role in the spread of infectious diseases, particularly in environments such as hospitals. In these settings, door handles, which are

frequently touched by individuals in various hospital areas including office spaces, wards, and surgery rooms, serve as critical points for microbial contamination. These door handles act as reservoirs for pathogenic microorganisms, transferring bacteria from one individual to another through touch. The transmission process often occurs when an individual touches a contaminated door handle and subsequently touches their face, eyes, mouth, or another person, facilitating the spread of infections.

Numerous studies highlight the importance of door handles as vectors for the transmission of bacteria in public places, including hospitals. A study by Itah *et al*. (2004) identified Gram-positive bacteria like *Staphylococcus aureus* and Gram-negative enteric bacteria such as *Escherichia coli*, *Klebsiella* spp., and *Citrobacter* spp. on various environmental surfaces, including door handles. These pathogens can remain on hard, non-porous surfaces, such as door handles, for extended periods, increasing the potential for transmission, especially in vulnerable populations such as immunocompromised individuals (Rusin, 2002).

The epidemiological concerns surrounding the spread of infectious diseases via door handles have led to increased efforts to improve hygiene practices, such as the use of hand sanitizers and hand-washing protocols. However, despite widespread awareness of these practices, compliance remains inadequate, often due to the inconsistent availability of sanitizing agents in public spaces, particularly in hospitals (Hansen, 2002). Consequently, even after washing hands, individuals can still transfer pathogens from contaminated surfaces to their bodies or to other individuals through direct contact, such as shaking hands or hugging.

The persistence of harmful microorganisms on door handles and other frequently touched surfaces is well-documented, with some studies indicating that these bacteria can survive for hours or even months on such surfaces (French *et al*., 2004). Cross-contamination between these environmental surfaces and hosts has been established as a significant concern in

hospital settings (Hardy *et al*., 2006). This highlights the need for proper cleaning and regular disinfection of fomites, including door handles, to mitigate the risk of hospital-acquired infections.

In hospitals, the type and material of door handles can influence the types of microorganisms found on them. Gram-positive bacteria, particularly *Staphylococcus aureus*, have been found to be more prevalent on door handles compared to Gram-negative bacteria, as these organisms are part of the normal flora of human skin and mucous membranes (Inweregbu *et al*., 2005). These bacteria can be transmitted through human contact or expelled from the respiratory tract, further complicating the spread of infections in hospital environments (Chikere *et al*., 2008).

The irregular cleaning practices in hospitals, such as the sporadic use of reusable cleaning cloths soaked in detergent, can exacerbate the risk of contamination, as they often fail to adequately address the bacterial load on door handles (Rutala and Weber, 2001). Regular cleaning and effective disinfection of frequently touched surfaces are essential measures to reduce the spread of infection. Studies have shown that improving hand hygiene practices and cleaning protocols significantly reduces the transmission of pathogens from contaminated environmental surfaces, including door handles. Therefore, the promotion of consistent sanitation practices, alongside the implementation of hygiene programs, is critical to improving hospital hygiene and preventing the spread of infectious diseases.

2.4. Factors Contributing to Microbial Contamination of Door Handles

Several factors contribute to the high level of microbial contamination found on door handles in financial institutions. The frequency of contact is the most obvious factor. Financial institutions typically serve large numbers of people each day, and door handles are among the

most commonly touched surfaces. Studies have shown that the more frequently a surface is touched, the higher the likelihood of it becoming contaminated (Russotto *et al*., 2017). Furthermore, door handles in high-traffic areas are particularly vulnerable, as they are constantly being touched by customers and employees who may not have washed their hands after touching potentially contaminated surfaces, such as cash, public transportation handles, or public restrooms (Choi *et al*., 2018).

In addition to human activity, environmental conditions also play a role in microbial contamination. Temperature and humidity affect the survival and persistence of microorganisms on surfaces. For example, studies have shown that warm and humid conditions favor the growth of bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, both of which have been found on door handles in financial institutions (Zhu *et al*., 2014). Non-porous surfaces, such as the metals or plastics commonly used for door handles, also tend to allow microbes to survive for extended periods, further increasing the potential for transmission.

Microorganisms can survive on surfaces for varying periods of time, depending on environmental conditions such as temperature, humidity, and the nature of the surface material. The persistence of bacteria on these surfaces plays a significant role in the potential for disease transmission. Some bacteria, particularly Gram-negative pathogens like *Escherichia coli* and *Pseudomonas aeruginosa*, can remain viable on surfaces for hours or even days (Boyce, 2007; Aiello *et al*., 2008). Studies have shown that *S. aureus* can persist on metal surfaces for up to 7 days, while *E. coli* and other enteric pathogens can survive for up to 24 hours or longer under certain conditions (Fletcher *et al*., 2009).

The survival rate of microorganisms on surfaces depends not only on the type of bacteria but also on the nature of the surface material. Non-porous surfaces, such as stainless steel or

plastic, provide an ideal environment for the survival of microorganisms, as they are less likely to lose moisture and nutrients compared to porous surfaces like wood (Aiello *et al*., 2008). The cleaning process also influences the persistence of bacteria on surfaces. Poor cleaning techniques or infrequent cleaning schedules contribute to the accumulation of biofilm—a slimy layer of microorganisms that can protect bacteria from being eradicated by cleaning agents (Boyce, 2007). Biofilm formation can significantly extend the survival of bacteria on door handles, making them more resistant to cleaning efforts.

2.5. Bacteria Contaminants Associated with Bank Door Handles

Fomites, such as door handles, are significant sources and vehicles for the transmission of common infections, serving as a bridge between contaminated hands and susceptible individuals. Publicly accessible surfaces, including those in banks, harbor a myriad of microorganisms due to frequent contact by diverse individuals, often under unsanitary conditions. Among the predominant bacteria isolated from door handles are *Staphylococcus aureus*, *Klebsiella* spp. *Escherichia coli*, *Proteus* spp., *Pseudomonas aeruginosa*, *Streptococcus* spp. and *Bacillus subtilis*. These bacteria possess diverse pathogenic potentials, ranging from skin infections to life-threatening systemic illnesses. Below is a detailed analysis of these contaminants, their characteristics, growth conditions, and implications for human health.

2.5.1 *Klebsiella* spp.

Klebsiella is a genus of non-motile, Gram-negative, rod-shaped bacteria distinguished by its prominent polysaccharide capsule, which enhances its resistance to phagocytosis and antimicrobial agents (Ryan and Ray, 2004). These facultative anaerobes thrive at temperatures between 35°C and 37°C and a pH of approximately 7.2. They can utilize citrate and glucose as their sole carbon sources and ammonia as their sole nitrogen source.

Klebsiella pneumoniae, the most common species associated with human infections, is a primary pathogen in nosocomial settings. It is implicated in pneumonia, urinary tract infections, septicemia, and soft tissue infections (Podschun and Ullmann, 1998). The emergence of multidrug-resistant strains, including those producing extended-spectrum beta-lactamases (ESBLs) and carbapenemases, poses a significant public health challenge (Nordmann *et al*., 2011).

2.5.2 *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive coccus that forms grape-like clusters. It is a facultative anaerobe and an opportunistic pathogen responsible for both community-acquired and hospital-acquired infections (Prescott *et al*., 2005). Found on the skin and mucous membranes, *S. aureus* can cause a range of illnesses, from minor skin infections to severe diseases such as toxic shock syndrome, sepsis, and pneumonia (Lakshmi and Harasreeramulu, 2011).

A major concern is the emergence of methicillin-resistant *S. aureus* (MRSA), which complicates treatment options. MRSA is commonly found on high-touch surfaces in healthcare and public settings (Dancer, 2009). Transmission occurs through direct contact or via fomites like door handles. Preventative measures, including proper hygiene and regular surface disinfection, are essential to curb its spread.

2.5.3 *Escherichia coli*

Escherichia coli (E. coli) is a Gram-negative, rod-shaped bacterium that is part of the natural gut flora in humans and animals. While many strains are harmless and beneficial, pathogenic strains can cause severe gastrointestinal diseases and urinary tract infections (Singleton,

1999). *E. coli* is frequently used as an indicator organism for fecal contamination in environmental microbiology due to its resilience outside the host body.

Pathogenic strains, such as *E. coli* O157, produce Shiga toxins, leading to hemorrhagic colitis and hemolytic uremic syndrome. These strains are a significant concern in public health due to their low infectious dose and severe outcomes in vulnerable populations (Fotadar *et al*., 2005).

2.5.4 *Bacillus subtilis*

Bacillus subtilis is a Gram-positive, endospore-forming bacterium commonly found in soil and the gastrointestinal tract. Its spores allow it to survive harsh environmental conditions, including extreme heat. While typically non-pathogenic, *B. subtilis* can cause opportunistic infections in immunocompromised individuals (Ryan and Ray, 2004). It has industrial and probiotic applications but is also known to cause food spoilage, such as the "ropiness" defect in bread due to its production of extracellular polysaccharides. Its presence on fomites like door handles indicates contamination from environmental sources.

2.5.5 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative, motile bacterium known for its metabolic versatility and resistance to antibiotics. It is an opportunistic pathogen associated with nosocomial infections, particularly in immunocompromised individuals (Todar, 2020).

This bacterium thrives in moist environments and can colonize a wide range of surfaces, including fomites. It is a leading cause of ventilator-associated pneumonia, wound infections, and bloodstream infections. Its ability to form biofilms on surfaces like door handles enhances its persistence and resistance to disinfectants.

2.5.6 *Streptococcus* spp.

Streptococcus species are Gram-positive, facultative anaerobic cocci that occur in chains or pairs. They are categorized into various groups, such as pyogenic, viridans, and enterococcus, based on their hemolytic patterns and habitat. Beta-hemolytic species, such as *Streptococcus pyogenes*, are significant human pathogens, causing diseases like pharyngitis, scarlet fever, and necrotizing fasciitis (Willey *et al*., 2011).

The presence of *Streptococcus* spp. on door handles indicates contamination from respiratory droplets or direct contact with infected individuals. Proper sanitation and personal hygiene can help reduce the risk of transmission.

2.5.7 *Proteus* spp.

Proteus species, including *P. mirabilis* and *P. vulgaris*, are Gram-negative, facultative anaerobic rods. They are urease-positive and capable of swarming motility, which contributes to their virulence (Willey *et al*., 2011). *Proteus mirabilis* is a common cause of urinary tract infections, particularly in catheterized patients. The presence of *Proteus* on door handles suggests contamination from fecal sources or infected individuals. Their ability to form biofilms makes them resilient to cleaning efforts, emphasizing the need for stringent hygiene practices.

2.6. Sources of Bacterial Contamination in Door Handles

Door handles in public spaces, including banks, are commonly contaminated with a wide variety of microorganisms, primarily due to the frequent handling by individuals and the environmental factors that influence microbial persistence on surfaces. The sources of bacterial contamination on door handles can be broadly categorized into human contact,

environmental conditions, and inadequate cleaning practices. This section explores the primary sources contributing to bacterial contamination on door handles, backed by relevant studies and literature.

2.6. 1. Human Contact

Human hands are the primary vector for bacterial transmission, making direct contact with door handles the most significant source of contamination. The human hand acts as a reservoir for both pathogenic and non-pathogenic microorganisms, which can be transferred to door handles through direct contact (Pittet, 2000). Studies have shown that hands can harbor a wide range of bacteria, including those that cause healthcare-associated infections (HAIs), gastrointestinal diseases, and respiratory infections. A study by Nworie *et al*. (2012) in Nigeria found that 47.2% of bacteria isolated from public door handles exhibited multidrug resistance, indicating the potential for transmission of harmful and resistant strains through human contact.

Moreover, hands carry bacteria from various sources such as the nose, mouth, skin, and other body parts. Contaminated hands are especially common after individuals engage in activities like touching face surfaces (mouth, nose, eyes), coughing or sneezing, and touching personal items (Levin, 2012). Pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp. and *Klebsiella pneumoniae* can easily be transferred from one individual to another by touching door handles, thus facilitating the spread of infectious diseases in public spaces (Ekhaise *et al*., 2010).

2.6.2. Inadequate Cleaning and Disinfection Practices

Inadequate or inconsistent cleaning practices are a significant source of bacterial contamination on door handles. Many high-traffic public places, such as banks, may not have

routine cleaning schedules for surfaces such as door handles, or the cleaning process may not effectively remove all microorganisms (Kandelaki *et al*., 2018). Research by Scott *et al*. (2015) indicated that insufficient disinfection protocols could lead to the proliferation of pathogens on surfaces in schools and daycare centers, similar to findings in other public spaces like banks.

Cleaning materials, if not properly selected or utilized, may also contribute to bacterial persistence on surfaces. For example, the improper dilution of cleaning agents or the use of inappropriate disinfectants may allow bacteria to survive on door handles (Widmer, 2000). Furthermore, reusable cleaning cloths, if not properly disinfected after use, can serve as vectors for microbial transfer across multiple surfaces, exacerbating contamination levels (Rutala and Weber, 2008).

In healthcare settings, door handles have been identified as high-risk areas for healthcare-associated infections (HAIs), such as those caused by *Clostridioides difficile*, *Methicillin-resistant Staphylococcus aureus (MRSA)*, and *Acinetobacter baumannii*. Kandelaki *et al*. (2018) highlighted that improper cleaning protocols in hospitals contributed to the spread of HAIs, emphasizing the importance of thorough and regular disinfection of high-touch surfaces.

2.6.3. Cross-Contamination from Other Sources

In addition to direct human contact and environmental factors, cross-contamination from other sources such as contaminated objects and surfaces can contribute to bacterial contamination on door handles. Items such as shopping bags, mobile phones, wallets, and keys can carry bacteria from one place to another, and when individuals touch door handles after handling these objects, bacteria are transferred to the handles (Davis *et al*., 2015).

Additionally, microorganisms from other surfaces, such as floors and tables, can be transferred to door handles through indirect contact.

One study by Otter *et al*. (2016) found that surfaces in healthcare environments, including door handles, were frequently contaminated with *MRSA* due to cross-contamination from patients' personal items and hospital equipment. Similarly, banks and other high-traffic public areas may experience similar forms of cross-contamination, with items such as pens, credit cards, and bags contributing to the transfer of bacteria to door handles.

2.7. Public Health Implications of bacterial Contamination of Door Handles

Door handles are among the most frequently touched surfaces in shared spaces, making them hotspots for microbial contamination. They are reservoirs for bacteria, viruses, and fungi, many of which are pathogenic. This contamination poses significant public health risks, particularly in high-traffic environments such as schools, healthcare facilities, markets, restaurants, and public transportation hubs. The role of door handles in facilitating the transmission of infectious diseases and contributing to the emergence of antimicrobial-resistant pathogens has been documented in numerous studies.

Microbial contamination of door handles promotes fomite-mediated disease transmission. Pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp.*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* have been frequently isolated from door handles in public spaces (Bright *et al*., 2010; Ekhaise *et al*., 2010). These pathogens can cause a wide range of infections, from mild skin infections to severe illnesses like sepsis, pneumonia, and gastrointestinal diseases.

Viruses, including influenza and coronaviruses, can also persist on surfaces like door handles, with studies showing that these viruses can remain viable for several hours to days depending

on environmental conditions (Otter *et al*., 2016). This persistence increases the risk of respiratory and other viral infections through indirect contact.

A concerning trend is the isolation of antimicrobial-resistant (AMR) organisms from door handles. Research shows that high-touch surfaces often harbor resistant strains such as Methicillin-Resistant *Staphylococcus aureus* (MRSA), Vancomycin-Resistant Enterococci (VRE), and Extended-Spectrum Beta-Lactamase (ESBL)-producing *E. coli* (Sharma *et al*., 2020). For example, a study by Nworie *et al*. (2012) in Nigeria found that 47.2% of bacteria isolated from door handles in public buildings exhibited multidrug resistance.

The spread of AMR pathogens through contaminated surfaces exacerbates the global AMR crisis. It leads to increased difficulty in treating infections, higher healthcare costs, prolonged hospital stays, and greater risk of complications. In healthcare facilities, door handles play a critical role in the transmission of healthcare-associated infections (HAIs), such as those caused by *Clostridioides difficile*, MRSA, and *Acinetobacter baumannii*. A study by Kandelaki *et al*. (2018) emphasized the role of inadequate cleaning protocols in amplifying the risk of HAIs, underscoring the need for stringent sanitation measures.

Schools and daycare centers are also high-risk settings. Studies have shown that pathogens like *Shigella* spp. *Rotavirus*, and *Norovirus* are often spread via contaminated surfaces, including door handles, in these environments, contributing to outbreaks of gastrointestinal and respiratory infections among children (Scott *et al*., 2015).

2.8. Prevention of microbial contamination of door handles

Preventing microbial contamination of door handles is crucial in reducing the risks associated with fomite-mediated transmission of infectious diseases. Given their frequent use and the variety of pathogens they harbor, implementing a combination of strategies is essential to

ensure public health safety. These strategies involve regular cleaning and disinfection, material engineering innovations, promoting hand hygiene, employing advanced technologies, and raising public awareness.

Routine cleaning and disinfection of door handles play a fundamental role in minimizing microbial load. Alcohol-based solutions (70–90% ethanol or isopropanol) and hypochlorite-based disinfectants are highly effective against a broad spectrum of pathogens, including bacteria, viruses, and fungi (Bright *et al*., 2010). Chlorine-based disinfectants, such as sodium hypochlorite, disrupt microbial membranes and denature proteins, leading to rapid inactivation. High-touch surfaces, such as door handles in public spaces, should be cleaned multiple times daily, particularly during outbreaks of infectious diseases like influenza or norovirus. Standardized cleaning protocols that detail disinfectant types, concentration, and contact time ensure consistent and effective microbial control. For example, a minimum contact time of 30 seconds is recommended for alcohol-based disinfectants to achieve optimum efficacy.

Incorporating antimicrobial materials into door handle designs provides a passive, long-term solution to microbial contamination. Metals such as copper, brass, and silver are well-known for their inherent antimicrobial properties: Copper ions disrupt bacterial cell membranes, leading to leakage of cellular contents and eventual cell death (Grass *et al*., 2011). Copper door handles have been shown to reduce microbial load by over 80% compared to stainless steel in hospital settings. These materials release silver ions, which interfere with microbial DNA and protein function, effectively preventing growth. Coating door handles with silver nanoparticles provides an additional layer of defense. These materials are not only effective but also durable, requiring minimal maintenance over their lifetime.

The hands of individuals are the primary medium through which pathogens are transferred from door handles to mucous membranes. Thus, promoting hand hygiene remains one of the most effective measures for breaking this chain of transmission. Washing hands with soap and water for at least 20 seconds effectively removes dirt, oils, and pathogens. Studies indicate that proper handwashing can reduce respiratory infections by 20% and diarrheal diseases by 30% (WHO, 2022). Alcohol-based hand sanitizers (ABHS) are highly effective in settings where access to soap and water is limited. Hand sanitizer dispensers placed near high-touch surfaces like door handles encourage use and reduce microbial transfer. Hand hygiene campaigns, incorporating visual aids, reminders, and incentives, foster long-term behavioral changes and compliance.

The advent of innovative technologies has provided additional solutions to mitigate contamination risks: Hands-free door-opening mechanisms, such as foot-operated or sensor-based systems, eliminate the need for direct hand contact with door handles. These systems are particularly beneficial in healthcare facilities and public restrooms. Coatings embedded with antimicrobial agents, such as quaternary ammonium compounds, zinc oxide, or titanium dioxide, create self-disinfecting surfaces. These coatings are durable and provide sustained microbial control, reducing the frequency of manual cleaning. UV-C light devices can be used to sterilize door handles periodically. UV-C light damages microbial DNA, rendering them nonviable, and has been shown to reduce bacterial load by over 90% in laboratory settings.

Educating the public about the risks of microbial contamination and the importance of hygiene practices is critical for reducing disease transmission: Public health campaigns, workshops, and media engagement can inform communities about proper hygiene and the role of contaminated surfaces in disease spread. Specific groups, such as schoolchildren,

healthcare workers, and food handlers, should receive tailored training to promote awareness and compliance. Placing posters near door handles, especially in public restrooms and healthcare settings, reinforces hand hygiene messages and reminds individuals to clean their hands before and after touching shared surfaces.

Addressing the issue of microbial contamination on door handles requires collaboration among public health officials, industrial designers, facility managers, and microbiologists. By combining cleaning protocols, engineering innovations, behavioral interventions, and advanced technologies, the risks associated with contaminated surfaces can be significantly mitigated.

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY AREA

The study was conducted in the University of Benin, located in Benin City, Edo State, Nigeria. Specifically, door handles from five banks within the campus were sampled. These banks were chosen due to their high patronage, ensuring a suitable environment for evaluating microbial contamination.

3.3 SAMPLE COLLECTION

Samples were collected from the door handles of five different banks located within the University of Benin campus. Swab samples were obtained from both the entrance and interior door handles of each bank to provide a comprehensive assessment of microbial contamination. The samples were collected during peak business hours (10:00 AM to 12:00 PM) when foot traffic and interactions with the door handles were expected to be highest, maximizing the likelihood of detecting bacterial contamination. Samples were taken once a week over a two-week period to assess bacterial variability over time and to capture potential differences in contamination levels that could be influenced by factors such as cleaning routines, foot traffic, and environmental conditions.

For each collection, sterile cotton swabs moistened with sterile normal saline were used to gently swab the surfaces of the door handles. The swabs were then immediately transferred to sterile containers containing 10 mL of sterile peptone water to preserve the viability of any bacteria present during transport. Each container was labeled with the respective bank name, date of collection, and sample number to ensure proper identification and tracking.The

samples were transported to the microbiology laboratory for analysis within one hour of collection to prevent the degradation of bacterial cultures and to ensure the accuracy of results.

3.4.Sterilization of Materials

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

3.3.1 Preparation and Sterilization of media

Materials used include; Glass wares such as test tubes, beakers, conical flasks, Petri-dishes, McCartney bottles, sterile cotton swabs, sterile gloves, Normal saline, Sterile sampling containers, stirring glass rod and measuring cylinder. Media and Biochemical test reagents and Gram's staining kit. All glassware which include MacCartney bottles, Petri dishes, test tubes, conical flasks, measuring cylinders and pipettes, were sterilized at 160 ^{o}C for 1 hr. in a hot-air-oven before use. The media used in this study were sterilized at 121 ^{o}C for 15 min in an autoclave. Agar media, agar slant and biochemical reagents were prepared freshly and refrigerated at 3-4 ^{o}C. Aseptic conditions were ensured during inoculation and subculturing.

3.3.1.1 Preparation of Nutrient agar

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 and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium will be the placed in an autoclave to sterilize it for 15 minutes at 121 °C. After sterilization, the flask will be allowed to cool.

3.3.1.2 Preparation of Citrate agar

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

3.3.1.3 Preparation of Triple Sugar Iron agar

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

3.4. ENUMERATION AND ISOLATION OF BACTERIAL ISOLATES

Upon arriving at the laboratory, each swab sample was subjected to serial dilution followed by the pour plate method for isolating bacterial and fungal microorganisms. This method was employed to ensure accurate colony counts and to minimize overcrowding on the plates. The swab sample was transferred into a sterile test tube containing 9 ml of sterile normal saline. The mixture was vortexed to ensure proper homogenization. A series of tenfold dilutions were prepared by transferring 1 ml of the initial solution into a second test tube containing 9 ml of sterile saline, thereby producing a 10^{-1} dilution. This process was repeated to create further dilutions ($10^2 10^3$, and 10^4). The pour plate method was used to isolate microorganisms from the diluted samples.

For each dilution, the following steps were carried out: 1 ml of each dilution (from 10^{-1} to 10^{-4}) was pipetted aseptically into sterile Petri dishes. Approximately 15-20 ml of molten

agar (cooled to about 45°C) was poured into each Petri dish and gently swirled to ensure even distribution of the inoculum. Plates containing Nutrient Agar, were incubated at 37°C for 24-48 hours for the isolation of bacterial colonies. After the incubation period, distinct colonies were counted using a colony counter. The number of colony-forming units (CFUs) per milliliter was calculated based on the dilution factor (Willey *et al.,* 2008)..

$$\frac{cfu}{ml} = \frac{number\ of\ colonies\ x\ dilution\ fold/series}{volume\ of\ inoculum}$$

3.4.1 Subculturing of Pure Isolates

After colony counting, well-isolated colonies with distinct morphologies were selected and subcultured onto fresh Nutrient Agar plates to obtain pure cultures. These pure cultures were then subjected to further identification tests, such as biochemical and morphological characterization. This pour plate method ensures a quantitative approach to assessing microbial load while isolating microorganisms from ATMs. It allows for a more accurate representation of the microbial population by minimizing colony crowding.

3.5 BACTERIAL IDENTIFICATION

The bacterial isolates were characterized based on colonial morphological characteristics such as colony shape, size, elevation, optical activity, margination and pigmentation on nutrient agar and MacConkey agar. Biochemical tests were also carried out to further identify the bacterial isolates. The fungal isolates were identified using colonial morphological characteristics such as size, texture colour and reverse colour. These parameters were evaluated by physical examination. Microscopy was also carried out using n-lactophenol cotton blue staining and a bright field microscope.

3.5.1 Gram staining

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

3.5.3 Potassium Hydroxide (KOH) test

Two drops of 3% solution of KOH were applied on a clean glass slide and a loopful of pure bacterial growth was stirred in a circular motion in the slide. The loop was occasionally raised and observed for the presence of a string of the mixture. The solution was observed to be of a viscous and mucoid consistency indicating a Gram-negative bacterium.No reaction (absence of stringing) indicates a Gram-positive bacterium (Roberts and Sandle, 2008).

3.6. BIOCHEMICAL TEST

3.6.1 Catalase Test

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

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

3.6.2 Oxidase Test

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

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

3.6.3 Citrate Utilization Test

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

3.6.4 Indole Test

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

3.6.5. Triple sugar iron (TSI) agar test

The Triple Sugar Iron (TSI) test is an ability to test an organism's capability to ferment sugars and to produce hydrogen sulphide (H_2S) or gas (O_2), or both. The test was used primarily to

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

3.7 Antibiotic susceptibility test

The identified colonies of bacteria were used to determine the susceptibility and resistance of bacterial isolates, which were subjected to standard antibacterial susceptibility testing (AST) to decipher their resistance or susceptibility to common antibiotics used for treatment within the locality. The standard discs were produced by Oxoid, UK, which was used to execute the disc diffusion method employed in this study. For this assay, a fully grown bacterial culture (from 18-24 hours) was cultured on MHA. The inoculum corresponding to 1.5 x 10^8 cells/ml McFarland standard was streaked using a sterile loop onto the MHA plates before the introduction of antibiotic discs and were added with extreme care to the plates with the aid of sterile forceps. The susceptibility results were recorded after incubation for 24 hours at 37 ºC.

Following the standard or rules of AST established in 2017 by CLSI (Clinical Laboratory Standards Institute). The inhibition zone around each disc (measured using a meter rule in diameter) was assessed and interpreted based on the 2020 CLSI standard as Resistant (R), Intermediate resistant (I) and Sensitive (S). The antibiotic discs used in the study with their corresponding codes and concentrations include

3.8. Multiple Antibiotic Resistance (MAR) Index

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

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

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

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

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

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

CHAPTER FOUR

RESULTS

Table 4.1 shows the Total Heterotrophic Bacterial Count (THBC) x 10^4 CFU/ml for door handles of selected banks over a two-week period. In Week 1, the bacterial counts ranged from 2.03 ± 0.03 x 10^4 CFU/ml (Bank D) to 4.17 ± 0.18 x 10^4 CFU/ml (Bank F). In Week 2, there was notable variation in the bacterial counts. The highest count was observed in Bank E (4.72 ± 0.31 x 10^4 CFU/ml), while the lowest was recorded in Bank B (2.10 ± 0.10 x 10^4 CFU/ml).

Table 4.2. Shows the biochemical test for identification of microorganisms isolated from the different parts of Automated Teller Machines in the different banks in University of Benin, Benin City. From the results, it was shown that three Gram positive bacteria and four Gram-negative bacteria were isolated from the studied machines. Detailed tests and observations including their growth features, biochemical reactions and sugar utilization as well as a crosscheck with existing Taxa in standard manuals Bergey's manual of determinative bacteriology and manual for identification of medical bacteria led to their identification as species of *Staphylococcus sp.*, *Bacillus* sp., *Streptococcus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Escherichia coli* and *Klebsiella* sp.

Table 4.4. Shows the occurrence of bacteria isolates door handles in the selected banks in University of Benin, Benin City. Several species of bacteria were isolated from the different machines. The isolates were found to have varying percentages of occurrence including *Escherichia coli* with an occurrence of 28%, *Staphylococcus* sp. 20%, *Enterobacter* sp. 14% and *Pseudomonas* sp 12%. *Streptococcus* sp. recorded 13% while d *Bacillus* spp. recorded 16% occurrence.

Table 4.3. Shows the antibiotic susceptibility patterns of bacterial isolates against ten antibiotics, including Cefotaxime (CTX), Ofloxacin (OFX), and Imipenem (IPM). Isolates are classified as resistant (R), intermediate (I), or susceptible (S) based on inhibition zone diameters. *Escherichia coli* demonstrates resistance to five antibiotics, indicating multidrug resistance.

Figure 4.2. Shows the multiple antibiotics resistance index of the bacterial isolates. The resistance index ranged from 0.3 to 0.5 the highest resistance index was recorded for *Escherichia coli.*

Table 4.1: Total Heterotrophic Bacterial Count x 10^4 (CFU/ml) for **door handles of selected banks Over Two Weeks**

Bank	Week 1	Week 2
A	3.43± 0.29	3.03 ± 0.03
B	2.93± 0.12	2.10 ± 0.10
C	3.10 ± 0.06	4.17 ± 0.27
D	2.03± 0.03	3.80 ± 0.43
E	3.0 ± 0.10	4.72 ± 0.31
F	4.17±0.18	3.50±0.15
G	3.10±0.53	3.33+0.19

Table 4.2: Cultural, Morphological, and Biochemical Characteristics of Bacteria isolates

Elevation	Raised	Raised	Flat	Flat	Raised	Flat
Margin	Entire	Entire	Undulate	Entire	Undulate	Entire
Color	Cream	Cream	Cream	Yellow	Green	White
Shape	Circular	Circular	Irregular	Circular	Irregular	Circular
Size	Medium	Medium	Large	Medium	Medium	Small
Gram Stain	-	+	-	-	-	+
Cell Type	Rod	Cocci	Rod	Rod	Rod	Cocci
Arrangement	Disperse	Clusters	Disperse	Disperse	Disperse	Chains
Color (Gram Reaction)	Pink	Purple	Pink	Pink	Pink	Purple
KOH String Test	+	-	+	+	-	-
Catalase	+	+	+	+	+	-
Indole	-	-	+	-	-	-
Citrate	+	-	-	+	+	-
Oxidase	-	-	-	-	+	-
Glucose	+	+	+	+	+	+
Sucrose	+	+	-	+	-	+
Lactose	+	+	+	+	-	+
Gas Formation	+	-	+	+	-	-
H_2S Formation	-	-	-	-	-	-
TSI (Slant/Butt) Reaction	A/AG	K/A	A/AG	A/A	K/AG	A/A
Identity	*Klebsiella* sp.	*Staphylococcus* sp.	*Escherichia coli*	*Enterobacter* sp.	*Pseudomonas* sp.	*Streptococcus* sp.

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

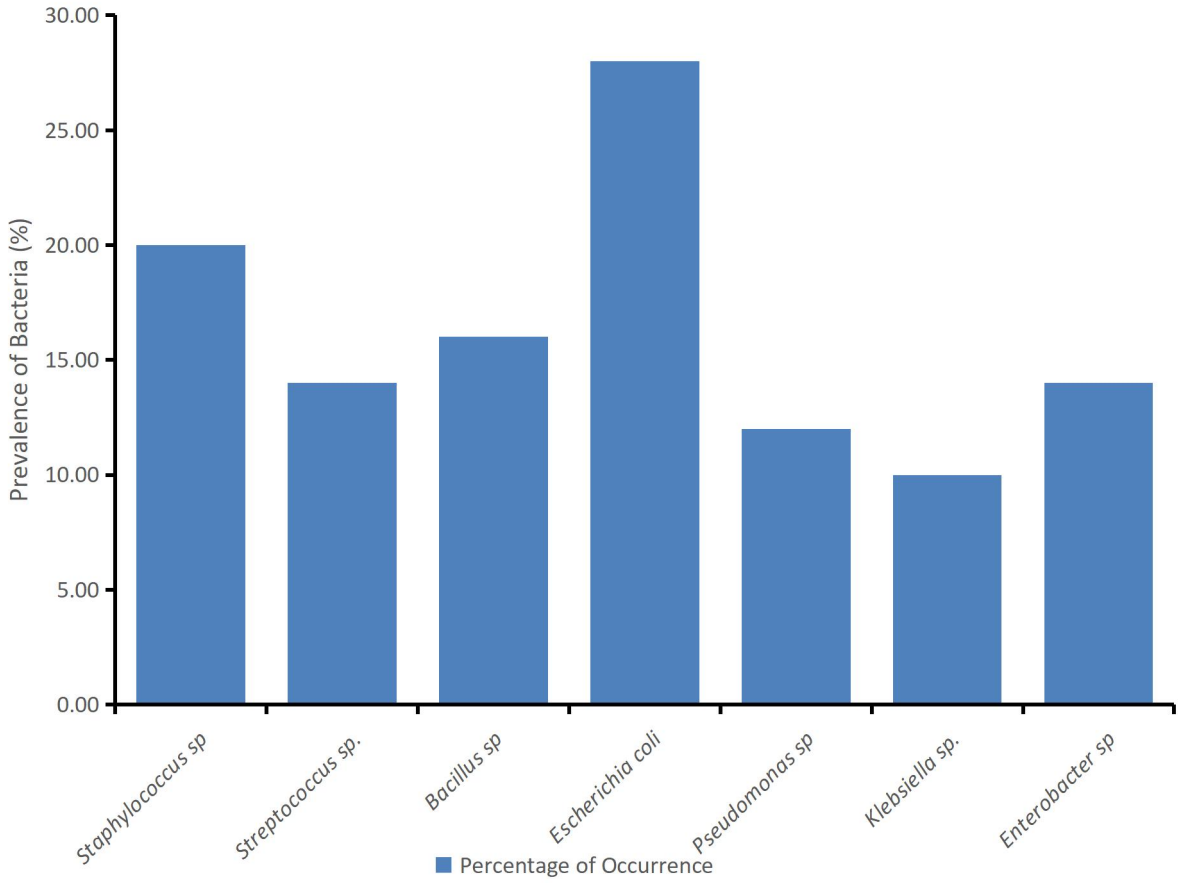

Figure 4.1:Percentage Frequency of Occurrence of Isolated Bacteria.

Table 4.5. : Antibiotics Susceptibility Pattern of bacteria Isolates from the door handles

Bacteria isolates	Antibiotics (Zone of Inhibition [mm])									
	PEF	**CN**	**APX**	**Z**	**AM**	**R**	**CPX**	**AZ**	**LEV**	**E**
Gram positive										
***Staphylococcus* sp**	18(S)	14(I)	0(R)	4(R)	0(R)	8(R)	12(I)	16(I)	16(I)	20(S)
***Streptococcus* sp.**	14(I)	14(I)	0(R)	9(R)	10(R)	12(I)	14(I)	24(I)	16(I)	18(S)
Bacillus cereus	18(S)	22(I)	0(R)	10(R)	0(R)	20(I)	18(S)	18(S)	16(I)	16(I)
Gram negative	**LEV**	**CF**	**SP**	**CPX**	**AM**	**AU**	**CN**	**PEF**	**OFX**	**AZ**
***Pseudomonas* sp.**	24(S)	10(R)	22(S)	18(S)	8(R)	10(R)	8(R)	16(I)	18(S)	20(S)
Escherichia coli	20(S)	10(R)	8(R)	14(I)	9(R)	10(R)	10(R)	16(I)	18(S)	18(S)
***Klebsiella* sp.**	16(I)	10(R)	18(S)	16(I)	8(R)	6(R)	14(I)	14(I)	10(R)	18(S)
***Enterobacter* sp.**	18(S)	16(I)	0(R)	0(R)	0(R)	16(I)	18(S)	18(S)	16(I)	16(I)

Resistant (R)= 0-10mm

Intermediate (I) = 11-16mm

Sensitive (S) =17mm and above

KEY: Resistance (R) = 0-10mm, Intermediate (I) = 11-16mm, Sensitive (S) = 17mm and above, PEF: Pefloxacin, CN: Gentamycin, APX: Ampiclox, Z: Zinnacef, AM: Amoxacillin, R: Rocephin, CPX: Ciprofloxacin, E: Erythromycin, LEV: Levofloxacin, AZ: Azithromycin, CF: Cefotaxim, SP: Saprifloxacin, OFX: Ofloxacin

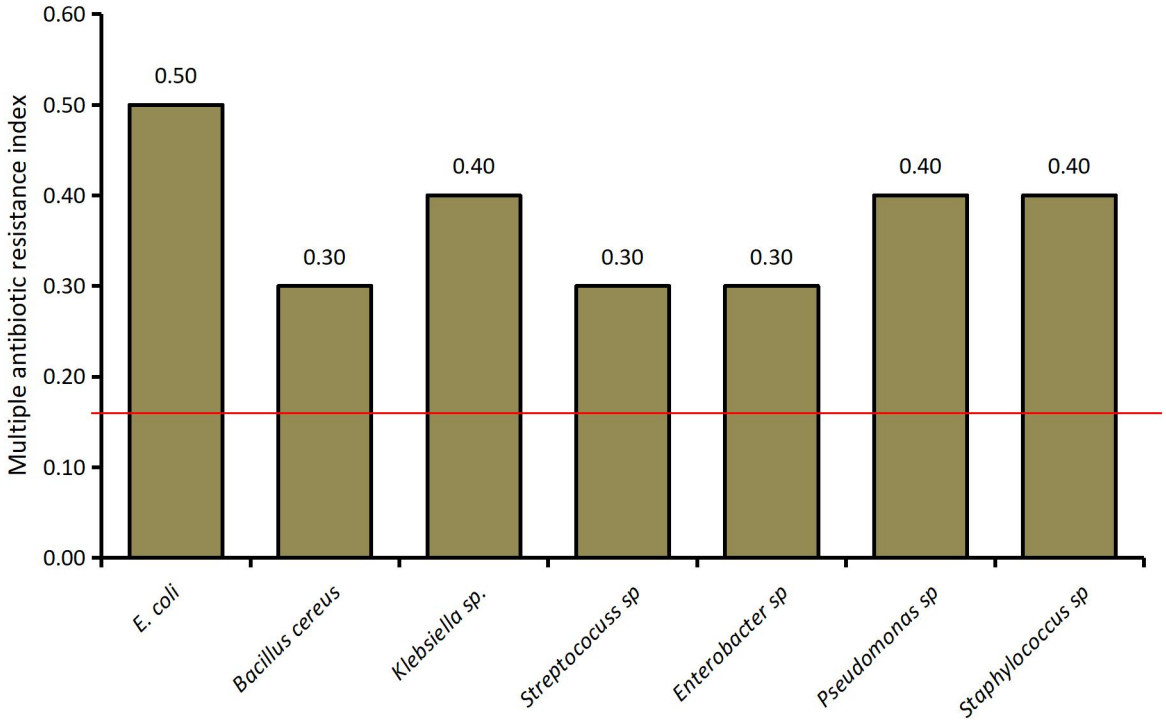

Figure 4.3: Multi-antibiotic Resistance Index of Isolated Bacteria.

CHAPTER FIVE

DISCUSSION

Door handles are high-contact surfaces commonly used to open or close doors in public and private spaces. They come in various designs, materials, and finishes, ranging from stainless steel and brass to plastic. Due to their frequent and repetitive use by multiple individuals, door handles serve as potential reservoirs for microorganisms, including bacteria, viruses, and fungi. These pathogens can be transferred through direct contact with contaminated hands, environmental exposure, or poor hygiene practices (Onwubiko and Chinyeaka, 2015). As a result, door handles are considered significant fomites, contributing to the transmission of infectious agents, especially in high-traffic areas such as banks, hospitals, schools, and public transportation hubs. This study seeks to investigate the isolation and identification of bacterial isolates from door handles in selected banks in the University of Benin, Benin City, Edo State.

The bacterial counts recorded during the study, as presented in Table 4.1, showed notable variations across the door handles of different banks. Week 1 recorded bacterial loads ranging from $2.03\pm0.03\times10^4$ CFU/ml (Bank D) to $4.17\pm0.18\times10^4$ CFU/ml (Bank F). By Week 2, the bacterial counts exhibited changes, with the highest count observed in Bank E ($4.72\pm0.31\times10^4$ CFU/ml) and the lowest in Bank B ($2.10\pm0.10\times10^4$ CFU/ml). These variations can be attributed to factors such as differences in foot traffic, cleaning practices, environmental conditions (e.g., humidity and temperature), and hygiene behaviors of bank customers (Odugie *et al*., 2017; Alonge *et al*., 2019). Similar findings have been reported in literature, where high-contact surfaces such as door handles and elevator buttons consistently exhibit significant microbial loads (Ngonda, 2017). Hasssan *et al*., (2022) similarly observed

high bacterial counts on public door handles in urban areas, emphasizing the urgent need for regular disinfection to curb microbial proliferation.

The cultural, morphological, and biochemical analyses of isolates identified seven bacterial species: *Staphylococcus sp.*, *Bacillus sp.*, *Streptococcus sp.*, *Pseudomonas sp.*, *Enterobacter sp.*, *Escherichia coli* and *Klebsiella sp.*. The frequency of occurrence analysis (Table 4.4) showed that *Escherichia coli* was the most prevalent isolate (28%), followed by *Staphylococcus sp.* (20%), this aling with the study of (Odugie *et al*., 2017). The finding of E. coli as the most frequent bacterial contaminant in this study is at variance with previous works in Nigeria that reported *S. aureus* as the most prevalent contaminants of door handles. In their study, Onwubiko and Chinyeaka, (2015), reported *S. aureus* 33(25.0%) as the most frequently isolated bacteria from door handles of a tertiary Institution at Umuahia, Abia State, Nigeria. The other isolates, such as *Bacillus sp.* (16%) *Streptococcus sp.* (13%), *Enterobacter sp.* (14%), and *Pseudomonas sp.* (12%), were also detected.

The predominance of *E. coli* indicates fecal contamination, likely stemming from inadequate hand hygiene or contaminated water sources. *Staphylococcus sp.* and *Streptococcus sp.*, commonly found on human skin and mucous membranes, reflect direct human interaction with the door handles. The isolation of species of *Staphylococcus* in the door sampled in this study is not surprising as they are known resident microflora of the skin (Hardy *et al*., 2006). *Staphylococcus aureus* is normally carried by 20-40% of healthy persons at any material time. The isolation of S. aureus agrees with the findings of Nwankwo and Afuruobi, 2015 (2015). Staphylococcus sp. is ubiquitous in nature which explains why it is usually frequent as a contaminant, as it can be easily discharged by several human activities including sneezing, talking, and contact with moist skin (Itah and Ben, 2004). The detection of environmental isolates such as *Pseudomonas sp.* and *Klebsiella sp.* is particularly concerning due to their

association with healthcare-associated infections and their intrinsic resistance mechanisms. These findings align with those of Onuoha and Kayode (2014), who reported similar bacterial profiles on high-touch surfaces in public settings. The presence of *Klebsiella sp.* and *Pseudomonas sp.* emphasizes the potential risk of opportunistic infections, especially among individuals with weakened immune systems.

The presence of *Pseudomonas sp. Pseudomonas sp.* and *Bacillus* sp. is also of public health significance. *P. aeruginosa* has been found to be a major opportunistic pathogen and reportedly identified as one of the vital causes of infection-related mortality among seriously ill and immunocompromised patients (Zaborina *et al*., 2006). These pathogens were found to be the leading causes of wound infection and diarrhea especially in developing countries. They are best described as classic opportunistic nosocomial pathogens which can cause a wide spectrum of infection and morbidity in immune compromised patient. Streptococcus sp. are also major pathogens that are often implicated in community-acquired pneumonia, sinusitis, otitis media and meningitis among the critically ill, the very young, and the elderly (Musher., 2000; Karlowsky *et al*., 2003). *Bacillus* species are mainly soil flora but have been implicated in some clinical manifestations such self-limiting food poisoning, ocular infections, meningitis, endocarditis, osteomyelitis, and bacteremia (Tuazon, 2017).

The antibiotic susceptibility testing (Table 4.5) revealed a resistance pattern among the isolates. *Escherichia coli* demonstrated resistance to multiple antibiotics, including Cefotaxime and Ampicillin, indicating a multidrug-resistant (MDR) phenotype. This resistance pattern is consistent with global reports of rising antimicrobial resistance (AMR) and underscores the challenge of treating infections caused by such pathogens (WHO, 2022).

Other isolates, such as *Staphylococcus sp.* and *Pseudomonas sp.*, displayed intermediate susceptibility to antibiotics like Gentamycin and Ciprofloxacin while retaining sensitivity to

Pefloxacin and Erythromycin. The multi-antibiotic resistance index (MARI) values, ranging from 0.3 to 0.5 (Figure 4.3), further highlight the significant prevalence of resistance among the isolates. High MARI values suggest prolonged exposure to antibiotics, either through clinical misuse or environmental contamination. The widespread resistance observed in this study is consistent with findings by Okoh *et al*. (2018), who documented high resistance indices among environmental bacteria. These observations emphasize the need for stringent antibiotic stewardship programs and the promotion of rational antibiotic use.

The isolation of these multidrug-resistant bacteria from door handles in banks has significant public health implications. Door handles, frequently touched by multiple individuals daily, serve as vectors for the transmission of infectious agents. Pathogens such as *E. coli* and *Klebsiella sp.* are associated with gastrointestinal and urinary tract infections, posing a greater risk to immunocompromised individuals.

The findings highlight the importance of implementing regular cleaning and disinfection of high-contact surfaces to reduce microbial loads. The use of disinfectants with broad-spectrum activity against bacteria, combined with public health campaigns promoting hand hygiene and the use of hand sanitizers, can significantly mitigate the risk of contamination and transmission. Additionally, educational programs aimed at improving hygiene behaviors, particularly in public settings, are essential. Banks and other public institutions should adopt stringent cleaning protocols and consider incorporating antimicrobial materials in frequently touched surfaces to minimize microbial survival.

CONCLUSION

This study underscores the microbial contamination of door handles in selected banks within the University of Benin and the associated risks of disease transmission. The identification of

multidrug-resistant bacteria, such as *E. coli* and *Klebsiella sp.*, emphasizes the urgent need for enhanced surveillance and effective control measures to safeguard public health. Future research should explore the molecular mechanisms driving antibiotic resistance in these isolates and investigate alternative disinfection methods, such as the use of natural antimicrobial agents.

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