ISOLATION AND IDENTIFICATION OF BACTERIAL ISOLATES FROM FACULTIES TOILET DOOR HANDLE

BY

Favour Osahon AMASOWAMWAN

LSC2009863

UNIVERSITY OF BENIN

BENIN CITY

FEBRUARY, 2025.

ISOLATION AND IDENTIFICATION OF BACTERIAL ISOLATES FROM FACULTIES TOILET DOOR HANDLE

BY

Favour Osahon AMASOWAMWAN

LSC2009863

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

FEBRUARY, 2025.

CERTIFICATION

This is to certify that the project work was carried out by Favour Osahon AMASOWAMWAN with Matriculation number **LSC2009863** in the Department of Microbiology, Life Sciences, University of Benin, Benin City.

PROF. F.O EKHAISE
(PROJECT SUPERVISOR)

5/03/25
DATE

PROF (MRS) F. I AKINNIBOSUN
(HEAD OF DEPARTMENT)

23/04/2025
DATE

Prof. C.J. Ogugbue
13-02-2025

26/2/25

DEDICATION

I dedicate this work to God Almighty for His unfailing love, help, strength and for being my source of wisdom and knowledge.

ACKNOWLEDGEMENTS

I wish to acknowledge God Almighty for His direction and help throughout this work, without Him, success would have been impossible.

To my amazing supervisor, PROF F.O EKHAISE, thank you Sir for your motivations, assistance, guidance, patience and understanding throughout this work.

To my Head of Department, Prof (Mrs) F.I Akinnibosun, thank you so much for your support and guidance. And to my amazing and beautiful course advisor Dr (Mrs)R. Adams, thank you for your constant counsel and love throughout my academic journey, to every member of staff of the Department of Microbiology who showed me love and support throughout this work, you are highly appreciated. I also want to specifically appreciate Dr(Mrs) I.B. IDEMUDIA for her love , support and guidance throughout this project work.

To the best parents in the world, Mr and Mrs AMASOWAMWAN, you are the greatest gift I have from God. Thank you for believing in me and supporting me through life. I love you dearly and God keep you to enjoy the fruits of your labour.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURE

ABSTRACT

Door handles are frequently touched surfaces that can serve as reservoirs for bacterial contamination, contributing to the spread of infectious diseases, especially in high-traffic environments such as universities. This study aimed to isolate and identify bacteria from door handles sampled at different faculties within the University of Benin, assessing microbial load and antibiotic resistance patterns. Total bacterial counts (TBC) varied across faculties, with the Faculty of Physical Sciences (FPS) exhibiting the highest count ($4.03 \pm 0.32 \times 10^{\square}$ cfu/cm²) in Week 1, while the Faculty of Life Sciences (FLS) recorded the highest count ($4.50 \pm 0.25 \times 10^{\square}$ cfu/cm²) in Week 2. Morphological and biochemical characterization identified *Escherichia coli*, *Bacillus* sp., *Streptococcus* sp., *Proteus* sp., *Salmonella* sp., *Staphylococcus* sp. and *Enterobacter* sp. as prevalent bacterial contaminants. *Staphylococcus* sp. was the most frequently isolated bacterium (24%), followed by *Escherichia coli* (18%) and *Bacillus* sp. (16%). Antibiotic susceptibility testing revealed high resistance levels among isolates, with *Escherichia coli* and *Staphylococcus* sp. exhibiting multidrug resistance. Ofloxacin, gentamicin, and levofloxacin demonstrated the highest efficacy, whereas cefotaxime and nitrofurantoin were largely ineffective. These findings highlight the need for enhanced hygiene practices and regular disinfection of high-contact surfaces to mitigate bacterial transmission within the university environment.

CHAPTER ONE

INTRODUCTION

1.1.Background of the study

Public health and sanitation have always been fundamental components of human well-being, especially in environments where large populations coexist, such as schools, hospitals, and universities. In densely populated settings, public spaces tend to be high-risk areas for the spread of infectious diseases. Universities, being hubs of student interaction, research, and communal living, present unique challenges in the maintenance of hygienic conditions. One particular area of concern is the microbial contamination of frequently touched surfaces such as toilet door handles. These surfaces are potential reservoirs for a wide array of microorganisms, including pathogenic bacteria that can cause various health issues (Greene, 2009). Door handles in restrooms, due to their unavoidable contact and infrequent disinfection, are prone to the accumulation of bacteria, thereby posing a significant risk to the university community (Otter *et al.*, 2013).

The critical nature of high-touch surfaces in disease transmission is well established in scientific literature. High-touch surfaces, like door handles, elevator buttons, and handrails, have been identified as sites where pathogenic microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, and other fecal coliforms can persist and spread from person to person (Tunc and Olgun, 2006). In a study by Reynolds *et al.* (2005), door handles in public spaces were found to harbor a significant bacterial load, including antibiotic-resistant strains. These bacteria can cause various infections, including skin infections, respiratory infections, and gastrointestinal diseases.

particularly in individuals with weakened immune systems or those who do not practice proper hand hygiene (Reynolds *et al.*, 2005).

Hygiene and sanitation are integral to disease prevention, particularly in institutional environments. The World Health Organization (WHO) emphasizes that proper sanitation and hygiene practices are essential in preventing the spread of infectious diseases, particularly in areas with high population densities like schools and universities (WHO, 2018). Poor hygiene practices, such as inadequate handwashing, contribute significantly to the spread of bacteria on high-touch surfaces like toilet door handles. This is a serious concern because contaminated hands transfer microorganisms from surfaces to other individuals, leading to potential outbreaks of infections (Chinakwe *et al.*, 2012).

Research has shown that restroom facilities, particularly in public institutions, are hotspots for bacterial contamination. The frequent touching of door handles by multiple individuals, combined with poor sanitation practices, leads to the buildup of microbial populations on these surfaces. In many cases, the cleaning schedules for restrooms are insufficient to manage the microbial load effectively. This results in the proliferation of bacteria that can potentially cause health issues (Gerba *et al.*, 2014). Public restrooms in universities, especially those with high traffic like the University of Benin, are often cleaned irregularly, which may contribute to an increased risk of bacterial transmission.

Studies on microbial contamination in public facilities have consistently highlighted restrooms as critical areas of concern. Improper cleaning practices and high foot traffic contribute to the accumulation of bacteria on surfaces. Inadequate sanitation measures, coupled with poor personal hygiene practices, exacerbate the issue. Public restrooms are often considered high-risk

areas because they facilitate the spread of various pathogenic microorganisms (Abney *et al.*, 2024).

Several studies have demonstrated that surfaces like door handles in public restrooms are frequently contaminated with pathogenic bacteria, posing a public health risk. For instance, a study conducted by Nworie *et al.* (2012) on public restrooms in Nigeria found that restroom door handles were contaminated with bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus vulgaris*. These bacteria can cause infections ranging from urinary tract infections to respiratory infections, especially in individuals who are immunocompromised. The study also noted that irregular cleaning schedules and poor hygiene practices among restroom users significantly contributed to the bacterial contamination of these surfaces (Nworie *et al.*, 2012). However, the risk of disease transmission through fomites is determined by; the frequency of site contamination and exposure; level of pathogen excreted by the host; likelihood of transfer of the infectious agent to a susceptible individual; virulence of organism; immune competence of the persons in contact; the practice of control measure such as disinfectant use and personal hygiene (Reynolds, 2005).

Indirect contact transmission, such as touching contaminated surfaces, is one of the most common ways in which bacteria spread in public spaces (Oranusi *et al.*, 2013). Aiello and Larson (2002) documented that bacteria present on high-touch surfaces could be transferred to individuals when they come into contact with these surfaces, and the risk of transmission increases when individuals fail to wash their hands after using the restroom. Once bacteria are transferred to an individual's hands, they can spread to other surfaces, thereby perpetuating the cycle of contamination. In a university setting, where students and staff frequently move between

different buildings and facilities, the potential for bacteria to spread quickly is particularly concerning.

Bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and other fecal coliforms have been identified as frequent contaminants on public restroom surfaces. These bacteria can lead to various health issues, from mild skin infections to more severe diseases like food poisoning and respiratory infections (Oranusi *et at.*, 2013). The risk is heightened in public restrooms where handwashing practices are often inadequate (Otter *et al.*, 2013). Furthermore, the growing problem of antibiotic resistance exacerbates the public health threat posed by these bacteria, as infections caused by resistant strains are harder to treat and control (Davies and Davies, 2010).

In the context of universities, restrooms are essential facilities used daily by a large population. However, due to limited cleaning schedules, overcrowding, and varying hygiene practices, these spaces can become heavily contaminated with bacteria. The University of Benin, a prominent institution in Nigeria, is no exception to this issue. With thousands of students and staff using restroom facilities daily, the potential for microbial contamination of high-contact surfaces such as toilet door handles is heightened. This study aims to isolate and identify bacterial species from toilet door handles in the university, providing valuable insights into the public health risks associated with poor sanitation practices in such environments.

1.2. Aim of the Study

The aim of this study was to **isolate and identify bacterial species from toilet door handles in the University of Benin, Benin City, Nigeria.**

The specific objectives were to:

1. enumerate, identify and isolate airborne bacterial present in the door handles of public toilets across selected Faculties in University of Benin, Benin City.
2. determine the antibiotics susceptibility pattern of the bacteria isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1. TOILET DOOR HANDLES

Toilet door handles are one of the most frequently touched surfaces in public restrooms, making them a significant point of contact for bacterial contamination. These surfaces are often exposed to a wide variety of microorganisms, including both harmless and potentially harmful bacteria, due to the high traffic of individuals using these facilities. Many public restroom users may not adhere to proper hand hygiene practices, which increases the likelihood of bacteria being transferred to door handles after using the toilet. This makes toilet door handles a critical surface for the transmission of pathogens (French *et al.*, 2004).

The types of bacteria commonly found on toilet door handles include *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus* spp., many of which originate from human skin or fecal matter. *Staphylococcus aureus* is frequently found on human skin and nasal passages, and while it is usually harmless, certain strains, such as methicillin-resistant *Staphylococcus aureus* (MRSA), can cause severe infections, especially in immunocompromised individuals (Best *et al.*, 2012). *Escherichia coli* is typically associated with fecal contamination and can cause gastrointestinal infections if transferred from hands to the mouth. Additionally, *Enterococcus* spp., which are often present in the gastrointestinal tract, can be transferred to door handles and pose a risk of urinary tract infections or other healthcare-associated infections, particularly in hospital settings (Reynolds *et al.*, 2005).

Studies have shown that public restroom facilities, especially those with high user traffic, such as those in airports, malls, and schools, often harbor significant bacterial loads on door handles. The bacteria present on these handles can survive for extended periods, especially in humid environments, which are common in restrooms (Gerhardts *et al.*, 2012). This persistence increases the potential for cross-contamination as each individual who touches the door handle can inadvertently pick up and transfer bacteria to other surfaces or individuals.

The risk of bacterial transmission through toilet door handles is further exacerbated by the fact that many individuals do not wash their hands properly after using the restroom. Incomplete or inadequate handwashing allows bacteria to remain on the hands, which are then transferred to door handles when exiting the restroom (CDC, 2020). This lack of proper hygiene creates a cycle of contamination, where bacteria can be passed between restroom users, contributing to the spread of diseases, especially in public spaces.

Moreover, toilet door handles can serve as reservoirs for bacteria that are resistant to antibiotics, such as (MRSA), which is particularly concerning in healthcare environments. In hospitals, where individuals are already vulnerable to infections, contaminated surfaces like door handles can facilitate the spread of healthcare-associated infections (HAIs), posing a serious risk to patient safety (Donskey, 2004). The transfer of bacteria from door handles to healthcare workers or visitors, who then interact with patients, is a significant pathway for the spread of infections in these settings.

2.2. BACTERIAL CONTAMINATION OF TOILET DOOR HANDLES

Toilet door handles are among the most frequently touched surfaces in public spaces, particularly in high-traffic areas such as shopping malls, schools, restaurants, hospitals, and transportation hubs. These high-touch surfaces can serve as reservoirs for a wide range of microorganisms, including pathogenic bacteria. The frequent contact with hands, often without prior handwashing, makes these surfaces critical points of contamination in public restrooms. This poses significant health risks, as the microbial load on these surfaces can include both harmless environmental bacteria and harmful pathogens capable of causing serious infections.

2.3. SOURCES OF BACTERIAL CONTAMINATION

Toilet door handles are subject to contamination primarily from human contact. Research has consistently shown that human hands are significant vectors for the transmission of bacteria, including fecal matter, skin flora, and respiratory pathogens (French *et al.*, 2004). Inadequate hand hygiene practices, such as insufficient handwashing after using the toilet, contribute to the transfer of microorganisms onto door handles.

In addition to direct human contact, bacteria can also be introduced via airborne droplets generated by toilet flushing, which can settle on various surfaces, including door handles (Best *et al.*, 2012). One of the most significant contributors to contamination on door handles is human contact. Many people fail to wash their hands after using the restroom or after coughing or sneezing. This can lead to the transfer of pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* and other microbes onto door handles (Otter *et al.*, 2011). Studies have demonstrated that bacteria from the skin, nasal cavity, and even fecal matter are frequently

found on door handles in public spaces (Scott *et al.*, 2009). In addition to human contact, bacteria can be transferred onto door handles via air, dust, or moisture. Poorly ventilated public restrooms, in particular, may allow aerosolized particles from flushing toilets to settle on surfaces, including door handles. Moisture from handwashing can also contribute to microbial growth on handles (Gerhardts *et al.*, 2012).

Public spaces such as hospitals, schools, airports, and restaurants have high foot traffic, which means door handles in these areas are touched by hundreds or thousands of people every day. This high usage increases the likelihood of contamination, especially when proper hygiene practices are not followed (Reynolds *et al.*, 2005).

2.4. MICROORGANISM ASSOCIATED WITH TOILET DOOR HANDLES

A pathogen or pathogenic organism is defined as a biological agent that can cause damage to its host. Damage can be inflicted directly by the microorganisms or indirectly through the host immune responses (Casadeval and Pirofski, 1999). The ability of the pathogen to cause diseases or infection is called its pathogenicity and this is expressed by means of their virulence, a term that refers to relative capacity of a microbe to cause damage in the host (Casadeval and Pirofski, 1999). There are various substrates including routes where pathogens can invade a host; water, soil, waste or fecal matter. Various bacteria and fungi are associated and isolated from toilet door handles. Examples of these microorganisms isolated from toilet door handles include; *Staphylococcus aureus, Escherichia coli, Salmonella, Shiigella, Candida* spp, *Penicillum* spp etc

2.4.1. *Staphylococcus aureus*

This is a Gram positive coccal bacterium that is a member of the *Firmicutes*. Although *S.aureus* is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory

infections and food poisoning. Pathogenic strains often promote infections by producing potent protein toxins and expressing cell-surface proteins that bind and inactivate antibodies.

An estimated 20% of the human population are long term carriers of *S.aureus* (Klutymans*et al.*, 1999) which can be found as part of the normal skin flora and in the nostrils (Cole *et al.*, 2001). *S.aureus* appears as grape-like clusters when viewed through a microscope and has large round, golden yellow colonies after with hemolysis when grown on blood agar plates (Ryan and Ray, 2004). *S.aureus* is responsible for many diseases but it may also occur as a commensal. The presence of *S.aureurs* does not always indicate infection. It can survive for months on dry environmental surface depending on strain (Cimolai, 2008).

Drug resistance by the organism is a major concern (Weinstein, 1998). Both methicillin (oxacillin or cefoxitin) and glycopeptide vancomycin and teicoplanin resistance may occur in *S.aureus.* it is found throughout the hospital environment, particularly around patients known to be colonized or infected with the bacterium (Dancer, 2009). *S.aureus* is transmitted through air droplet or aerosol when an infected person coughs or sneeze he or she release numerous small droplets of saliva that remains suspended in the air. These contain strains that can cause diseases and infect other persons.

2.4.2. *Escherichia coli*

Escherichia coli commonly abbreviated as (*E.coli*) is a Gram-negative, facultative anaerobic, rod-shaped bacterium of the genus *Escherichia* that is mostly found in the lower intestine of warm-blooded organisms (Singleton, 1999). Most *E.coli* strains are harmless but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination (Corner *et al.*, 2010). The strains that are harmless forms part of the normal flora of the gut and can benefit their hosts through their production of vitamin K_2

and preventing colonization of the intestine with pathogenic bacteria (Corner *et al.*, 2010). *E.coli* and other facultative anaerobes constitute about 0.1% of the gut flora and faecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them ideal indicator organism to test environmental samples for faecal contamination.

E.coli is the most important widely studied prokaryotic model organism and is an important species in the fields of biotechnology and microbiology. The optimal growth of *E.coli* occurs at 37°C (98.6°F) but some laboratory strains can multiply at temperatures of up to 49°C or 120°F (Fotadar *et al.*, 2005). Different strains of *E.coli* are often host specific, making it possible to determine the source of faecal contaminant in environmental samples. Most virulent strains of *E.coli* cause serious illness or death in the very young children, in the elderly and immune-compromised. Such strains include 0157:H7. The genus of *E.coli* belongs to a bacterial group informally known as "Coliforms" and also the Enterobacteriaceae family "the enterics" of the gammaproteobacteria (Brenner *et al.*, 2005). The general categories of *E.coli* include *Enterotoxigenic E.coli* (ETEC),*Enteroinvasive E.coli (*ETEC), *Enteropathogenic E.coli (*EIEC), *Enterohemorrhagic E.coli* (EHEC).

2.4.3. *Candida albicans*

Candida is a genus of yeast and is the common cause of fungal infections (Manolakaki *et al.*, 2010). *Candida albicans* is the most commonly isolated species and can cause infections in humans and other animals. It appears as large, round, while or cream colonies with a yeasty odor agar plates at room temperature.

Candida albicans ferments glucose and maltose to acid and gas sucrose to acid and does not ferment lactose which helps to distinguish it from other *Candida* species. (Jawetz *et al.*, 1978).

Candida albicans is the most commonly isolated species and can cause infections in humans and other animals. Many species of *Candida* are found in gut flora including *Candida albicans* in mammalian hosts, whereas others live as endosymbionts in insect hosts (Nguyen *et al.*, 2007).

Antibiotics promote yeast infections including gastrointestinal *Candida* spp overgrowth and penetration of the gastrointestinal mucosa (Kennedy *et al.*, 1987). Women are more susceptible to genital yeast infects than men. People with diabetics or impaired immune systems such as those with HIV are more susceptible to yeast infection.

Candida albicans ferments glucose and maltose to acid and gas, sucrose to acid and does not ferment lactose which helps to distinguish it from other *Candida* spp. Amongst *Candida* spp., *Candida albicans* which is a normal constituent of the human flora, a commensally of the skin and gastrointestinal and genitourinary tracts is responsible for the majority of *Candida* bloodstream infections (Candidemia).

2.4.4. *Salmonella* spp.

Salmonella is a rod shaped bacteria of the family *Enterobacteriaceae*. There are two species of *Salmonella, Salmonella bongori and Salmonella enteric*.

Strains of *Salmonella* cause illness such as typhoid fever, paratyphoid fever and food poisoning (Salmonellosis). *Salmonella* are non-spore forming predominantly motile Enterobacteria with peritrichous flagella (Fabrega, 2013)

They are chemoorganotrophs obtaining their energy from oxidation and reduction reactions using organic sources and are facultative anaerobes capable of surviving with or without oxygen. Most subspecies of *Salmonella* produce hydrogen sulphide which can readily be detected by growing them on media containing ferrous sulphate such as triple sugar iron test. Most isolates

exist in two phases, motile and non-motile phase. *Salmonella* species are facultative intracellular pathogens (Jantsch *et al.*, 2011). Many infections are due to ingestions of contaminated food.

2.4.5. *Klebsiella* spp.

Klebsiella is a genus of non-motile, Gram negative, oxidase negative rod shaped bacteria with a prominent polysaccharide-based capsule (Ryan and Ray, 2004). *Klebsiella* species are found everywhere in nature. They tend to be rounder and thicker than other members of the *Enterobacteriaceae* family. They occur as straight rods with rounded or slightly pointed ends. They cause a variety of diseases, pneumonia, urinary tract infection, septicemia, meningitis, diarrhea and soft tissue infection (Podshin and Ullmann, 1998).

2.5. BACTERIAL CONTAMINATION OF FOMITES

It is generally acknowledged that inanimate objects can carry microorganisms originating from the surrounding environment. These deposited microorganism posses bio-transfer potential, i.e. the ability to be transferred to another substratum where growth is possible — for example on food, or on the human body (Joanna, 2012). The spread of infectious diseases through hand contact has been an area of major concern. Itah and Ben. (2004) states that Enteric bacteria such as *Escherichia coli, Klebsiella* spp and *Citrobacter* spp. were found to contaminate various contact surfaces including door handles and many other common house hold fixtures. Fomites consist of either porous or non porous surfaces or inanimate objects that when contaminated with pathogenic microorganism can be transferred to a new host thereby serving as vehicles in transmission (Greene, 2009). Fomites when in constant contact with humans or natural habitats of pathogenic organism which represent a major source of spread of infection diseases (Osterholm *et al.*, 1995). Such fomites include door handles of conveniences, showers, toilet

seats and sinks, lockers, chairs, tables especially those found in public offices, hospitals, hotels, restaurants and restrooms (Bright *et al.*, 2010). Microorganism that cause infections can be found in any environment include soil, air, water and food as well as environmental surfaces or objects (Neely and Sittig, 2002). Most of the bacteria found by researchers are normal flora of the skin, mouth and nasal passages that can pass to our hands. Although many of these bacteria won't hurt unless the immune system becomes weak because of illness (oluduro *et al.*, 2011).

Baadhaim *et al.* (2011) indicated that the door handles may aid in the spread of microbes between individuals and that they may be a reservoir of microbial contamination. In their experiments, they assessed the prevalence of Gram negative bacteria that were found on door handles of Olin Hall. It was hypothesized that during times where the building was near its peak usage, a larger percentage of the bacteria sampled from the door handles of Olin Hall would be Gram negative. The results showed that of total microbial colonies observed as 49% were Gram negative bacteria.

Another study held by Nworie *et al.* (2012) recognized that the increased incidence of outbreaks of certain diseases and its rate of spread from one community to the other had become a major health concern. The sample collected from the Door handles/knobs of public conveniences of selected public offices, motor parks, and markets in Abuja metropolis were investigated for bacterial contamination. Total of 180 swab samples cultured, 156 (86.7%) were positive. The most positive samples from female toilet handles/knobs (41.7%) and bathroom door handles/knobs than males (11.5%). The study also found that toilet door handles/knobs in markets, motor parks and restaurants had higher rate of contamination compared to Government offices, and banks. Contamination was also higher in toilet door handles/knobs (87.2%) than in bathroom door handles/knobs (85%). Most of the bacteria contaminants were coliforms. The

isolated bacterial contaminants were *Staphylococcus aureus* (30.1%), *Klebsiella pneumoniae* (25.7%), *Escherichia coli* (1%), *Enterobacter* spp (11.2%), *Citrobacter* spp. (7.1%), *Pseudomonas aeruginosa* (5.9%), and *Proteus* spp . (4.5%). This shows that the city's convenient places harbours highly pathogenic bacteria which have the potentials of causing epidemics in the near future.

The prevalence of bacterial organisms on toilet door handles in secondary schools in Bokkos Local Government; Jos Plateau State, Nigeria was evaluated by Maori *et al.* (2011). A total of 120 samples were collected and cultured, 40 from each of the schools, Government Secondary School Bokkos (G.S.S.B), All Nation Academy and Government secondary School Mushere. Out of the 120 samples that were collected 60 (50%) yielded growth and 60 showed no growth at all. The following organisms were isolated *Staphylococcus* species (43.3%), *Candida species* (10%), *Escherichia coli* (16.7%), *Citrobacter* spp (1.7%), *Klebsiella* spp (20%), *Proteus* spp (6.7%) and *Salmonella* spp (1.7%). The result showed that (G.S.S.B) has the highest contamination (48.3%) followed by All Nations Academy (30%) and then (G.S.S.M) (21%).

A study was carried out by Sabra, (2013) on public female restrooms at Taif, Kingdom of Saudi Arabia; Restrooms (RR) from different buildings, in order to characterize the locality of contamination and bacterial loads. 260 sample collected from different rest room (RR) like (RR Door, RR Handle; RR sink; RR Toilet door; RR Toilet handle). Incidence of bacterial growth or positive culture was 187/260 (71.9%). The predominant positive was from RR Toilet Handle in 73/80 (91.3%), then followed by RR Toilet Door in 59/80 (73.8%), RR Sink in 38/60 (63.3%), RR Handle in 10/20 (50%), finally less positive from RR Door in 7/20 (35%). Different isolated bacteria arranged according to their percentage as *Staphylococcus aureus* 76/187 (40.6%),

Escherichia coli 42/187 (22.5%), *Bacillus* spp. 40/187 (21.4%), *Klebsiella pneumoniae* 25/187 (13.4%), *Enterococcus faecalis* 18/187 (9.6%), *Citrobacter* spp. 16/187 (8.6%), *Pseudomonas aeruginosa* 13/187 (7%) and *Proteus mirablilis* 10 /187 (5.3%) as well as known harmful microorganisms can be transferred to hands from contaminated surfaces.

These contaminated hands can transmit disease to oneself as well as to others according to a study that was carried out to determine the extent the hand hygiene practices and toilet door knobs contribute to the bacterial load of hands of toilet users in a medical school. Swabs were taken from a randomly selected sample of 60 medical students for bacterial count from both hands before and after toilet use and from door knobs of six toilets. Only 40 (66.7%) claimed they washed their hands with soap. Significantly more females (83%) used soap to wash hands compared to males (50%). Bacterial load in the hands of both males and females showed an increase after toilet use. The increase was significant among male students. The dominant hand had a significantly higher bacterial load than the other. The mean bacterial loads of male toilet door knobs (12 CFU/cm^2) were significantly higher than of female toilet door knobs (2.5 CFU/cm^2). *Staphylococcus aureus* was isolated from the hands of 21 students (De Alwis *et al.*, 2012).

Toilet door handles are among the most frequently touched surfaces in public spaces, particularly in high-traffic areas such as shopping malls, schools, restaurants, hospitals, and transportation hubs. These high-touch surfaces can serve as reservoirs for a wide range of microorganisms, including pathogenic bacteria. The frequent contact with hands, often without prior handwashing, makes these surfaces critical points of contamination in public restrooms. This

poses significant health risks, as the microbial load on these surfaces can include both harmless environmental bacteria and harmful pathogens capable of causing serious infections.

2.6. HEALTH RISKS AND IMPLICATIONS

The presence of pathogenic bacteria on toilet door handles presents serious public health risks, particularly due to the high level of human contact these surfaces experience in public and communal spaces. When individuals come into contact with contaminated surfaces such as door handles, they can easily transfer bacteria to other parts of their body—particularly to mucous membranes such as the mouth, eyes, or nose—or to open wounds. This process can lead to a wide range of infections, from minor skin infections to more severe systemic illnesses (French *et al.*, 2004).

The transmission of bacteria from door handles to humans typically occurs via direct contact. Once bacteria are picked up by hand, they can be transferred to the face or other surfaces that are then touched, facilitating the spread of pathogens (Aiello and Larson, 2002). This becomes especially problematic in environments where individuals may not practice proper hand hygiene after using restroom facilities, increasing the likelihood of contamination. In particular, public facilities like airports, shopping malls, and schools experience a high volume of traffic, making it difficult to ensure that all users maintain adequate hygiene, which, in turn, increases the risk of bacterial transmission.

The risk of disease transmission from contaminated toilet door handles is particularly high in environments that house vulnerable populations, such as hospitals, nursing homes, and daycare centers. In these settings, individuals often have weakened immune systems, making them more

susceptible to infections caused by pathogens commonly found on door handles, such as *Staphylococcus aureus* (including methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumonia* and *Escherichia coli* (Best *et al.*, 2012). Healthcare-associated infections (HAIs) are a significant issue, particularly in hospitals, where pathogens on door handles and other frequently touched surfaces can easily spread through the facility (Otter *et al.*, 2013).

Multidrug-resistant organisms, including MRSA and carbapenem-resistant *Klebsiella pneumoniae*, pose particular challenges in healthcare settings due to their resistance to common antibiotics (Donskey, 2004). These organisms can be picked up by healthcare workers or visitors from contaminated door handles and subsequently spread to patients, leading to infections that are difficult to treat. The World Health Organization (WHO) has highlighted the global threat posed by antimicrobial resistance (AMR), noting that inadequate infection control measures in hospitals and public spaces contribute to the spread of resistant bacteria (WHO, 2017).

Healthcare-Associated Infections (HAIs) are a major concern in hospitals, where multidrug-resistant bacteria such as MRSA and *Klebsiella pneumoniae* can spread easily through contact with contaminated surfaces, including toilet door handles. According to Donskey (2004), door handles in hospitals can serve as fomites, or objects capable of carrying infectious organisms, contributing to the transmission of pathogens from healthcare workers and visitors to patients. HAIs not only increase patient morbidity and mortality but also significantly elevate healthcare costs. In the United States, the Centers for Disease Control and Prevention (CDC) estimates that HAIs lead to approximately 1.7 million infections and 99,000 associated deaths annually (Klevens *et al.*, 2007).

In addition to hospitals, nursing homes and long-term care facilities are at heightened risk for the spread of bacteria through door handles. These facilities house populations that are particularly vulnerable to infections due to age-related immune system decline and underlying chronic conditions. A study by Otter and French (2009) demonstrated that door handles in nursing homes are frequently contaminated with bacteria, and that poor hand hygiene practices among both staff and residents contribute to the spread of pathogens.

Public restrooms in high-traffic areas such as airports, train stations, schools, and shopping malls can serve as reservoirs for community-acquired infections. Healthy individuals who use these facilities are at risk of coming into contact with bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*—organisms that are commonly found on door handles and other restroom surfaces (Best *et al.*, 2012). These bacteria are often introduced by restroom users who do not wash their hands after using the toilet, leading to fecal contamination on door

handles. In addition, bacteria can spread through contact with respiratory droplets, further contaminating surfaces (Aiello and Larson, 2002).

The spread of community-acquired infections from restroom door handles poses significant public health risks, as individuals who contract these infections can subsequently spread the bacteria to others in their household or community. In particular, schools and daycare centers are environments where bacteria can spread rapidly due to the close proximity of children and the likelihood of inadequate hand hygiene (Gerhardts *et al.*, 2012). Children are more susceptible to infections due to their developing immune systems, and outbreaks of gastrointestinal illnesses caused by bacteria such as *E. coli* are common in schools where restroom hygiene is not adequately maintained (Aiello and Larson, 2002).

2.7. PREVENTIVE MEASURES

To minimize bacterial contamination on toilet door handles and other high-contact surfaces, several preventive measures can be implemented. These strategies are particularly effective in reducing the spread of infectious agents in public restrooms, ensuring a safer environment for all users.

2.7.1. Proper Hand Hygiene

Encouraging proper hand hygiene is one of the most effective ways to reduce bacterial contamination on toilet door handles. The Centers for Disease Control and Prevention (CDC) recommend washing hands with soap and water for at least 20 seconds, especially after using the restroom. Proper hand hygiene can reduce the transfer of harmful microorganisms from hands to surfaces such as door handles, significantly limiting the spread of infectious agents (CDC, 2020).

Hand sanitizers with at least 60% alcohol can also be an effective alternative when soap and water are unavailable (Boyce and Pittet, 2002).

2.7.2. Regular Cleaning and Disinfection

Routine cleaning and disinfection of high-touch surfaces, including toilet door handles, is crucial in reducing microbial load. The use of EPA-approved disinfectants, such as those containing alcohol, chlorine, or hydrogen peroxide, has been shown to effectively eliminate bacteria, including pathogens that are resistant to common cleaning agents (Rutala and Weber, 2013). Studies indicate that regular disinfection schedules can prevent the accumulation of bacteria on surfaces, decreasing the risk of cross-contamination between restroom users (Gerhardts *et al.*, 2012). High-frequency touchpoints, including door handles, should be cleaned multiple times daily in high-traffic areas to maintain low levels of bacterial contamination (Otter *et al.*, 2013).

2.7.3. Touchless Technology:

The installation of touchless restroom fixtures, such as automatic doors, soap dispensers, and hand dryers, reduces the need for physical contact with contaminated surfaces, thereby lowering the risk of bacterial transmission. Automatic doors and sensor-based technology prevent direct hand contact with surfaces, mitigating contamination from unwashed hands (Ekrami & Kalantar, 2011). Research has demonstrated that the implementation of touchless systems can significantly reduce the presence of bacteria on restroom surfaces (Knobloch *et al.*, 2017). In addition,

touchless technologies have been shown to improve user compliance with hygiene practices, such as handwashing, by making these actions more convenient (Boyce and Pittet, 2002).

2.7.4. Public Education and Awareness

Educating the public on the importance of hygiene in public restrooms is vital in promoting better sanitation practices. Simple interventions, such as placing posters and signage in visible locations that remind individuals to wash their hands properly, have been shown to improve hygiene behavior in public spaces (Aiello *et al.*, 2008). Studies suggest that increased awareness of the health risks associated with poor hygiene can lead to more consistent and thorough handwashing, helping to reduce the spread of bacteria (Prüss-Ustün *et al.*, 2019). Additionally, training cleaning staff on best practices for disinfecting high-touch surfaces can further enhance restroom hygiene and limit bacterial contamination (Rutala and Weber, 2013).

2.7.5. Enhanced Ventilation

Improving the ventilation in public restrooms can also contribute to lowering bacterial contamination. Poor ventilation may lead to the persistence of airborne pathogens, which can settle on surfaces like door handles. Adequate airflow and the use of high-efficiency particulate air (HEPA) filters can reduce the presence of airborne bacteria and viruses in enclosed spaces, thereby improving overall indoor air quality (Azimi and Stephens, 2013).

2.7.6. Hand Hygiene Facilities

Ensuring the availability of hand hygiene facilities, such as soap, clean water, and paper towels or hand dryers, is critical for supporting proper hygiene behavior. A lack of these resources has been shown to decrease handwashing compliance, leading to higher levels of bacterial contamination on restroom surfaces (Aiello *et al.*, 2008). The placement of these facilities near restroom exits encourages individuals to wash their hands before touching door handles or other shared surfaces.

Implementing preventive measures such as proper hand hygiene, regular disinfection, touchless technology, public education, and enhanced ventilation can significantly reduce bacterial contamination on toilet door handles in public restrooms. These strategies, supported by effective public health education, play a crucial role in minimizing the transmission of pathogenic bacteria and improving overall sanitation in communal spaces. Ensuring that these measures are consistently applied, especially in high-traffic areas, is essential to safeguarding public health and preventing the spread of infections.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Study Area

The study was conducted at various faculties toilet door handle of University of Benin, located in Benin City, Edo State, Nigeria. The university has several public restrooms located in various faculties, libraries, and administrative buildings. This restrooms are frequently used by students, staff, and visitors, providing a high volume of traffic and frequent use of toilet door handles. The climate in Benin City is typically tropical, characterized by heavy rainfall and high humidity, which may influence the microbial growth on surfaces.

3.2. Sample Collection

A total of **10 toilet door handles** from different restrooms across various faculties buildings over a two-week period, with sampling occurring **once a week.** The samples were collected with Sterile swab sticks moistened with sterile physiological saline were used to collect samples from the surfaces of toilet door handles. Each handle was swabbed by rubbing the sterile swab across the surface of the door handle in a standardized manner to ensure consistency in sample collection. The swabs were immediately placed in sterile transport media and labeled with specific identifiers, including the location of the restroom, date, and time of sampling to ensure accurate tracking of samples. Samples were transported to the microbiology laboratory for analysis within two hours of collection.

3.3. Sterilization of Materials

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

3.4.1. 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 °C for 1 hr in a hot-air-oven before use. The media used in this study were sterilized at 121 °C for 15 min in an autoclave. Agar media, agar slant and biochemical reagents were prepared freshly and refrigerated at 3-4 °C. Aseptic conditions were ensured during inoculation and subculturing.

3.4.1.1 Preparation of Nutrient agar

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

3.4.1.2 Preparation of Citrate agar

An amount of 24.28grams of agar was suspended 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.

3.4.1.3 Preparation of Triple Sugar Iron agar

An amount of 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.

3.5. ISOLATION AND ENUMERATION OF BACTERIAL ISOLATIES

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, 103, 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.

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

(Willey *et al.*, 2008).

3.5.1 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.

3.6. 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.

3.6.1 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.

3.6.2. Potassium Hydroxide (KOH) test

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

stringing indicates a Gram-positive bacterium (Roberts and Sandle, 2008).

3.7. BIOCHEMICAL TEST

Several biochemical test were carried out to further identify the various bacterial isolates.

3.7.1 Catalase Test

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

$$2\,H_2O_2 \rightarrow 2\,H_2O + O_2 \quad \text{---------- equation 1}$$

3.7.2 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.

$$NH_2CO.NH_2 + H_2O \rightarrow 2NH_3 + CO_2 \quad \text{---------- equation 2}$$

3.7.3 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.

3.7.4 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.

3.7.5. 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).

3.8. 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 °C. Following the standard or rules of AST established in 2017 by Clinical Laboratory Standards Institute (CLSI). 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

3.9. 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 areas where antibiotics are abused.

This tool becomes necessary for health risk assessment. According to Davis and Brown (2016), an index of ≥ 0.2 and above is indicative of a 'high-risk' contamination source. In this study the MAR index was determined by employing the methods delineated by Chitanand *et al.* (2010). The formula below was used to decipher MAR index of bacterial isolates.

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

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

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

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

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

3.10. Statistical analysis

Data obtained in this study were collected and analyzed using Microsoft excel and by statistical package for social scientist (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA).

CHAPTER FOUR

RESULTS

Table 4.1. presents the total bacterial counts ($\times 10^4 \pm$ SD cfu/g) obtained from door handles sampled at different faculties within the University of Benin over two weeks. The bacterial counts were recorded as mean values with their corresponding standard deviations. In Week 1, the Faculty of Physical Sciences (FPS) exhibited the highest bacterial count of 4.03 ± 0.32, while the Faculty of Life Sciences (FLS) had the lowest count of 3.10 ± 0.06. Similarly, in Week 2, the highest bacterial count was observed at the Faculty of Life Sciences (FLS) with 4.50 ± 0.25, while the lowest count was recorded at the Faculty of Law (FL) with 2.93 ± 0.094.

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

Figure 4.1. present provides a summary of bacterial isolates identified from the sampled door handles, along with their respective numbers and percentage occurrences. The most frequently isolated bacterium was *Staphylococcus aureus,* accounting for 24% of the total isolates. This was followed by *Escherichia coli* (18%) and *Bacillus* sp. (16%). Other isolates included *Streptococcus* sp. (13%), *Proteus* sp. (11%), *Salmonella* sp. (10%), and *Enterobacter* sp. (8%).

Table 4.3: The bacterial isolates showed varying levels of resistance, intermediate sensitivity, and susceptibility across the antibiotics tested: Ofloxacin, Gentamicin, and Levofloxacin demonstrated the highest efficacy, with most isolates showing susceptibility (≥17 mm). Ampicillin andCefuroxime showed mixed results, with intermediate sensitivity in some isolates (11–16 mm). Cefotaxime and Nitrofurantoin were largely ineffective, with most isolates exhibiting resistance (≤10 mm). *Escherichia coli* and *Staphylococcus* sp. showed considerable resistance to multiple antibiotics.

Table 4.1. Total bacterial counts of door handles at different sampling sites in UNIBEN ($\times 10^4 \pm$ SD cfu/g).

Sample Location	WEEK 1	WEEK 2
FSS	3.90 ±0.10	3.66 ± 0.31
FPS	4.03 ±0.32	3.80 ± 0.12
FLS	3.10 ±0.06	4.50 ± 0.25
FL	3.63 ±0.09	2.93 ± 0.094

Values represented in mean ±standard deviation

KEY:

FSS - Faculty of Social Sciences

FPS - Faculty of Physical Sciences

FLS - Faculty of Life Sciences

FL - Faculty of Law

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

Shape	**Irregular**	**Circular**	**Circular**	**Circular**	**Irregular**	**Circular**	**Circular**
Size	Medium	Small	Large	Medium	Medium	Medium	Small
Colour	Golden yellow	Cream	Red	Cream	Cream	Red	White
Cell type	Rod	Rod	Rod	Cocci	Rod	Rod	Cocci
Cell arrangement	Disperse	Clusters	Cluster	Disperse	Disperse	Disperse	Chains
Gram	-	+	-	+	-	-	+
KOH	+	-	+	-	+	+	-
Gas formation	+	-	+	-	+	+	-
ndole	+	-	-	-	-	-	-
Citrate	-	+	-	-	+	+	-
Oxidase	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	-
H□S ormation	-	+	+	-	+	-	-
ucrose	+	+	-	+	+	+	-
lucose	+	+	+	+	+	+	+
actose	+	-	-	+	+	+	-
SI reaction lant/butt)	A/A	K/A	K/A	A/A	A/A	A/A	A/A
lentity	*Escherichia* coli	*Bacillus* sp.	*Proteus* sp.	*Staphylococcus* sp.	*Salmonella* sp.	*Enterobacter* sp.	*Streptococcus sp.*

KEY:

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

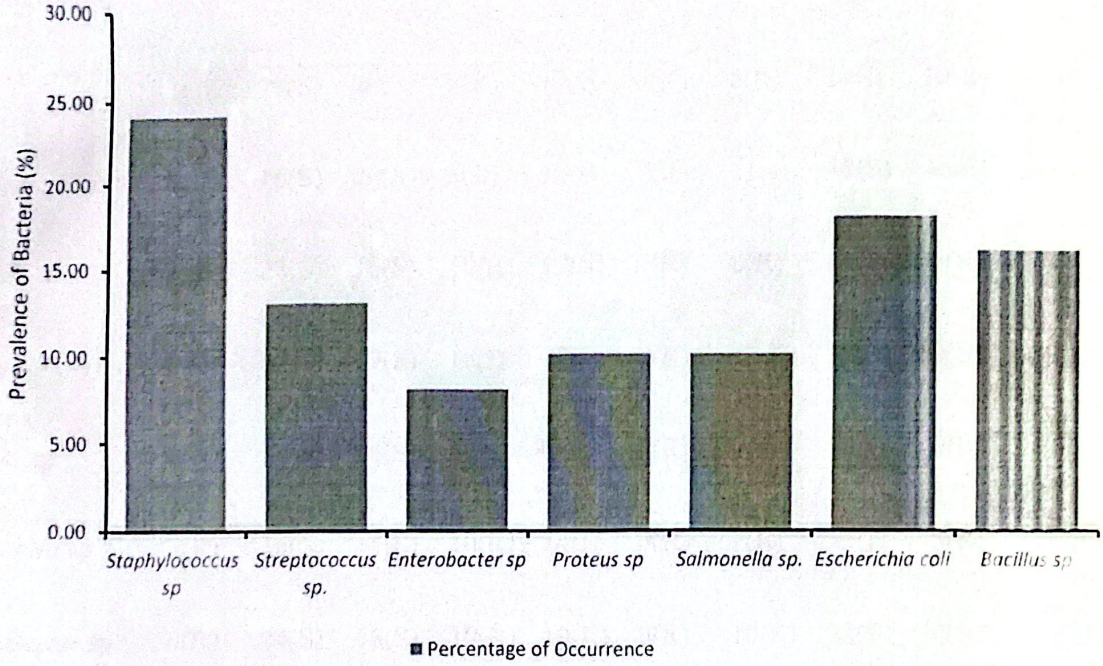

Figure 4.1: Percentage occurrence of bacterial isolates

Table 4.3. Antibiotics Sensitivity Test for Bacterial Isolates In Diameter (mm)

ISOLATES	CTX	OFX	GEN	CFX	AMP	NIT	CFM	LEV	IPM	NAI
E.coli	10(R)	18(S)	20(S)	9(R)	14(I)	0(R)	6(R)	14(I)	10(R)	12(I)
Staphylococcus sp	3(R)	16(S)	20(S)	8(R)	10(R)	7(R)	11(I)	18(S)	16(S)	14(I)
Bacillus sp	10(R)	22(S)	16(S)	10(R)	17(S)	7(R)	0(R)	14(I)	0(R)	17(S)
Streptococcus sp	0(R)	24(S)	19(S)	10(I)	12(I)	0(R)	10(R)	18(S)	0(R)	12(I)
Proteus sp	0(R)	16(S)	16(S)	3(R)	0(R)	9(R)	0(R)	14(I)	0(R)	9(R)
Salmonella sp	3(R)	16(S)	14(S)	10(R)	14(I)	0(R)	7(R)	12(I)	3(R)	9(R)
Enterobacter sp.	0(R)	24(S)	18(S)	10(R)	10(R)	0(R)	10(R)	22(S)	0(R)	16(S)

Key:

Resistant (R) = 0 – 10mm

Intermediate (I) = 11 – 16mm

Susceptible (S) = 17mm and above.

CTX=Cefotaxime AMP=Ampicillin

OFX=Ofloxacin CFM=Cefixime

GEN=Gentamicin LEV=Levofloxacin

CFX=Cefuroxime IPM=Imipenem

NIT=Nitrofurantoin NAL=Nalidixic acid

CHAPTER FIVE

5.0. DISCUSSION

Bacterial contamination on high-touch surfaces, such as restroom door handles, is a significant public health concern. These surfaces serve as reservoirs for diverse microbial communities, facilitating the transmission of opportunistic and pathogenic bacteria (Abiose, 2019). Variations in bacterial load across different faculties often reflect factors such as population density, sanitation practices, and environmental conditions. Moreover, the increasing prevalence of antibiotic-resistant bacteria on these surfaces underscores the urgent need for effective hygiene practices and antimicrobial resistance monitoring. This study investigates the bacterial load, diversity of isolates, and antibiotic resistance patterns across faculties, providing insights into the implications for public health and hygiene interventions.

The total bacterial counts observed across the faculties revealed significant variations, reflecting the interplay of multiple factors influencing microbial contamination. The Faculty of Life Sciences recorded the highest bacterial count ($4.50 \pm 0.25 \times 10\square$ cfu/g) during Week 2, whereas the Faculty of Law had the lowest count ($2.93 \pm 0.094 \times 10\square$ cfu/g) within the same period. These findings are consistent with those of Nworie *et al.*,(2012), who reported that toilet environments usually contain higher microbial loads than other facilities within any public centres. Faculties with larger populations, such as Life Sciences, exhibit higher bacterial loads due to increased contact with surfaces like door handles. Odigie *et al.* (2017) described high-traffic areas as hotspots for bacterial accumulation. The lower bacterial count in the Faculty of Law likely reflects more effective cleaning routines or reduced frequency of use. Ekanem *et al.* (2021) demonstrated that cleaning frequency and effectiveness significantly impact

contamination levels. Humidity and temperature variations within restrooms play a critical role in bacterial survival and proliferation. Onyeka *et al.* (2020) noted that such environmental factors profoundly influence microbial viability on surfaces.

A total of seven bacterial species were identified through cultural, morphological, and biochemical analyses. These included *Staphylococcus* sp., *Escherichia coli*, *Bacillus* sp., *Proteus* sp., *Salmonella* sp., *Enterobacter* sp. and *Streptococcus* sp., illustrating the diversity of microorganisms present on restroom door handles. This is in collaboration with the study of Bashir *et al* (2016), who reported the presence of *S. aureus, Bacillus* sp., *Micrococcus* sp., *E. coli, Klebsiella* spp. and *Salmonella* sp. from door handles of public toilets in Federal University, Dutse, Jigawa State, Nigeria (Bashir *et al.*, 2016).

Staphylococcus aureus was the most frequently isolated bacterium (24%). This finding aligns with Odigie *et al.* (2017), who reported its prevalence on selected door handles in University of Benin Teaching Hospital, Benin City, Edo State. This result also align with that of (Maori *et al.*, 2013; Onwubiko and Chinyeaka, 2015), who reported *S. aureus* 33(25.0%) as the most frequently isolated bacteria from door handles of a tertiary Institution at Umuahia, Abia State, Nigeria. As a known pathogen causing skin infections, respiratory illnesses, and food poisoning, its presence emphasizes the potential for nosocomial infections in communal environments. The presence of *E. coli* (18%) indicates fecal contamination, pointing to poor hand hygiene practices. Similar findings were reported by Odigie *et al.* (2017), who detected fecal coliforms on restroom surfaces in door handles. This bacterium is associated with gastrointestinal diseases, posing significant public health concerns. *Bacillus* species (16%) were isolated, consistent with their ability to form resilient spores. Nworie *et al.* (2012) highlighted their environmental adaptability.

which enables survival on surfaces in public restrooms. Pathogens such as *Proteus* sp., *Salmonella* sp. and *Enterobacter* sp. are associated with urinary tract infections and foodborne illnesses (Onuoha *et al.*, 2021). *Streptococcus* sp., though less common, has been linked to respiratory tract infections, posing risks particularly to immunocompromised individuals (Musher, 2000). These findings underscore the role of fomites in the transmission of both opportunistic and pathogenic bacteria, necessitating targeted public health interventions.

The antibiotic susceptibility profiles of the isolates revealed varying resistance patterns, raising critical public health concerns: Ofloxacin, Gentamicin, and Levofloxacin exhibited high efficacy, with most isolates showing susceptibility. Antibiotics like Ampicillin and Cefuroxime showed intermediate effectiveness, reflecting their reduced potency in treating certain bacterial infections. Cefotaxime and Nitrofurantoin exhibited pronounced resistance, particularly among *E. coli* and *Staphylococcus aureus*. Multidrug-resistant strains of *E. coli* and *Staphylococcus aureus* were identified this phenomenon could be as a result of overuse and misuse of antibiotics in low-resource settings.

These findings emphasize the urgency of implementing antimicrobial stewardship programs to mitigate resistance. The presence of fecal coliforms like *E. coli* underscores the need for enhanced hand hygiene. Educational campaigns promoting proper handwashing could significantly mitigate disease transmission risks. Regular and effective disinfection of high-touch surfaces, such as toilet door handles, is crucial. Public health campaigns targeting hygiene practices among students and staff can reduce microbial contamination risks. Addressing the high prevalence of antibiotic-resistant bacteria requires stringent regulations on antibiotic use and distribution, coupled with the promotion of alternative approaches like biocides.

5.1. CONCLUSION

This study has revealed that toilet door handles in selected Faculties in the University Of Benin are contaminated by a variety of pathogenic and non-pathogenic microorganisms. The most frequently isolated bacterium was *Staphylococcus aureus,* followed by *Escherichia coli* and *Bacillus* sp. Other isolates included *Streptococcus* sp., *Proteus* sp., *Salmonella* sp. and *Enterobacter* sp. Hence, door handle surfaces within could therefore act as potential fomites for communicable diseases dissemination. Staff and Students are encouraged to pay strict attention to personal hygiene practices to avoid the incidence and spread of bacterial infections.

REFERENCES

Abiose, O. F. (2019). Bacterial contamination of selected public toilet door handles within Adekunle Ajasin University campus, Akungba-Akoko, Ondo State, Nigeria. *International Journal of Sciences: Basic and Applied Research (IJSBAR),* **43**(1):76-86.

Abney, S. E., Higham, C. A., Wilson, A. M., Ijaz, M. K., McKinney, J., Reynolds, K. A. and Gerba, C. P. (2024). Transmission of Viruses from Restroom Use: A Quantitative Microbial Risk Assessment. *Food and Environmental Virology* **16**(1): 65-78.

Aiello, A. E., Coulborn, R. M., Perez, V. and Larson, E. L. (2008). Effect of hand hygiene on infectious disease risk in the community setting: A meta-analysis. *American Journal of Public Health* **98**(8):1372-1381.

Aiello, A. E., Larson, E. L. and Sedlak, R. (2008). Hidden heroes of the health revolution: Sanitation and personal hygiene. *American Journal of Infection Control* **36**(10):128-151.

Azimi, P. and Stephens, B. (2013). HVAC filtration for controlling infectious airborne disease transmission in indoor environments: Predicting risk reductions and operational costs. *Building and Environment* **70**:150-160.

Baadhaim M., Kusner, D. and Ahmed H. (2011). "Distribution and Prevalence of Bacteria Found on the Door Handles of Olin Hall, Drake University". *Conference Poster.*

Bashir, S. F., Muhammad, H., Sani, N. M. and Kawo, A. H. (2016). Isolation and identification of bacterial contaminants from door handles of public toilets in Federal University Dutse, Jigawa State, *Continental Journal of Medical Research* **6**(1):7-11.

Best, E. L., Parnell, P. and Wilcox, M. H. (2012). Microbiological comparison of hand-drying methods: the potential for contamination of the environment, user, and bystander. *Journal of Hospital Infection* **80**(1):1-7.

Boyce, J. M., & Pittet, D. (2002). Guideline for hand hygiene in healthcare settings. *Infection Control & Hospital Epidemiology*, 23(S12), S3-S40. https://doi.org/10.1086/503164

Brenner, Don, J., Kreig, Noel, R., Stanley, J.T., Williams, W. and George, M. G. (2005). The Gammaproteobacteria *Bergey'sManual of Systematic Bacteriology.*2nd edition. New York: Springer. p1108.

Bright K. R., Boone S. A. and Gerba C. P. (2010). Occurrence of bacteria and viruses on elementary classroom surfaces and the potential role of elementary classroom hygiene in the spread of infectious diseases. *Journal of School of Nursing* **26** (1):33-36

CDC (2020). Handwashing: Clean hands save lives. Centers for Disease Control and Prevention.

Chinakwe E. C., Nwogwugwu N. U., Nwachukwu I. N., Okorondu S. I., Onyemekara N. N. and Ndubuisi-Nnaji, U. (2012). Microbial quality and public health implication of hand-wash Water samples of public adults in Owerri, South-East Nigeria. *Internattional Research Journal of Microbiology* **3**(4):144-146.

Cimola, N (2008). *MRSA* and the enivroment; implications for comprehensive control measures. *European journal clinical microbiology infections disease* **27**(7):481-493.

Cole, A.M., Tahks S. Orien, A. Yoshioka, D. Kim, Y.H. Parks, A and Ganz, T (2001). Determination of *Staphylococcus aureus* nasal carriage. *Clinical diagnostic laboratory immunology* **8** (6):1064-1094.

Corner,Y., Heather, A. and Haizal, H. (2010). Bacterial infections and pregnancy.*European Journal of Medical Research* **22**:88-166.

Dancer, S.J. (2009). The role of environmental cleaning in the control of hospital acquired infection. *Journal of Hospital Infection;* **73**:378-385.

Davies, J., and Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and molecular biology reviews* **74**(3), 417-433.

De Alwis, W. R., Pakirisamy, P., Wai San, L. and Xiaofen, E. C. (2012). A Study on Hand Contamination and Hand Washing Practices among Medical Students. *ISRN Public Health* **3**:1- 5.

Donskey, C. J. (2004). The role of the intestinal tract as a reservoir and source for transmission of nosocomial pathogens. *Clinical Infectious Diseases* **39**(2):219-226.

Ekrami, A. and Kalantar, E. (2011). Bacterial infections in burn patients at a burn hospital in Iran. *Indian Journal of Medical Research* **134**(5):724–728

Eltablawy S. Y. and Elhinfnawi H.N. (2009). Microbial contamination of some computer keyboards and mice in National center for Radiation Research and Technology. *Journal World Applied Sciences* **6**(4):162-7.

Fabrega, A and Villa, J (2013). *Salmonella entirica, serova* and *tyhumurium;* skills to succeed in the host; virulence and regulation. *Clinical microbiological reviews* **26**(2): 308-341.

Fotadar, U., Zaveloff, P. and Terracio, L. (2005). Growth of *Escherichia coli* at elevated temperatures.*Journal Basic Microbial* **45**(5):403-404.

French, G.L., Otterb, J.A., Shannona, K.P., Adams, N.M.T., Watling, D. and Parks, M.J. (2004).Tackling contamination of the hospital environment by methicillin-resistant *Staphylococcus aureus*(MRSA): a comparison between conventional terminal cleaning and hydrogen peroxide vapour decontamination.*Journal of Hospital Infections* **57**:31-37.

Gerba, C. P., Sobsey, M. D. and Pepper, I. L. (2014). Microbial contamination of high-touch surfaces in public environments. *Environmental Microbiology* **5**(7): 891-898.

Gerhardts, A., Hammer, T. R., Balluff, C., Mucha, H. and Hoefer, D. (2012). A model of the transmission of micro-organisms in a public setting and its correlation to pathogen infection risks. *Journal of Applied Microbiology* **112**(3):614-621.

Itah B. and Ben A. E. (2004). Incidence of Enteric Bacteria and *Staphylococcus aureus* in Day Care Centers in Akwalbom State, Nigeria. *Journal of Tropic Medical & Public Health*; **35**(1):202 9.

Jantsch, J., Chikkaballi, D and Hensel, M (2011). Cellular aspects of immunity to intracellular *Salmonella enterica. Immunological Reviews* **240** (1): 185-195.

Jawetz, M and Adebergs (1978). "Medical mycology" *Review of medical microbiology* (13th ed) 276-278

Joanna V. (2012). School of Healthcare Science, Manchester Metropolitan University, Manchester, the Microbial Contamination of Mobile Communication Devices. *Journal Microbiology and Biological Education* **13**(1): 59–61.

Klevens, R. M., Edwards, J. R., Richards, C. L., Horan, T. C., Gaynes, R. P., Pollock, D. A. and Cardo, D. M. (2007). Estimating health care-associated infections and deaths in US hospitals, 2002. *Public Health Reports* **122**(2):160-166.

Kluytmans, J, Van-belkum, A. and Verbrugh, H (1997). Nasal carriage of *Staphylococcus aereus*; epidemiology, underlying mechanisms and associated risks. *Clinical microbiology revised* **10**(3): 505-520

Knobloch, J. K.-M., Stange, C., & Stuermer, E. K. (2017). Hygiene requirements in the operating theatre and infection control. *Wound Healing Southern Africa* **9**(1): 6-10.

Kramer, A., Schwebke, I., & Kampf, G. (2006). How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infectious Diseases* **6**(1):130-132.

Manolakaki, D., Velmahos, G., Kourkoumpelis, T., Chang, Y., Alam, H.B., De moya, M.M. and Mylonakis, E (2010). *Candida* infection and colonization among trauma patients. *Virulence* **1**(5): 367-375

Maori, C. L., Agbor, V. O. and Ahmed, W. A. (2013). The prevalence of bacterial organisms on toilet door handles in secondary schools in Bokkos L. G. A., Jos, Plateau State, Nigeria. *IOSR Journal of Pharmacy and Biological Sciences* **8**(4):85-91.

Maori, L., Agbor, V. O. and Ahmed, W. A. (2011). The prevalence of bacterial organisms on toilet door handles in Secondary Schools in Bokkos LGA, Jos, Plateau Sate, Nigeria. *Journal of Pharmacy Biological sciences* **8**(4); 85-91.

Musher, D. M. (2000). Streptococcus pneumoniae. In G. L. Mandell, J. E. Bennett, & R. Dolin (Eds.), *Mandell, Douglas, and Bennett's principles and practice of infectious diseases* (5th ed.) Churchill Livingstone. pp. 2128–2147.

Neely A. and Sittig D. F. (2002). Basic microbiologic and infection control information to reduce the potential transmission of pathogens to patients via computer hardware. *Journal American Medical Information Association* **9**(5):500-508.

Nguyen, N. H, such S.O. and Blackwell, M (2007). Five novel Candida species in insect associate yeast clades isolated from neuropteran and other insect. *Mycology* **99**(6):842-858.

Nworie, A., Ayeni, J. A., Eze, U. A., & Azi, S. O. (2012). Bacterial contamination of door handles/knobs in selected public conveniences in Abuja metropolis, Nigeria: A public health threat. *Continental Journal of Medical Research* **6** (1): 7-11.

Nworie. A., Ayeni. J. A., Eze U. A., and Azi. S. O. (2012). Bacterial contamination of door handles/knobs in selected public conveniences in Abuja metropolis, Nigeria; a public health threat. *Continental Journal Medical Research* **6**(1): 7-11.

Oluduro A.O., Ubani E.K., and Ofoezie I .E, (2011). Bacterial assessment of electronic hardware user in interface in Ile-Ife, Nigeria. *Journal of Applied pharmacy Science*; **32**(3):323-334.

Onwubiko, B. N. E. and Chinyeaka, A. H. (2015). Isolation and identification of bacterial contaminants from door handles in a tertiary institution in Umuahia, Abia State, Nigeria. *Nigerian Journal of Microbiology* **29**:3139-3147.

Oranusi S.U., Dahunsi S.O., Owoso O.O., and Olatile T. (2013). "Microbial profile of hands, food, easy contact surfaces and food surfaces: A case study in a University Campus" *Journal of Nov International Biotechnology and Bioscience* **2**(1):30-38.

Osterholm M. T., Hederg C. W. and MacDonald K. l. (1995). Epidemiology of infectious diseases. In: *Mandell, Douglas and Bennett's principles and practice of infectious diseases.* Vol.1, 4th edition, Churchill-Livingstone, NewYork, p.165.

Otter, J. A. and French, G. L. (2009). Survival of nosocomial bacteria and fungi on hospital fabrics and plastic items. *Journal of Hospital Infection* **72**(1):71-78.

Otter, J. A., Yezli, S., Salkeld, J. A. and French, G. L. (2013). Evidence that contaminated surfaces contribute to the transmission of hospital pathogens and an overview of

strategies to address contaminated surfaces in hospital settings. *American Journal of Infection Control* **41**(5):6-11.

Prescott, M.L, John, P.H and Donald, A.K. (2005).*A Textbook of Microbiology, 6th Edition.* New York: McGraw Hill;pp 970-985.

Prüss-Ustün, A., Wolf, J., Bartram, J., Clasen, T., Cumming, O., Freeman, M. C. and Cairncross, S. (2019). Burden of disease from inadequate water, sanitation, and hygiene for selected adverse health outcomes: An updated analysis with a focus on low- and middle-income countries. *International Journal of Hygiene and Environmental Health* **222**(5):765-777.

Reynolds, J.M. (2005). Bacteria contamination of door handles/knobs in selected public conveniences in Abuja metropolis, Nigeria. *Journal of Medical Research* **6**(1):7-11.

Rutala, W. A. and Weber, D. J. (2013). Disinfection and sterilization in healthcare facilities: An overview and current issues. *Infection Control & Hospital Epidemiology* **34**(5):508-517.

Ryan, K.J. and Ray, C.G. (2004).*Sherri's Medical Microbiology*; 4th edition.McGraw Hill.pp 370.

Sabra, S. M. (2013). Bacterial public Health Hazard in the public Female Restrooms. *Middle-East Journal Scientific Research* **14**(1):63-68.

Saldanha, T., Hora, V. P., Oliveira, P. A., & Andrade, C. F. (2020). Public surfaces in high-traffic areas as a potential source of microbial contamination. *Journal of Public Health and Hygiene* **62**(2):98-106

Scott E., Bloomfield S. F. and Barlow C. G., (1982). An investigation of microbial contamination in the home. *Journal of Hygiene* **89**(2):279-293.

Scott, I. S. and Bloomfield, S. (1990). The survival and transfer of microbial contamination via cloths, hands and utensils. *Journal of Applied Bacteriology* **68**(3): 27 1-278.

strategies to address contaminated surfaces in hospital settings. *American Journal of Infection Control* **41**(5):6-11.

Prescott, M.L, John, P.H and Donald, A.K. (2005).*A Textbook of Microbiology, 6th Edition.* New York: McGraw Hill;pp 970-985.

Prüss-Ustün, A., Wolf, J., Bartram, J., Clasen, T., Cumming, O., Freeman, M. C. and Cairncross, S. (2019). Burden of disease from inadequate water, sanitation, and hygiene for selected adverse health outcomes: An updated analysis with a focus on low- and middle-income countries. *International Journal of Hygiene and Environmental Health* **222**(5):765-777.

Reynolds, J.M. (2005). Bacteria contamination of door handles/knobs in selected public conveniences in Abuja metropolis, Nigeria. *Journal of Medical Research* **6**(1):7-11.

Rutala, W. A. and Weber, D. J. (2013). Disinfection and sterilization in healthcare facilities: An overview and current issues. *Infection Control & Hospital Epidemiology* **34**(5):508-517.

Ryan, K.J. and Ray, C.G. (2004).*Sherri's Medical Microbiology*; 4th edition.McGraw Hill.pp 370.

Sabra, S. M. (2013). Bacterial public Health Hazard in the public Female Restrooms. *Middle-East Journal Scientific Research* **14**(1):63-68.

Saldanha, T., Hora, V. P., Oliveira, P. A., & Andrade, C. F. (2020). Public surfaces in high-traffic areas as a potential source of microbial contamination. *Journal of Public Health and Hygiene* **62**(2):98-106

Scott E., Bloomfield S. F. and Barlow C. G., (1982). An investigation of microbial contamination in the home. *Journal of Hygiene* **89**(2):279-293.

Scott, I. S. and Bloomfield, S. (1990). The survival and transfer of microbial contamination via cloths, hands and utensils. *Journal of Applied Bacteriology* **68**(3): 27 1-278.

Singleton, P. (1999).Bacteria in Biology, *Biotechnology and Medicine*; 5th edition.Willey.pp. 444-454

Sleigh D. J. and Timbury M. C. (1998). *Notes on Medical Microbiology*, 5th edition. Churchill Livingstone, New York. p173.

Tunç, K., and Olgun, U. (2006). Microbiology of public telephones. *Journal of Infection* **53**(2): 140-143.

Weinstein, R.A. (1998). Nosocomial infection update. *Journal of Emergent Infection Discovery* 4:416-420.

World Health Organization (2017). WHO guidelines on hand hygiene in health care: a summary. World Health Organization

Willey, J.M., Sherwood, L.M. and Woolverton, C.J. (2011).Epidemiology and public health microbiology.*Prescott's Microbiology;* 8th ed., McGraw Hill, New York; pp. 884-886.

World Health Organization. (2018). Public health and sanitation guidelines. *WHO Report*. Retrieved from