BACTERIA CONTAMINATION OF WASH HAND BASINS IN UNIVERSITY OF BENIN COMMUNITY, BENIN CITY, EDO STATE, NIGERIA.

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BMS 1601792

TO

THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE,

SCHOOL OF BASIC MEDICAL SCIENCES,

UNIVERSITY OF BENIN,

BENIN CITY,

EDO STATE.

SUPERVISED BY: PROF I.N. IBEH

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IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, UNIVERSITY OF BENIN FOR THE AWARD OF ''BACHELOR OF MEDICAL LABORATORY SCIENCE''

CERTIFICATION

This is to certify that this project work titled "**BACTERIA CONTAMINATION OF WASH HAND BASINS IN UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA**" was carried out by **ADEKUNLE VICTORIA DANIEL**, in the Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City, Edo State.

_____ **Date** _____

Supervisor:Prof. I.N. Ibeh

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DEDICATION

This project work is dedicated to the Almighty God; the giver of life and also to my beloved parents and my lovely siblings.

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ABSTRACT

Microorganisms are living things ordinarily too small to be seen without magnification, in terms of numbers and range of distribution, microbes are the dominant organisms on earth. The hands are probably the most exposed organs of the human body, to environmental bacterial contamination. They are the central organs for the physical manipulation and control of the surrounding environment. Wash hand basins are common amenities provided in toilets and other facilities used primarily for washing of hands and other purposes. They can become contaminated thereby putting the general public at a risk of infection. The aim of this study was to evaluate the bacterial contamination of wash hand basins in University of Benin Community, Benin city, Edo state, Nigeria.A total of 100 samples were collected asceptically from wash hand basins in toilets and other public facilities in University of Benin using sterile swab sticks moistened in normal saline. Swab samples were collected specifically from the knob of the taps and the surface area where hands are rested on and analyzed by culturing on chocolate and MacConkey media and biochemical tests were carried out on isolates.The result from the study shows the prevalence of bacterial contaminating wash hand basins was 58.6%. A total of eight (8) bacteria isolated, which are *Klebsiella* spp*, Providencia* spp*, Pseudomonas* spp*, Streptococcus faecalis, Staphylococcus aureus, Enterobacter* spp*, Escherichia coli* and *Proteus mirabilis.* However, *Staphylococcus aureus* has the highest prevalence rate of 36.6% which was followed by *Klebsiella* spp. With 19.5%, *Escherichia coli* with 14.6%, *Enterobacter spp.* With 12.2%, *Providencia* spp. *Pseudomonas* spp. and *Proteus mirabilis* has 4.9% each, and lastly *Streptococcus faecalis*with 2.4% respectively. In conclusion, this study determined the distribution of bacterial transfer rates between various site of sampling surfaces of wash hand basins commonly encountered during hand washing in the University environment. The results shows a relatively high prevalence that bacterial transfer rates among student hands and wash

hand basins surfaces are highly variable, and faucet spigots may be a significant source of cross-contamination. Therefore, daily cleaning and disinfection in conjunction with a regular hygiene service are recommended to reduce cross-infection risks in washrooms and toilets.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

The hands are probably the most exposed organs of the human body to environmental bacterial contamination.. They are the central organs for the physical manipulation and control of the surrounding environment. It is impossible for the hands to be free of microorganisms and that is why they serve as a means of transmission of microorganisms from an individual to another and from one place to another. In addition to the normal flora of the hand, the human hands usually harbors transient microorganisms contracted from the environment (Dodrill *et al*., 2011).The most prominent bacteria present on every healthy hand include: *Staphylococcus epidermidis*, *Corynebacterium* spp, *Micrococcus* spp, in addition to some members of the family Enterobacteriaceae (Leyden *et al*., 1991). Transient Pathogenic bacteria that may be present on the hand include: *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella* spp *Enterobacter* spp*, Streptococcus* spp and *Staphylococcus aureus* (Orskov *et al*., 1997). Some of these transient bacteria can sometimes become notorious pathogens that may lead to acute or even chronic illness.

Scientific studies have revealed that commonly used surfaces such as telephones, computers, books, door handles, ATM and vending machines, desks and numerous other items can be a major source of potentially pathogenic bacteria (Dakroub and Nawas, 2017). Microorganisms that colonizes parts of the body without causing infection, these ones are called normal body flora, but can cause infection when found in another site of the body. The normal flora of gastrointestinal tract which are the *enterobacterieaceae* are passed out through excreta, faecal matter remains a major reservoir source of human pathogens, which in adverse situation may

bring about outbreaks of infection example shigellosis (Francesco, 2010). The occurrence of this may be attributed to the unhygienic use of the toilet facilities, which results to the gross contamination of the place including wash hand basins, which individuals are less likely to see as contaminated (Francesco, 2010). These objects upon contamination serve as transmission vehicles of infection, such that the user may succeed in picking these pathogens even after performing the practice of hand washing. The organism thus picked can be introduced into such individual either orally/topically or can be transferred to another person.

A wash hand basin is a bowl or basin that is typically fixed to a wall or pedestal for washing one's hands and face (Oxford, 2022). Given their warm and humid environment, wash hand basins provide an ideal setting for the survival of microorganisms. Many pathogenic organisms such as bacteria and norovirus can survive on environmental surfaces for weeks or months (Kramer *et al*., 2014). Wash hand basins can be classified as a formite since it's an inanimate object that can become colonize by bacteria. Fomites can be described as non-living objects that are capable of carrying infectious organisms and can transmit them from one person to another (Cramer *et al*., 2018). Fomites has been shown to play a role in transmission of human pathogen either directly, by surface to mouth contact, or indirectly, by contamination of fingers and subsequent hand to mouth contact (Butz *et al*., 2004). In the 21st century, fomites have higher role than ever in transfer of disease in human history because of human lifestyle (Cook and Nigel, 2015).

As of 2017, no fewer than 120 million people in Nigeria lacked access to improved sanitation facilities, thereby exposing them to public health hazards (UNICEF, 2017). Inadequate access to sanitation and hygiene facilities is known to be a leading cause of morbidity and mortality, particularly in low-income countries (Cook and Nigel 2015). In fact, approximately 10% of the

global burden of disease is thought to be attributed to inadequate water, sanitation, and hygiene(WASH), which is largely driven by increased exposure to human pathogens transmitted via the fecal-oral route (UNICEF, 2022). A lack of safely managed WASH infrastructure may drive exposure to enteric pathogens through improper hand hygiene, or through direct contact with contaminated surfaces (WHO, 2022).

Pathogenic organisms, that is viruses, bacteria and protozoa, may be excreted in large numbers in biological substances including blood, mucus, saliva, faeces and urine (Islam *et al*., 2004). Pathogens are readily transferred to hands from contaminated fomites and to the mouth from contaminated hands (Rusin *et al*., 2009) hence putting the health of students at risk; if the transfer remain unchecked. The risk of disease transmission through surfaces involves a number of factors including 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 the organism, immuno-competence of the persons in contact; and the practice of control measures (disinfectant use and personal hygiene) and other factors (Kelly *et al*., 2009).

Most individuals who make use of the wash hand basins provided in public restroom and in other facilities are of the impression that these amenities are hygienic. However, such facilities may be potential sites for the transmission of pathogenic bacteria. Little attention has been paid to the potential risks of recontamination of hands from contaminated washbasin surfaces (Lorna *et al*., 2019). Certain conditions such as temperature, moisture, carbon, nitrogen, pH e.t.c, when all this conditions are met in the wash hand basins bacteria can proliferate. Bacteria are ubiquitous and are found more frequently to be the cause of community acquired infections in immune compromised patients (Pessi *et al*., 2005). The hand is a common means of direct contact with

inanimate objects and surfaces, the process of touching surfaces and objects the hand could pick up infectious agents from the surfaces and objects (Pittet *et al*., 2000).

1.2 Justification of Study

Wash hand basins are common amenities provided in toilets and other facilities used primarily for washing of hands and other purposes. Wash hand basins can become contaminated with pathogenic organisms for example, bacteria excreted in large numbers into biological substances such as blood, mucus, saliva, thereby contaminating the wash hand basin and putting the general public at a risk of infection (Islam *et al*., 2004). These pathogens are readily transferred to hands from contaminated surfaces and to the mouth from contaminated hands (Rusin*et al*., 2009) hence putting the health of individuals at risk if the transfer remain unchecked. However, in our environment today where wash hand basins in public facilities are not well taken care off, individuals can become infected with multi drug resistant organisms such as Methicilin Resistant *Staphylococcus aureus* (MRSA). Hence, this study is aimed at determining and evaluating the bacterial contamination of wash hand basins in University of Benin Community, Benin city, Edo state, Nigeria.

1.3 Aim of the Study

The aim of this study was to evaluate the bacterial contamination of wash hand basins in University of Benin Community, Benin city, Edo state, Nigeria.

1.4 Specific Objectives

- To identify the common types of bacteria present in wash hand basins used in the university of Benin community
- To determine the prevalence of bacteria contaminating wash hand basins in University of Benin community.

- To determine the relationship of the bacteria isolated and the microbial contamination of the wash hand basins.
- To determine the antibacterial sensitivity pattern of the various organisms isolated.

1.5 Research Hypothesis

HA: There is high bacteria contamination of wash hand basins in the university of Benin community which can cause transmission of disease to individuals that comes in contact with t.

HB: There is low bacteria contamination of wash hand basins in the university of Behemnin community which cannot cause transmission of disease to individuals that comes in contact with them.

CHAPTER TWO

2.0 LITERATURE REVIEW

The wash hand basin is known by many other names and they include; sink, washbowl, hand basin wash basin and simply basin (Collins, 2021). It is a bowl-shaped plumbing fixture used for washing hands, face and other purposes. The wash hand basin has faucet (taps handle) that supply water, it also include a drain for removal of used water and may also have integrated soap dispenser. Given their warm and humid environment, wash hand basins provide an ideal setting for the survival of microorganisms. Many pathogenic organisms such as bacteria and norovirus can survive on environmental surfaces for weeks or months (Gunter, 2020) Contaminated environments may also serve as vehicles for the acquisition and spread of methicillin resistant *Staphylococcus aureus* (MRSA) to the nose, eyes or mouth of washroom and public wash hand basin users via indirect contact (Walsh *et al* 2017). Contaminated areas not only include toilet facilities and their immediate environment (Sheppard *et al*., 2019) but also hand-drying facilities. Little attention has been paid to the potential risks of recontamination of hands from contaminated wash-room facilities and surfaces. This study aimed to examine the cleanliness of the washroom environment and in particular the wash hand basin of public washrooms .Given that countless people use public washrooms on a daily basis, the findings of this study may have significance public health.

2.1 History of Wash Hand Basin and Hand Washing

2.1.1 18th Century Wash Hand Basin

Looking over history, wash hand basins originated from washstands. A washstand or basin stand is a piece of furniture consisting of a small table or cabinet, usually supported on three or four legs, and most commonly made of mahogany, walnut, or rosewood, and made for holding a wash basin and water pitcher. In the 16th Century, the wealthy would have wooden washstand in their bedrooms, which would hold an iron pitcher and bowl to wash their face and hands (Hugh, 1911). In its 18th-century form the washstand was called a basin stand or basin frame, and is still sometimes described as a wash hand stand. Its direct, but remote, ancestor was the monastic lavabo, ranges of basins of stone, lead or marble fed from a cistern (tank). They were usually of primitive conception, and a trough common to all was probably more frequent than separate basins (Hugh, 1911) Great numbers of these stands were made to fit into corners, and a corner wash stand is still a common object in old furniture shops. (Fig. 2.1)

Figure 2.1: 18th Century Wash basin.

WIKIPEDIA, 2021

2.1.2 19th and early 20th Century Wash Hand Basin (Fig 2.2)

With the beginning of the 19th century and the expansion of ideals of personal cleanliness, the washstand grew in size and importance. It acquired the form of an oblong wooden table provided, like its smaller predecessors, with orifices for basins and fitted with a brad shelf-like stretcher upon which the jugs were placed when they were removed from the basins. Ample space was provided for soap-dishes and water bottles. It was supported upon a tripod; a circular orifice in the top received the basin and smaller ones were provided for a soap dish and a water-bottle. Sometimes a stand for the water-jug when the basin was in use was provided (Hugh, 1911). In the first quarter of the 20th century, the wooden top of the washstand was replaced by marble, unpierced, the basins being placed upon the slab, which, in the beginning almost invariably white, were later made of red or other warm-tinted marble (Hugh, 1911). The larger ones, which possessed receptacles for soap-dishes, were the predecessors of the modern bathroom wash basin, or sink. Both varieties, often of very elegant form, were in extensive use throughout a large part of the 18th century and early-19th century, eventually disappearing with the advent of modern indoor plumbing. As personal hygiene became more important, so did washstands. These grew in size and possessed more accommodating basins and space for soap.

Figure 2.2: 19th Century Wash basins.

WIKIPEDIA, 2021.

2.1.3 History of Hand Washing

Hand washing with soap and water has been considered a measure of personal hygiene for centuries (Rotter, 2000 and Jumaa, 2005) and has been generally embedded in religious and cultural habits. Nevertheless, the link between hand washing and the spread of disease was established only two centuries ago, although this can be considered as relatively early with respect to the discoveries of Pasteur and Lister that occurred decades later. In the mid-1800s, studies by Ignaz Semmelweis, a Hungarian working in Vienna General Hospital, Austria, and Oliver Wendell Holmes in Boston, USA, established that hospital-acquired diseases were transmitted via the hands of health care works (WHO, 2009). In 1847, Semmelweiss was appointed as a house officer in one of the two obstetric clinics at the University of Vienna Allgemeine Krankenhaus (General Hospital).Semmelweis, 1861 observed that maternal mortality rates, mostly attributable to puerperal fever, were substantially higher in one clinic compared with the other (16% versus 7%). He also noted that doctors and medical students often went directly to the delivery suite after performing autopsies and had a disagreeable odour on their hands despite hand washing with soap and water before entering the clinic. He hypothesized therefore that "cadaverous particles" were transmitted via the hands of doctors and students from the autopsy room to the delivery theatre and caused the puerperal fever. Following the implementation of hand hygiene, the mortality rate fell dramatically to 3% in the clinic most affected and remained low thereafter (WHO, 2009)

A few years later in Scutari, Italy, the Crimean War brought about a new handwashing champion, Florence Nightingale. At a time when most people believed that infections were caused by foul odors called miasmas, Florence Nightingale implemented hand washing and other hygiene practices in the war hospital in which she worked. While the target of these practices

was to fight the miasmas, Nightingale's hand washing practices achieved a reduction in infections (Global hand washing, 2020).

Sadly, the hand hygiene practices promoted by Semmelweis and Nightingale were not widely adopted. In general, hand washing promotion stood still for over a century. It was not until the 1980s, when a string of food borne outbreaks and healthcare-associated infections led to public concern that the United States Centers for Disease Control and Prevention identified hand hygiene as an important way to prevent the spread of infection. In doing so, they heralded the first nationally endorsed hand hygiene guidelines, and many more have followed (Global hand washing, 2021).

2.1.4 Proper Hand Washing

Hand washing is one of the most effective ways to prevent the spread of bacterial and viral illnesses. Throughout the day, people's hands accumulate germs and dirt as they touch objects and other people. Individuals can then transfer these substances to others, or infect themselves when touching their face(Jayne, 2020). Regular hand washing can limit the transfer of microbes, such as bacteria and viruses. However, many people do not wash their hands properly or long enough to get rid of germs (jayne *et al*., 2020. It is recommend that washing and scrubbing the hands is done for at least 20 seconds (CDC, 2022). In the absence of a timer, they suggest singing the "Happy Birthday" song twice while washing (CDC, 2022). Different temperature of water does not have a significant effect on the number of bacteria that hand washing removes it is the thorough washing technique is what removes the bacteria (Dane *et al*., 2017). However, warm water can be more pleasant than cold water, especially when washing the hands for 20 seconds (Dane *et al*., 2017). Plain soap and water are very effective at removing microbes from the skin. It does not matter what kind of soap a person uses. There is not enough evidence to

show that over the counter antibacterial soaps are any better at killing microbes than regular soaps (FDA, 2019).

The Food and Drug Administration (FDA) has announced the recalls of several hand sanitizers due to the potential presence of methanol. Methanol is a toxic alcohol that can have adverse effects, such as nausea, vomiting, or headache, when a significant amount is used on the skin. More serious effects, such as blindness, seizures, or damage to the nervous system, can occur if methanol is ingested. Drinking hand sanitizer containing methanol, either accidentally or purposely, can be fatal (FDA, 2019).

2.1.5 Why wash hands?

Proper hand washing is necessary to reduce the risk of: transferring dirt and microbes to surfaces and other people colds and flu, coronavirus (COVID-19), viral infections that cause diarrhea, including norovirus, eye infections, pathogen such as Methicilin-resistant *Staphylococuss aureus.* Proper hand washing can prevent the spread of illness and infection. It is a simple yet effective technique that anyone can use (Jayne, 2020). To properly wash the hands, use soap and water, and rub every surface of the fingers and hands for at least 20 seconds as seen in fig 2.3 . Thoroughly dry the hands on a disposable or clean towel, or allow to dry in the air (WHO 2021).

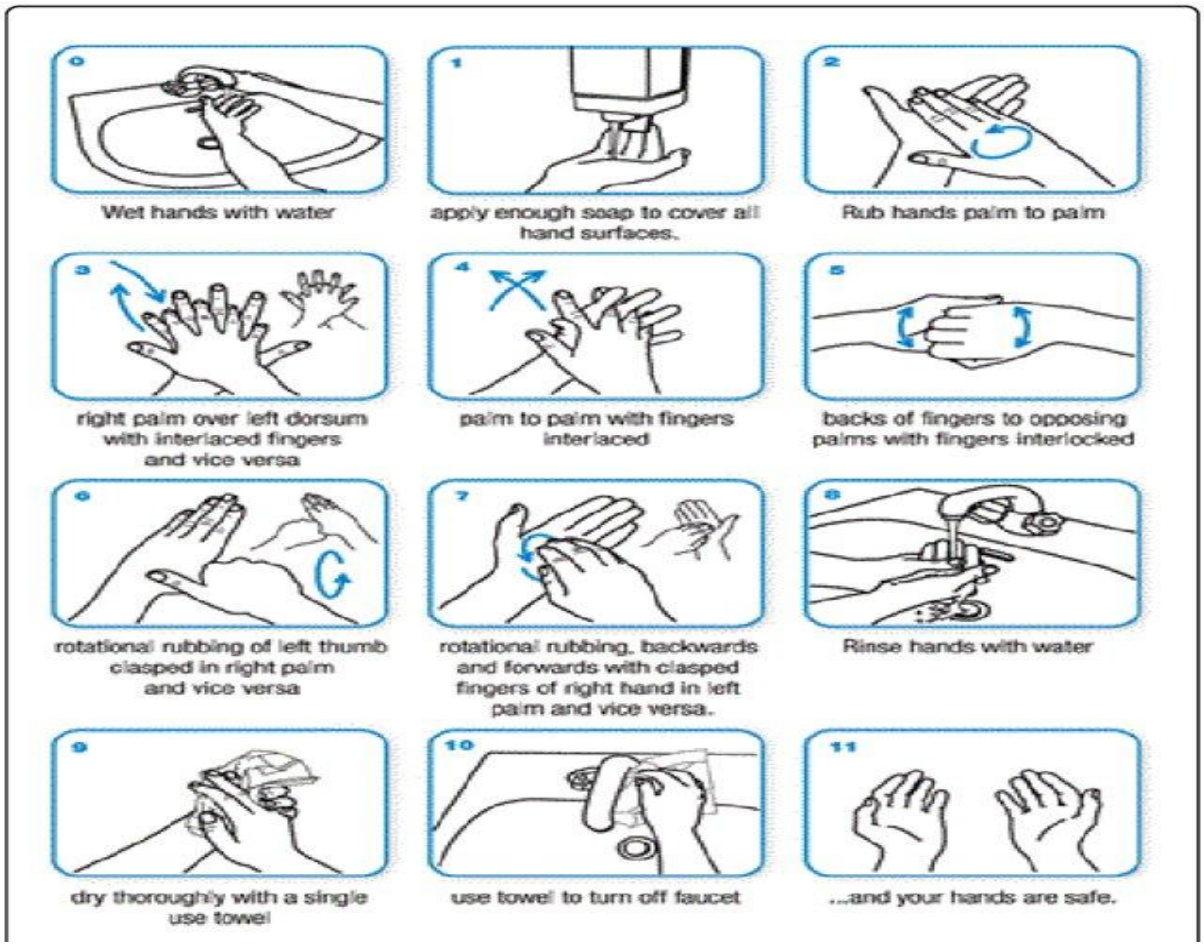

Figure 2.3: Hand Washing Technique by WHO.

WHO, 2021.

2.2 Contamination of Wash Hand Basin

For decades we've been taught that hand hygiene is the most critical aspect of infection control. Although that may be true, what about the wash hand basin and faucets (tap). These oft overlooked areas can easily pose infection control risks. How clean can your hands really be if the sink and faucet are heavily contaminated and dirty (Saskia, 2019).

Blake *et al* 2019 identified sink proximity to toilets as a risk factor for contamination. Bacteria like *Staphylococcus aureus*, *Klebsiella pneumoniae*, producing organisms tend to be prolific in moist environments and are often pervasive in intensive care unit sinks and drains. Researchers found that sinks near toilets were 4-times more likely to host the organisms than those further from toilet (Saskia, 2019).

2.3 Public Places and Bacteria

The human skin is the main organ of contact with microbes. The skin on its own houses many bacteria which could be beneficial or harmless. Even though most bacteria found on the human skin are harmless, *Staphylococuss aureus* (Kloos and Mussel, 1975), *Corynebacterium minutissimum,* and *Pseudomonas aeruginosa* could cause skin diseases, such as atopic dermatitis and erythrasma (Ross and Neufeld, 2015). Studies have shown that bacteria, such as *Acinetobacter, calcoaceticus* and *Staphylocuss aureus,* commonly occur on hospital surfaces. "Hospital" bacteria lead to nosocomial infections which are infections acquired in hospital settings. In this research, the life span of the bacteria found on dry hospital surfaces was found to be 13 days,which could be an additional factor in transmission of nosocomial infections (Getchell-White and Groschel, 1989). In the past years, much attention has been paid to nosocomial infections as the pathogens causing these infections became resistant to antibiotics. In comparison, there are few studies focusing on infections originating on public surfaces, such

as public telephones, shopping carts, buses, shopping carts, office furniture, and even on surfaces in university campuses. Public telephones, which are commonly used in some parts of the world, have been investigated and are found to harbor pathogens. A particular study carried out in Melbourne, Australia, identified certain potentially pathogenic bacteria on telephones, such as *Acinetobacter anitratus, Enterobacter cloacae, Pantoea agglomerans,* and *Staphylococcus aureus* (Ferdinandus *et al.,* 2001). From this study, it was ascertained that telephones could be labelled as potential reservoirs for pathogens. Similarly, transportation systems such as buses and trains harbor pathogens. Transport systems in Portland, Oregon, USA, were investigated for the presence of pathogens on seats, floors, and railings (Yeh *et al., 2011*). The research further analyzed the pathogens for any resistance in certain antibiotics. Another "public" place, university campuses, may have high population densities, which makes it easier for bacteria to spread. Students may be exposed to many health risks on campuses, which can be contracted from unclean surfaces, cafeteria food, or bathrooms. Communicable diseases spread easily in places with many people as is the case with universities. The presence of harmful bacteria in a university can pose a threat to the health of students, staff, and faculty (Morris, 2016). These bacteria can be easily passed around because as people interact in congregated areas, they may pick up bacteria which could potentially infect them as well as people around them (Shanks and Kelly, 2009). Students in a university normally make use of computers, library study tables, restrooms, and other utility. All these surfaces may harbor bacteria and could potentially harm people.

2.3.1 Bacteria in Universities

Previous studies on bacteria in university campuses have confirmed the presence of bacteria on surfaces. For example, pathogens such as *Escherichia coli* have been found on university tables

in clinics, laboratories, and libraries (Burnham and Haas, 2009). Disinfection protocols may have an impact on the level of contamination. However, pathogens could still be present regardless of the cleaning being done on surfaces. Health risks to students on university campuses could result from the use of public computers, ingestion of cafeteria food, use of bathrooms, or contact with railings, and door handles. Cafeteria may be cleaned frequently, but may still harbor bacteria. Food particles could fall off on the tables in a cafeteria, and if the tables are not probably cleaned, could be the cause of microbes being present. Contact between students and these tables could transfer pathogens to people. Bathrooms are generally known to harbor bacteria. Public restrooms are visited by people with different hygiene behaviors, which can influence the type of bacteria found on restroom surfaces (Flores *et al.,* 2011).

Depending on the cleaning schedule of a facility, the level of contamination can be minimal. In addition to restrooms, computer keyboards in computer labs could also be infected with bacteria. Campus computers are often accessed by many people daily, and frequent contact could contribute to high microbial activity. Keyboards may not be routinely cleaned, as well (Anderson and Palombo, 2008). Microorganisms, such as *Staphylococuss aureus*, Enterobacteriaceae, *Enterococcus faecalis,* and *Bacillus cereus*, were found on computer keyboards in a university computer lababoratory with *Staphylococuss aureus* having the highest colony growth on computers used by multiple people. Another study conducted in a university in New York City sampled different surfaces in lecture halls, restrooms, libraries, and cafeterias (Shanks and Kelly, 2009). The findings revealed that the bacteria found on these surfaces, including *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, were resistant strains. This poses a greater risk to people on campuses as the diseases caused by these pathogens will be harder to treat (Shanks & Peteroy-Kelly, 2009).This shows that students may potentially become infected and that these

infections might be hard to curtail due to the resistant strains. The health risk to students may be even higher if bacteria found on campuses have developed a resistance. Such resistance is often due to a mutation or frequent use of an antibacterial agent to clean the surfaces. The bacteria could adapt to the substance being used to clean and natural selection will take place. The only way to curtail diseases caused by bacteria resistant to antibiotics is by prescribing the right dosage of antibiotics. This is a pressing public health issue in addition, the rise in antimicrobial resistance has also been attributed to the frequent use of antimicrobial agents while cleaning. Some bacteria found on public surfaces that have resistant strains include *Escherichia coli*, *Salmonella* spp, and *Enterococcus* spp (Conly, 2002). The bacteria mentioned are often found on campuses. The different types of bacteria found on surfaces depends on seasons and the different uses of the buildings (Dunn *et al*., 2013). The level of microbial contamination in a university depends on several factors. These include the cleaning schedule practiced by the Facilities Management and, cleaning materials and products being used (e.g., types of chemicals). The surfaces of a university campus can be seen as non-critical since the surfaces come in contact mostly with human skin and is not necessarily contaminatedby blood and bodily fluids (Rutala and Weber, 2001). The U.S based Center for Disease Control and Prevention (CDC) has emphasized the need to disinfect surfaces even though they have not been contaminated with blood or body fluids (Rutala and Weber, 2001). If the surfaces get cleaned frequently with a strong disinfectant, the bacterial load will be minimal or negligible.

2.4 Antibiotic Resistance

Antimicrobial resistance has become a major global concern. Aside from bacteria and viruses, other microorganisms are rapidly developing a resistance to antimicrobial agents being used to kill them. This evolution makes the treatment of infectious diseases less effective and, in the long

run, may cause death. Though sometimes viewed as an apocalyptic fantasy, antibiotic resistance, which might allow even minor injuries to kill, is indeed a reality in the 21stCentury (WHO, 2014). Antimicrobial resistance is a global concern because it will make the treatment of infectious diseases less effective and prolong illnesses (WHO, 2016). The increasing global concern for antimicrobial resistance has urged scientists to further research this issue. Projections made by scientists showed deaths that could be attributable to antimicrobial resistance yearly by 2050 will be high. Most deaths will most likely occur in Asia and Africa. Although antimicrobial resistance is increasing, less research have been carried out on new drugs to curtail these resistant pathogens.

2.4.1 Resistance in Bacteria

Dangerous, antibiotic resistant bacteria have been observed with increasing frequency over the past several decades (Richard, 2014). Bacterial resistance to antibiotics has been a recognized reality almost since the dawn of the antibiotic era, but only within the past twenty years has the emergence of dangerous, resistant strains occurred with a disturbing regularity. This escalating evolution of resistance coupled with a diminished antibiotic pipeline has led some to claim that a post-antibiotic era is eminent (Appelbaum, 2012).

Various bacteria have developed a resistance to particular antibiotics.To name a few, bacteria such as *Mycobacterium tuberculosis* and *Staphylococcus aureus* have developed a resistance to certain antibiotics (Shanks and Kelly, 2009). *Klebsiella pneumoniae*, a bacterium known for causing intestinal infections, is resistant to carbapenem antibiotics and has spread globally, leading to several deaths (WHO, 2016). Additionally, *Escherichia coli* which causes intestinal infections, is resistant to fluoroquinolone antibiotics. Currently, many countries around the world have no effective treatment for these bacteria.

2.5 Role of Fomite in Disease Transmission

Conceptualized as early as the 1500s, fomites (or fomes) werefirst thought of as "seeds of disease"found in the clothing ofinfected individuals that spread contagion long distances by indirect human contact (Nutton *et al*.,1990). Today, fomites are generally considered any inanimate object that, when contaminated with infectious organisms, can serve as a means of transferring disease causing agents to a new human host. Because people in industrialized countries spend approximately 90% of their time indoors (Klepeis *et al*., 2002), the most important fomites for contamination and transmission tend to be those found in the built environment and those that humans frequently come into direct contact with, such as doorknobs, countertops, medical equipment, handrails, clothing, and mobile phones. As our understanding of microbes in the built environment has greatly expanded in the last decade, so has our understanding of fomites and their role in the transmission of infectious agents and other microbial matter to and from humans. Here, we review the recent body of literature on fomite contamination and microbial survival in the built environment, factors that affect transmission of microbes between fomites and humans, and the implications for human health.

2.5.1 Indoor Microbiomes

We live in a microbial world. Viruses, bacteria, protists, fungi,and archaea exist in all of our inhabited environments(Gilbert *et al*.,2018). In buildings, we shed microbes directly to the indoor air and onto building surfaces (Adams *et al.,* 2015; Hospodsky *et al*., 2012), microbes are transported indoors from outdoors, and we also acquire microbes from our surroundings (Lax *et al*.,2017; Lai *et al*., 2017). Human occupancy and activity, the outdoor environment, and building design and operation each influence the abundance and diversity of microbes in buildings or what is collectively referred to as the indoor microbiome (Danne miller *et al*., 2016;

Stephens *et al*., 2016). Many molecular analyses have identified considerable microbial diversity on built surfaces. Most microbes found in indoor environments appear to be dormant, inactive, or dead (Gibbons *et al*., 2016), and either has no known impact on human health or are possibly even beneficial to human health (O'Connor *et al*., 2018). For example,early life exposures to particular microbes or assemblages of microbes have been shown to shape our innate immune responses to protect against allergy and asthma (Stein *et al*., 2016).However, potentially pathogenic organisms can also reside within the microbial milieu of our built worlds, which can have a variety of negative health consequences.

2.5.2 Microbes on Surfaces

Inanimate objects in the built environment can serve as reservoirs of microbial matter. Each of these objects is host to an entire community composed of a wide variety of bacterial,viral, archaeal, protistan, and fungal organisms, including potential pathogens and microbial metabolic products harmful to humans.

2.6 Microbial Community Ecology on Fomite Surfaces

On indoor surfaces that lack abundant moisture and nutrient availability, most microorganisms that arrive from other environments (such as from human occupants) are generally considered unlikely to survive, and those viable microbes that do survive are generally considered to be inactive or dormant until transferred to other host locations or until they experience an influx of moisture and nutrients that help them proliferate (Chase *et al*., 2016; Hegarty *et al*., 2018; Hu *et al*., 2019). Surveys of fungal communities in indoor environments, conducted using high throughput molecular sequencing, have shown that they tend to be driven primarily by transport from the local outdoor environment (Adams *et al*., 2013). However, similar surveys of bacterial communities in the built environment have revealed high abundances of skin-associated bacteria

(e.g. *Propionibacterium acnes, Corynebacterium* spp, and *Streptococcus* spp), particularly in buildings and on surfaces with high human occupancy and frequency of interactions (Adams *et al*., 2015). Source-tracking efforts have also provided insight into the origin of the bacteria that reside on various indoor surfaces. For example, urine- and feces-associated bacteria have been shown to be more common on toilet seats and toilet handles than on other surfaces (Flores *et al*., 2012); bacteria associated with fresh produce have been shown to be more common on kitchen countertops and inside refrigerators (Flores *et al*., 2014); and bacteria associated with leaves and soil have been shown to be more common on the interior and exterior trim of doors that open to the outside than other (more interior) home surface locations (Dunn *et al*., 2015). Conversely, on surfaces that frequently have high moisture levels, such as those in bathrooms and kitchens (e.g., shower curtains, sinks, and countertops), rich microbial biofilms can form community assemblages that closely resemble those found in plumbing systems and water reservoirs (Adams *et al*., 2017).

Investigating differences both within and between homes, Lax *et al*. (2014) demonstrated that bacterial communities on different surfaces in an individual homes have strong similarities for some surfaces (e.g. kitchen floors were similar to bedroom floors and both were similar to human feet; and kitchen light switches were similar to the front doorknob, which were also similar to occupants's hands) but not for others (e.g., kitchen countertops and human noses were distinct from doorknobs) (Lax *et al*., 2014). Moreover, when a family moved homes, the bacterial community composition on surfaces in the new home rapidly converged toward the composition of bacteria from surfaces in the previous home, suggesting that the new occupants quickly deposited their own unique signatures of human-associated bacteria to the new space. While much has been revealed about bacterial and fungal communities in indoor environments in

recent years, much less is known about viral communities and total viral abundance on surfaces in buildings (Prussin *et al*., 2015) However, much has been learned about the presence, abundance, and survival of specific viruses and other potential pathogens that cause concern for infectious disease transmission and other emerging microbial hazards.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was carried out on wash hand basins sited at different locations within the University of Benin metropolis, Ugbowo campus, Benin City, Edo State, Nigeria. University of Benin (UNIBEN) is situated in Benin in Egor/Ovia North East Local Government area of Edo State. Benin City is the administrative headquarter of Oredo Local Government Area of Edo State which lies between latitude 6º20.022'N of the Equator and longitude 5°36.009`E of the Greenwich Meridian. University of Benin (UNIBEN) has a population of 40,000-44, 999 in 2010 and 75,000 in 2012 (NPCN, 2012). The University of Benin (UNIBEN) is a research University located in Benin City, Edo State, Nigeria. It is among the university owned by the Federal government of Nigeria and was founded in 1970 (MEN, 2019).

3.2 Study Population

Fifteen (15) sites consisting of student's hostels, some faculties, and some public facilities within the University of Benin Community, Ugbowo campus, Benin City, Edo State having wash hand basins were randomly selected and used in this study. A minimum of three samples were collected from three (3) wash hand basins located within each site

3.2.1 Inclusion Criteria:

Wash hand basins currently in use and located within the University of Benin, Benin city were included for this study.

3.2.2 Exclusion Criteria:

Wash hand basins located outside the University of Benin metropolis were excluded from the study.

3.3 Ethical Approval:

Approval for this research was sought and obtained from the Ethics and Research Committee, University of Benin, Benin City, Edo state, Nigeria.

3.4 Sample Size Calculation:

The sample size (N) for this research was calculated using prevalence from bacterial contamination on public surfaces in Abuja, Nigeria, which was 5 % (Alonge *et al*., 2019). The sample size for this study was then obtained using the formula described by (Daniel *et al*., 1999).

$$N = \frac{Z^2 P (1 - P)}{C^2}$$

N= required sample size

Z= confidence level at 95% (standard value of 1.96)

P = estimated prevalence of bacterial contamination on public surfaces in Abuja, (5%) (Alonge *et al*., 2019).

C = margin of error at 5% (standard value = 0.05)

$$N = \frac{1.96^2 \text{ X } 0.05(1-0.07)}{(0.05)^2} = \frac{3.8416 \text{ X } 0.05(0.95)}{0.0025}$$

N = 71

However a minimum of 70 swab samples were used.

3.6 Sample Collection

A total of 70 swab samples were collected asceptically using sterile swab sticks moistened in normal saline from wash hand basins in toilets and other public facilities in University of Benin community using sterile swab sticks moistened in normal saline (Reynolds *et al*., 2005). The swab samples were collected specifically from the faucet of the taps and the surface area were hands are rested on. The swab sticks were properly labeled and transferred immediately to the medical microbiology laboratory for microbiological analysis.

3.7 Sample Processing

Culture: Three Culture media, that is; Basal media (Nutrient agar), Enriched media (Blood agar) and Differential media (MacConkey agar) are used and prepared according to standard procedures (Cheesbrough, 2010).

3.7.1 Micro Organisms Identification from Isolation

The swabs sticks were properly inoculated on the agar plate, forming a well and a sterile wire loop was used to streak out on the various agar plates to give discrete colonies of organisms. The various culture media was incubated at 37 °C for 24 hours for visible growth After 24 hours of incubation, the growth on the cultured plates was examined and the appearance, size, colour and morphology were observed and recorded.

3.7.2 Gram stain: Gram staining reactions will also be carried out to identify the Gram morphology of bacteria cells and the Gram staining reaction will also be recorded. Biochemical identification of the colonies on the culture plates were carried out using Catalase, Coagulase, Indole, Oxidase, urease and Citrate utilization tests as described by Cheesbrough (2010) to identify the organisms isolated.

3.8 Antibiotic Sensitivity Test

Antibiotic sensitivity test was carried out using Kirby-Bauer disc diffusion technique. The degree of sensitivity of the organism to the drugs was determined by measuring the visible zones of inhibition of growth produced by the diffusion of the antibiotic from the disc into the surrounding medium.

3.9 Statistical Analysis

Data obtained from the analysis was analyzed using the statistical software SPSS for Windows, version 17.0 (SPSS, Chicago, IL, USA). Correlations between variables was calculated with Spearman's rank correlation test. $P = 0.05$ was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

Table 4.1 Prevalence of bacteria contamination of wash hand basins sited at various locations within the University Benin benin city the prevalence of bacteria contaminating wash hand basins sited at various locations in university of Benin metropolis was 41%, among the total number of sample examined.

Table 4.2 shows the biochemical reaction of microorganism isolated in the study. A total of 8 different organisms were identified which are *Klebsiella* spp, *Providencia* spp, *Pseudomonas* spp, *Streptococcus faecalis, Staphylococcus aureus*, *Enterobacter* spp, *Escherichia coli* and *Proteus mirabilis.*

Table 4.3 shows the Frequency Distribution of Bacterial contaminating wash hand basins sited at various locations in University of Benin metropolis, Benin City. The overall percentage occurrences of the isolated bacteria were: *Staphylococcus aureus* 15 (36.6%), *Klebsiella* spp 8 (19.5%), *Escherichia coli* 6 (14.6%), *Enterobacter* spp 5 (12.2%), *Providencia* spp, *Pseudomonas* spp. and *Proteus mirabilis* has 2 (4.9%) each, and *Streptococcus faecalis* 1 (2.4%) respectively. *Staphylococcus aureus* has the highest prevalence rate of 36.6% among all the isolated bacteria which was followed by *Klebsiella* spp with 19.5%, this was followed by *Escherichia coli* with 14.6%, *Enterobacter* spp, with 12.2%, *Providencia* spp, *Pseudomonas* . and *Proteus mirabilis* has 4.9% each, and *Streptococcus faecalis* with 2.4% respectively.

Table 4.4 shows the Frequency distribution of organisms across the various wash hand basins sited at various locations in the University of Benin metropolis. Hall I, Hall II and Faculty shows the highest frequency distributions of bacteria contaminant with 4 different organisms, this was followed by Physical Science, Life Science, NDDC, Hall III, MLS and Management Science with 3 different organism. Thus, St. Albert, PG Hostel, Education, Hall V and BMS faculty has 2 different organism; while Main Gate has only 1 organism**.**

Table 4.5 Sensitivity Pattern of Bacteria contaminating wash hand basins in University of Benin. *Escherichia coli* shows resistance to most of the antibiotic disc (Piperacillin (PRL), Amoxicillin (AMC), Gentamycin (CN), Cefotaxime (CTX)) used, while *Proteus mirabilis* was sensitive to all the antibiotic disc used. *Staphylococcus aureus, Enterobacter* spp, *Klebsiella* spp. and *Providencia* spp. was resistant to Piperacillin (PRL) and Amoxicillin (AMC), then sensitive to the others respectively. *Streptococcus faecalis* was only resistant to Piperacillin (PRL) and sensitive to the others. *Pseudomonas* spp were resistant to Piperacillin (PRL), Amoxicillin (AMC) and Gentamycin (CN) while others were Sensitive.

Table 4.1: Prevalence of Bacteria contaminating wash hand basins sited at various locations in University of Benin , Benin City.

Variables	Total No. Examined	Prevalence	Percentage (%)
Positive	70	41	58.6
Negative	70	29	41.4

Table 4.2: Identification of organisms using biochemical reaction of isolated bacterial in the study

Biochemical Tests													
Fermentation	Gram	Mot	Ox	Ind	Cit	Urea	KIA				Cat	Coa	Organisms Suspected
							Glu	Lact	H_2S	Gas			
LF	GNB	+	-	-	+	+	+	+	-	+	NA	NA	*Klebsiella* spp.
LF	GNB	+	-	+	-	-	+	+	-	+	NA	NA	*Escherichia coli*
NLF	GNB	+	-	+	+	+	+	-	+	+	NA	NA	*Proteus mirabilis*
LF	GNB	+	-	-	+	-	+	+	-	+	+	NA	*Enterobacter* spp.
NLF	GNB	+	-	+	+	-	+	-	-	-	+	NA	*Providencia* spp.
NLF	GNB	+	+	-	+	-	-	-	-	+	+	-	*Pseudomonas* spp.
NLF	GPC	-	-	-	-	-	+	+	-	-	-	-	*Streptococcus faecalis*
NLF	GPC	-	-	-	+	+	+	+	-	-	+	+	*Staphylococcus aureus*

(+) = Positive, (-) = Negative,

Key:

GNB = Gram Negative Bacilli, GPC = Gram Positive Cocci, LF = Lactose Fermenters

NLF = Non-Lactose Fermenters, Mot = Motility, Ox = Oxidase, Ind = Indole, Cit = Citrate,

Glu = Glucose, Lact = Lactose, H_2S = Hydrogen Sulphate Production, Gas = Gas Production,

KIA = Kligler Iron Agar, NA = Not Available, Cat = Catalase Test,Coa = Coagulase Test.

Table 4.3: Frequency Distribution of Bacterial contaminating wash hand basins sited at various locations in University of Benin, Benin City.

Bacterial Isolated	No. Positive	Percentage (%)
Staphylococcus aureus	15	36.6
Streptococcus faecalis	1	2.4
Enterobacter spp.	5	12.2
Klebsiella spp.	8	19.5
Proteus mirabilis	2	4.9
Pseudomonas spp.	2	4.9
Providencia spp.	2	4.9
Escherichia coli	6	14.6
	41	**100**

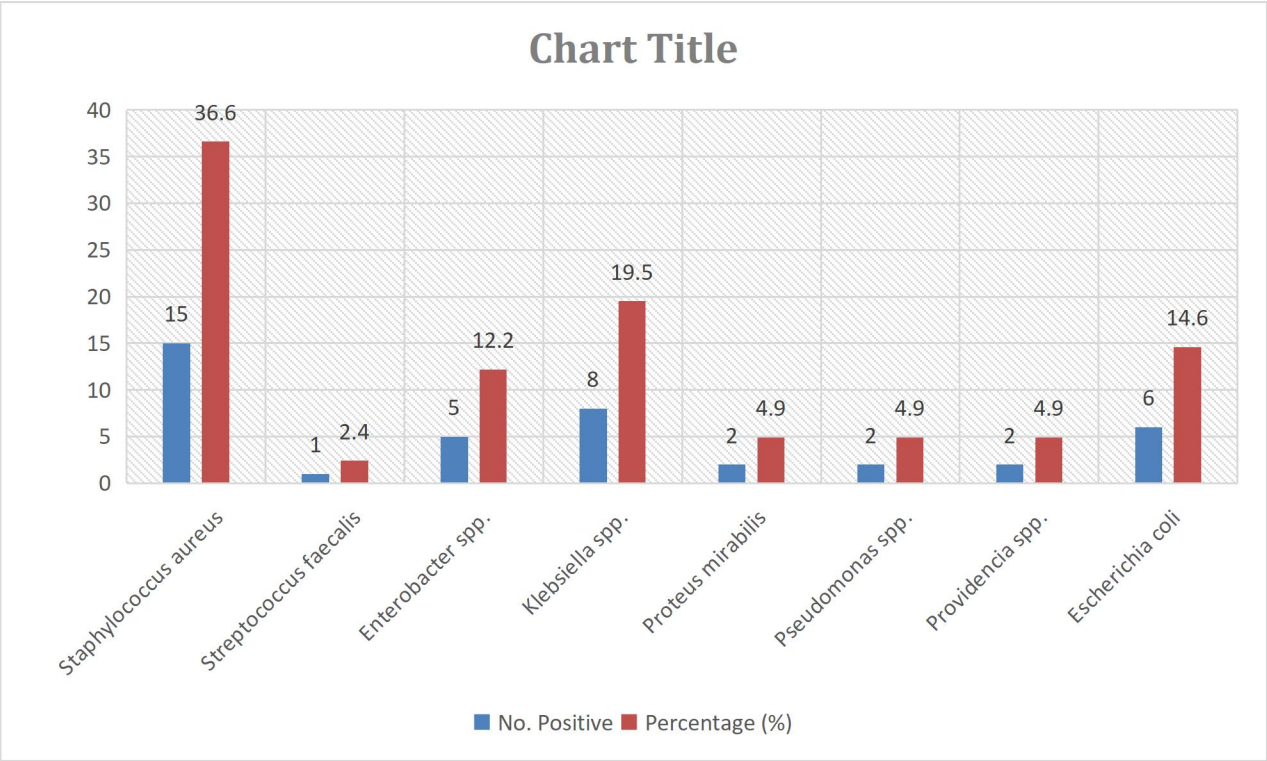

Fig 4.1: Bar-chart representation of the frequency distribution of Bacterial contaminating wash hand basins sited at various locations in University of Benin, Benin City.

Table 4.4: Frequency Distribution of Organisms Isolated across the Various Wash Hand Basins sited at Various Locations in the University of Benin, Benin City.

Site Locations	Total No. Positive (%)	Bacterial Isolated
St. Albert	2	*Streptococcus faecalis* *Staphylococcus aureus*
Main Gate	1	*Staphylococcus aureus*
Physical Science	3	*Klebsiella* spp. *Enterobacter* spp. *Staphylococcus aureus*
Life Science	3	*Klebsiella* spp. *Proteus mirabilis* *Staphylococcus aureus*
NDDC	3	*Enterobacter* spp. *Providencia* spp *Staphylococcus aureus*
PG Hostel	2	*Escherichia coli* *Staphylococcus aureus*
Hall I	4	*Klebsiella* spp. *Enterobacter* spp. *Providencia* spp *Staphylococcus aureus*
Hall II	4	*Klebsiella* spp. *Escherichia coli* *Enterobacter* spp. *Staphylococcus aureus*
Hall III	3	*Klebsiella* spp.

		Enterobacter spp. *Staphylococcus aureus*
MLS	3	*Escherichia coli* *Pseudomonas* spp. *Staphylococcus aureus*
Education	2	*Escherichia coli* *Staphylococcus aureus*
Faculty	4	*Klebsiella* spp. *Escherichia coli* *Pseudomonas* spp. *Staphylococcus aureus*
Hall V	2	*Klebsiella* spp. *Staphylococcus aureus*
BMS Hostel	2	*Klebsiella* spp. *Staphylococcus aureus*
Management Science	3	*Escherichia coli* *Proteus mirabilis* *Staphylococcus aureus*

Table 4.5: Sensitivity Pattern of Bacterial contaminating wash hand basins in University of Benin.

Bacterial Isolated	No. Isolated	CN	CTX	OFX	LEV	AMC	PRL
Staphylococcus aureus	15	S – 9 R – 6	S – 13 R – 2	S – 13 R – 2	S – 15 R – 0	S – 0 R – 15	S – 0 R – 15
Streptococcus faecalis	01	S – 1 R – 0	S – 1 R – 0	S – 1 R – 0	S – 1 R – 0	S – 1 R – 0	S – 0 R – 1
Enterobacter spp.	05	S – 5 R – 0	S – 5 R – 0	S – 5 R – 0	S – 5 R – 0	S – 4 R – 1	S – 5 R – 0
Klebsiella spp.	08	S – 7 R – 1	S – 6 R – 2	S – 7 R – 1	S – 7 R – 1	S – 0 R – 8	S – 0 R – 8
Proteus mirabilis	02	S – 2 R – 0	S – 2 R – 0	S – 2 R – 0	S – 2 R – 0	S – 0 R – 2	S – 0 R – 2
Pseudomonas spp.	02	S – 0 R – 2	S – 2 R – 0	S – 2 R – 0	S – 0 R – 2	S – 0 R – 2	S – 0 R – 2
Providencia spp.	02	S – 2 R – 0	S –2 R –0	S –2 R –0	S –2 R –0	S –0 R –2	S –0 R –2
Escherichia coli	06	S – 0 R – 6	S – 5 R – 1	S – 6 R – 0	S – 5 R – 1	S – 0 R – 6	S – 0 R – 6

Key:

S = Sensitive, R = Resistant, PRL= Piperacillin, AMC= Amoxicillin, CN= Gentamycin

CTX= Cefotaxime, OFX= Ofloxacin, LEV= Levofloxacin

CHAPTER FIVE

5.0 Discussion, Conclusion and Recommendations

5.1 Discussion

After the outbreak of Covid 19, the use of wash hand basins increased (Prashansha, 2020). Wash hand basins have been recognized as important reservoirs for pathogens in public settings, but the distribution of pathogens within wash hand basins and the individual/University students has been poorly understudied. In this study, samples from the different wash hand basins varied in the level of target organism contamination because the wash hand basins are cleaned regularly, the wash hand basins surfaces and drain covers were not as heavily contaminated as the faucet.

The result from this study shows that the prevalence of bacterial contaminating wash hand basins was 58.6%. However, *Staphylococcus aureus* has the highest prevalence rate of 36.6% which was followed by *Klebsiella* spp with 19.5%, *Escherichia coli* with 14.6%, *Enterobacter* spp. With 12.2%, *Providencia* spp, *Pseudomonas* spp. and *Proteus mirabilis* has 4.9% each, and lastly *Streptococcus faecalis* with 2.4% respectively. Findings from this study also has identified areas of high bio-burden within wash hand basins and the associated premise plumbing that pose a risk for dispersion of pathogens to the surrounding environment.

The proximity of high concentrations of target organisms in the tail pipe sample below the drain cover is one locale within wash hand basins that could be the focus of a future infection control strategy. The dynamics of drain cover colonization, disinfection through daily cleaning, and re-colonization from the tail pipe biofilm is a process that should be further explored to better understand and prevent dispersion of pathogens from wash hand basins to the student environment. Replacement or harsh cleaning of sink plumbing and fixtures has been shown to be ineffective and therefore aiming to completely eradicate potential pathogens from the sink drain

environment may not be a suitable approach (Gbaguidi-Haore, 2018). A heat and vibration device installed on the p-traps in one study reduced the proportion of carbapenemase positive sink drain cultures by 80% (Mathers, 2018). Focusing infection control measures on smaller areas, such as the drain cover or tail pipe directly below the drain cover may be just as effective and much less costly.

The Frequency distribution of organisms across the various wash hand basins sited at various locations in the University of Benin metropolis shows that the different organisms isolated from the wash hand basins in Hall I, Hall II, Hall III and BMS toilet varied significantly and this variation was associated with the presence or absence of faucet aerators. This was in line with Denton *et al*., (2000) and Cristina, (2014). Faucet aerators have been previously documented as reservoirs for Health Associated Infection pathogens and this finding further highlights the role that faucet aerators can play in promotion of biofilm growth (Denton *et al.,* 2000; Cristina, 2014). While there was a significant difference in bacteria count of isolates for first catch samples, no difference was observed in two-minute flush samples between faucets with and without aerators, indicating that the overall water quality was not compromised by the presence of aerators. The faucet samples taken also showed a large amount of variation in the concentration of target organisms present when compared among the various collection site. This variation may be due to wash hand basins usage including flushing, cleaning, or disposal of liquid waste materials (carbon or energy sources for microorganisms) down the sink drain (Grabowski, 2018).

The current study isolatedeight (8) bacteria, which are Klebsiella spp, Providencia spp, *Pseudomonas* spp, *Streptococcus faecalis, Staphylococcus aureus, Enterobacter* spp, *Escherichia coli* and *Proteus mirabilis* (Table 4.3).This was not in line with previous studies by

Amala and Monsi, (2017) who isolated five (5) bacterial isolates; *S. aureus, E. coli, Bacillus* spp *Proteus* spp. and coagulase negative *Staphylococci* spp were tagged as potential pathogens. From the study also Sensitivity Pattern of Bacteria contaminating wash hand basins in University of Benin shows that *Escherichia coli* was resistant to most of the antibiotic disc (PRL, AMC, CN) used, while *Proteus mirabilis* was sensitive to all the antibiotic disc used, others shows varied sensitivity pattern. The result was similar to the results gotten from Franco *et al.* (2020); their findings shows that the Percentage of Enterobacteriaceae and *Pseudomonas spp,* isolates were resistant (R), intermediate (I) resistance and sensitive to the antibiotics tested. ATM – aztreonam, CAZ – cefazidime, MEM – meropenem, ERT – ertapenem, CTX – cefotaxime.

The *in situ* nature of this study and the focus specification on wash hand basins allowed us to identify areas of high pathogen concentration and previously unknown differences between wash hand basins in stations situated at university environ. Hence, this study capture the microbial community in its entirety and report concentrations of pathogens rather than relative abundance, which is useful for estimating risk of transmission to students, visitors, or other surfaces. Future work should include culture-independent based analyses of samples similar to those discussed in this study.

5.2 Conclusion

This study determined the distribution of bacteria transfer rates between various site of sampling surfaces of wash hand basins commonly encountered during hand washing in the University environment. By collecting samples from the various site. Results from this study shows a relatively high prevalence that bacterial transfer rates among student hands and wash hand basins surfaces are highly variable, and faucet spigots may be a significant source of cross-contamination. Bacteria occur in large numbers on surfaces which users of washrooms and toilets readily contact. Pathogens, if present, can similarly be transmitted. Daily cleaning and disinfection in conjunction with a regular hygiene service are recommended to reduce cross-infection risks in washrooms and toilets.

5.3 Recommendations

- Paper towel dispensers should be placed far from the toilet seat given that the closer the toilet seat from paper towel dispenser is, the greater the risk of contamination by splashes from contaminated water when flushing the toilet.
- Bins should be closed and pedal operated, and should undergo sanitary cleaning to reduce bacterial transfer and prevent cross-contamination.
- Recycled paper towels should be replaced by virgin paper towels, because recycling papers might sometimes be contaminated with pathogens
- Water should not only be filtered but should be treated for pathogens and an improved storage system should be applied to successfully prevent the growth of pathogens and avoid food poisoning through contaminated hands.
- Alcohol Based Hand Sanitizer should be used combined with the hand washing procedure to increase compliance to the sanitary hand hygiene.

- A scheduled plan to perform microbiological tests for all hand hygiene tools is required; this procedure should be implemented at least once a year to minimize the risk of cross-contamination from pre-contaminated hand hygiene tools.

5.4 Limitations of study

One limitation of this study is the time at which wash hand basins were cleaned relative to when the swab samples of the wash hand basin were collectedwas unknown, but the variation in faucets contamination between samplings suggests that the tap-handles can become contaminated between cleanings and therefore may be an important locale in which to focus infection prevention practices, especially given the previous finding suggesting that bacteria must be present on the wash hand basins surfaces or tap- handles in order for dispersion to occur (Kotay *et al.,* 2017; Kotay, 2019).

Another limitation of this study is that it is unknown whether the source of faucets contamination is from the biofilm in the plumbing below, or from students, or visitor inputs during wash hand basins usage. Based on the findings of Kotay *et al.*,(2017), demonstrating green fluorescent protein-labeled *E. coli* growing from p-trap water to the drain cover in a span of seven days (eight-inch distance), contamination of the drain cover by the biofilm growing in the plumbing between daily cleanings is plausible (Kotay *et al.*, 2017).

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APPENDIX 1

Media

The media used includes commercially dehydrated products and laboratory prepared media.

Macconkey Agar (CM7, Oxiod, England)

Preparation

- 52 grams of MacConkey agar powder was weighed and suspended aseptically in 1liter of sterile distilled water and was allowed to dissolve for 10 minutes.
- It was sterilized by autoclaving at 121^{o}C for 15 minutes.
- The agar was cooled at 50^{o}C, mixed and then poured aseptically into the petri dish.
- It was allowed to set and stored at 4^{o}C for 2 weeks.

Mueller Hinton Agar (LabMal, Academy)

Preparation

- 38grams of Mueller Hinton agar powder was weighed and suspended aseptically in 1 liter of sterile distilled water and was allowed to dissolve for 10 minutes.
- It was sterilized by autoclaving at 121^{o}C for 15 minutes.
- The agar was cooled at 50^{o}C, mixed and then poured aseptically into the petri dish.
- It was allowed to set and stored at 4^{o}c for 2 weeks.

Mueller Hinton Agar Slant (LabMal, Academy)

Preparation

- 38grams of Mueller Hinton agar powder was weighed and suspended aseptically in 1 liter of sterile distilled water and was allowed to dissolve for 10 minutes.
- It was sterilized by autoclaving at 121^{o}C for 15 minutes.
- The agar was cooled at 50^{o}C, mixed and then poured aseptically into the sterile bijou bottles.
- It was allowed to set in a slanted position.
- It was then stored at 4^{o}C for 2 weeks.

Citrate Koser Medium (Himedia (M069) Laboratories, India)

Preparation

- 5.7 grams of sodium citrate powder was weighed and suspended aseptically to 1 liter of sterile distilled water.
- This was allowed to dissolve for 10 minutes and then mixed.
- Equal volume of the broth was dispensed into sterile bijou bottles.
- It was then sterilized by autoclaving at 121^{o}C for 15 minutes.
- It was allowed to cool and then stored at room temperature.

Urea Agar Base (CM53, Oxiod, England)

Preparation

- 2.4 grams of urea agar base powder was suspended in 95ml of sterile distilled water.
- It was heated to boil to dissolve completely.
- It was sterilized by autoclaving at 121^{o}C for 15 minutes.
- It was cooled to 50^{o}C, 5ml of sterile 40% urea solution was added aseptically and mixed.
- It was then dispensed into sterile bijou bottles and was allowed to set in a slanted position.

- It was then stored at 4°C for 2 weeks.

Peptone Water (CM9, Oxiod, England)

Preparation

- 10 grams of peptone and 5 grams of sodium chloride were weighed and added aseptically to 1 liter of sterile distilled water.
- It was mixed and dispensed into sterile bijou bottles.
- It was sterilized by autoclaving at 121°C for 15 minutes.
- It was allowed to cool and stored at room temperature.

Blood agar (CM9, Oxiod, England)

Preparation

- 28 grams of nutrient were weighed and added aseptically to 1 liter of sterile distilled water.
- It was mixed while stirring to fully dissolve components.
- It was sterilized by autoclaving at 121°C for 15 minutes.
- It was allowed to cool to about 45°C to 50°C, 5% vol/vol of sterile defribinated sheep blood that has been warmed to room temperature is added and mixed gently and then poured aseptically into the petri dish.
- It was allowed to set and stored at 4°C for 2 weeks.

Media Constituent

Macconkey Agar (CM7, Oxiod, England)

Constituents

Peptone	20.0grams
Lactose	10.0grams
Neutral red	0.075grams
Bile salt	5.0grams
Sodium chloride	5.0grams
Agar	12.0grams
Distilled water	1000ml

pH 7.4 ± 0.2 at 25ºC

Mueller Hinton Agar (LabMal, Academy)

Constituents

Casein hydrolysate	17.5grams
Beef infusion	2.0grams
Starch	1.5grams
Agar	17.0grams
Distilled water	1000ml

pH 7.3 ± 0.1 at 25ºC

Citrate Koser Medium (Himedia (M069) Laboratories, India)

Constituents

Sodium ammonium phosphate	1.5grams
Potassium dihydrogen phosphate	1.0grams

Magnesium sulphate	0.2grams
Sodium citrate	3.0grams
Bromothymol blue	0.016grams
Distilled water	1000ml

pH 6.7 ± 0.2 at 25°C

Peptone Water (CM9, Oxiod, England)

Constituents

Peptone	10grams
Sodium chloride	5.0grams
Distilled water	1000ml

pH 7.2 ± 0.2 at 25°C

Urea Agar Base (CM53, Oxiod, England)

Constituents

Peptone	1.0grams
Glucose	1.0grams
Sodium chloride	5.0grams
Disodium phosphate	1.2grams
Potassium dihydrogen phosphate	0.8grams
Phenol red	0.012grams
Agar	15.0grams
Distilled water	95ml

pH 6.8 ± 0.2 at 25ºC

Chemical Reagent

All chemicals used in this study were of analytical grade and they include;

Gram Stain Reagent

Constituent

Crystal violet	0.2grams
Ethyl alcohol	2ml
Distilled water	80ml
Ammonium oxalate, 1% aqueous	18grams
Lugol' s iodine	2.0grams
Iodine	1.0grams
Distilled water	100ml

Potassium was dissolved in water and iodine was added

Acetone	95%
Neutral red	1.0gram
Distilled water	100ml

1gram of neutral red was dissolved in a small amount of water and was made up to 100ml

Oxidase reagent

Constituent

Tetramethyl-p-phylene diamine dihydrochloride	0.1gram

Distilled water	10ml

Kovac's reagent

Constituent	
Sodium chloride	0.85grams
Distilled water	100ml

APPENDIX II

Materials

Slides

Cover slips

Grease pencil

Wire loop

Straight wire

Test tubes

Petri dish

Maccartney bottles

Forceps

Equipment

Microscope

Hot air oven

Incubator

Autoclave