ISOLATION AND IDENTIFICATION OF BACTERIAL ISOLATES FROM STUDENTS LECTURE TABLES IN UNIVERSITY OF BENIN

BY

SAMUEL EFOSA LAWANI

LSC1906909

UNIVERSITY OF BENIN
BENIN CITY

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

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CERTIFICATION

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

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DR. (MRS) I.B. IDEMUDIA **DATE**

(Project Supervisor)

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PROF. F.I. AKINNIBOSUN **DATE**

(Head of Department)

DEDICATION

This work is dedicated to God Almighty for his, grace, guidance and favor throughout the project work and compilation of this report.

Secondly, this project is dedicated to my Late Father (MR PULLEN LAWANI), my family and everyone who supported me.

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ABSTRACT

Fomites are generally considered as any inanimate object that can serve as a means of transferring pathogens to a new host. Table surfaces which are fomites can be a major source of spread of infectious diseases. The main aim of the study is to enumerate, isolate and identify bacteria from Environmental Management and Toxicology (EMT) students lecture tables at University of Benin as well as to determine the antimicrobial susceptibility test of the bacterial isolates. Samples were collected twice on a weekly basis using a sterile swab. Methods involved in the isolation and identification of the bacteria were serial dilution, Gram staining, biochemical test which include oxidase, catalase, indole, urease, sugar fermentation and potassium hydroxide test, as well as antimicrobial sensitivity test. The probable isolate identified were *Staphylococcus aureus, E.coli, klebsiella* spp., *pseudomonas* spp. and *Enterobacter aerogenes*, with the second week having a higher number of bacterial population and isolates. Unhygienic practices by the students accounts for these isolates.

CHAPTER ONE

INTRODUCTION

Background to the study

It's widely recognized that various routes exist for the transmission of microorganisms throughout a community. Air, water, and soil serve as typical conduits. Additionally, these microorganisms can be directly transferred between individuals via physical interaction, or indirectly through contact with infected surfaces or items. Microorganisms are omnipresent and possess the capability to adjust to new surroundings and proliferate considerably on a range of surfaces within a constrained timeframe (Ogilvie and Hirsch, 2012). Their knack for adaptation and rapid multiplication in various conditions is crucial to their presence in diverse locations, such as soil surfaces, wastewater, deep within the earth's crust, organic matter, and within the organisms of plants and animals (Balkwill *et al.*, 1997). Given this intriguing observation, it's essential to conduct empirical research to demonstrate the existence and transmission methods of these microorganisms, especially given their common association with inanimate objects such as tables.

Dating back to the 1500s, the concept of fomites, also known as fomes, emerged, initially portraying them as "seeds of disease" present in the garments of infected individuals. It was believed that these fomites facilitated the transmission of contagion over long distances through indirect human contact (Nutton, 1983). In contemporary times, fomites are generally understood as inanimate objects that, when contaminated with infectious organisms, can act as vehicles for transferring disease-causing agents to a fresh host. The continuous interaction between humans or the natural environments where pathogenic organisms reside and fomites represents a significant means by which infectious diseases are disseminated. In developed nations, individuals typically allocate around 90% of their time indoors (Klepeis *et al.,* 2001). Consequently, the primary sources of contamination and transmission through fomites are often those present in the built environment and frequently touched by humans. These encompass various items such as doorknobs, handles, handrails, tables, chairs, cups, dishes, cutlery, trays, surfaces in washrooms, computer input devices, electronic devices with buttons, phones, office supplies, and children's toys.

The majority of individuals assess cleanliness based on the outward condition of surfaces and objects, without recognizing the potential presence of numerous microbes on them. There is a common misconception that microbes are only associated with medical equipment and specific surfaces within healthcare facilities like research labs, hospitals, and clinics (Reynolds *et al.*, 2005).

The environment plays a crucial role in various aspects of the infection transmission cycle. While surfaces that are contaminated with microorganisms can act as reservoirs for pathogens, they are usually not directly implicated in the transmission of infections. Factors such as the presence of organic material, exposure to sunlight, temperature, and humidity are significant in determining the survival of pathogens on surfaces (Taylor *et al.,* 2013). According to a literature review, several types of Gram-positive bacteria like *Enterococcus* spp., *Staphylococcus aureus*, and *Streptococcus pyogenes*, as well as Gram-negative bacteria including *Acinetobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Shigella* spp., have the ability to endure on surfaces for extended periods, potentially lasting months (Kramer *et al*., 2006). Different pathogens exhibit varying survival rates, with *mycobacteria* and *Clostridium difficile* able to persist for several months. In contrast, *Bordetella pertussis*, *Haemophilus influenza*, and *Vibrio cholera* can only survive for a few days (Wagenvoort and Penders, 1997; Webster *et al.*, 2000). Certain bacteria like *Salmonella* spp. and *Escherichia* coli have been implicated in the transmission from hands to raw, processed, and cooked foods, even in minimal quantities present on the fingers (Nworie *et al.,* 2012). Bacteria can readily cross-contaminate fingers through the continuous handling of unclean currency notes (Umeh *et al.,* 2007). Additionally, reports have indicated the presence of fungal microbial contamination associated with the use of ATMs (Ozkhan, 2016). Several studies have demonstrated that once microbes come into contact with hands or certain hard surfaces, they can easily establish habitats and become challenging to eliminate (Hood and Zottola, 1997). Microorganisms are primarily transmitted from environmental surfaces to students through hand contact with those surfaces. While practicing good hand hygiene is crucial in minimizing this transfer, it is equally important to properly clean and disinfect environmental surfaces to effectively reduce their potential role in the occurrence of healthcare-associated infections.

Historically, it was widely believed that bacterial diseases were primarily transmitted through direct contact, with little to no emphasis on the role of the environment in disease transmission. However, perspectives on bacterial transmission have evolved over time, now encompassing a more comprehensive and dynamic model of disease propagation (Cozad and Jones, 2013). The transmission of microbial infections now encompasses the involvement of contaminated fomites or surfaces (Springthorpe and Sattar, 2010).

Hence, the main inquiry is to determine the specific types and susceptibility of opportunistic bacteria present on fomites, with a particular focus on lecture tables within a selected post-secondary educational institution. Consequently, this study aims to isolate and characterize the opportunistic bacteria in order to gain a better understanding of their nature.

1.1 AIM AND OBJECTIVES

The main aim of the study is to isolate and identify bacteria from Environmental Management and Toxicology (EMT) students lecture tables at University of Benin.

The specific objectives were to;

1. enumerate, isolate and identify the bacteria from the lecture tables
2. determine the antimicrobial susceptibility test of the bacterial isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1 Fomites Transmission of Infectious Diseases

It is commonly accepted that inanimate objects have the capacity to harbor microorganisms derived from their surrounding environment. These deposited microorganisms have the potential to be transferred to other surfaces, such as food or the human body, where they can thrive and grow (Joanna, 2012). The transmission of infectious diseases through hand contact has been a significant area of concern.

According to Itah and Ben (2004), Enteric bacteria like *Escherichia coli, Klebsiella* spp., and *Citrobacter* spp. were discovered to be present on different surfaces that come into contact with people, such as door handles and various household items. Fomites refer to objects or surfaces, whether porous or non-porous, that can harbor pathogenic microorganisms. When these fomites are contaminated, they can transfer the microorganisms to a new host, acting as vehicles for transmission. According to Osterholm *et al*. (1995), fomites that come into regular contact with humans or natural habitats of pathogenic organisms are a significant source of infectious disease transmission. These fomites include various surfaces such as door handles in public places like restrooms, showers, toilet seats, sinks, lockers, chairs, and tables found in public offices, hospitals, hotels, restaurants, and restrooms, as mentioned by Bright *et al.* (2010). Pathogenic microorganisms that cause infections can be present in any environment, including soil, air, water and food as well as environmental surfaces or objects (Neely and Sittig, 2002). Researchers have discovered that the majority of bacteria they have found are actually part of the normal microbial communities found on the skin, in the mouth, and in the nasal passages. These bacteria can be transferred to our hands. However, they typically do not cause harm unless the immune system is weakened due to illness, as noted by Oluduro *et al*. (2011).

To assess the occurrence of fecal contamination, which may contain disease-causing parasites, a study was conducted in two daycare centers in Atlanta. The researchers examined various surfaces in these centers to determine the prevalence of fecal coliform bacteria, which is commonly used as an indicator of fecal contamination, as well as a potential presence of pathogens.

Out of a total of 398 samples taken from various surfaces, furniture, and objects in daycare centers, fecal coliforms were detected in 17 samples, representing a prevalence rate of 4.3%. These contaminated surfaces have the potential to contribute to the transmission of enteric diseases among children. Hence, it is crucial to not only emphasize proper hand hygiene but also ensure disinfection of inanimate objects as a means of controlling the spread of enteric diseases in daycare centers. Specifically, one center had 10 positive plates, accounting for a 5.0% positivity rate, while the other center had 7 positive plates, accounting for a 3.5% positivity rate. Weniger *et al.* (1983) identified positive samples from various sources including toilets, diapering items, floors, furniture, and a refrigerator handle. On the other hand, Omololu-Aso *et al.* (2011) conducted a study at the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) where they collected two hundred swabs from specific locations. These locations included doctors' stethoscope diaphragms, cell phones of healthcare workers (HCWs), patients' bed linen, pillows, and door knobs. Swab cultures were analyzed to detect the presence of *Staphylococcus aureus.* The findings revealed that 18.70% of the doctors' stethoscopes, 20.33% of the doctors' cell phones, and 20.33% of the doorknobs were contaminated with *S. aureus*, as reported by Omololu-Aso *et al.* (2011). Similarly, Oranusi *et al.* (2013) conducted a study where they collected a total of 130 samples from various locations within the university campus. These samples included 40 hand swabs, 20 samples each from food sources and food contact surfaces, and 10 samples each from banisters, table tops, door handles, tap handles, and toilet flushers. Their findings revealed that approximately 98% of hand swabs, samples from food contact surfaces, and other frequently touched surfaces were contaminated with microorganisms. Specifically, hand swabs taken from the halls of residence and library exhibited higher levels of contamination, with counts of 2.1×105 and 1.9×105 colony-forming units (cfu) respectively. Toilet flushers and banisters showed even higher levels of contamination, with counts of 8.3×106 cfu. The microorganisms identified in their study included *Bacillus* spp., *Staphylococcus* spp., *Streptococcus* spp., *Escherichia* coli, *Salmonella* spp., and *Klebsiella* spp.

Baadhaim *et al.* (2011) suggested that door handles could contribute to the transmission of microorganisms among individuals and serve as a potential source of microbial contamination. In their study, they investigated the presence of Gram-negative bacteria on the door handles of Olin Hall. The researchers hypothesized that during periods of high building occupancy, a greater proportion of the bacteria collected from the door handles of Olin Hall would be Gram-negative. The findings indicated that 49% of the observed microbial colonies were identified as Gram-negative bacteria.

Nworie *et al.* (2012) acknowledged the growing occurrence of disease outbreaks and their rapid transmission between communities, which has become a significant public health issue. The researchers conducted an investigation into bacterial contamination by collecting samples from door handles/knobs in public restrooms, motor parks, and markets within the Abuja metropolis. Out of a total of 180 swab samples that were cultured, 156 of them (86.7%) tested positive for bacterial presence. The highest percentage of positive samples was found on female toilet handles/knobs (41.7%) and bathroom door handles/knobs, as compared to males (11.5%). The research also revealed that toilet door handles/knobs in markets, motor parks, and restaurants exhibited a higher level of contamination compared to government offices and banks. Additionally, the contamination rate was higher on toilet door handles/knobs (87.2%) compared to bathroom door handles/knobs (85%). Coliform bacteria were found to be the most prevalent contaminants. The identified bacterial contaminants included *Staphylococcus aureus* (30.1%), *Klebsiella pneumoniae* (25.7%), *Escherichia coli* (1%), and *Enterobacter* spp. (11.2%), *Citrobacter* spp. (7.1%), *Pseudomonas aeruginosa* (5.9%) and *Proteus* spp. (4.5%). This indicates that the easily accessible locations within the city contain dangerous bacteria that have the capacity to trigger widespread disease outbreaks in the coming times.

The study conducted by Maori *et al.* (2011) assessed the occurrence of bacterial organisms on restroom door handles in secondary schools situated in Bokkos Local Government, Jos Plateau State, Nigeria. A total of 120 samples were obtained and subjected to laboratory cultivation, with 40 samples collected from each of the three schools, namely Government Secondary School Bokkos (G.S.S.B), All Nation Academy, and Government Secondary School Mushere. Among the 120 samples, 60 (50%) exhibited microbial growth, while the remaining 60 showed no growth whatsoever. The isolated organisms included *Staphylococcus* spp. (43.3%), *Candida* spp. (10%), *Escherichia coli* (16.7%), *Citrobacter* spp. (1.7%), *Klebsiella* spp. (20%), *Proteus* spp. (6.7%), and *Salmonella* app (1.7%). The findings indicated that

Government Secondary School Bokkos exhibited the highest level of contamination at 48.3%, followed by All Nations Academy at 30%, and Government Secondary School Mushere at 21%. In a separate study conducted by Scott et al. (1982), they examined the bacterial flora in more than 200 households. Out of the collected samples, 60 were obtained from bathrooms, toilets, and kitchens, while 9 samples were taken from living rooms. The results of bacterial contamination, expressed as percentages, observed in the 200 homes were as follows: *E. coli* 64.5%, *Klebsella Pneumoniae* 29.5%, *Klebsiella* spp., 6%, *Proteus mirabilis* 4%, *Salmonella* spp.. 1.5%, *Citrobacter freundii* 42%, *Citrobacter* spp. 29%, *Enterobacter cloacae* 26%, *Enterobacter* agglomerans 7.5%; *Pseudomonus aeruginosa* 4%, *Staphylococcus aureus* 31.5%, *Streptococcus* spp.. 16%, the majority of homes were contaminated with *enterobacteria* spp. and *Pseudomonus* spp., many of which are potentially pathogenic. Other potential Pathogens included *Staphylococcus aureus* and *Streptococcus* spp.

Sabra (2013) conducted a research in Taif, Kingdom of Saudi Arabia, focusing on public female restrooms in various buildings. The objective was to analyze the distribution of contamination and bacterial levels within these restrooms. A total of 260 samples were collected from different areas within the restrooms, including the restroom door, restroom handle, restroom sink, restroom toilet door, and restroom toilet handle. Among the samples, 187 out of 260 (71.9%) showed bacterial growth or positive cultures. The most prevalent positive results were observed in the RR Toilet Handle, with 73 out of 80 samples (91.3%) showing bacterial growth. This was followed by the Restroom Toilet Door, where 59 out of 80 samples (73.8%) displayed positive cultures. The RR Sink exhibited a lower but still notable incidence of bacterial growth, with 38 out of 60 samples (63.3%) yielding positive results. The Restroom Handle had a positive rate of 10 out of 20 samples (50%), while the Restroom Door had the least positive findings, with 7 out of 20 samples (35%) showing bacterial growth. 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 known that harmful microorganisms can be transferred to hands from contaminated surfaces. These contaminated hands can transmit disease to own self as well as to others according to a study that done to determine to which 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 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).

Fomites refer to non-living objects that can act as carriers for transmitting pathogens. In Nigeria, Maryam *et al.* (2014) conducted a research study in a teaching hospital with the aim of identifying pathogenic bacteria present on fomites. A total of 35 samples were collected for analysis. Out of these samples, 23 isolates (65.7%) were obtained, with a ratio of 12 Gram-positive organisms to 11 Gram-negative organisms. The bacteria isolated were *Staphylococcus aureus* (21.7%), *Staphylococcus epidermidis* (8.7%), *Streptococcus* spp. (8.7%), *Bacillus* spp. (13.0%), *Escherichia coli* (26.1%), *Pseudomonas* spp. (8.7%) and *Klebsiella* spp. (13.0%). Other a study was conducted to determine the prevalence of some pathogenic bacteria and the general hygienic status on the interior surfaces of some domestic refrigerators (n = 150). *Campylobacter* spp*.,* and *Salmonella* spp. were not recovered from any refrigerators, but *Staphylococcus aureus* was recovered from 9.54%, *Listeria monocytogenes* 3.8%, *Escherichia* coli from 2.1% from 1.6% of examined refrigerators. That indicated very poor standards of consumer refrigerator management and hygiene, and posing risks to consumer health (Abdulla *et al.*, 2008).

The presence of enteric bacteria in kitchen sponges and dish cloths indicates their potential involvement in the transfer of foodborne pathogens, leading to cross-contamination of foods, surfaces, and hands. In a study conducted by Koenig (2014), the focus was on examining the occurrence of bacteria in kitchen towels commonly utilized for drying dishes, hands, and other surfaces within household kitchens.
A grand total of 82 samples of kitchen hand towels were gathered from households in five prominent cities in the United States and Canada. The towels were analyzed to determine the quantities of heterotrophic bacteria, coliform bacteria, and *Escherichia coli* present in each one. Coliform bacteria were found in 89.0% of the samples, while E. coli was detected in 25.6% of the total coliform bacteria discovered in the towels.

According to Adams *et al.* (2015), humans are responsible for introducing microbes into indoor areas through direct contact, as well as by bringing them in from the outside (Adams *et al.*, 2013). Additionally, microbes can be transferred from our surroundings (Adams *et al.,* 2013; Lax *et al.*, 2017) and enter the indoor air. The composition and diversity of the indoor microbiome, which refers to the microbial population in buildings, are influenced by human activities, external environment, architectural factors, and building management (Adams *et al.,* 2015). Various molecular analyses have revealed a significant range of microbial species on constructed surfaces. The majority of microbes present indoors are typically dormant, inactive, or deceased (Gibbons, 2016). Furthermore, many of these microbes either have no known impact on human health or are actually beneficial for human well-being (Lynch *et al.,* 2014). Inanimate objects within the built environment can serve as reservoirs for various types of microorganisms, including bacteria, viruses, archaea, protists, and fungi. Among these microorganisms, there is a potential presence of pathogens and microbial metabolic byproducts that may pose risks to human health.

Microorganisms that come from external environments are typically believed to be unable to survive on indoor surfaces that lack sufficient moisture and nutrients. Those microorganisms that do manage to survive under such conditions are usually considered to be inactive or dormant until they encounter moisture and nutrients that facilitate their growth or until they are transported to different locations within their host environment (Gibbons *et al.,* 2015). Studies using advanced molecular sequencing techniques to analyze fungal populations in indoor environments have revealed that the majority of these populations primarily originate from the local outdoor environment, indicating transportation as the main source (Adams *et al.,* 2013).

Similarly, investigations focusing on buildings and surfaces with higher levels of human occupancy and frequent human contact have reported increased levels of bacteria associated with the skin (Adams *et al.,* 2015). Efforts have been undertaken to identify the origins of bacteria found on various indoor surfaces. Research has revealed that bacteria originating from urine and feces are more commonly found on toilet seats and lavatory handles compared to other surfaces (Flores, 2011). Additionally, bacteria commonly found on fresh produce have been observed to be more abundant on kitchen counters and in refrigerators (Flores, 2011).

Bacteria commonly associated with leaves and soil are more commonly found on the interior and exterior door trims of doors that open to the outside in residential areas (Dunn *et al.,* 2013). On the other hand, in bathrooms and kitchens, where surfaces often experience high humidity levels, rich microbial biofilms can form communities that closely resemble those found in plumbing systems and water reservoirs (Kelley *et al.,* 2004). In a study conducted by Lax *et al.* (2014), it was clearly demonstrated that bacterial communities on various surfaces within a single household exhibited distinct similarities on certain surfaces but not on others. Furthermore, when families relocated to new homes, the bacterial composition of the surfaces in the new house rapidly converged with that of the surface bacteria, suggesting that the new inhabitants quickly introduced their own unique collection of human-related bacterial signatures into the new environment. Although significant progress has been made in understanding microbial communities in indoor environments in recent years, our knowledge regarding bacterial communities and the specific types of bacteria found on fomites remains limited (Prussin *et al.*, 2015). However, it is crucial to gain a better understanding of the bacterial species present on fomites, as they can potentially contribute to the transmission of infectious diseases and pose new microbial threats.

2.2 Potential Pathogenic Bacteria on Fomites

Marks *et al.* (2014), detected viable *Streptococcus pyogenes* and *Streptococcus pneumoniae* in samples from a daycare and then verified in laboratory tests that isolates of both organisms remained viable over extended periods of time and remained infectious in a mouse model when present as a biofilm (rather than as desiccated cells on surfaces). These findings suggest that fomite transmission in the environment could be an important pathway if fomites are contaminated with oropharyngeal secretions containing biofilm *streptococci*.In a study conducted by Jones and Lutz in 2014, they determined that the average length of time that *Pseudomonas aeruginosa* could survive on laminate, glass, and stainless steel surfaces was 3.75, 5.75, and 6.75 hours, respectively. In a separate investigation carried out by Malcolm and colleagues in 2017, they examined the growth and endurance of *Mycobacterium abscessus,* a non-tuberculous *mycobacterium*, in the presence of various mineral particles, including kaolin, halloysite, silicon dioxide, and house dust. *Mycobacterium abscessus* showed interaction with the particulate matter, and its survival rates were found to be higher when exposed to house dust. In fact, it was able to endure desiccation for a period of up to two weeks.

Numerous studies have reported the isolation of pathogenic microorganisms from children's hands, indicating inadequate handwashing practices. In a study conducted in Greece, which involved the analysis of 1956 hand swab samples, it was revealed that 52.9% of children's hands were found to be contaminated with fecal Streptococci

(Kyriacou *et al.,* 2009). In a separate investigation conducted by Tambekar and Shirsat in 2009, the hands of 400 students were sampled, revealing the presence of bacterial pathogens in all the collected hand swabs. Another study, conducted by Itah *et al.* in 2004, demonstrated that various Gram-negative enteric bacteria, including *Klebsiella* spp., *Escherichia coli*, *Citrobacter* spp., as well as Gram-positive *Staphylococcus aureus,* were detected on numerous frequently touched surfaces such as door handles, tables, windows, chairs, and various other common household furniture.

The presence of microbial contaminations is frequently documented in various indoor and outdoor settings. In a study conducted by Tunc and Olgun in 2006, the bacterial contaminations of 50 public telephones in Afyon City, Turkey, were examined. The researchers identified twelve different types of bacteria, including *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus,* on the surface of the telephones. Namias *et al*. (2000) documented comparable findings regarding microbial contamination on hospital telephones and personal pagers. Rutala *et al.* (2006) conducted a study to assess the level of microbial contamination on computer keyboards, as well as the effectiveness of various disinfectants and their potential effects on the appearance and functionality of the keyboards.

The primary results indicated that microbial contamination is widespread on keyboards, but it can be effectively eliminated through the use of disinfectants. Narmeen *et al.* (2009) conducted a study in Azadi General Hospital, where they isolated and identified the pathogen *Staphylococcus aureus* in various areas, including patients, healthcare staff, and the hospital environment. They employed both bacteriological methods and molecular markers for identification purposes. Out of the 224 specimens collected from different sites, only 52 isolates were found to be *S.*

aureus, which accounted for 23.21% of the total isolates. *S. aureus* can cause infections habitually in newborns, surgical, burns, diabetic patients, and persons who are taking drugs suppressing immunodeficiency diseases.

In their study, Harrison *et al.* (2003) investigated the potential for cross-contamination between hands, towels, and dispensers when any one of them is contaminated. They utilized *Micrococcus luteus* and *Serratia marcescens,* both of which exhibit a unique colonial morphology on the plate count agar utilized. The findings revealed that there is a possibility of bacteria being transferred back and forth between paper towel dispensers and hands in a zig-zag pattern if any of these elements are contaminated.

These studies and others confirm that potentially pathogenic bacteria are present in the built environment and that they can survive on fomites for long periods of time.

2.3 Transmission Rate of Bacteria between Surfaces

The transfer rate of bacteria between surfaces can be influenced by various factors. These factors encompass the type of bacteria, the surfaces involved (both the source and destination), the time elapsed since inoculation, and the level of moisture present (Rusin *et al.,* 2002). In a study conducted by Montville *et al.* (2001), the researchers examined the rates at which bacteria are transferred between food and hands, as well as between hands and food, with and without the use of gloves as a barrier.

The study conducted by Montville *et al.* (2001) revealed that the use of gloves as a barrier can slow down the transfer rate of microorganisms from food to hands. However, it was noted that most gloves are still permeable to bacteria when tested under conditions that mimic real-world usage. Furthermore, the researchers speculated that the effectiveness of the glove barrier may be influenced by the number of bacteria present (inoculum size). The study conducted by Tunc and Olgun (2006) identified frequent usage of public telephones as a significant factor contributing to cross-contamination. It has been observed that higher quantities of bacteria are typically found on porous surfaces and in moist environments (Rusin *et al.,* 2002). On the other hand, transmission rates of microorganisms to hands are more efficient from nonporous, hard surfaces like stainless steel (Rusin *et al.,* 2002). Rusin *et al. (*2002) found that bacterial transfer rates ranging from 27.6% to 40.0% occurred from a sink faucet handle, while rates of 38.5% to 41.8% were observed from the telephone to the hand with minimal contact durations. A study by Kwan *et al*. (2018), showed that the human microbiome (skin, oral cavity, and gut) was the main source of bacteria on desks at school, where this study also demonstrated that desks cleaning physically removed around 50% of bacteria, fungi, and human cells and a full recovery of the surface microbial concentrations occurred within 2-5 days. Additional research has been conducted to demonstrate the occurrence of bacteria in school-age children. In India, a study was carried out to assess the quantity of bacteria present on the hands of school children. Out of the children tested, 61% had swabs that revealed the presence of potential harmful bacteria. Among these samples, *Staphylococcus aureus* was the most frequently identified pathogen, found in 44% of the cases.

Furthermore, a minority of less than 70% believed that web spaces harbor dirt, whereas a larger proportion (78%) considered palms to be potentially dirtier. Nearly 86% of the participants claimed to wash their hands before having lunch. Among them, 47.3% admitted to never using soap, while only 21.3% reported always using soap. Interestingly, 18.4% of students mentioned that soap was consistently available at all times in their school (Ray *et al.,* 2011). In a separate study conducted in Lebanese schools, samples were collected from the surfaces of classroom desks, water taps of bathrooms and air. Public schools exhibited significantly higher levels of contamination compared to private schools. Among the three educational levels, namely primary, preparatory, and secondary, it was found that the elementary level had the highest degree of contamination (Malaeb *et al.,* 2016).

2.4 Transmission of Pathogenic Bacteria from Fomites to Food

Maintaining cleanliness in the environment where food is both prepared and consumed is crucial to prevent the risk of acquiring foodborne illnesses. Additionally, ensuring food safety is particularly vital for managers of food establishments. While the significance of keeping food contact surfaces clean is widely acknowledged in terms of food safety, the cleanliness of non-food contact surfaces, such as tables and salt-shakers, tends to be underestimated. Staff members and food consumers frequently come into contact with these surfaces, making them potential breeding grounds for the transmission of harmful microorganisms.

Numerous instances of foodborne disease outbreaks have identified several contributing factors, including the transfer of bacteria to cooked food through contact with hands or contaminated surfaces and equipment. Assessing the microbial condition of food service establishments is typically not a routine part of the inspection process, and even when conducted, it may not occur with the desired frequency. It is important to note that bacterial or viral contamination of surfaces cannot be detected through visual assessment alone. Indeed, research utilizing sterilized swabs and agar plates has demonstrated that relying solely on visual inspection of surfaces is an inadequate measure of cleanliness (Moore and Griffith, 2002).

The primary tool utilized in food preparation is typically the hands of the person responsible for preparing the food. As a result, the hands of both food handlers and consumers can serve as a means for bacterial or viral transmission to non-food contact surfaces. For this reason, it is crucial that staff and customers in food service establishments thoroughly wash their hands with soap and water before handling any food or after cleaning surfaces that do not come into direct contact with food.

The cleaning protocols for surfaces, equipment, and furniture in food establishments typically vary depending on the specific procedures implemented by the establishment. Several factors come into play when deciding which cleaning methods to employ, including time constraints, cost considerations, ease of use, and the characteristics of both food contact and non-food contact surfaces. However, a particular concern is the consistent cleaning of certain surfaces located outside the kitchen, as this may not be consistently performed across all food service establishments. This may be true especially on furniture, equipment and any other frequently used items such as salt-shakers. Proper cleaning of these surfaces may also be lacking. Findings from a prior study revealed that tables in restaurants and bars exhibited higher bacterial counts after being cleaned with a dishcloth compared to their pre-cleaning state (Yepiz-Gomez *et al.,* 2006).

2.5 Bacterial Survival and Persistence on Fomites

Most Gram-positive bacteria, such as *Enterococcus* spp. including vancomycin-resistant *Enterococcus* (VRE), *S. aureus* including methicillin-resistant *Staphylococcus aureus* (MRSA), or *Streptococcus pyogenes* survive for months on dry surfaces. Overall, there is no apparent distinction in survival rates between multi-resistant and susceptible strains of *S. aureus* and *Enterococcus* spp. A solitary study by Wagenvoort and Penders (1997) hinted at a potential variance in survival time between antibiotic-resistant and susceptible bacteria; however, the susceptible strains exhibited only a slightly shorter survival time on surfaces, which was not statistically significant.

Several Gram-negative species, including *Acinetobacter* spp., *Escherichia coli, Klebsiella* spp., *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Shigella* spp., have the ability to survive on inanimate surfaces for extended periods, sometimes even months. These species are frequently isolated from patients with Hospital-acquired infections (HAI), as reported by Kramer *et al.* (2006). In contrast, other Gram-negative bacteria such as *Bordetella pertussis*, *Haemophilus influenzae*, *Proteus vulgaris*, and *Vibrio cholerae* only persist for days.

Mycobacteria, including *Mycobacterium tuberculosis,* and spore-forming bacteria, such as *C. difficile,* can survive for many months on surfaces.

Due to the continued prevalence of paper in healthcare facilities around the world, Hübner *et al.* (2011) conducted a study to examine the survival of different types of bacteria, both Gram-positive and Gram-negative, such as *E. coli, S. aureus, P. aeruginosa,* and *Enterococcus hirae*, on office paper. The paper samples were deliberately contaminated with a standardized number of bacterial suspensions, containing approximately 2.8 × 107cfu/mL. In contrast to *E. coli*, the other organisms examined in the study showed greater resilience under room conditions and experienced only a 3 log10 reduction on paper after 7 days. However, *E. coli* demonstrated a more rapid reduction of 5 log10 within 24 hours. Additionally, the study found evidence of bacteria being transmitted between hands and paper for all strains. Similar investigations have shown that paper currency can serve as a potential reservoir and means of transmission for pathogens (Vriesekoop *et al.,* 2010).

2.6 Factors Influencing the Survival of Microorganisms in the Environment

2.6.1 Relative Humidity (RH)

Staphylococcus aureus can persist longer at low humidity. However, for *Enterococcus faecalis* the survival kinetic is decreased at 25 % RH compared to 0 % RH (Robine *et al*., 2000). According to Tang (2009), the viability of aerosolized Gram-negative bacteria, including *Pseudomonas* spp., *Enterobacter* spp., and *Klebsiella* spp., was found to improve under conditions of higher relative humidity and lower temperature. On the other hand, studies conducted on airborne Gram-negative bacteria such as *S. marcescens, E. coli, Salmonella pullorum, Salmonella derby*, and *Proteus vulgaris* demonstrated reduced survival rates when exposed to intermediate (approximately 50-70% RH) to high (approximately 70-90% RH) relative humidity levels.

According to Tang (2009), certain airborne Gram-positive bacteria such as *Staphylococcus epidermidis*, *Streptococcus haemolyticus*, *Bacillus subtilis,* and *Streptococcus pneumoniae* exhibited decreased survival rates when exposed to intermediate relative humidity levels ranging from 50-70% RH. In indoor air, Gram-positive cocci were found to be the most prevalent, followed by Gram-positive rods (e.g., *Bacillus* spp. and *Actinomycetes* spp.), Gram-negative rods, and Gram-negative cocci (Tang, 2009). The variation in bacterial behavior can be attributed to the structural composition of the bacterial cell wall. Gram-positive organisms are better equipped to withstand dry conditions compared to Gram-negative organisms due to their cell wall design. Gram-positive bacteria have a cell wall consisting of a lipid double-layer structure with a thin peptidoglycan (Murein) layer made up of alternating residues of β-(1,4) N-acetylglucosamine and N-acetylmuramic acid. This structural composition provides better protection against physical stress. In contrast, Gram-negative bacteria have a less robust cell wall structure, making them more vulnerable to environmental challenges, and requiring higher relative humidity to survive.

2.6.2 Temperature

According to Tang (2009), it is widely observed that airborne bacterial survival is consistently reduced when exposed to constant temperatures above 24°C.

2.6.3 Biofilm

In a nutrient-rich environment, biofilm serves as the predominant life form for microorganisms. When microorganisms adhere to surfaces and form biofilms, a sigma factor is activated, leading to the suppression of numerous genes. As a result, bacteria within the biofilm exhibit significantly higher tolerance, at least 500 times, to antimicrobial agents (Costerton *et al.,* 1995) and physical cold plasma (Matthes *et al.,* 2013). The enhanced tolerance observed in biofilms can be attributed to the secretion of extracellular substances such as polysaccharides, proteins, and DNA following attachment to surfaces. Biofilm formation requires a certain level of humidity to occur. The biofilm matrix serves to retain water and nutrients, providing protection to microorganisms against environmental factors (Flemming and Wingender, 2010). As a result, biofilms play a significant role in the long-term survival of microorganisms on various surfaces, both in natural environments and in industrial or medical settings (Bryers, 2008). The persistence of microorganisms on inanimate surfaces is extended and influenced by environmental conditions, with humidity being particularly important. Biofilms have been observed on various objects and surfaces in hospital settings, including sterile supply buckets, opaque plastic doors, venetian blind cords, and sink rubbers, with viable bacteria being cultivable from these biofilms. However, the impact of biofilm presence or absence on the risk of transmission or cross-transmission is not well understood due to limited research in this area. In addition to providing protection, biofilms may serve as a mechanism for the persistence of multi-drug resistant bacteria within the hospital environment (Vickery *et al.*, 2012). Moreover, these bacteria within biofilms can exchange virulence factors with other species present in the biofilm, as well as within their own species (Tribble *et al.,* 2012).

2.7. Measures Required in Preventing Cross contamination from Surfaces

Various measures to maintain hygiene were identified for preventing the transfer of contaminants from one surface to another. Effective hand hygiene is considered a crucial method for minimizing and averting surface-to-surface cross-contamination (Kendall *et al.,* 2003). According to a study by Cogan *et al.* (1999), no instances of Campylobacter jejuni were found on cutlery that had been washed by hand. On the other hand, certain studies (Cogan *et al.,* 2002) provide evidence that consumer-style hand-washing methods are not effective. Therefore, it is necessary to either enhance the hand-washing procedure or minimize hand contact with the meat to prevent contamination. Disinfectants like bleach are utilized to eliminate bacteria and other harmful microorganisms on surfaces, thereby preventing contamination and reducing the likelihood of infection.

In a recent investigation, the impact of hygiene practices on the transfer of *C. jejuni* and *L. casei* from chicken meat in domestic settings was explored by De-Jong *et al.* (2008). The study revealed that cross-contamination could be notably diminished by washing cutting boards and cutlery with hot water at a temperature of 68°C. Nonetheless, the author acknowledged that relying solely on this method is inadequate for preventing cross-contamination. Rather than relying solely on one method, it is recommended to employ separate cutting boards for raw meat and other ingredients, while also taking measures to prevent direct contact between hands and meat. If contact does occur, thorough hand cleaning with soap is essential. To prevent cross-contamination on computer keyboards, Rutala *et al. (*2006) advised disinfecting them on a daily basis, or when they are visibly dirty or come into contact with blood, as a precautionary measure. Other researchers recommended that disinfection must be applied on computer keyboards that are in us on patient care areas (Man *et al.,* 2002; Schultz *et al.*, 2003). To minimize bacterial contamination associated with public telephones, Tunc and Olgun (2006) proposed the adoption of hands-free telecommunication devices equipped with magnetic card or voice activation. Additionally, alternative approaches to reduce bacterial contamination include incorporating antimicrobial additives into the manufacturing of telephones, door knobs, fabrics, and various building materials (Lin *et al.,* 2002).

2.8 Antibiotic-Resistant Bacteria on Fomite surfaces

Extensive research has been conducted on antibiotic-resistant bacteria, surpassing the level of scrutiny given to potentially harmful bacteria. In 2013, the analysis of the primary antibiotic-resistant risks in the United States was published by the Centers for Disease Control and Prevention (CDC, 2013). A review conducted by Davis *et al.* (2012) examined existing literature on the transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) and other *Staphylococci* within households. The findings suggested that household microbial communities might play a role in the dissemination of antimicrobial resistance genes and could serve as reservoirs for the recolonization of humans. The significance of public transit settings in the transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) was highlighted in two studies conducted in Portugal. Healthcare environments are among the most critical areas for the transmission of antibiotic-resistant bacteria. Numerous objects, known as fomites, in these settings have the potential for contamination and subsequent transmission. Although there has been a hypothesis suggesting that numerous fomites serve as significant sources of transmission, a more detailed examination often uncovers a more nuanced perspective. To illustrate this, Julian *et al.* (2012) conducted a study where they collected samples from the surfaces of cellular phones carried by personnel at a veterinary hospital. The purpose was to detect the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP). The study found that out of 123 phones sampled, only 2 phones tested positive for MRSP, and only 1 phone tested positive for MRSA. Likewise, Missri *et al.* (2018) conducted a study to evaluate bacterial colonization on mobile phones belonging to healthcare workers in a hospital. The phones were sampled both before and 5 minutes after being sanitized with bactericidal wipes. The findings revealed that all phones were colonized with bacteria, with healthcare workers exhibiting higher levels of bacterial colonization compared to administrative staff. However, potential pathogens, primarily *Staphylococcus aureus,* were detected on approximately one-third of the phones, while only one phone was found to be colonized with MRSA. No multi-drug resistant bacteria were detected. Smibert *et al.* (2018) swabbed medical staff personal mobile phones, departmental phones, and ICU keyboards and cultured for 94 multi-drug resistant organisms (MRDOs) that had been previously cultured from ICU patients, including 11 MRSA, 2 VRE, and 81 Gram-negative bacteria. MRSA was isolated from only two phones, and whole-genome sequencing of mobile phone isolates demonstrated the isolates on mobile phones had different single nucleotide polymorphism (SNPs) compared with the clinical isolates, which suggests that these fomites are unlikely to contribute to hospital-acquired MRDOs. Studies that solely focus on characterizing bacterial colonization in the built environment are generally less effective in providing in-depth understanding of mechanisms or health-related implications compared to studies that specifically target particular pathogens and other microbial hazards. This is due to the widespread presence of bacteria in the built environment.

In addition to MRSA, other major microbial hazards in healthcare environments include *Clostridioides difficile*, carbapenem-resistant *Enterobacteriaceae,* vancomycin-resistant *Enterococcus* (VRE), and a number of single- and multi-drug-resistant organisms (Conceicao *et al.,* 2013). In their study, Haun *et al. (*2016) examined 72 studies that investigated the presence of pathogens on inanimate objects (fomites) in healthcare environments. They discovered that contamination rates varied significantly depending on the type of fomite, the specific microbial agent being studied (such as MRSA, Gram-negative rods, *enterococci*, and *C. diff*), as well as the methods used for sampling and analyzing microbiological samples.

In a study conducted by Grimmond et al. in 2018, they examined 50 disposable sharps containers and 50 reusable sharps containers from seven hospitals to investigate the presence of *C. difficile*. The results showed that 8% of disposable containers and 16% of reusable containers had detectable, but not infectious, amounts of *C. difficile.* These findings indicate that sharps containers are unlikely to be a source of *C. difficile* transmission.

In a study conducted by Jackson *et al.* in 2019, they examined the number of bacteria present on various body sites of intensive care unit (ICU) patients who were colonized with vancomycin-resistant Enterococcus (VRE), as well as the healthcare workers (HCWs) who provided care to those patients.

The presence of vancomycin-resistant Enterococcus (VRE) on the body sites of patients, such as perianal, stool, and skin swabs, was found to be associated with contamination of personal protective equipment (PPE) worn by healthcare workers (HCWs), specifically gloves and gowns. This suggests that ICU patients with a higher bacterial load were more likely to transmit VRE to HCWs through their PPE.

2.9 Methods for Controlling Antibiotic Resistant Bacteria on Fomite Surfaces

Several approaches have been studied to manage antibiotic-resistant bacteria and other microbial risks on surfaces that can transmit infections, such as fomites. Such methods include the use of UV light, disinfectant cleaners, coatings, and various others. In a recent study conducted by Mitchell *et al.* in 2019, they specifically focused on determining the necessary amounts of UV light to deactivate antibiotic-resistant bacteria such as MRSA, VRE, *C. difficile*, and murine norovirus on fomite surfaces made of stainless steel and Formica laminate. Reitzel and his colleagues conducted a study in 2014 where they tested a new antiseptic coating made of chlorhexidine and gentian violet to determine its effectiveness in killing bacterial and fungal pathogens on disposable medical gloves. They discovered that the coating effectively eliminated bacteria such as MRSA, VRE, and multi-drug resistant *Pseudomonas aeruginosa* from the surface of the gloves. However, despite the success of antimicrobial cleaners, other research suggests that caution should be exercised in their use.

For instance, Hartmann *et al.* (2016) found antibiotic resistance genes (ARGs) in settled dust from athletic and educational facilities. They observed that the abundance of ARGs was positively correlated with the concentration of antimicrobial chemicals present in the same dust samples. This implies that the use of antimicrobial cleaners may contribute to the development of antibiotic resistance.

Similarly, Mahnert *et al.* (2019) compared the microbial communities and their resistomes (the total profile of antibiotic resistance genes) on surfaces in clinical settings. By utilizing metagenomic genome and plasmid reconstruction, they discovered that highly maintained environments had distinct resistomes and a greater diversity of resistance genes compared to other built environments. How these results are best applied is still an active area of research, as ARGs are also natural components of environments rich with bacteria (e.g., soils), and their role in shaping bacteria in indoor environments is not yet well understood.

A promising field of study that has gained traction recently could provide an alternative to conventional cleaning techniques. Instead of antimicrobials that destroy microbes, probiotic cleaners containing spores from *Bacillus* species (*B. subtilis, B. pumilus, and B. megaterium*) are believed to function mainly through biological competition, inhibiting the survival and growth of pathogenic bacteria (Falagas and Makris, 2009; Vandini *et al.,* 2014). Probiotic cleaners have shown superior effectiveness compared to traditional cleaning methods, with various research revealing they reduced the pathogen presence on surfaces by an average of 90% more than standard chemical cleaners, with variations between 70 and 99% (Caselli *et al.,* 2016: Caselli *et al.,* 2018). Moreover, a study by Caselli *et al.* (2019) indicated that in hospitals where probiotic cleaners were employed, there was up to a 99% decrease in the prevalence of antibiotic resistance genes on treated surfaces. Importantly, a study by Caselli *et al.* (2016) confirmed their safety in healthcare settings by tracking the infection rate among over 30,000 patients in seven facilities, finding no infections caused by *Bacillus* species, even in patients at high risk of infection by opportunistic pathogens. The efficient sterilization or elimination of antibiotic-resistant bacteria on inanimate object surfaces continues to be a vibrant research field.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Study area

This study was conducted at the Department of Environmental Management and Toxicology (EMT) student's lecture tables in the University of Benin. This is where students (200 - 500 levels) of the EMT department receives lectures. The tables and chairs are made of wooden materials, which are arranged in three columns and sixteen rows and are capable of accommodating about 250 students.

3.2 Sample collections

Three swab samples were collected from the lecture tables in a systematic manner from the first, middle and last row, (each from a column). The tables were measured at $20cm^2$ by $20cm^2$ using a meter rule. With the aid of a sterile swab, the samples were collected within the measured area and the swab sticks were then transferred to the laboratory immediately for bacteriological analysis. These sampling processes were carried out in duplicates on a weekly interval.

3.3 Materials

The materials used include glass wares such as conical flasks and measuring cylinder, inoculating wire loop, aluminum foil, cotton wool, swab sticks, Bunsen burner, spirit lamp.

Nutrient agar was used for the work. Reagents include; distilled water, methylated spirit, ethanol and stains such as safranin, crystal violet and Gram iodine.

3.4 Washing and Sterilizing of Materials

All glass wares were washed with detergent and were air dried. The glass wares were packed with aluminum foil into canisters and were placed into hot air oven for sterilization at 16˚C for 2 hours. The wares were brought out of the oven and were allowed to cool after sterility has been achieved. They were kept for storage when needed. Work surfaces were cleaned and sterilized by swabbing with 95% ethanol. Aseptic working environment was achieved with the use of Bunsen burner and spirit lamp.

3.5 Preparation of Media

Nutrient agar) were weighed and prepared according to manufacturer's specification. The prepared media was carefully packed into the autoclave and sterilized at 121˚C for 15 minutes. Prior to use, the media were cooled to about 45˚C.

3.6 Serial Dilution

The swab sticks used for the collection of samples was immersed into a test-tube containing 10ml of sterile water to form a stock solution. 1ml of the stock solution was transferred to another test-tube containing 9ml of sterile water to a dilution factor 0f 10^{-1}, this was done serially to a dilution factor of $10^{-5.}$

10^{-2}, 10^{-3} and 10^{-4} dilution factors were cultured on the nutrient agar using the pour plate method.

3.7 Isolation and Identification of Bacterial Isolates

Growths on the plates were noticed after 24 hours incubation and the colonial characteristics were observed, the distinct isolates were sub-cultured on fresh media plates to obtain a pure culture. The pure cultured isolates were stocked into

MacCartney bottles. The isolates were identified based on their morphological appearance, Gram reaction and biochemical characteristics.

3.8 Gram Staining Techniques

A thin smear was made by emulsifying a little portion of organisms picked from stocked colony of 18–24 hours old pure culture into a drop of sterile distilled water on a grease free slide. The smear was air dried and heat fixed by passing it slightly over flame. The slide was carefully placed on the staining rack and was flooded with primary stain (crystal violet) for 30–60 seconds. Gram's iodine was added (mordant) for 30 seconds. The smear was gently rinsed with tap water. 70% ethanol was applied as decolorizer for 10 seconds; it was then stained with the secondary stain (safranin) for 30 seconds before rinsing with tap water and was allowed to dry. The smear was examined under the microscope using oil immersion objective (x100). Gram positive organisms appeared purple while Gram negative appeared red.

3.9 Biochemical Characterization of the Isolates

These tests were carried out to further identify and classify the isolates. They include; Catalase test, oxidase test, indole test, urea hydrolysis (urease test), sugar fermentation test (glucose, sucrose, lactose) respectively.

3.9.1 Oxidase Test

This test is used to identify microorganisms containing the enzyme cytochrome oxidase (important in the electron transport chain). It is commonly used to distinguish between oxidase negative *Enterobacteriaceae* and oxidase positive *Pseudomadaceae*. A piece of filter paper was soaked with a few drops of oxidase reagent (Tetramethyl-p-phenylenediaminedihydrochloride). A colony of the test organism was then smeared on the soaked filter paper. If the organism could produce oxidase, the phenylenediamine in the reagent will be oxidized to deep purple color. The change of color within 10 seconds indicates positive result.

3.9.2 Sugar Fermentation Test

The carbohydrate fermentation test is used to determine whether or not bacteria can ferment a specific carbohydrate. Carbohydrate fermentation patterns are useful in differentiating among bacterial groups or species. It tests for the presence of acid and/or gas produced from carbohydrate fermentation. Basal medium containing a single carbohydrate source such as glucose, lactose, sucrose or any other carbohydrate is used for this purpose. A pH indicator bromothymol blue (BTB), is also present in the medium; which will detect the lowering of the pH of the medium due to acid production. It's a positive test for all members of *Enterobacteriaceae*. The black colour change indicates presence of H_2S and presence of bubbles indicates presence of CO_2 gas.

3.9.3 Catalase Test

This test is used to identify organisms that produce the enzyme catalase. This enzyme detoxifies hydrogen peroxide (H_2O_2) by breaking it down into water and oxygen gas. This test demonstrates the presence of catalase, an enzyme characterized with the release of oxygen from hydrogen peroxide. A drop of 3% hydrogen peroxide solution was added to the sterile slide containing a loopful of the organism. Foaming or bubble indicates a positive result.

3.9.4 Indole Test

This test is used to identify microbes that can break down tryptophan to indole. It is used to identify bacteria of the family *Enterobacteriaceae*. Inocuate sterilized tubes containing tryptophan broth (4 ml) and incubate tubes for 24–28 hrs. After which 0.5 ml of Kovac's reagent is added. Presence/absence of ring indicates positive/negative test.

3.9.5 Urease Test

This is used to identify those organisms that are capable of hydrolysing urea (bacteria that produce urease) to produce ammonia and carbon dioxide. It is primarily used to distinguish urease-positive protease from other *Enterobacteriaceae*. Organisms that hydrolyze urea rapidly (*Proteus* spp.., *Morganella morganii,* and some *Providencia stuartii* strains) will produce strong positive reactions within 1 or 6 hours of incubation; delayed positive organisms (*Klebsiella* spp. and *Enterobacter* spp.) will produce weak positive reactions in the slant in 6 hours of incubation which will be intense during further incubation. The culture medium will remain a yellowish color if the organism is urease negative e.g., *Escherichia coli.* If organism produces urease enzyme, the color of the slant changes from light orange to magenta. If organism do not produce urease the agar slant and butt remain light orange (medium retains original color).

3.9.6 Potassium Hydroxide Test

The KOH String Test relies on the differential resistance to 3% potassium hydroxide between gram positive and negative cells, where a portion of a colony is mixed with a small volume of 3% KOH. If the cells lyses, the liberated cellular DNA makes the mixture viscous or "stringy." The positive string test indicates a gram-negative organism. Hence the alternative name for the test is "String Test".

The potassium hydroxide test may thus aid in differentiation between Gram positive and Gram-negative organisms and is a useful complement to the Gram stain and the antibiotic disc test.

Expected Results:

For positive results, the isolates become thick, stringy and form long strands within the first 30sec. This is seen in Gram negative bacteria. While for negative results, the isolates leave the suspension unaltered or absence of stringing. This is seen in Gram positive bacteria.

3.9.7 Antimicrobial Sensitivity Test

The antimicrobial sensitivity test method used was the disk diffusion method, which requires the use of antibiotic-containing wafers or disks to test whether particular bacteria are susceptible to specific antibiotics. First, pure bacteria isolate was cultured. Then, a known quantity of bacteria was grown overnight on agar (solid growth media) plates in the presence of a thin wafer that contains a known amount of a relevant antibiotic. If the bacteria are susceptible to the particular antibiotic from a wafer, an area of clear media where bacteria are not able to grow surrounds the wafer, which is known as the zone of inhibition. A larger zone of inhibition around an antibiotic-containing disk indicates that the bacteria are more sensitive to the antibiotic in the disk.

CHAPTER FOUR

RESULTS

Figure 4.1 shows the total heterotrophic bacterial count from the table surfaces at two different weeks. There were 3 samples collected twice (1^{st} and 2^{nd} rounds) on a weekly basis from EMT student lecture table, making a total of 6 samples collected. 4 isolates were obtained in the first round and 5 isolates in the second round of the sampling collection.

Fig 4.2 shows the different bacteria population between the first and second week of sample collection. The bacteria population in the second week appeared more than that of the first week, indicating a higher level of contamination.

Table 4.1 shows the cultural, morphological and biochemical Characteristics of the bacteria Isolates as well as their probable identity.

Table 4.2 shows the antibiotic sensitivity of the bacteria isolates, indicating their susceptibility, intermediate and resistivity ranges against certain antibiotics.

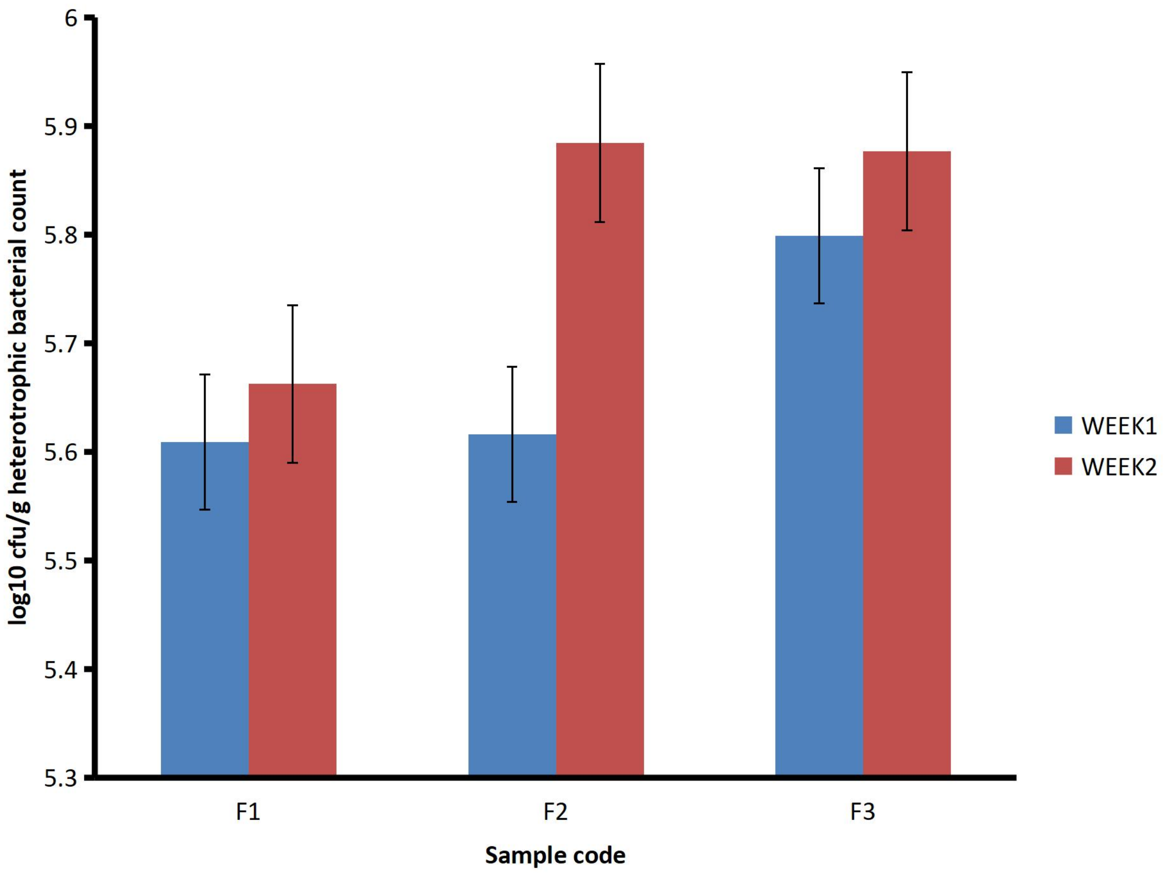
6
5.9
5.8
5.7
5.6
5.5
5.4
5.3
log10 cfu/g heterotrophic bacterial count
F1
F2
F3
Sample code
WEEK1
WEEK2

Figure 4.1: Total Heterotrophic Bacterial Count (log_{10}cfu/g)

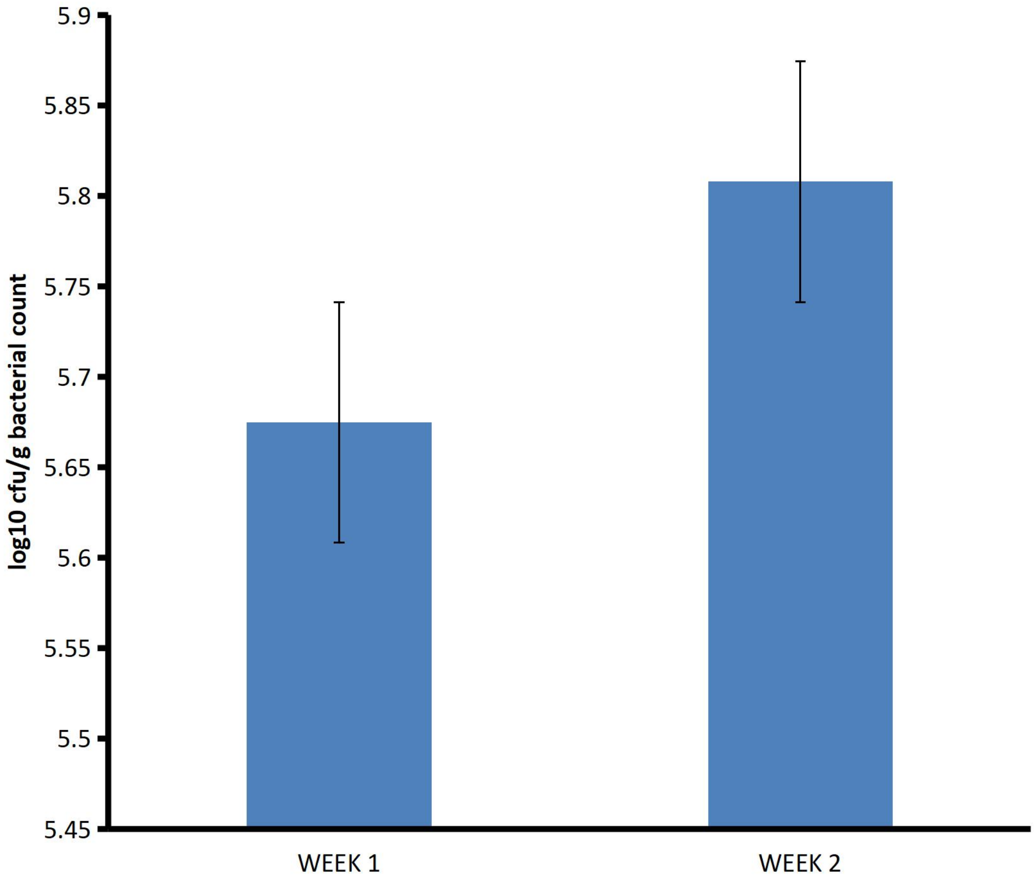

Figure 4.2: Difference in Bacteria population between week 1 and week 2

Table 4.1: Cultural, Morphological and Biochemical Characteristics of Isolates

Colony	1	2	3	4	5
Elevation	Flat	Flat	Flat	Raised	Raised
Margin	Undulate	Undulate	Entire	Entire	Undulate
Color	Cream	Cream	Cream	Lemon	Cream
Shape	Irregular	Irregular	Circular	Circular	Irregular
Size	Large	Large	Small	Medium	Small
Differential agar	EMB	EMB	EMB	PCA	MSA
Colour	Purple	Green	Pink	Green	Yellow
Morphological					
Gram stain	-	-	-	-	+
cell type	Rod	Rod	Rod	Rod	Cocci
Arrangement	disperse	disperse	disperse	disperse	clusters
Color	pink	pink	pink	Pink	Purple
Biochemical					
KOH test	+	+	+	+	-
Indole	-	+	-	-	-
Citrate	+	-	+	-	+
Oxidase	-	-	-	+	-
Urease	-	-	+	+	+
Glucose	+	+	+	-	+
Sucrose	-	+	-	-	+
Lactose	-	+	-	-	+
Mannitol	-	-	-	-	-
Gas formation	+	+	+	-	-
H2S formation	-	-	+	-	-
Probable identity	*Enterobacter aerogenes*	*E. coli*	*Klebsiella oxytoca*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*

Table 4. 2: Antibiotic sensitivity test

ISOLATES	CS	CIP	GEN	E	TE	M	CD	AUG
E.coli	0(R)	16(S)	14(S)	0(R)	0(R)	0(R)	9(R)	12(I)
Staphylococcus sp.	0(R)	14(S)	15(S)	8(R)	0(R)	7(R)	11(I)	14(S)
Enterobacter sp.	0(R)	22(S)	15(S)	10(I)	17(S)	7(R)	0(R)	9(R)
Klebsiella sp.	0(R)	24(S)	19(S)	10(I)	10(R)	0(R)	10(R)	15(S)
Pseudomonas sp.	4(R)	14(S)	17(S)	2(R)	0(R)	0(R)	4(R)	17(S)

Key:

CS - Ceftriaxone Sodium, CIP - Ciprofloxacin, GEN - Gentamicin, E - Erythromycin, TE - Tetracycline, M - Metronidazole, CD - Clindamycin, AUG - Augmentin

Range for interpretation:

1- 9 (Resistant)

10-13 (Intermediate)

14- 24 (Susceptible)

CHAPTER FIVE

DISCUSSION

This study aimed in isolation and identification of bacteria from EMT lecture tables in the University of Benin. The lecture tables are commonly touched with hands, which may act as a source of hand transfer of disease. Various bacterial spp. was found to coexist on the table surfaces and on the hands of the students. These surfaces harbour a community of bacteria with varying virulence and pathogenicity, thereby increasing the risk of infection and also the severity of infections. In this study, *Staphylococcus aureus, E. coli, klebsiella* spp., *pseudomonas* spp. and *Enterobacter aerogenes* were isolated from the lecture tables.

The samples which were collected twice on a weekly basis had different results as more bacteria isolate were obtained in the second week due to the full resumption of the students and lectures rather than the first week where very few students were present, this was revealed in Fig 1 and 2.

Kramer *et al.* (2006), concluded in their study that the common nosocomial pathogens may well survive or persist on surfaces for months and can thereby be a continuous source of transmission if no regular prevention surface disinfection is performed. Although Ayeliffe (1991), remarked that the inanimate environment has little relevance to the spread of infection, other workers noted that the fomites are involved in the transmission of pathogens in health care environments. The similarity in the bacterial loads recorded on interfaces studied can be attributed to frequent dermal contact and sharing by numerous individuals with differing hygiene practices and health conditions. The prevalence of microorganisms present on a surface is one of the microbe-associated factors that determine whether an infection will occur or not. The bacterial load on a fomite also determines the survival of bacteria on that fomite;

the higher the concentration of a microorganism on a fomite the longer it survives and this invariably increases the chances of picking up the microbe from the environment. In addition, Neely and Maley (2000), showed that microorganisms can survive for longer on plastics, the main material of which most accessible components of user interfaces are composed, than on other surfaces such as fabrics or steel. Thus, the ability of microorganisms to survive long on plastic user interfaces suggests the possibility of their serving as reservoirs for microorganisms and as a vehicle for their transfer. Rutala and Weber (2004) had similar results with their bacterial counts on keyboards at a university health-care system. They found out that student's hands had more contamination than the surfaces themselves. The magnitude of the bacterial load on hands shows that students hands are probably a major source of bacterial contamination on the table surfaces, since on daily basis hands typically touch a continuous sequence of surfaces, substances, objects, skin, food and body fluids. In spite of the number of bacteria present at any one time, the type and quality of microorganism found on a surface is also an important determinant of whether an infection will occur or not. Most skin flora bacteria are Gram-positive, which would account for their predominance on the table surfaces.

Staphylococcus aureus, was found to being the most frequent bacterial contaminant on the lecture table surfaces. *Staphylococcus aureus* is a major component of the normal flora of the skin and nostrils, which probably explains its high prevalence as a contaminant, the prevalence of *S. aureus* lies on its easy dissemination resulting from human activities like sneezing, talking and contact with moist skin. *Enterobacter aerogenes,* was the least frequent bacterial contaminants in most studies, although *Enterobacter aerogenes* have also been isolated from environmental objects. The conducive environment provided by the students as a result of their unhygienic practices may account for this problem. This could be related to the fact that multiple contaminations are influenced by the level of personal hygiene exhibited by the students, since most display a poor level of hygienic practice during their break.

The findings of this study do not necessarily mean that the table surfaces are consistently safe. The abundance of *Staphylococcus aureus* which is one of the skin florae in this study is an indicator that transmission of pathogenic bacteria from the skin of infected persons to the table surfaces is more than plausible. In this case, such surfaces will be highly dangerous media for transferring pathogenic bacteria from infected persons to others coming into contact with these contaminated surfaces.

5.1 CONCLUSION

This study indicated the various bacteria present on EMT student lecture tables, which were *Staphylococcus aureus, E.coli, klebsiella* sp, *Pseudomonas* sp. and *Enterobacter aerogenes.* The table surfaces can serve as a medium for infection transfer, as there were pathogenic bacteria present. The comparison between the first and second week indicates that the contamination of the table surfaces occurred more from the activities and poor hygiene of the students rather than the environment, having noted that the first week with fewer students and their activities resulted into fewer numbers of microorganisms when compared to the second week with more numbers.

5.2 RECOMMENDATIONS

Proper disposal of dirt and hygiene practices should be carried out by the students as well as regular cleaning of the table surfaces before use. If possible, hand wash basins and soap could be situated close to the lecture room and the students should be sensitized on the importance of observing proper personal hygiene especially while having meals.

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