ASSESSMENT OF PATHOGENIC MICROORGANISMS FROM SANITARY FACILITIES AND ANTIMICROBIAL SENSITIVITY PATTERNS IN REFERRAL HOSPITALS AT ABAKALIKI, EBONYI STATE

BY

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A PROJECT SUBMITTED TO THE
DEPARTMENT OF PUBLIC HEALTH
SCHOOL OF HEALTH TECHNOLOGY
FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI

IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE AWARD OF
MASTERS IN PUBLIC HEALTH (MPH) IN PUBLIC HEALTH

AUGUST, 2024

CERTIFICATION

This is to certify that the thesis- **Assessment of Referral Hospital Sanitary Facilities as Reservoirs of Pathogenic Microorganisms and their Antimicrobial Susceptibility Patterns In Abakaliki, Ebonyi State**, was carried out by **Nwigwe Romanus Rushell (Reg No: 20194196918)** has been certified as meeting the requirement for the award of Master's Degree in Public Health (Epidemiology and Disease Control Option) in the Department of Public Health, School of Health Technology, Federal University of Technology Owerri, Imo State.

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DEDICATION

I sincerely dedicate this work to my parents who gave me startling fundamental foundation in education and their unreserved moral and financial supports.

ACKNOWLEDGEMENTS

My inexpressible and unquantifiable gratitude goes to God Almighty for the grace to finish this programme with good success.

I want to express my profound gratitude to my Supervisors, Dr. U.G. Ekeleme and Dr. J.C. Nwaokoro, for giving me the thorough supervision and finding time to guide me to complete this work. May God bless you all abundantly, Amen.

I also acknowledge the Head of Department, Public Health, Federal University of Technology, Owerri, Dr U.M. Chukwuocha, and the Dean, School of Health Technology (SOHT), Rev. Sr. (Prof.) E.T. Oparaocha.

I say a big thank you to my dedicated lecturers and non-academic staff in the Department of Public Health; Prof. A.N. Amadi, Prof. (Mrs.) E.A. Nwoke, Prof. (Mrs.) S.N.O. Ibe, Dr. (Mrs.) B.O. Nworu, Dr. U.M. Chukwuocha, Dr. C.C. Okereke, Dr. C.C. Iwuala, Dr. O.G. Udujih, Dr. C.I.C. Ebirim, Dr. (Mrs.) U.W. Dozie, Dr. (Mrs.) S.M. Orji, Mr. G.N.U. Iwuoha, Miss J.C. Ezelote, Mrs. Rita Chukwu, Mrs. J.C. Obi, Mrs. M.O. Okorie, Mrs. S. Mbachu and Miss. V. Akam, for their professional, outstanding and update knowledge they imparted on me. God bless you all for your sacrifice.

I appreciate my beloved wife, Mrs. Nwigwe Chinwe, and my children, for their love, encouragement, moral and financial supports throughout this work.

I am grateful to Dr. (Mrs.) Ngozi Okonkwo, for her encouragement and supports. God bless you Dr.

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ABSTRACT

This study assessed referral hospital sanitary facilities in Abakaliki, Ebonyi State as reservoirs of pathogenic microorganisms and their antimicrobial susceptibility patterns. A cross-sectional descriptive and experimental study designs were adopted for this study. A purposive sampling technique was adopted to select two (2) Referral Hospitals. For the cross-sectional descriptive study, a checklist by the Joint Monitoring Program (JMP) under the World Health Organization was adopted to assess the nature of the sanitary facilities. For the experimental study design, swabbing techniques were adopted. A total of one hundred and forty (140) samples were collected from bathroom floors, toilet seats, door handles, flush knobs, toilet covers and wash-hand basins within the hospital. Results revealed that borehole water was the major water source in the hospitals. Flush toilet facilities were major toilets used. Total viable bacterial counts of the sanitary facilities ranged from 2.0×10^1 cfu/swab to 7.2×10^2 cfu/swab; total coliform count ranged from 4.0×10^1 cfu/swab to 8.0×10^1 cfu/swab while total fungal counts ranged from 2.0×10^1 cfu/swab to 1.2×10^2 cfu/swab. The microbial load of the sanitary facilities showed that toilet seats had the highest total viable bacterial count, followed by bathroom floors and toilet covers. Bathroom floors had the highest coliform count while highest fungal count was recorded with door handles. Bacterial isolates from the sanitary facilities were *Pseudomonas*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Bacillus*, *Micrococcus*, *Streptococcus* and *Staphylococcus* species. Fungal isolates were *Candida* species, *Rhizopus*, *Aspergillus*, *Penicillium* and *Mucor s*pecies. Out of 24 isolates of *Staphylococcus* species, 9(37.5%) produced beta-haemolysis, while 7(29.2%) produced gamma-haemolysis. Out of 16 isolates of *Streptococcus* species recovered, 2(33.3%) produced alpha, while 2(33.3%) produced beta haemolysis. Out of 16 isolates of *Aspergillus* species recovered, 5(31.2%) produced alpha, while 3(18.7%) produced beta haemolysis. Zones of inhibition of antimicrobial drugs ranged from 12mm to 36mm, with *Bacillus*, *Escherichia*, *Streptococcus*, and *Micrococcus* species being resistant to some antibiotics. *Aspergillus* species and *Rhizopus* species showed resistance to fluconazole and ketoconazole. These microorganisms could be vehicles for hospital-acquired infections in the hospital. The provision of safe and accessible water sources and toilet facilities in the hospitals is necessary to ensure effective cleaning of the sanitary facilities.

Keywords: Hospital, Microorganisms, Pathogenic, Referral, Reservoir, Sanitary facilities,

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

A hospital is a healthcare facility where the diagnosis and treatment of patients are carried out with the help of specialized health science and auxiliary healthcare staff and medical equipment (Ezekiel, 2018). However, hospitals can also be the source of infections due to contamination by antimicrobial-resistant pathogens (Mulvey & Simor, 2009). The World Health Organization noted in 2011 that going to the hospital was far riskier than flying. Globally, the chance of a patient being subject to an error was about 10%, and the chance of death resulting from an error was about 1 in 300. In developing countries, 7% of hospitalized patients and 10% in developing countries acquire at least one healthcare-associated infection (World Health Organization, 2019).

Sanitary facilities are designated area for use by the general public that includes a toilet, urinal, sink or shower. At minimum, sanitary facility such facility must include a toilet and sink. Hospital sanitary facilities include toilets, urinals, sinks and showers used by both out-patients and in-patients in the hospital environment. The absence of basic facilities such as water, sanitation and hygiene (WASH) in healthcare facilities such as hospital sanitary facilities makes them a source of infection transmission to the users (Mughal, 2015).

Hospital sanitary facilities should be cleaned regularly to prevent the build-up of pathogens (WHO, 2021). When cleaning these areas, it is important to use effective disinfectants that are

known to kill the specific pathogen in question (Traverse & Aceto, 2015). In some cases, steam cleaning or ultraviolet light may also be necessary. It is important to follow all manufacturers' instructions when using cleaning products or equipment (Traverse & Aceto, 2015). When cleaning hospital sanitary facilities, it is also important to pay special attention to high-touch surfaces, such as door handles, countertops, and sinks. These surfaces should be cleaned more frequently than in other areas, as they are more likely to come into contact with hands and other body parts that can transfer pathogens (Koutsoumanis et al., 2021).

The role of hospital sanitary facilities in the spread of antimicrobial-resistant pathogens is well-documented. Antimicrobial resistance (AMR) occurs when microorganisms evolve mechanisms that protect them from the effects of antimicrobials. Clinical conditions due to AMR cause millions of deaths each year. Infections caused by resistant microbes are more difficult to treat, requiring higher doses of antimicrobial drugs, or alternative medications which may prove more toxic (Tanwar, 2014; Saha & Sarkar, 2021).

There is a clear link between poor sanitation and the transmission of antimicrobial resistance. Hospitals must, therefore, improve their sanitary facilities to reduce the spread of these dangerous pathogens (Mulvey & Simor, 2009). One of the most important things hospitals can do is ensure their sanitary facilities are properly cleaned and disinfected regularly. All surfaces, equipment, and materials that come into contact with patients or their bodily fluids must be disinfected according to protocol. This includes countertops, floors, bed rails, door handles, IV poles, and any other frequently touched surfaces (Traverse & Aceto, 2015).

In addition to proper cleaning and disinfection, hospital staff must practice good hygiene. This means washing their hands thoroughly and often, using personal protective equipment when

necessary, and avoiding contact with contaminated surfaces. By taking these simple precautions, hospital staff can help to prevent the spread of antimicrobial resistance (WHO, 2021). In the last few years, the spread of antimicrobial resistance has become a major public health concern. Antimicrobial-resistant pathogens can cause serious and even life-threatening infections, and efforts to contain them have been largely unsuccessful (WHO, 2021).

Recent research has indicated that hospital sanitary facilities may be reservoirs of antimicrobial-resistant bacteria, putting patients at an even greater risk of infection (Prestinaci et al., 2015). These pathogens can cause serious infections and are often difficult to treat. Sanitary facilities can become contaminated with these pathogens through various means, including contact with contaminated surfaces, equipment, or patients (Koutsoumanis et al., 2021). Once present in the environment, these pathogens can spread to other patients, staff, or visitors (Gerba, 2015). These pathogens can cause infections that are difficult to treat and may lead to death. Antimicrobial resistance can also make it more difficult to control the spread of infections in healthcare settings.

Antimicrobial-resistant infections that require the use of second- and third-line treatments can harm patients by causing serious side effects, such as organ failure, and prolonged care and recovery, sometimes for months (CDC, 2022). Infections caused by antimicrobial-resistant pathogens can result in longer hospital stays, higher medical costs, and increased mortality (WHO, 2022). The latest joint monitoring programme report by the World Health Organization and the United Nations Children's Fund has revealed that 32% of healthcare facilities in Nigeria had no basic sanitation services as of 2021. The report noted that patients,

visitors and staff members are at risk of infections that could spread through faecal routes in health facilities without sanitation infrastructure.

The report read, "In sub-Saharan Africa, the coverage of basic sanitation services was four times higher in urban health care facilities (24%) than in rural (7%), but there was little difference between government (11%) and non-government facilities (14%). By contrast, in fragile contexts, coverage in non-government facilities (25%) was nearly twice that in government facilities (13%). The rate at which patients contract infections from hospital facilities (nosocomial infection) has reached an alarming crescendo, resulting in multiple public health problems. The WHO Director, Department of Environment Climate Change and Health, Dr. Maria Neira, said, "Hygiene Facilities and practices in health care settings are non-negotiable. Their improvement is essential to pandemic recovery, prevention and preparedness (WHO, 2021).

1.2 Statement of the Problem

The mission of all hospitals and health systems, regardless of size and location, is to provide quality care to patients and advance health in their communities. Hospital sanitation is a critical issue in the fight against antimicrobial resistance. Poor sanitation can lead to the spread of pathogens that are resistant to antibiotics, making it difficult to treat patients with infections. Researchers have long reported that hospital sanitary facilities may serve as reservoirs of persistent antimicrobial-resistant pathogens.

Nosocomial infections comprise infections that are acquired from hospitals or healthcare centres. In underdeveloped and developed countries, they affect 7 and 10% of hospitalized

patients, respectively. HAI affects around 3.2 million patients in Europe per year. The intensity and frequency of the infection are strongly proportional to the patient's immune condition. The most vulnerable categories are patients in burn units, intensive care units (ICUs), organ transplant recipients, and infants. In Sub-Saharan Africa and South-East Asia, HAIs are also responsible for three out of every four neonatal deaths. Surgical site infections (2-5% incidence rate), catheter-related bloodstream infections (12-25% incidence rate), catheter-related urinary tract infections (12% incidence rate), and ventilator-associated pneumonia (9-27% incidence rate) are the most prevalent HAI types. Therefore, improving WASH conditions can help establish trust in health services, thereby reducing the rate of nosocomial infections among patients and healthcare workers in the hospitals. The current study assessed referral hospital sanitary facilities in Abakaliki, Ebonyi State as reservoirs of pathogenic microorganisms and their antimicrobial susceptibility patterns.

1.3 Objectives of the study

1.3.1 General Objective

This study aims to assess referral hospital sanitary facilities in Abakaliki, Ebonyi State, as reservoirs of pathogenic microorganisms and their antimicrobial susceptibility patterns.

1.3.2 Specific objectives

The following specific objectives shall be achieved:

1. To assess the availability of sanitary facilities in the referral hospitals in Abakaliki, Ebonyi State.

2. To determine the microbial load of the referral hospital sanitary facilities.

3. To isolate and identify microorganisms associated with the referral hospital sanitary facilities.

4. To determine the *in vitro* pathogenicity of the isolated microorganisms.

5. To determine the antimicrobial susceptibility patterns of the microorganisms isolated from the referral hospital sanitary facilities.

1.4 Research questions

1. What are the available hospital sanitary facilities in the referral hospitals in Abakaliki, Ebonyi State?

2. What is the microbial load of the referral hospital sanitary facilities?

3. What are the microorganisms associated with the referral hospital sanitary facilities?

4. What is the *in vitro* pathogenicity of the isolated microorganisms?

5. What are the antimicrobial susceptibility patterns of the microorganisms isolated from the referral hospital sanitary facilities?

1.5 Research Hypothesis

Ho: There is no association between hospital sanitary facilities and pathogenic microorganisms.

1.6 Significance of the Study

The study is seen to have the following significance

(i) To future researchers: This study on assessing hospital sanitary facilities as reservoirs of resistant pathogens in hospitals will add to existing literature, thereby guiding upcoming researchers to provide a basis and backup for further research.

(ii) To hospital: It will serve as a reference point to hospitals in the practice of environmental and personal hygiene or hospital sanitation, as well in the prevention and control of hospital-acquired infections (Nosocomial infections)

(iii) Government and non-governmental agencies: The research will serve as an essential tool and compass for planning and executing health activities in the hospital environment. The non-governmental agencies will also find this very significant, especially in their intervention programmes.

1.7 Scope of the Study

The study on the assessment of hospital sanitary facilities has its scope in seeking to analyse and provide data or information on the study's objectives. However, the study is limited to referral hospitals' sanitary facilities in Abakaliki Metropolis.

CHAPTER TWO

LITERATURE REVIEW

2.1 Conceptual Review

2.1.1 Sanitation

Sanitation refers to public health conditions related to clean drinking water and treatment and disposal of human excreta and sewage. Preventing human contact with faeces is part of sanitation, as is hand washing with soap. Sanitation systems aim to protect human health by providing a clean environment that will stop the transmission of disease, especially through the faecal–oral route (World Bank, 2019). A range of sanitation technologies and approaches exits. These include community-led total sanitation, container-based sanitation, ecological sanitation, emergency sanitation, environmental sanitation, onsite sanitation and sustainable sanitation. A sanitation system includes capturing, storing, transporting, treating, disposing, or reusing (World Bank, 2019).

2.1.2 Sanitary facility

A sanitary facility is a designated area for use by the general public that includes a toilet and sinks and may include a urinal and/or shower. Sanitary facilities are everyday features which are of great importance, especially in situations where people are deprived of their liberty, when detainees find themselves in a situation of total dependence on the authorities. Also, in hospitals, patients are confined to sanitary facilities during their admission to the hospital for a period of time (Kodriyah et al., 2022).

2.1.3 Hospital

A hospital provides patient treatment with specialized health science, auxiliary healthcare staff, and medical equipment. Hospital is a health service facility, a gathering place for both sick and healthy people. The interaction of various components in a hospital, such as buildings, equipment, people (staff, patients and visitors) and health service activities, can have a good or bad impact. On the other hand, the existence of a hospital can give a negative impact in the form of bad effects on humans, such as its garbage and waste which can cause environmental pollution, a source of disease transmission (Duvernay et al., 2020).

2.1.4 Hospital sanitary facilities

Hospital sanitary facilities refer to the physical infrastructure and amenities within healthcare settings specifically designed and dedicated to promoting hygiene, cleanliness, and infection prevention. These facilities are critical in maintaining a safe and healthy environment for patients, healthcare workers, and visitors. They encompass a range of areas and components, each with distinct characteristics (Smith, 2022).

2.1.4.1 Characteristics of hospital sanitary facilities

Patient Rooms: Patient rooms are individual spaces where patients receive care and recover. They typically include a bed, bedside table, storage cabinets, and a bathroom or adjacent washroom facility. These rooms are equipped with hand hygiene stations, waste disposal bins, and surfaces designed to be easily cleaned and disinfected.

Bathrooms/Washrooms: Bathrooms or washrooms within hospital facilities are dedicated areas for personal hygiene activities, such as handwashing, showering, and toileting. They

have sinks, toilets, showers, and appropriate fixtures and fittings. These facilities should be easily accessible, well-maintained, and designed to minimize the risk of microbial contamination and transmission (Clark & John, 2006).

Sinks and Faucets: Sinks and faucets are essential components of hospital sanitary facilities, providing a water source for handwashing, instrument cleaning, and general cleaning purposes. They should be strategically located throughout the facility, including patient care areas, corridors, and workstations. Sinks and faucets should be designed for easy cleaning, with smooth surfaces and no areas that can harbour microbial growth.

Surfaces and Equipment: Hospital sanitary facilities comprise various surfaces and equipment that require regular cleaning and disinfection to prevent the accumulation and transmission of pathogens. This includes countertops, tables, medical equipment, door handles, light switches, and other high-touch surfaces. These surfaces should be constructed of durable, non-porous materials, and resistant to damage from cleaning agents.

Waste Management Systems: Effective waste management is crucial in hospital sanitary facilities to minimize the risk of contamination and spread of infectious agents. Facilities should have designated waste disposal bins and containers for different types of waste, including general waste, infectious waste, sharps containers, and recycling bins. Proper segregation, handling, and disposal of waste are essential to prevent the transmission of antimicrobial-resistant pathogens.

Ventilation and Air Quality: Hospital sanitary facilities should have adequate ventilation systems to maintain good air quality and minimize the spread of airborne contaminants. Proper airflow and filtration systems help control the dissemination of pathogens and maintain a clean and safe environment. The ventilation systems should comply with

regulatory standards and guidelines to ensure efficient air exchange and filtration (Smith, 2022).

2.1.5 Sanitary facilities as important reservoirs of pathogens

Sanitary facilities, including hospitals, healthcare facilities, and community settings, are important reservoirs of antimicrobial-resistant pathogens due to several key factors:

1. High density of potentially infected individuals: Sanitary facilities often house a large number of individuals, including patients, healthcare workers, visitors, and even healthy carriers of pathogens. This high density of people provides ample opportunities for the transmission and dissemination of pathogens. Infected individuals can shed resistant pathogens through various means, such as respiratory droplets, contaminated surfaces, or faecal-oral routes, increasing the likelihood of Pathogens being present in these facilities (Rodriguez-Bano et al., 2021).

2. Frequent use of antibiotics: Sanitary facilities, particularly healthcare settings, often involve the administration of antibiotics to patients as part of treatment regimens. The extensive use of antibiotics creates a selective pressure that favours the survival and proliferation of antibiotic resistance platform (ARPs). The presence of antibiotic resistance platform (ARPs) in these facilities is further amplified by the potential for inappropriate prescribing, inadequate dosing, or incomplete treatment courses, all of which contribute to the development and spread of resistance.

3. Presence of immunocompromised individuals: Sanitary facilities frequently provide care to immunocompromised individuals, such as those undergoing chemotherapy, organ transplantation, or intensive care. These individuals are more susceptible to infections and, consequently, more likely to acquire ARPs. The compromised immune system of these

patients makes them particularly vulnerable to the serious consequences of infections caused by ARPs, leading to prolonged hospital stays, increased morbidity and mortality rates, and additional healthcare costs.

4. Frequent contact with healthcare interventions: Sanitary facilities, especially healthcare settings, involve various invasive procedures and medical interventions, such as surgeries, catheterizations, and the use of medical devices. These interventions create opportunities for ARPs to colonize and infect patients. The presence of ARPs on medical equipment, surfaces, and healthcare workers' hands can result in cross-contamination and subsequent transmission to vulnerable individuals (Rodriguez-Bano et al., 2021).

5. Inadequate Infection Control Measures: Despite the implementation of infection control practices, lapses in adherence can occur in sanitary facilities, allowing ARPs to persist and spread. Factors such as improper hand hygiene, inadequate cleaning and disinfection of surfaces, and insufficient compliance with isolation protocols can contribute to the survival and transmission of ARPs. In addition, the formation of biofilms on surfaces can further protect ARPs from the effects of disinfectants and hinder effective cleaning (Murray et al., 2022).

6. Environmental Reservoirs: Sanitary facilities provide favourable environments for the survival and persistence of ARPs. Moisture, warmth, and organic matter present in these settings can support the growth and survival of resistant pathogens. For example, wastewater systems, drains, and plumbing networks can harbour ARPs and serve as reservoirs for their dissemination. The role played by sanitary facilities as reservoirs of ARPs requires robust infection control measures, including strict adherence to hand hygiene protocols, proper cleaning and disinfection practices, effective waste management systems, and implementation

of antimicrobial stewardship programs. Surveillance programs, antibiotic resistance monitoring, and research into innovative interventions and technologies are also necessary to mitigate the spread and impact of ARPs in sanitary facilities (Murray et al., 2022).

2.1.6 Factors that facilitate the spread and growth of pathogens within sanitary facilities

1. **High antibiotic usage**: The extensive use of antibiotics in sanitary facilities, such as hospitals and healthcare settings, plays a significant role in the emergence and spread of ARPs. The over-prescription, misuse, and unnecessary use of antibiotics create selective pressure, leading to the survival and proliferation of resistant strains. Inadequate prescribing practices, including broad-spectrum antibiotics, incomplete courses, and inappropriate durations, contribute to the development of resistance (Holmes et al., 2016).

2. Close proximity and crowding: Sanitary facilities often have a high density of individuals, including patients, healthcare workers, and visitors, in close proximity. This close contact increases the opportunities for the transmission of ARPs through person-to-person interactions. The sharing of spaces, common areas, and healthcare equipment further facilitates the spread of resistant pathogens (Mestrovic et al., 2022).

3. Inadequate infection control practices: Lapses in infection control practices within sanitary facilities can contribute to the spread of ARPs. Inadequate hand hygiene compliance among healthcare workers, patients, and visitors allows for the transmission of resistant pathogens. Insufficient cleaning and disinfection of surfaces and medical equipment can lead to the persistence and transfer of ARPs. Ineffective implementation of isolation precautions

for infected or colonized individuals can contribute to the spread of resistant pathogens to susceptible individuals (Murray et al., 2022).

4. Transmission via contaminated surfaces and fomites: Sanitary facilities contain numerous surfaces and objects that can become contaminated with ARPs. Infected individuals can shed resistant pathogens onto surfaces such as doorknobs, bedrails, bedside tables, and medical devices. Other individuals who come into contact with these contaminated surfaces can acquire the resistant pathogens and contribute to their spread. Shared equipment and objects, including stethoscopes, thermometers, and electronic devices, can act as fomites for the transmission of ARPs (Holmes et al., 2016).

5. Cross-contamination: The movement of patients, healthcare workers, and visitors within sanitary facilities can lead to cross-contamination and the spread of ARPs. Resistant pathogens can be transferred from one area to another, from one patient to another, or from healthcare workers to patients. This movement can occur through contaminated hands, clothing, or medical equipment, contributing to the dissemination and growth of ARPs within the facility.

6. Invasive medical procedures: Sanitary facilities often perform invasive medical procedures that can introduce ARPs into the body or facilitate their spread. Surgeries, catheter insertions, intravenous lines, and other invasive interventions provide opportunities for resistant pathogens to enter vulnerable sites, causing infections and potentially disseminating within the facility. Invasive procedures that breach natural barriers can increase the risk of colonization or infection by ARPs (Holmes et al., 2016).

7. Environmental reservoirs: Sanitary facilities provide environments where ARPs can survive and persist. Moisture, warmth, and the presence of organic matter create favourable conditions for the growth and survival of resistant pathogens. Plumbing networks, wastewater systems, and contaminated water sources can act as reservoirs for ARPs. Inadequate wastewater treatment or the use of contaminated water for various purposes can contribute to the dissemination of ARPs into the environment.

8. Traveling patients and visitors: Sanitary facilities often receive patients and visitors from diverse geographic locations. These individuals may have acquired ARPs from other healthcare facilities, community settings, or during travel. Patients transferred from other hospitals or long-term care facilities may carry resistant pathogens, introducing new strains of ARPs into the facility. Similarly, visitors from the community can bring in resistant bacteria, increasing the diversity and spread of ARPs within the facility (Michael et al., 2014).

2.1.7 Specific areas prone to pathogen transmission

In sanitary facilities, several specific areas and activities are more prone to the transmission of antimicrobial-resistant pathogens (ARPs):

1. Patient rooms: Patient rooms, particularly those housing patients with known or suspected ARPs, are areas where transmission is likely to occur. Close contact between patients and healthcare workers in these settings provides ample opportunities for the transfer of resistant pathogens. High-touch surfaces in patient rooms, such as bed rails, call buttons, bedside tables, and remote controls, can become contaminated and act as sources of transmission (Michael et al., 2014).

2. Intensive Care Units (ICUs): ICUs are high-risk areas due to the acuity and severity of illnesses, the frequent use of invasive procedures, and the prolonged stays of patients. Intensive care patients are more vulnerable to infections, including those caused by ARPs. The presence of ventilators, central lines, and urinary catheters further increases the likelihood of colonization or infection with resistant pathogens.

3. Surgical Suites: Surgical suites are areas where invasive procedures are performed, making them potential sites for the acquisition and transmission of ARPs. Surgical procedures breach natural barriers and create opportunities for resistant pathogens to enter the body. Inadequate cleaning and sterilization of surgical instruments or equipment can contribute to the transmission of ARPs during surgical interventions (Michael et al., 2014).

4. High-Traffic Areas: Areas with high traffic and frequent interactions among patients, healthcare workers, and visitors pose a higher risk of transmission. These areas include nursing stations, waiting rooms, hallways, and communal spaces. The movement of individuals and the potential for close contact increase the chances of cross-contamination and the spread of ARPs (Larsen et al., 2022).

5. Hand Hygiene Stations: Hand hygiene stations, including sinks and hand sanitizer dispensers, are important for infection prevention. However, if they are not appropriately utilized or become contaminated, they can contribute to the transmission of ARPs. Inadequate hand hygiene practices or contaminated soap or hand sanitizers can compromise their effectiveness in reducing the spread of resistant pathogens.

6. Shared Equipment and Devices: Equipment and devices shared among patients, such as blood pressure cuffs, stethoscopes, thermometers, and mobile devices, can act as fomites for transmitting ARPs. Failure to adequately clean and disinfect these items between uses can transfer resistant pathogens from one individual to another (Larsen et al., 2022).

7. Toilets and Bathrooms: Sanitary facilities, including toilets and bathrooms, can serve as reservoirs and transmission sources for ARPs. Contaminated surfaces, inadequate cleaning practices, and the potential for faecal-oral transmission contribute to the spread of resistant pathogens in these areas. Improper hand hygiene after using the toilet or before exiting the bathroom further amplifies the risk of transmission.

8. Food Preparation Areas: Foodborne transmission of ARPs is possible in facilities where food is prepared and served, such as hospitals and long-term care facilities. Contamination of food or food-contact surfaces with resistant pathogens can lead to infections in individuals consuming the contaminated food (Larsen et al., 2022).

It is important to note that the specific areas and activities where transmission is likely to occur can vary depending on the type of facility, patient population, and local infection control practices. Implementing rigorous infection control measures, including proper cleaning and disinfection, adherence to hand hygiene protocols, and appropriate isolation precautions, can help mitigate the risk of transmission and prevent the spread of ARPs within these areas.

2.1.8 Possible illnesses associated with pathogen contamination in sanitary facilities

Several key illnesses are associated with antimicrobial-resistant pathogens (ARP) contamination in sanitary facilities (Rodriguez-Bano et al., 2021). Some examples include:

1. Healthcare-Associated Infections (HAIs): HAIs are infections that result from receiving healthcare treatment in a facility such as a hospital or long-term care facility. ARPs can cause various types of HAIs, including:

a. Methicillin-resistant *Staphylococcus aureus* (MRSA) Infections: MRSA is a common ARP associated with HAIs. It can cause skin and soft tissue infections, surgical site infections, bloodstream infections, and pneumonia. MRSA is particularly concerning because it is resistant to many commonly used antibiotics (Samuel, 2019).

b. *Clostridium difficile* Infections: *Clostridium difficile* is a bacterium that can cause severe gastrointestinal infections. ARPs of *C. difficile* have emerged, leading to increased resistance to antibiotics used to treat these infections. *C. difficile* infections can be challenging to manage, leading to recurrent or severe diarrhoea.

2. Urinary Tract Infections (UTIs): UTIs are infections that affect the urinary system, including the bladder and kidneys. ARPs can cause UTIs, and these infections are commonly associated with healthcare settings. Examples of ARPs causing UTIs include extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae and carbapenem-resistant Enterobacteriaceae (CRE) (Samuel, 2019).

3. Respiratory Infections: ARPs can also cause respiratory infections, particularly in vulnerable populations. Examples include:

a. Multidrug-resistant *Pseudomonas aeruginosa* Infections: *Pseudomonas aeruginosa* is an opportunistic pathogen that can cause respiratory infections, especially in individuals with compromised immune systems or underlying lung conditions. Resistance to multiple antibiotics, including those commonly used to treat *Pseudomonas* infections, makes treatment challenging.

b. *Acinetobacter baumannii* Infections: *Acinetobacter baumannii* is another opportunistic pathogen associated with respiratory infections, particularly in healthcare settings. It is known for its ability to develop resistance to a wide range of antibiotics, including carbapenems.

4. Surgical Site Infections (SSIs): SSIs are infections that occur at the site of a surgical incision or operation. ARPs can cause SSIs, leading to complications and prolonged hospital stays. Examples include MRSA, ESBL-producing Enterobacteriaceae, and vancomycin-resistant *Enterococcus* (VRE).

5. Bloodstream Infections: ARPs can also cause bloodstream infections, which can be life-threatening. Examples of ARPs associated with bloodstream infections include MRSA, VRE, and multidrug-resistant Gram-negative bacteria, such as *Klebsiella pneumoniae* and *Escherichia coli* (Rodriguez-Bano et al., 2021).

6. Gastrointestinal Infections: ARPs can cause gastrointestinal infections, leading to symptoms such as diarrhoea, abdominal pain, and vomiting. Examples include:

a. Extended-Spectrum Beta-Lactamase (ESBL) - producing Enterobacteriaceae Infections: ESBL - producing Enterobacteriaceae, such as *Escherichia coli* and *Klebsiella pneumoniae*, are resistant to a wide range of antibiotics, including penicillin and cephalosporin. These ARPs can cause severe gastrointestinal infections, particularly in healthcare settings.

b. Vancomycin-Resistant *Enterococcus* (VRE) Infections: VRE is a group of ARPs resistant to vancomycin. They can cause gastrointestinal, urinary tract, and bloodstream infections, especially in immunocompromised individuals or those with prolonged hospital stays (Rodriguez-Bano et al., 2021).

7. Skin and Soft Tissue Infections: ARPs can lead to skin and soft tissue infections, including:

a. Multidrug-Resistant Gram-Negative Bacteria Infections: Gram-negative bacteria, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, can cause skin and soft tissue infections, particularly in healthcare settings. These ARPs are often resistant to multiple antibiotics, making treatment challenging.

b. Methicillin-Resistant *Staphylococcus aureus* (MRSA) Infections: MRSA can also cause skin and soft tissue infections, including abscesses, cellulitis, and surgical site infections. MRSA strains are resistant to methicillin and other beta-lactam antibiotics.

8. Neonatal Infections: Newborns in healthcare facilities are particularly susceptible to ARPs, and infections in this population can have severe consequences. Examples include:

a. Extended-Spectrum Beta-Lactamase (ESBL)-producing *Klebsiella pneumoniae* Infections: ESBL-producing *Klebsiella pneumoniae* can cause serious infections in neonatal intensive care units (NICUs), including bloodstream infections and pneumonia. These ARPs pose a significant threat to vulnerable newborns (Samuel, 2019).

b. Carbapenem-Resistant Enterobacteriaceae (CRE) Infections: CRE infections in neonates can be life-threatening. CRE strains, such as *Klebsiella pneumoniae* and *Escherichia coli*, are resistant to carbapenem antibiotics, which are typically reserved as last-resort treatments.

9. Central Line-Associated Bloodstream Infections (CLABSIs): ARPs can cause bloodstream infections associated with using central venous catheters or central lines. Examples include MRSA, VRE, and multidrug-resistant Gram-negative bacteria. CLABSIs are a significant concern in healthcare facilities and can lead to severe complications (Rodriguez-Bano et al., 2021).

10. Postoperative Wound Infections: ARPs can cause infections at surgical sites, leading to postoperative complications. Examples include MRSA, VRE, and multidrug-resistant Gram-negative bacteria (Murray et al., 2021).

2.1.9 Possible control or elimination measure of the transmissions of pathogens in hospital systems

Reducing or eliminating the transmission of antimicrobial-resistant pathogens (ARPs) via sanitation systems requires a comprehensive approach that includes various strategies and there are:

1. Improved Sanitation Practices: Enhancing sanitation practices is crucial to prevent the spread of ARPs. This includes promoting proper hygiene measures such as handwashing with soap and water or using alcohol-based hand sanitizers. Implementing rigorous cleaning and disinfection protocols for surfaces, medical equipment, and shared facilities can help reduce ARP contamination.

2. Effective Wastewater Treatment: Effective wastewater treatment is essential to reducing the release of ARPs into the environment. Wastewater treatment facilities should adopt advanced treatment technologies that can effectively remove or inactivate ARPs. This includes employing processes such as activated sludge, membrane filtration, ultraviolet disinfection, and advanced oxidation (Samuel, 2019).

3. Antibiotic Stewardship: Implementing antibiotic stewardship programs is crucial to reducing the overall prevalence of ARPs. These programs promote responsible and appropriate antibiotic use, aiming to minimize unnecessary antibiotic prescriptions and the development of resistance. Proper prescribing practices, education for healthcare professionals and patients, and surveillance of antibiotic use can all contribute to reducing ARP transmission.

4. Enhanced Infection Prevention and Control: Robust infection prevention and control measures are essential in healthcare facilities to prevent the spread of ARPs. This includes implementing strict protocols for hand hygiene, personal protective equipment use, and isolation precautions. Regular surveillance and monitoring of ARPs and targeted interventions can help identify and control outbreaks (Samuel, 2019).

5. Public Awareness and Education: Raising awareness among the general public about the risks of ARPs and the importance of infection prevention and control practices is crucial. Education campaigns can promote proper hand hygiene, appropriate antibiotic use, and understanding the role of sanitation in preventing ARP transmission.

6. Research and Development: Continued research is needed to develop innovative solutions for reducing ARP transmission via sanitation systems. This includes studying the effectiveness of different disinfection methods, exploring new technologies for wastewater treatment, and investigating alternative sanitation approaches that can minimize the spread of ARPs (Samuel, 2019).

7. International Cooperation and Policy Implementation: Collaboration among countries and international organizations is vital in addressing ARP transmission via sanitation systems. This includes sharing best practices, harmonizing regulations and guidelines, and promoting the adoption of effective strategies globally. Governments can play a significant role by implementing and enforcing policies prioritising infection control and antimicrobial stewardship.

8. Water Treatment and Quality: Ensuring safe and clean water is essential to minimize the risk of ARP transmission. Implementing effective water treatment processes, such as filtration, chlorination, and ultraviolet disinfection, can help remove or inactivate ARPs in the water supply. Regular monitoring and testing of water quality are also crucial to detect any ARP contamination (Samuel, 2019).

9. Enhanced Surveillance and Monitoring: Strengthening surveillance systems to track the prevalence and spread of ARPs within sanitary facilities is vital. This includes routine monitoring of surfaces, wastewater, and patient samples to identify emerging resistance patterns and monitor the effectiveness of infection control measures. Timely and accurate data can inform targeted interventions and response strategies.

10. Development of New Sanitation Technologies: Investing in research and development of new sanitation technologies can contribute to reducing ARP transmission. Innovative approaches, such as self-cleaning surfaces, antimicrobial coatings, and advanced disinfection techniques, can help minimize microbial contamination and prevent the spread of ARPs within sanitary facilities (Samuel, 2019).

11. Collaboration between Healthcare and Sanitation Sectors: Promoting collaboration between the healthcare and sanitation sectors is crucial to address ARP transmission effectively. Sharing information, expertise, and best practices can lead to integrated approaches that encompass both healthcare-associated and community-associated ARPs. This collaboration can facilitate coordinated efforts in surveillance, prevention, and control of ARPs in healthcare and sanitation settings.

12. Risk Communication and Public Engagement: Effective communication strategies are essential to educate and engage the public in preventing ARP transmission. Providing clear and accurate information about the risks of ARPs, proper hygiene practices, and the importance of sanitation can empower individuals to take necessary precautions. Public engagement initiatives, such as community workshops, educational campaigns, and targeted

messaging, can foster behaviour change and promote responsible actions (Murray et al., 2022).

13. Sustainable Antibiotic Manufacturing and Disposal: Promoting sustainable antibiotic manufacturing and disposal practices can help reduce ARP transmission. This includes promoting responsible antibiotic production processes, minimizing antibiotic waste and pollution during manufacturing, and encouraging proper disposal of unused antibiotics to prevent environmental contamination (Samuel, 2019).

14. International Collaboration and Harmonization: Global cooperation and harmonization of efforts are essential to effectively address ARP transmission via sanitation systems. This involves sharing knowledge, best practices, and research findings across countries and regions. International organizations can play a pivotal role in facilitating collaboration, establishing global guidelines, and fostering a coordinated response to mitigate the spread of ARPs (Murray et al., 2022).

Implementing these solutions can significantly reduce the transmission of ARPs within sanitary facilities and contribute to combating the global challenge of antimicrobial resistance.

2.1.10 Nosocomial infections

Nosocomial infections (also known as hospital-associated/acquired infections) are those infections that develop in a patient during his/her stay in a hospital or other clinical facilities and are not present at the time of admission. Hence, pathogens that cause such infections are termed nosocomial pathogens. The hospital environment is a potential reservoir of infectious agents since it houses patients with diverse pathogenic microorganisms and many

susceptible/immunocompromised individuals (Monegro et al., 2020). The nosocomial pathogens that cause infections can come from endogenous or exogenous sources.

The hospital exists as a closed community, so it is not surprising that certain microorganisms become predominant and cause diseases. The pathogens can be expelled from an infected or colonized patient either through the healthcare workers or even by the patients. Therefore, environmental surfaces in healthcare centres act as reservoir for bacteria and can as well serve as vectors of the bacteria pathogens. Depending on the environmental conditions, these pathogens may remain infectious on the surfaces for weeks after the contamination (Burke, 2018).

Nosocomial infections have continued to be an important cause of morbidity, mortality, prolonged hospital stay and extra financial burden to patients. The type of infection vary, the most common infection being urinary tract infection and pneumonia accounted for 60% of total hospital infections. Different bacterial species have been isolated from various hospitals across the world. Some of them are resistant to many antimicrobial agents. They are opportunistic pathogens and hence pose a challenge to patients, especially those who are immunocompromised (Akbari et al., 2015). Nosocomial infection is a crucial problem for patients, especially in high-risk departments such as the intensive care unit (ICU). The patients admitted in ICU are at high risk as a result of mechanical ventilation, prolonged catheterization and improper antimicrobial treatments (Conway et al., 2022).

Nosocomial infections are one of the most important issues regarding the surety of patients at healthcare institutions. Every hospitalized patient may acquire one nosocomial infection (Qyli, 2017). Nosocomial infections are major challenges to patient safety. It is estimated that

a total of 1.7 million nosocomial infections (4.5 per 100 admissions) occurred in 2002, and almost 99 000 deaths resulted from or were associated with the infection. Nosocomial infection is one of the leading causes of death. At any time, 1.4 million people suffer from hospital-acquired infection and its complications. The prevalence of hospital-acquired infection is roughly 5 – 10% in developed countries and up to10 – 30% in developing countries which varies between countries, states and districts. This is 2 - 20 times higher than developed countries (Qyli, 2017).

2.1.11 Modes of transmission of nosocomial infections in the hospital

The transmission of microorganisms from environmental surfaces to patients is largely via hand contact with surfaces, usually directly but sometimes indirectly by means of secretions from the body. Many ordinary surfaces such as upholstery, side table/bench, floors, carpets and many other areas in the hospital environment may not adequately be contaminated and can become reservoirs of pathogens (Weber, 2016). Otter et al. (2011) reported that surfaces can play important role in the epidemic and endemic transmission of the major pathogens linked to healthcare associated infections. Nosocomial infection caused by the nosocomial pathogens has posed problems of enormous magnitude globally. Hospital localities have proven favourable in transmission of infection due to existing suitable pathogens-host-environment relationship.

Nosocomial pathogens shed by patients can contaminate surfaces in hospitals at concentrations sufficient for transmission. It is constantly reintroduced into the hospital environment by visitors and patients transferred from other facilities. Spread occurs from patient to patient on the hands of hospital personnel, by direct patient contact with

contaminated reservoirs, and by the ingestion of contaminated foods and water. *Pseudomonas aeruginosa* has been recognized as an emerging opportunistic pathogen of clinical relevance i.e. it causes infections among immuno-compromised patients (Wang, 2019).

Air can also be a route of transmission of air-borne nosocomial pathogens (e.g., in droplet nuclei and aerosols) that infect the respiratory tract. The faecal-oral route is a portal of entry for food-borne and water-borne infections. Unfortunately, commonly used disinfection techniques are sometimes incapable of eradicating the fomite reservoir of nosocomial pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Hammuel et al., 2014).

2.1.12 Microorganisms associated with nosocomial infections in the hospital

In the past, most nosocomial infections were caused by gram-positive microbes, in which *Staphylococcus aureus* was the primary cause of nosocomial infection (Hammuel et al., 2014). Gram-negative bacteria, such as *E. coli* and *Pseudomonas aeruginosa* that can cause opportunistic skin infections are also the major cause today. The degree of occurrence of one or two of these organisms over others depends on the environment.

The environment significantly influences multiple factors in the chain of infection (Ekrami et al., 2011). Microorganisms are transmitted from environmental surfaces to patients largely via contact with the surfaces. Contamination of hospital equipment, medicines, and water supplies with hospital pathogens is a well-recognized cause of common-source outbreaks of infection. This can be especially troublesome in hospital environments where patients with

immunodeficiency are at enhanced risk for contracting nosocomial infections (Hammuel et al., 2014).

The bacteria that commonly cause nosocomial infections include *Staphylococcus aureus*, *Streptococcus* species, *Bacillus cereus*, *Acinetobacter* species, coagulase negative staphylococci, enterococci, *Pseudomonas aeruginosa, Legionella* and members of the Enterobacteriaceae family such as *Escherichia coli*, *Proteus mirabilis*, *Salmonella* species, *Serratia marcescens* and *Klebsiella pneumoniae*. *Pseudomonas aeruginosa* which is one the major nosocomial pathogens is capable of multiplying in two days, it can swim from one site to the next as motile cell or it multiplies as an adherent microcolony biofilm by producing a slimy layer (Jernigan et al., 2020; Muhammad, 2021).

2.1.13 Origin of antimicrobial resistance in the environment

The primary source of AMR bacteria is the environment. Most of the genes responsible for AMR did not evolve in clinical or agricultural settings, but rather long before this, in the natural environment due to the presence of naturally occurring antibiotics (including penicillin, streptomycin, tetracycline and chloramphenicol) derived from soil-dwelling bacteria and fungus as a means to compete with other bacteria for limited resources (Nelson & Levy, 2011; Gaynes, 2017). However, as these antibiotic-producing bacteria are physiologically similar to the bacteria they compete with, there is potential for them to be susceptible to others' toxic metabolic compounds and therefore they had to develop defence mechanisms to protect themselves.

Many AMR genes may have originated from these antibiotic producers, disseminated to other bacterial species via horizontal gene transfer over time. Antibiotic resistance may have also arrived from spontaneous mutations that have altered the antibiotic target sites, as well as pre-existing genes encoding the enzymes or transported by efflux pumps and later evolving as a resistance mechanism (Chen et al., 2015; Blanco et al., 2016; Dickinson et al., 2019).

2.1.14 The rise of antimicrobial-resistant bacteria

AMR is not a modern phenomenon; it is a complex challenge and while much attention has been given to Alexander Fleming's prescient warning of its emergence in his acceptance speech for the 1945 Nobel Prize for Medicine, resistance far precedes that observed in the first mass-produced antibiotics. Genes encoding resistance to several antibiotics have been found in 30,000-year-old permafrost sediments (D'Costa et al., 2011), and β lactamases, the enzymes that render penicillin-like antibiotics ineffective, are estimated to be 1–2 million years old. Antibiotics are found in fungi and bacteria as a natural defence mechanism (D'Costa et al., 2011). They may have become a severe problem only as a result of human activities, including their misuse and overuse in healthcare and agriculture (Shallcross & Davies, 2014).

In addition to evolution and random mutations driven by selection due to antibiotic pressure or other environmental stress factors, AMR can arise through horizontal gene transfer between species, including the exchange of pathogenicity islands via plasmids, transposons, chromosomal cassettes or prophages (Munita & Arias, 2016). Resistance genes can then be transmitted to other areas, including where antibiotic usage is significantly lower, via the movement of humans or through the food chain (Sivaraman et al., 2021a).

2.1.15 The challenge of antimicrobial-resistant (AMR) bacteria surveillance in public settings

The prevalence and distribution of AMR bacteria are on the rise (Barros et al., 2012; Lee et al., 2019), but due to limited resources, including time, cost, expertise and the heavy focus on observing clinically important pathogens, it has not been possible to implement a comprehensive AMR surveillance system in public settings for all bacterial species. Surveillance is mainly limited to human and animal bacterial pathogens in healthcare facilities and agriculture. However, due to the nature of horizontal transfer, even non-pathogenic bacteria that possess AMR genes need to be considered a public health threat as they risk transferring the AMR genes to known pathogens (Sivaraman et al., 2021b). In addition, AMR bacteria have been known to spill over from hospitals and agriculture into public settings such as restaurants, street food stalls and markets (Manyi-Loh et al., 2018; Sivaraman et al., 2020, 2021a).

Moreover, inappropriate antibiotic use and self-prescribing within low-income and middle-income countries (LMICs), particularly in lower socioeconomic communities, may also contribute to increased AMR in the community and in public settings (Erku et al., 2017; Saleem et al., 2019) including the carriage of mobile genetic elements by houseflies (Sobur et al., 2019). There is, however, a serious knowledge gap about prevalence in public settings, much of which has gone unreported. The current methodology is the main limiting factor preventing better surveillance of AMR bacteria in public settings.

Biases in traditional culturing methods select only those organisms that can grow within laboratory conditions. This limits the number of studies to a specific group of microorganisms

that have been previously shown to be a public health threat; are culturable in the lab; are easily speculated using molecular typing methods (e.g. multilocal sequence typing (MLST), pulsed-field gel electrophoresis (PFGE) and *spa* typing for *Staphylococcus aureus*); are known to contain clinically relevant AMR genes; are detectable by PCR; and can be profiled by a standardised antibiotic susceptibility test (Maugeri et al., 2019).

More advanced culture techniques known as culturomics have been used for high throughput analysis to identify and characterise microorganisms in their habitats, including unknown species that were previously known as uncultivable (Greub, 2012). Culturomics uses a wide range of culture conditions and techniques, such as sequencing, for rapid identification of species. Although this technique can expand the repertoire of culturable bacteria, many bacterial species within a habitat are still not detected, and its execution still remains labour-intensive, costly and time-consuming (Nowrotek et al., 2019). Due to more recent advancements and a sharp drop in the cost of next-generation sequencing technologies, however, it is becoming increasingly possible to perform culture-independent metagenomic studies (Forbes et al., 2017). This approach is highly comprehensive and has high precision in bacterial species identification, quantification, and functional characterisation of genes (Thompson et al., 2017).

The level of detail obtained from metagenomics analysis offers opportunities to use the technology for the development and management of ‘smart cities’, potentially linking urban design with public health, for instance, in the design of more hygienic subways and public transport systems (Mason et al., 2016). Using the information from metagenomics, we can use an interdisciplinary collaborative approach of experts in public health, microbiology,

bioinformatics, data science, architecture, material science and social science to better understand the spread of infectious disease and AMR and how to prevent it. The only limiting factor of metagenomic culture-independent studies is that it contains unknown sequences from unidentified microbes and lack of knowledge regarding the phenotypes of isolates that display antibiotic resistance as characterising antibiotic genes alone cannot always correlate to antibiotic resistance phenotype as antibiotic resistance phenotypes can alter due to environmental changes or be influenced by the genetic context of resistance determents (Hughes & Andersson, 2017; Jaillard et al., 2017; Nowrotek et al., 2019). Therefore, culturing methods are still preferred to link antibiotic resistant phenotypes to the bacterial strain and genotype.

Although culture-independent metagenomics studies within public settings remain very limited, a body of knowledge is emerging on, for example, mass transit systems, including in Hong Kong (Kang et al., 2018), Boston (Hsu et al., 2016), and opportunistic pathogens on the New York subway (Afshinnekoo et al., 2015); in athletic facilities (Fahimipour et al., 2018) and classrooms (Hartmann et al., 2016).

However, most studies that investigate built-up environments and non- healthcare public settings still use culture-dependent methods to detect staphylococci and enteric bacteria such as *Enterobacteriaceae*, as these are the most frequently recovered bacteria from such areas that are known to cause infections in humans (Roberts et al., 2013). This perpetuates an incomplete knowledge of not only the microbiome of such environments but also the best ways to design out vulnerabilities to disease transmission and spread. Such understanding could offer benefits to the control of other diseases, including influenza and COVID-19 as

well as those caused by AMR bacteria, by identifying where targeted hygiene interventions might be best introduced.

2.1.16 Factors contributing to the transmission of antimicrobial resistant bacteria in public settings in built environments

Multiple studies have indicated that AMR bacteria can be transmitted to humans in public settings including on buses (Conceiçao et al., 2013); at railway stations (Lin et al., 2017); on subway trains (Mason et al., 2016) and in university classrooms (Li et al., 2015). These areas typically see high population movement and throughput and, at times, high population density (Batista et al., 2020).

Public settings such as mass transport systems (buses and trains) contain surfaces that are frequently touched by multiple people and so too do shops and leisure facilities, all posing a particular challenge to AMR spread. High- frequency hand-touched surfaces can act as a vector for AMR transmission from person-to-person if they either touch these surfaces directly or via bacterial shedding from one's skin onto a surface which is then transmitted to others who touch the same surface (Conceicao et al., 2013; Plipat et al., 2013; Cave et al., 2019).

Surfaces that are not sanitized regularly and are touched multiple times a day, particularly if this is by people who have poor hand hygiene, could further the transmission of AMR bacteria within the population (Cobrado et al., 2017). Moreover, within overcrowded public settings, AMR bacteria can be transmitted directly, either via the air from bacterial shedding from skin, by direct contact, or through food (Li et al., 2018; Siva-Raman et al., 2021). Some

of these factors, including direct contact and aerosolized transmission have been key for transmission of SARS-CoV-2, the virus that has caused the COVID-19 pandemic (Meyerowitz et al., 2020). To successfully combat the transmission, governments across the world have taken measures to educate the public with regards to hand hygiene, to improve cleaning and hygiene practices relating to frequently- touched surfaces to prevent fomite transmission, and have introduced social distancing rules to prevent transmission via aerosols (Chu et al., 2020).

Maintaining long-term social distancing in the post-COVID-19 era may the easiest task to undertake due to its adverse physical and mental effects (Di Corrado et al., 2020), along with the economic (Nicola et al., 2020) and social compliance challenges (Hills & Eraso, 2021), however keeping up with good hand hygiene and cleaning/infection control practices will help prevent transmission of infectious diseases including those caused by antimicrobial resistant bacteria.

2.1.17 Antimicrobial resistant *Staphylococcus aureus* from fomite surfaces and air in public settings

Increasingly, studies are showing that MRSA can be found outside of clinical settings, in several places frequented by the general public. Studies have, for example, looked at university campuses and recreational beaches in the USA (Roberts et al., 2013); public buses in Portugal (Conceiçao et al., 2013), the USA (Lutz et al., 2014) and Ethiopia (Kahsay et al. 2019); transport hubs and metro systems in China (Lin et al., 2017; Peng et al., 2015); and the handles of shopping baskets in Japan (Domon et al., 2015).

Transmission of HA-MRSA onto surfaces found in public buses has been linked to bus routes connected with hospitals, suggesting unintentional spread by patients and hospital workers (Conceiçao et al., 2013; Lutz et al., 2014). Between May 2011 and May 2012, the authors (Conceiçao et al., 2013) screened hand touched surfaces of 199 buses in Lisbon, Portugal; 32% were contaminated with MRSA, whereas 15 of the 575 passengers screened carried MRSA on their hands.

2.2 Theoretical Framework

There are many theories of behavioural change, some of which synthesise multiple theoretical components into a single framework. This study will adopt behavioural modification theory. Behaviour modification uses empirically demonstrated methods to improve or change behaviour. There are generally three primary levels of influence related to behaviour modification and infection control in healthcare facilities: 1) Intrapersonal factors (e.g., individual characteristics – healthcare personnel knowledge, skills, attitudes, perceptions); 2) Interpersonal factors (e.g., friends, peers, role-models); and 3) Institutional or Organizational factors (e.g., rules, policies, norms, culture). Including these three levels of influence in infection control interventions is key to prevention efforts in healthcare facilities (Scott et al., 2022).

2.2.1 Principles of behaviour modification for effective infection control

On an individual level, healthcare personnel (HCP) should have the necessary knowledge, skills, and abilities to implement effective infection control practices. Research suggests that increasing the knowledge base of HCP may influence their perceptions and motivate them to

change their behaviour – this includes providing access to information that supports learning and problem-solving activities. However, increasing knowledge alone may not be sufficient for effective infection control and may be insufficient to effect sustained change especially considering the multi-factorial nature of the problem of healthcare-associated infections (Lutz et al., 2014).

The use of skill building, peer-to-peer role modelling, and champions on an interpersonal level have been shown to influence the implementation of key infection control practices positively. On an institutional level, healthcare facilities should work to foster organizational attributes such as leadership support, interdisciplinary teamwork, and communication. In addition, efforts should be made to promote HCP job satisfaction. Efforts also should be made to engineer and implement systems throughout the organization that prompt, reinforce, and facilitate best practices (e.g., prescribing practices) so that infection control and prevention is embedded in all aspects of care. This includes providing the necessary resources so that HCP have reliable and easy access to supplies (e.g., alcohol-based rub) (Lutz et al., 2014).

Healthcare institutions need to implement sustainable systems that promote shared knowledge and provide HCP with an architecture that prompts and facilitates appropriate behavior (e.g., antimicrobial stewardship decision systems).On the inter-institutional level, there is some evidence that interfacility collaboration, such as participation in multicenter quality improvement collaboratives, can influence organizational learning and organizational culture in ways that facilitate improved infection control practices among HCP (Wise et al., 2011).

2.2.2 Consequences of ignoring behavior modification and infection control

Studies have found that HCP factors such as knowledge, attitudes, and perceptions (e.g., perceived benefits and barriers) are likely to influence changes in behavior and practices of HCP. If HCP do not have the necessary knowledge to perform appropriate infection control practices and/or perceive that there is not a problem or they are not at risk (personally or to their patients), then infection prevention practices may be compromised. If a healthcare facility lacks role models or champions, appropriate HCP infection control skills may be limited, and best practices to prevent healthcare-associated infections may not be consistently implemented. Weak or poor organizational culture factors (e.g., leadership, teamwork, communication) also have been shown to be risks for infection control problems and adverse patient outcomes (Mayer et al., 2011).

2.2.3 Information that supports the conclusions of behaviour modification and infection control

There is limited quantitative data regarding behavioral modification and infection control published to date. There is however a few large studies that purport success for preventing infections through behavior modification but the causal link and association between the modified behavior and the infection control area of interest are not well established. For example, data from a large multicenter collaborative cohort study which included daily goals sheets to improve clinician-to-clinician communication and a comprehensive unit-based safety program to improve organizational culture resulted in a sustained reduction in the rate of catheter-related bloodstream infection of 66% at 16-18 months after implementation (Wise et al., 2011). In addition, a program of universal surveillance, contact precautions, hand

hygiene, and institutional culture change was associated with a significant and sustained decrease in healthcare-associated MRSA transmissions and infections.

2.3 Empirical Review

2.3.1 Microorganisms associated with contact surfaces in different environments

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

Nworie et al. (2012) investigated bacterial contamination of door handles/ knobs in selected public conveniences in Abuja Metropolis, Nigeria. Door handles/ knobs of public conveniences such as public offices, motor parks, and markets in Abuja metropolis were investigated for bacterial contamination. Out of the 180 samples, 156 (86.7%) were found positive. More positive samples were found in female toilet handles/knobs (41.7%) and bathroom door handles/knobs (11.5%) than males. In this study most of the bacterial contaminants were found coliforms. The isolated organisms were *Staphylococcus aureus*

(30.1%), *Klebsiella pneumoniae* (25.7%), *Escherichia coli (*1%), *Enterobacter* species (11.2%), *Citrobacter* species (7.1%), *Pseudomonas aeruginosa* (5.9%), and *Proteus* species (4.5%). The study found higher rate of contamination in toilet door knobs/handles of markets, motor parks and restaurants compared to government offices and banks.

Lorina et al. (2015) carried out a research on potential pathogenic bacteria contaminants of shared utility devices in a university setting at Al-Hofuf, Saudi Arabia. Samples were collected from office and toilet doors handles/knobs, washroom tap heads, elevator buttons and computer keyboards. Antibiotic susceptibility test as well as minimum inhibitory concentrations (MICS) were also determined. The bacterial contaminants found were *Staphylococcus aureus* (4.02%), *Staphylococcus haemolyticus* (18.59%), *Staphylococcus epidermidis* (21.10%), other *Staphylococcus* species (51.76%), *Enterococcus faecalis* (2.01%), *Enterococcus* species (1.51%), *Klebsiella pneumoniae* (0.50%), *Streptococcus sanguins* (0.50%), *Pseudomonas aeruginosa* (14.03%), *Pseudomonas stutzeri* (3.5%), *Pseudomonas luteola* (10.53%), and *Pantoea* species (72%). The study also found multidrug resistant organisms among the isolates which indicate the source of infection in the university community.

Augustino et al. (2014) determined the bacterial load and antibiotic susceptibility of bacteria isolated from students toilets at Sokoine University of Agriculture, Morogoro, Tanzania. About 60 samples were collected from 30 different toilets in different surfaces including toilet seats, toilet bowls, door handles in and out of the restrooms, faucet handles, toilet flush handles and the restroom floors. The following bacteria species were isolated from this research; *Staphylococcus aureus* (25%), *E. coli* (36.7%), *Pseudomonas aeruginosa* (13.3%),

Streptococcus pyogenes (6.7%), *Proteus mirabilis* (6.7%) and *Klebsiella pneumoniae* (11.6%). The results indicated that surfaces routinely touched with hands had the highest bacterial contamination compared to restroom floor and toilet seats. Results of antibiotic susceptibility testing indicated that all bacterial isolates were resistant and intermediate resistant to at least one antibiotic.

Opere et al. (2013) conducted a study on antibiotic susceptibility and plasmid profile analysis of pathogenic bacteria isolated from environmental surfaces in public toilets. Samples were collected from door handles, tap handles and flush handles of a public toilet. A total of eight (8) organisms were isolated which include *Bacillus* species (4.35%), *Staphylococcus aureus* (34.72%), *Staphylococcus epidermidis* (34.72%), *Micrococcus* species (4.35%), *Pseudomonas* species (8.70%), *Enterococcus faecalis (4.35%), Salmonella typhi* (4.35%) and *Shigella dysenteriae* (4.35%). Results of antibiotic susceptibility testing revealed that *Salmonella typhi* had the highest level of multidrug resistance, showing resistance to all eight antibiotics tested. *Bacillus* species and *Pseudomonas* species showed susceptibility to five of the eight antibiotics. Plasmid profile analysis of the isolates revealed the absence of plasmids.

Omar et al. (2016) conducted a research on microbial contamination of door knobs in public toilets during Hajj. Samples were taken from door knob surfaces of 224 randomly selected toilets in Arafat, Muzdalifah and Mina places. Bacterial Contamination was found in (78.3%) of doorknobs. The highest number of contamination was found in Muzdalifah (100%) followed by Arafat (73.3%). The study revealed the presence of both Gram positive (49.2%) and Gram negative bacteria (35.0%). The isolates that were found include *Staphylococcus aureus* (22%), coagulase-negative Staphylococci (CoNS) (17.3%) and *Acinetobacter* (10%).

Out of 42 *S. aureus* isolates (16.7%) were found to be MRSA (positive for mecA genes) and (31 %) were positive for PVL. The mecA and PVL genes of *Staphylococcus* isolates were detected by PCR.

Sabra (2013) investigated bacterial public health hazards in the public female restrooms at Taif, KSA. A total of 260 specimens were collected from each restroom (RR) differentiated as follows: RR Door (No. =20), RR Handle (No. =20), RR Sink (No. =20X3=60), RR Toilet Door (No. =20X4=80) and RR Toilet Handle (No. =20X4=80). Positive results were found in 187 samples, including RR Toilet Handle in 73/80 (91.3%), RR Toilet Door in 59/80 (73.8%), RR Sink in 38/60 (63.3%), RR Handle in 10/20 (50%), finally lower positive were found from RR Door in 7/20 (35%). The isolated bacteria arranged according to their percentage as *Staphylococcus aureus* 76/187 (40.6%), *Escherichia coli* 42/187 (22.5%), *Bacillus* species 40/187 (21.4%), *Klebsiella pneumoniae* 25/187 (13.4%), *Enterococcus faecalis* 18/187 (9.6%), *Citrobacter* species16/187(8.6%), *Pseudomonas aeruginosa* 13/187 (7%) and *Proteus mirablilis*10 /187 (5.3%).

Maryam et al., (2014) aimed at characterization and determination of antibiotic susceptibility pattern of bacteria isolated from some fomites (tables, chairs, pens, stethoscopes, uniforms, doorknobs and IVF stands) in a teaching hospital in northern Nigeria. 35 samples were used for this study among which 23 (65.7%) isolates were obtained. The ratio of Gram positive to Gram negative bacteria was 12:11. The bacteria isolated were *Staphylococcus aureus* (21.7%), *Staphylococcus epidermidis* (8.7%), *Streptococcus* species (8.7%), *Bacillus* species (13.0%), *Escherichia coli* (26.1%), *Pseudomonas* species (8.7%) and *Klebsiella* species

(13.0%). The isolated bacteria were found susceptible to the antibiotics used and all were susceptible to erythromycin and streptomycin.

Adewoyin et al. (2013) evaluated antibiotic resistance profile of microbial isolates of toilet bowl of some students' hostels in Ogbomoso, Nigeria. A total of fifteen bacterial isolates were identified from toilet bowl of six prominent student hostels. Out of the fifteen bacteria isolates, the genus *Streptococcus* was highly dominant with highest prevalence (19.36%) followed by *Streptococcus faecium* and least (3.23%) by *S. pyogenes* and *S. zymogenes*. *Pseudomonas aeruginosa* showed highest resistance (90%) and *Streptococcus zymogenes* showed lowest resistance (20%) to antibiotics tested. About 80% of all the isolates were found resistant to augmentin while 26.67% resisted pefloxacin and chloramphenicol. The highest and lowest total bacterial counts (TBC) were 33.90±4.23 x 10 and 9.00±1.80 x10CFU/ml, respectively.

2.3.2 Prevalence and antibiogram pattern of some nosocomial pathogens isolated from hospital environment

Hammuel et al. (2014), in their study on the prevalence and antibiogram pattern of some nosocomial pathogens isolated from hospital environment in Zaria, Nigeria using 160 swab samples from ten different surfaces including nurses' hand swab, nurses' table top, door knob/handle, toilet seat, operation table, sink, stretcher, floor, bedrail, and cupboard. The total percentage prevalence of *Staphylococcus aureus* was 50.80%, *Pseudomonas aeruginosa* 28.60% and *Escherichia coli* 20.60%. Out of 20.60% of *E. coli* isolates 7.7% were found to be *E. coli* O157:H7. *S. aureus* isolates were highly resistant to ampicillin and cefoxitin *P. aeruginosa* and *E. coli* were resistant to tetracycline. The multiple antibiotic resistance

indexes of the pathogens were more than 0.2mg/ml. Among the isolates, *S. aureus* showed more multidrug resistance (31.30%) and *E. coli* had the least multidrug resistant. Frequently touched surfaces within the hospital environment are contaminated by *Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus.*

Chikere et al. (2018) reported the distribution of probable nosocomial pathogens in a government hospital in Nigeria using thirty swabs and air samples from patients, hospital personnel, fomites and air in four wards namely orthopaedic (OW), paediatric (PW), surgical (SW) and medical (MW). For the patients and personnel, skin and nasal samples were used. A total of 56, Gram positive (45) and Gram negative (11) bacteria were isolated. Gram positive cocci were the highest number of isolates of which *Staphylococcus epidermidis* (22; 39.2%) occurred the most especially from the skin in all the wards. This was followed by *Staphylococcus aureus* (16; 28.5%) and the least being *Streptococcus* species (5; 8.9%). Among the Gram negative bacilli, *Escherichia coli* was the highest (4; 7.1%). Others were *Klebsiella pneumonia* (3; 5.3%), *Proteus* spp. (2; 3.5%) and *Enterobacter aerogenes* (2; 3.5%). The only Gram positive bacilli isolated were *Bacillus cereus*. Orthopaedic ward (22) had the highest number of isolates followed by paediatric ward (15). Surgical and medical wards had 10 and 9 isolates, respectively.

Anyadoh-Nwadike et al. (2011) reported the prevalence of *Staphylococcus aureus* (*S. aureus)* in the hospital environment (General Hospital, Umuguma Owerri, Imo State, Nigeria) using forty-five (45) swab samples. Twenty-five (25), representing 55.6% of samples from the hospital environment, were suspected of harbouring *S. aureus*. Two samples i.e. 4.0% had non-significant growth of the organism while the rest 13 i.e. 28.9% did not seem to harbour

the organism. The morphological characteristics of the organisms on both media used (Mannitol salt agar and MacConkey agar) were used as presumptive identification, while standard biochemical tests were used to confirm the isolates as *S. aureus.* The analysis revealed a significant prevalence of *Staphylococcus aureus* within the hospital environment.

Nsofor et al. (2015) reported the distribution and antibiotic susceptibility pattern of *Staphylococcus aureus* isolates from Health Care Workers in Owerri, Nigeria using 156 nasal swab specimens collected from the healthcare workers (HCW). Nasal swab specimens from 78 males and 78 females were cultured on mannitol salt agar. *S. aureus* was identified by using standard microbiological techniques. Antibiotic susceptibility testing was carried out by Kirby-Bauer disk diffusion method. The overall carrier rate of *S. aureus* among the HCW was 47.4%. The antibiotic susceptibility test result shows that erythromycin was most active drug while highest resistance was observed in oxacilin. Statistical analysis showed that average number of resistance phenotypes per isolate was not significantly different in any of the sampled hospitals ($P<0.05$). The high carrier rate of *S. aureus* and antibiotics resistant observed in this study emphasizes the need for continues surveillance of the antibiotic susceptibility of *S. aureus* aimed at recommending appropriate and effective therapy in the treatment of staphylococcal infections.

Nwokorie and Nwachukwu (2015) reported on the prevalence and antibiotic susceptibility of *Staphylococcus aureus* in suppurative lesions of the surgical ward and outpatients of Imo State Teaching Hospital Nigeria. The prevalence of *Staphylococcus aureus* in 122 patients sampled was 59.4% for the surgical inpatients and 48.3% for outpatients giving an average prevalence of 53.9% for both groups of patients. The average antibiotic susceptibility patterns

for the 8 antibiotic tested were: ampicillin (75.0%), chloramphenicol (34.4%), ciprofloxacin (1.6%), erythromycin (7.8%), gentamycin (0%), methicillin (1.6%), tetracycline (45.3%) and co-trimoxazole (50.0%). The resistance in surgical inpatients was significantly higher than outpatients ($t=1299$, $p<0.05$) and Methicillin resistance was confirmed by PCR. *Staphylococcus aureus* is highly prevalent and more resistant in inpatients. There is a higher risk of acquiring drug resistant *Staphylococcus aureus* infection in inpatients of the hospital with a Methicillin resistance of 0% and 2.6% for out and inpatients respectively.

Aloma et al. (2016), reported on isolation, characterization and antibiotic susceptibility patterns of *Pseudomonas aeruginosa* and *Staphylococcus aureus* from hospital environment in Kaduna metropolis, Kaduna State using four different hospitals. One hundred and sixty (160) swab samples (40 each) were taken from environmental surfaces of the four hospitals. Their results showed that one hundred and seventy (170) bacterial pathogens were isolated, of which 138(81.2%) were identified as *Staphylococcus aureus*, and 32(18.8%) were identified as *Pseudomonas aeruginosa* and all were confirmed with polymerase chain reaction. The total percentage distribution of this pathogen on the surfaces was 53.12%. *S. aureus* was isolated from sinks (43.8%), floor (90.6%), bedrails (96.9%), door knobs (100%) and table tops (100%). *P. aeruginosa* was isolated from sinks (59.4%), floor (34.4%), table tops (6.3%) with none isolated from door knobs and bedrails. The statistical analysis showed that there was significant difference in the prevalence rate of the organisms on the surfaces. (P=0.000*). Of all the *Staphylococcus aureus* isolates, 41.3% were found to be multi-drug resistant (MDR), while 21.9% of the *P.aeruginosa* isolates were MDR.

Hammuel and Yadock (2017) in their study on prevalence and antibiotic susceptibility pattern of *Pseudomonas aeruginosa* and *Escherichia coli* O157:H7 isolated from two hospital environments in Zaria, Nigeria using 310 samples from two hospitals (Major Ibrahim B. Abdullahi memorial hospital and St. Luke Anglican hospital). The results of their study at Major Ibrahim B. Abdullahi memorial hospital showed that 16 (69.6%) and 7 (30.4%) were *P. aeruginosa,* and *E. coli* respectively, while from St. Luke Anglican hospital, 13 (61.9%), *P. aeruginosa* and 8 (38.1%) *E. coli* were isolated. There was zero (0) prevalence of *Escherichia coli* O157:H7 in the two hospitals selected for the study. *Pseudomonas aeruginosa* was 100% susceptible to ceftazidime and imipenem, *E. coli* was also 100% susceptible to gentamicin, cefoxitin and ceftazidime. *P. aeruginosa* and *E. coli* were more resistant to tetracycline. The multidrug resistant of the isolates from Major Ibrahim B. Abdullahi memorial hospital showed that 12.5% of *P. aeruginosa* and 14.3% of *E. coli* were also multidrug resistant. There was no multidrug resistant isolates among *E. coli* from St. Luke's Anglican hospital but 30.8% of *P. aeruginosa* were multidrug resistant isolates.

Zhinzela (2017) in their study on prevalence of potential nosocomial pathogens isolated from environments of regional Hospital of Korca, Albania. The study revealed that the prevalence of microorganisms isolated was as following: *Staphylococcus aureus* 237 (60.3%) of isolates, *E. coli* 124 (31.6%), *Klebsiella* species 1 (0.3%), *Pseudomonas* species 13 (3.3%), *Proteus* species 1 (0.3%), *Staphylococcus epidermidis* 4 (1.0%), Saprophytes 13 (3.3%).

Kumar and Singh (2015) in their study on incidence and antimicrobial resistance among potential nosocomial bacteria isolated from indoor environment of hospital, using 165 clinical samples, revealed that a total of eighty one bacterial isolates were obtained from 165 patients

(those admitted in hospital). The most common organism came to be *E. coli.* The sensitivity pattern of *Klebsiella pneumoniae* showed that 87.5% of the isolates were sensitive to polymyxin B, followed by 75% to amikacin, 62.5 % sensitive to both netimycin and Ciprofloxin. As much as 43.7% of *Staphylococcus* were resistant to amikacin and cefaperazone plus sulbactum while 50% percent of them were sensitive to netimycin and polymyxin B. However, 56.3% were sensitive to ceftazidime.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

Cross-sectional descriptive and experimental study designs were adopted for this study. A cross sectional study is a type of study that analyzes data from a population, or a representative subset, at a specific point in time. Experimental study design involves conducting research in an objective and controlled fashion so that precision is maximized and specific conclusions are drawn.

3.2 Area of Study

The study area of this study was Referral Hospitals in Abakaliki Metropolis, the Capital City of Ebonyi State, and southern eastern Nigeria. Abakaliki City lies on the geographical coordinates of 6^0 20'ON', 8^0 6'O'E latitude of Nigeria. It lies at the intersection of roads from Enugu, Afikpo and Ogoja located 64 kilometers Southeast of Enugu. The capital city is surrounded with the following towns: Effium, etc. The last known population of Abakaliki was nine hundred and fifteen thousand, four hundred thirty eight (915,438) person (year 2019).

Abakaliki people, like other south-eastern Nigerians, are predominantly Christians. Other religious faiths like Traditionalist, Islam, Jewish, etc, are practiced by a handful of the natives as well as non-natives from other parts of the country. Roman Catholic, Presbyterian, Anglican and other Pentecostal missions are the dominant Christian faiths. Abakaliki

Metropolis's residents are primarily civil servants, public servants, farmers, traders, and politicians. The capital city is richly blessed with natural deposits or resources such as lead, limestone, zinc and salt where the salt of nation for Ebonyi State originated from. Culturally, Abakaliki is richly blessed with a lot of cultural heritages. The diverse cultural heritages are integrated into a unit known as cultural knot of Abakaliki town.

The former Federal Medical Center Abakaliki now Federal Teaching Hospital Abakaliki was established in 1930s by the then colonial administration to serve as a casualty control post for soldiers wounded in the Cameroon theatre of the 2nd World war. The Alex Ekwueme University Teaching Hospital as it is known today was established as one of the Frontline tertiary health institutions in Nigeria dedicated to delivering efficient, effective and qualitative services comparable with internationally acceptable best practices.

The then Federal Medical Center was upgraded to Teaching Hospital in December 7,2011 by the then president Ebele Goodluck Jonathan in fulfilment of his campaign promises. He directed that Ebonyi State University Teaching Hospital be absorbed into the new mega Teaching Hospital comprising FETHA 1(former FMC) and FETHA 11(Former EBSUTH) under the leadership of Prof. Paul Ezeonu as Chief Medical Director, (CMD). The hospital is primarily assigned with the responsibilities of providing quality, accessible and affordable health care services The hospital since it's inception has had several and reputable Chief Medical Directors(CMD) till now. It has several departments in operational as full-fledged University Teaching Hospital. The Alex Ekwueme Federal University Teaching Hospital Abakaliki, (AEFUTHA 1 & 11) is located within the capital city of Ebonyi State, Abakaliki The hospital is proximate to each other not more than one kilometer away. The hospital is

world-class with a retinue of over 5,000 staff comprising over 200 consultants in various specialities,602 beds, wards complex distributed in various departments, 500 doctors undergoing residency training and a capacity for training of over 250 House officers every year. It has over five thousand employees (5,000), staff nominal roll. It is the only referral hospital in Abakaliki, Ebonyi State and has several annexes operational within the State. The Federal Teaching Abakaliki was renamed Alex Ekwueme University Teaching Hospital Abakaliki in 2019.

3.3 Study Population

In Abakaliki, there are only two Referral Hospitals (FETHA 1 and FETHA 2). This study comprised two (2) Referral Hospitals in Abakaliki, Ebonyi State, Nigeria. The sanitary facilities in the Referral Hospitals were swabbed using sterile swab sticks moistened in normal saline were used to swab the surfaces of bathroom floors, toilet seats, door handles, flush knobs, toilet covers and wash-hand basins.

3.4 Sample Size and Sampling Method

3.4.1 Sample size

A total of one hundred and forty (140) samples were collected from the sanitary facilities of the two (2) Referral Hospitals in Abakaliki, Ebonyi State. Sample size was derived using Leslie Kish formula used for an unknown population.

$$n = \frac{Z^2 Pq}{d^2}$$

Where: N=Minimum sample size

Z^2=Standard Normal Deviate set at 1.96

P= 10.2% [proportion of sanitary facilities in the referral hospitals

Q= 1-p = 89.8%

d= Level of precision set at 0.05

$n = \frac{1.96^2 * 0.102 * 0.898}{0.052}$

n = 140

3.4.2 Sampling Technique

A purposive sampling technique was adopted in the selection of the Referral Hospitals in Abakaliki, Ebonyi State. Also, the study adopted an experimental technique in the collection of samples from different sanitary facilities in the hospitals. Seven different sanitary facilities were sampled. A total of one hundred and forty (140) samples comprising of seventy (70) each from the two (2) Referral Hospitals in Abakaliki, Ebonyi State using swab stick technique. Thereafter, sterile swab sticks moistened with sterile water were used in the swabbing of the sanitary facilities in the hospitals.

3.5 Instrument for Data Collection

3.5.1 Checklist

The instrument for data collection was a checklist for health facility assessment by Joint Monitoring Program (JMP) under World Health Organization. The checklist was used to assess the nature of the sanitary facilities in the Referral Hospitals (WHO, 2021 (See Checklist in Appendix).

3.5.2 Microbiological instruments

Swabbing technique using swab sticks were adopted in the collection of samples from the sanitary facilities in the hospitals. Sterile swab sticks moistened with sterile water were used in the swabbing of the sanitary facilities in the hospitals. The hospital sanitary facilities such as; bathroom floors, toilet seats, door handles, flush knobs, toilet covers and wash-hand basins were swabbed and analyzed for the presence of pathogens.

3.6 Validity of the Instrument

The checklist as the instrument of data collection was developed and submitted to the researcher's supervisor and two other experts in the field of Public Health for proper scrutiny to ensure that the checklist was valid to assess the objectives of the study. For the microbiological instrument, already existing methods described by Hammuel et al. (2014) was adopted.

3.7 Reliability of Instrument

The reliability of the instrument was determined using the test re-test method. The checklist was used by recruited research assistants to obtain relevant data from a study location different from the study area. These facilities shared characteristics similar to those of the selected hospitals in Abakaliki, Ebonyi State, used for this study. The reliability of the instrument was tested using Chronbach Alpha Coefficient of Reliability test, and a coefficient of (r=0.82) was deemed reliable.

3.8 Method of Data Collection

Data were obtained using a Checklist and sterile swab sticks. This was done with the aid of two (2) field assistants who was trained to aid the researcher in the data collection process. The checklist was used to assess the different sanitary facilities in the hospitals. This was done by checking all the availability and nature of the sanitary facilities and each available were indicated as well as the conditions of the sanitary facilities. For the sterile swab sticks, they were moistened with sterile water were used in the swabbing of the sanitary facilities in the hospitals. Each of the sanitary facilities was carefully swabbed by gently rubbing the swab stick on the surfaces of the sanitary facilities.

3.9 Method of Data Analysis

3.9.1 Descriptive Study

Data generated from this study was analyzed using SPSS statistical software, chi-square test and analysis of variance (ANOVA). The variables will be expressed in mean and standard deviation. A p-value of less than 0.05 ($P < 0.05$) will be considered statistically significant.

3.9.2 Experimental Study

Preparation of media and sterilization of glasswares and materials

The media used in this study included nutrient agar, eosin methylene blue agar, sabouraud dextrose agar, peptone water, triple sugar iron agar, Simmon's citrate agar, Mannitol salt agar and Mueller-Hinton agar. Nutrient agar served as general purpose media for the isolation of different types of aerobic bacteria. Eosin methylene blue agar served for the isolation of coliform bacteria while sabouraud dextrose agar was used in the isolation of fungi (yeasts and molds). Triple sugar iron agar was used for the identification of sugar fermentation test, acid production, gas production and hydrogen sulphide production. Peptone water was used together with Kovac's reagent for identification of indole producing bacteria while Simmon's citrate agar was in the identification of citrate utilizing bacteria. Mannitol salt agar was used for the isolation of *Staphylococcus* species. All the glassware used in this study was sterilized using a laboratory hot air oven at 160 ^{0}C for 1 hour, and the media used in this study was sterilized using the autoclave at a temperature of 121 ^{0}C at 15 psi for 15 minutes. After the sterilization, the media were brought together with the glassware and kept on a clean laboratory bench. The media were poured into the Petri dishes when cooled to 45 ^{O}C and were allowed to solidify (Cheesbrough, 2010).

Isolation of microorganisms from the swabs collected from the hospital sanitary facilities

The method described by Hammuel et al. (2014) was adopted in the isolation of microorganisms from the surfaces of the hospital sanitary facilities. Swabs from sampled

surfaces were inoculated in 10ml of peptone water by cutting the swabs aseptically into the peptone water, shaking and was allowed to stand for 20 minutes. The spread plate technique as described by Cheesbrough (2010) was used in the inoculation of the plates. 0.1 milliliter aliquot and was dropped onto the different media in the plates. A sterile bent glass rod was used to spread the aliquot evenly on the media (nutrient agar, eosin methylene blue agar and sabouraud dextrose agar). The plates were labeled accordingly. The inoculated plates were inverted and incubated in the incubator at a temperature of 37 ^{0}C for 24 hours except Sabouraud Dextrose agar plates were incubated at room temperature (28 ^{0}C) for three days.

Microbial plate count

After the incubation of the different plates, the different colonies formed on the media were counted using the digital colony counter. The total population of the colonies were expressed as colony forming unit per swab (cfu/swab).

Colonial morphology identification

The method described by Cheesbrough (2010) was adopted in the identification of colonial morphology. Presumptive identification of the colonies were done by observing their individual shape, colour, elevation, edge, surface, consistency and appearance on the media used for isolation. Colonies with characteristic metallic sheen on EMB agar and lactose fermenters on MacConkey agar was noted. The colonies were preserved in sterile agar slants in test tubes. Purified colonies were further characterized using Gram stain and biochemical tests.

Purification and preservation of isolates

Bacterial isolates were picked up with a sterile wire loop based on their morphological appearances. The picked colonies were subcultured onto freshly prepared nutrient agar plates to obtain pure cultures. They were further incubated for 24h at 37^0C. After incubation, pure cultures were stored in McCartney bottle in a refrigerator at 4^0C. Fungal isolates were subcultured onto freshly prepared Sabouraud dextrose medium.

Gram staining

The Gram staining technique described by Cheesbrough (2010) was adopted. Gram staining techniques is used to characterize bacteria according to their Gram's reaction. A smear of each of the bacterial isolates was made on clean grease-free glass slides. Thereafter, the smears were allowed to dry and heat-fixed to denature proteins. The smears were stained with crystal violet stain for 60 seconds and rapidly were washed off with water thereafter. The smears were stained with Lugol's iodine for 60 seconds and were washed off with water. The smears were decolorized with acetone alcohol and were washed off after 10 seconds. The smears were finally flooded with safranin for 2 minutes and were washed- off with clean water. The back of the slides were then wiped and placed in a draining rack for the smear to dry before they were viewed with x40 and x100 (oil immersion objective) lenses. Gram-positive bacteria gave purple colouration, while gram-negative bacteria gave pinkish colouration.

Biochemical tests with the bacterial isolates

The method described by Cheesbrough (2010) and Ochei and Kolhatkar (2010) were adopted in the biochemical characterization of the bacterial isolates. The biochemical tests were carried out will include; catalase, oxidase, coagulase, citrate utilization, indole production, sugar fermentation and motility test.

Citrate utilization test

This test determines the ability of a bacterium isolate to utilize citrate as a sole source of carbon and ammonia as a sole of hydrogen and nitrogen for metabolism. It is therefore a useful test in the identification of organisms in the Enterobacteriaceae and other genera. Simon's citrate agar medium was used. Tube slants of the medium was prepared and lightly inoculated by streaking the isolates on the surface. Inoculated slants were incubated at 37 ^{0}C for 24 hours and citrate utilization indicated by alkaline production, which change the color of the medium from green to blue (Cheesbrough, 2010).

Motility test

This test is used to identify those bacteria that are motile with the help of flagellar. The semi-solid agar of nutrient agar used for this study. The media was prepared in slants and the organisms were inoculated by stabbing technique. Zig-zag growth along the line of stabs indicated a positive result while none indicated a negative result (Cheesbrough, 2010).

Catalase test

This test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase-producing bacteria, such as streptococci. Five (5ml) of hydrogen peroxide was poured into a tube and a sterile glass rod was used to collect a colony of the pure culture from the agar slant tube. It was dipped into the tube containing the hydrogen peroxide. Active bubbling indicated positive catalase test while none indicated catalase negative test (Ochei & Kolhatkar, 2010).

Indole test

Indole production test is used to identify those bacteria that are capable of fermenting the amino acid tryptophan present in peptone water to give indole on addition of Kova'c reagent. The test organisms were suspended in sterile peptone (about 3ml) preparation in sterile test tubes and incubated at 37 ^{0}C for 48 hours after which 0.5ml of Kovac's reagent was added and shaken gently. A red coloration in the surface layer within 10 minutes was an indication of a positive test while none was an indication of a negative test (Ochei & Kolhatkar, 2010).

Oxidase test

This test is used to identify those bacteria that are capable of producing the enzyme oxidase. A piece of filter paper was placed in a clean petri-dish and three drops of fleshly prepared oxidase reagent was added in each case of the test organism. With a sterile piece of stick, each colony of the test organism was removed and smeared on each oxidase reagent drop on the filter paper. The development of a blue-purple coloration was an indication of a positive test while none was an indication of a negative test (Ochei & Kolhatkar, 2010).

Coagulase test

This test is used to identify those bacteria that are capable of producing the enzyme coagulase. A drop of distilled water was placed on each end of a slide for each of the test organisms. Thereafter a colony of each of the test organism was emulsified in each of the drops to make two thick suspensions. A loopful of plasma was then-added to one of the suspension and mixed gently for each of the test organism. Clumping within 10 seconds was an indication of positive test while none was an indication of a negative test (Cheesbrough, 2010).

Sugar fermentation test

This test is used to identify those bacteria that are capable of fermenting the three sugars; mannose, sucrose and glucose. The fermentation of these sugars results in acid production, gas production and hydrogen sulphide. Each colony of the different test organisms was inoculated onto sterile agar slopes of triple sugar iron agar using stab inoculation. After this, the inoculated, agar slopes were incubated at 37 °C for 24 hours. The different colours of the slopes and butts in addition to the presence of gas production and hydrogen sulphide (H_2S) blackening were indication of the type of bacteria present (Ochei & Kolhatkar, 2010).

Identification of the fungal isolates

The method described by Ajayi and Ekozien (2014) was adopted in the identification of the fungal isolates. The fungal isolates were identified by morphological characteristics on Sabouraud dextrose agar (SDA) and microscopic examination after lacto-phenol cotton blue staining technique. Each of the fungal isolates were separately collected with a sterile inoculating needle and transferred to a glass slide, it was then emulsified with a sterile

inoculating needle and then covered with a cover slip gently, to avoid air bubbles. Observation under low and high power objective lens was carried out, the observation include, searching for different features of fungi including, the hyphae, conidia, sporangiophore (reproductive structure), and identification was carried out microscopically by examining the colonies using x10 objective lenses.

3.10 *In vitro* pathogenicity test

The method described by Sagar (2019) was adopted in the determination of the *in vitro* pathogenicity test. The different bacterial and fungal isolates were streaked onto freshly prepared nutrient agar and sabouraud dextrose agar enriched with blood and glucose using decontaminated wireloop. The plates were incubated for 5 days at 30±2 ^{0}C. The pathogenicity of the fungi was confirmed by determining alpha and beta zones of growing fungi on the plates.

3.11 Standardization of inocula

The standardization of the inocula was carried out as described by Ede et al. (2017). The test organisms were inoculated by transferring representative organisms from fresh culture plate into sterile saline bottle. The mixture was shaken to achieve homogenous suspension. The homogenous suspension was adjusted to 0.5 McFarland's standard.

3.12 Antibiotics susceptibility patterns of the isolated bacteria

Antibiotic susceptibility profiles of the bacterial isolates were evaluated using single disc diffusion assay. The antibiotic discs were: tarivid (10mcg), riflacine (10mcg), ciproflox

(10mcg), augmentin (30mcg), gentamycin (10mcg), streptomycin (30mcg), ceporex (10mcg), ceftazidime (30mcg), cefepime (30mcg) and cefuroxime (30mcg). The discs were aseptically placed on the surface of Mueller-Hinton agar (MHA) plates that has already been seeded with 0.5 McFarland standard of the test isolates and were incubated at 37°C for 18-24hrs. After incubation, diameters of zone of inhibitions were observed and measured in millimeters accordingly. The interpretation of the measurement as sensitive and resistant was made according to the manufacturer's standard zone size interpretative table (CLSI, 2010).

3.13 Antifungal susceptibility testing of the fungal isolates

Antifungal susceptibility profiles of the fungal isolates were evaluated using agar well diffusion assay. Antifungal drugs such as; Ketoconazole® (200 mg) and Fluconazole® (200 mg) was used in testing the antifungal effect against the fungal isolates. Each of 200mg of Ketoconazole® and Fluconazole® were dissolved homogenously in 200 ml of dimethylsulphoxide (DMSO) to obtain 1 mg/ml for Ketoconazole® and Fluconazole®. The turbidity of the suspended cells were adjusted to match the turbidity standard of 0.5 McFarland's standard was prepared by mixing 0.6ml of 1% barium chloride dehydrate ($BaCl.2H_2O$) and 99.4ml of 1% concentrated tetraoxosulphate (VI) acid (Conc. H_2SO4). The turbidity was standardized using spectrophotometer at 660 nm was equivalent to approximately 10^8 cells per millilitre (Eduardo et al., 2018). Each of the fungal isolates were carefully streaked onto the surface of Mueller-Hinton agar (MHA) plates supplemented with glucose that has already being seeded with 0.5 McFarland standard of the test isolates. Thereafter, 6 mm cork borer was used to make a 6 mm agar well in the streaked petri-dishes containing the test fungal isolates. 0.1 ml each of the antifungal drug suspensions were

pipetted and dropped into each of the wells made. The plates were labeled accurately and incubated at 30±2 ^{0}C for 5 days. After incubation, diameters of zone of inhibitions were observed and measured in millimeters accordingly. The interpretation of the measurement as sensitive and resistant was made according to the manufacturer's standard zone size interpretative table (Chidi-Onuorah et al., 2017).

3.14 Ethical Considerations/Informed Consent

An introductory letter was obtained for conducting the study and the confidentiality was attached to the cover page of the check list. The approval to carry out the study was approved by the Research Ethical Committee of Public Health Department, School of Health Technology, Federal University of Technology, Owerri. A written informed consent was given to the Chief Medical Directors (CMDs) in the different Referral Hospitals for approval to collect samples (See Appendix).

CHAPTER FOUR

RESULTS AND DISCUSSION

This chapter presents the results of the study on assessment of hospital sanitary facilities in Abakaliki, Ebonyi State as reservoirs of pathogenic microorganisms and their antimicrobial susceptibility patterns. The results include; availability of the hospital sanitary facilities, microbial load of the sanitary facilities in the referral hospital, microorganisms associated with the referral hospital sanitary facilities, *in vitro* pathogenicity of the isolated microorganisms and antimicrobial susceptibility patterns of the microorganisms isolated from the referral hospital sanitary facilities. The obtained results were discussed.

4.1 Results

4.1.1 Availability and nature of the sanitary facilities in the Referral Hospitals

Table 1 shows the availability and nature of the sanitary facilities in the Referral Hospitals. Borehole water was the major sources of water in the hospitals. The borehole water was connected to different taps which served the patients in the hospitals. Flush toilets facilities were major toilets used in the study area. Most of the toilet facilities were classified as basic. Most of the toilet facilities were dedicated toilet for staff and were sex-separated. However, toilet facilities with menstrual supplies and accessible to people with limited mobility were lacking.

Table 1: Availability and nature of the sanitary facilities in the Referral Hospitals

Facilities	Referral Hospitals/Nature of facilities	
	FETHA 1	**FETHA 2**
Water source type		
Borehole	√	√
Hand-dug well	-	-
Rainwater	-	-
River	-	-
Water source service type		
Basic service	√	√
Limited service	√	√
No service	-	-
Toilet facility type		
Flush toilets	√	√
Pit toilets	-	-
Mobile toilets	-	-
Toilet facility service type		
Basic service	√	√
Limited service	√	√
No service	-	-
Hand wash stations		
Yes	√	√
No	-	-
Hand wash facility service type		
Basic service	√	√
Limited service	√	√
No service	-	-

Key: A – B = Different Referral hospital
√ = Availability of facility
- = Unavailable facility

4.1.2 Microbial load of the sanitary facilities in the referral hospitals

Table 2 shows the microbial load of the sanitary facilities available in the referral hospitals. For the bathroom floor, total viable bacterial counts ranged from 2.4×10^2 to 7.2×10^2 cfu/swab, total coliform count ranged from 4.0×10^1 to 8.0×10^1 cfu/swab while total fungal count ranged from 6.0×10^1 to 1.2×10^2 cfu/swab.

For toilet seats, total viable bacterial counts ranged from 4.0×10^2 to 8.2×10^2 cfu/swab, total coliform count ranged from 2.0×10^1 to 6.0×10^1 cfu/swab while total fungal count ranged from 4.0×10^1 to 9.0×10^1 cfu/swab.

For door handles, total viable bacterial counts ranged from 1.8×10^2 to 2.8×10^2 cfu/swab, total coliform count ranged from 2.0×10^1 to 4.0×10^1 cfu/swab while total fungal count ranged from 6.0×10^1 to 1.4×10^2 cfu/swab.

For flush knobs, total viable bacterial counts ranged from 2.2×10^2 to 4.2×10^2 cfu/swab, total coliform count ranged from 3.0×10^1 to 6.0×10^1 cfu/swab while total fungal count ranged from 2.0×10^1 to 4.0×10^1 cfu/swab.

For toilet covers, total viable bacterial counts ranged from 4.4×10^2 to 6.8×10^2 cfu/swab, total coliform count ranged from 4.0×10^1 to 6.0×10^1 cfu/swab while total fungal count ranged from 3.0×10^1 to 1.2×10^2 cfu/swab.

For tap knobs, total viable bacterial counts ranged from 1.0×10^2 to 1.8×10^2 cfu/swab, total coliform count ranged from 2.0×10^1 to 4.0×10^1 cfu/swab while total fungal count ranged from 2.0×10^1 to 8.0×10^1 cfu/swab.

For wash hand basins, total viable bacterial counts ranged from 4.0×10^2 to 4.8×10^2 cfu/swab, total coliform count ranged from 4.0×10^1 to 6.0×10^1 cfu/swab while total fungal count ranged from 3.0×10^1 to 8.0×10^1 cfu/swab.

In all, toilet seats had the highest total viable bacterial count followed by bathroom floors and toilet covers. Bathroom floors had the highest coliform count followed by flush knobs of the sanitary facilities. Highest fungal count was recorded with door handles followed by toilet covers.

Table 2: Microbial load of the sanitary facilities in the referral hospitals

Samples (cfu/swab)	Total plate count (10^2)	Faecal coliform count	Total fungal count
FETHA 1			
Bathroom floors	7.2±0.28	8.0±0.32	1.2 ±0.28
Toilet seats	8.2±0.18	6.0±0.22	9.0 ±0.22
Door handles	2.8±0.52	4.0 ±0.32	1.4 ±0.32
Flush knobs	4.2±0.22	6.0±0.42	4.0±0.42
Toilet covers	6.8±0.52	6.0±0.20	1.2±0.40
Tap knobs	1.8±0.52	4.0±0.20	8.0±0.40
Wash hand basins	1.2±0.52	4.0±0.20	8.0±0.40
FETHA 2			
Bathroom floors	5.2±0.28	5.0±0.32	1.1 ±0.28
Toilet seats	7.2±0.18	1.0±0.22	6.0 ±0.22
Door handles	1.8±0.52	7.0 ±0.32	1.2 ±0.32
Flush knobs	3.2±0.22	4.0±0.42	3.0±0.42
Toilet covers	5.2±0.52	8.0±0.20	1.0±0.40
Tap knobs	1.2±0.52	3.0±0.20	6.0±0.40
Wash hand basins	1.0±0.52	8.0±0.20	6.0±0.40

Key: ND = Not detected

cfu/swab = Colony forming unit per swab

4.1.3 Cultural morphology and biochemical characteristics of the bacterial isolates associated with the sanitary facilities in the referral hospital

Table 3 shows the cultural morphology and biochemical characteristics of the bacterial isolates associated with the sanitary facilities in the referral hospital. A total nine (9) bacteria comprising five (5) Gram negative and four (4) Gram positive bacteria were isolated from the sanitary facilities. The Gram bacteria were; *Pseudomonas, Escherichia, Klebsiella, Enterobacter* and *Proteus* species. The Gram positive bacteria were; *Bacillus, Micrococcus, Streptococcus* and *Staphylococcus* species.

Table 3: Cultural morphology and biochemical characteristics of the bacterial isolates from the sanitary facilities

Morphological Characteristics	Gram reaction	Oxidase test	Indoletest	Spore test	Catalase test	Citrate test	Coagulase test	Motility test	S FT				
									S	B	G	H_2S	Possible bacteria
Bluish-green, flat, non-mucoidcolonies	Gram negative rods in diploids	+	-	-	+	+	-	+	R	R	-	-	*Pseudomonas* species
Milkish, flat, rhizoid-like dry-surface colonies	Gram positive rods in short chains	-	-	+	+	-	-	+	Y	Y	+	-	*Bacillus* species
Pinkish, raised, mucoid Colonies with green-metallic sheen	Gram negative rods in diploids	-	+	-	-	+	-	+	R	Y	+	-	*Escherichia coli*
Pinkish, raised, mucoid enlarged colonies	Gram negative rods in short chains	-	-	-	+	+	-	-	R	Y	-	-	*Klebsiella* species
Pale, flat, non-mucoid elongated colonies	Gram negative rods in short chains	-	+	-	-	+	-	+	R	Y	+	+	*Proteus* species
Milkish, raised, non-mucoid circular colonies of about 3mm in size	Gram positive cocci in pairs	-	-	-	+	-	-	-	No Reaction		-	-	*Micrococcus* species
Milkish, raised, non- mucoid colonies in clusters	Gram positive cocci	-	-	-	+	-	+	-	No Reaction		-	-	*Staphylococcus* species
Pinkish, raised, non-mucoid colonies of about 3mm	Gram negative rods in short chains	-	-	-	+	+	-	-	R	Y	-	-	*Enterobacter* species
Milkish, raised, non- mucoid colonies, with clear zones	Gram positive cocci in long straight chains	-	-	-	-	-	-	-	No Reaction		-	-	*Streptococcus* species

Key: -= Negative += Positive S = color of slope B = color of butt G= Gas production H_2S= Hydrogen sulphide production (blackening)
R= Reddish coloration (alkaline production) Y= Yellow coloration (Acidic production) SFT= Sugar fermentation test

4.1.4 Cultural morphology and microscopic characteristics of the fungal isolates from the sanitary facilities used in the referral hospitals

Table 4 shows the cultural morphology and microscopic characteristics of the fungal isolates from the sanitary facilities used in the referral hospitals. A total of five (5) fungal isolates comprising one (1) yeast and four (4) moulds were isolated. They were; *Candida* species (yeast), *Rhizopus, Aspergillus, Penicillium* and *Mucor* species.

Table 4: Cultural morphology and microscopic characteristics of the fungal isolates from the sanitary facilities used in the referral hospitals

Cultural morphology	Microscopy	Possible fungi
Whitish, raised, fluffy, cotton like colonies that covered the plate after three days.	Non-septate hyphae	*Rhizopus* species
Whitish, circular, colonies with light yellow reverse.	Septate hyphae	*Aspergillus* species
Whitish, circular, colonies with light green powdery centre.	Septate hyphae with tiny mycelium joining	*Penicillum* species
Whitish, circular, colonies with grey centre.	Non-septate with spores at both ends	*Mucor* species
White, creamy, circular colonies of about 3mm.	Budded yeast cells in diploid	*Candida* species

4.1.5 Frequency and percentage occurrence of the bacterial isolates from the sanitary facilities used in the referral hospital (FETHA 1)

Table 5 shows the frequency and percentage occurrence of the bacterial isolates from the sanitary facilities used in the referral hospitals (FETHA 1). *Staphylococcus* species 27(19.4%) had the highest occurrence, followed by *Pseudomonas* and *Micrococcus* species 23(16.5%). *Enterobacter* and *Streptococcus* species 6(4.3%) were the least occurring bacteria. Bathroom floors had the highest bacterial isolates followed by toilet seats while wash hand basins had the least bacterial isolates.

Pseudomonas species 10(26.3%) occurred highest with bathroom floors, *Klebsiella* species 4(12.1%) occurred highest with toilet seats while *Escherichia* species 8(24.2%) occurred highest with toilet covers. *Enterobacter* species 3(11.5%) had highest occurrence with toilet covers, *Proteus* 4(10.5%), *Streptococcus* 3(7.9%) and *Bacillus* species 8(21.0%) had highest occurrence with bathroom floors while *Staphylococcus* species 7(21.2%) had the highest occurrence with toilet seats.

Table 5: Frequency and percentage occurrence of the bacterial isolates from the sanitary facilities used in the referral hospital (FETHA 1)

Bacterial isolates	**Sanitary facilities/Occurrence of bacteria**								
	BF	**TS**	**DH**	**FK**	**TC**	**WHB**	**TK**	**Total**	**%**
Pseudomonas spp	10(26.3)	3(9.1)	0(0)	3(17.6)	5(19.2)	2(2.5)	2(2.5)	23	16.5
Escherichia spp	2(5.3)	8(24.2)	2(11.7)	1(5.9)	6(23.1)	1(12.5)	0(0)	20	14.4
Klebsiella spp	3(7.9)	4(12.1)	0(0)	1(5.9)	2(7.7)	0(0)	0(0)	10	7.2
Enterobacter spp	2(5.3)	1(3.0)	0(0)	0(0)	3(11.5)	0(0)	0(0)	6	4.3
Proteus spp	4(10.5)	3(9.1)	0(0)	1(5.9)	2(7.7)	0(0)	0(0)	10	7.2
Bacillus spp	8(21.0)	4(12.1)	5(29.4)	2(11.7)	3(11.5)	1(12.5)	5(29.4)	23	16.5
Micrococcus spp	2(5.3)	3(9.1)	5(29.4)	2(11.7)	1(3.8)	1(12.5)	2(5.3)	14	10.1
Streptococcus spp	3(7.9)	0(0)	0(0)	0(0)	1(5.9)	2(7.7)	0(0)	6	4.3
Staphylococcus spp	4(10.5)	7(9.1)	5(29.4)	6(35.3)	2(7.7)	3(37.5)	6(37.5)	27	19.4
Total	**38(100.0)**	**33(100.0)**	**17(100.0)**	**17(100.0)**	**26(100.0)**	**8(100.0)**	**15(100.0)**	**139**	**100.0**

Key: BF = Bathroom floors TS = Toilet seats DH = Door handles FK = Flush knobs TC = Toilet covers
WHB = Wash hand basins TK = Toilet knobs % = Percentage

4.1.6 Frequency and percentage occurrence of the bacterial isolates from the sanitary facilities used in the referral hospital (FETHA 2)

Table 6 shows the frequency and percentage occurrence of the bacterial isolates from the sanitary facilities used in the referral hospitals (FETHA 2). *Pseudomonas* species 26(22.4%) had the highest occurrence followed by *Staphylococcus* species 24(20.7%). Bathroom floors had the highest bacterial isolates followed by toilet seats while wash hand basins had the least bacterial isolates.

Pseudomonas species 7(22.5%) occurred highest with bathroom floors, *Klebsiella* species 4(12.9%) occurred highest with bathroom floors while *Escherichia* species 5(20.8%) occurred highest with bathroom floors. *Proteus* species 5(16.1%) had highest occurrence with bathroom floors while *Staphylococcus* species 6(25.0%) had the highest occurrence with toilet seats. *Bacillus* species 6(19.3%) occurred highest with bathroom floors.

Table 6: Frequency and percentage occurrence of the bacterial isolates from the sanitary facilities used in the referral hospital (FETHA 2)

Bacterial isolates	**Sanitary facilities/Occurrence of bacteria**								
	BF	**TS**	**DH**	**FK**	**TC**	**WHB**	**TK**	**Total**	**%**
Pseudomonas spp	7(22.5)	4(16.6)	2(15.4)	2(12.5)	4(30.7)	3(42.8)	4(33.3)	26	22.4
Escherichia spp	4(12.9)	5(20.8)	1(7.7)	3(18.7)	4(30.7)	0(0)	0(0)	17	14.6
Klebsiella spp	4(12.9)	2(8.3)	0(0)	0(0)	1(7.7)	0(0)	0(0)	7	6.0
Proteus spp	5(16.1)	4(16.6)	0(0)	0(0)	3(23.1)	0(0)	0(0)	12	10.3
Bacillus spp	6(19.3)	2(8.3)	3(23.1)	4(25.0)	1(7.7)	2(28.6)	3(25.0)	21	18.1
Micrococcus spp	3(9.7)	1(4.2)	3(23.1)	2(12.5)	0(0)	0(0)	0(0)	9	7.7
Staphylococcus spp	2(6.4)	6(25.0)	4(30.7)	5(31.2)	0(0)	2(28.6)	5(41.7)	24	20.7
Total	**31(100.0)**	**24(100.0)**	**13(100.0)**	**16(100.0)**	**13(100.0)**	**7(100.0)**	**12(100.0)**	**116**	**100.0**

Key: BF = Bathroom floors TS = Toilet seats DH = Door handles FK = Flush knobs TC = Toilet covers

WHB = Wash hand basins TK = Toilet knobs % = Percentage

4.1.7 Frequency and percentage occurrence of the fungal isolates from the sanitary facilities used in the referral hospital (FETHA 1)

Table 7 shows the frequency and percentage occurrence of the fungal isolates from the sanitary facilities used in the referral hospitals (FETHA 1). *Mucor* species 18(27.3%) had the highest occurrence followed by *Rhizopus* species 16(24.2%) and *Aspergillus* species 16(24.2%). Bathroom floors had the highest fungal isolates followed by door handles while tap knobs had the least fungal isolates.

Rhizopus species occurred highest with door handles 4(30.8%) and wash hand basins 4(57.1%), *Aspergillus* species occurred highest with bathroom floors 6(30.0%) while *Penicillium* species 5(25.0%) occurred highest with bathroom floors. *Mucor* species had highest occurrence with door handles 5(38.5%) while *Candida* species had the highest occurrence with toilet seats 3(50.0%).

Table 7: Frequency and percentage occurrence of the fungal isolates from the sanitary facilities used in the referral hospital (FETHA 1)

Bacterial isolates	**Sanitary facilities/Occurrence of fungi**								
	BF	**TS**	**DH**	**FK**	**TC**	**WHB**	**TK**	**Total**	**%**
Rhizopus spp	3(15.0)	0(0)	4(30.8)	0(0)	3(30.0)	4(57.1)	2(50.0)	16	24.2
Aspergillus spp	6(30)	1(16.7)	2(15.4)	3(50.0)	4(40.0)	0(0)	0(0)	16	24.2
Penicillium spp	5(25.0)	0(0)	1(7.7)	0(0)	2(20.0)	0(0)	0(0)	8	12.1
Mucor spp	4(20.0)	2(33.3)	5(38.5)	2(33.3)	0(0)	3(42.9)	2(50.0)	18	27.3
Candida spp	2(10.0)	3(50.0)	1(7.7)	1(16.7)	1(10.0)	0(0)	0(0)	8	12.1
Total	**20(100.0)**	**6(100.0)**	**13(100.0)**	**6(100.0)**	**10(100.0)**	**7(100.0)**	**4(100.0)**	**66**	**100.0**

Key: BF = Bathroom floors TS = Toilet seats DH = Door handles FK = Flush knobs TC = Toilet covers

WHB = Wash hand basins TK = Toilet knobs % = Percentage

4.1.8 Frequency and percentage occurrence of the fungal isolates from the sanitary facilities used in the referral hospital (FETHA 2)

Table 8 shows the frequency and percentage occurrence of the fungal isolates from the sanitary facilities used in the referral hospitals (FETHA 2). *Mucor* species 16(33.3%) had the highest occurrence followed by *Aspergillus* species 12(25.0%). Bathroom floors had the highest fungal isolates followed by door handles while tap knobs had the least fungal isolates.

Rhizopus species occurred highest with door handles 5(41.7%), *Aspergillus* species occurred highest with bathroom floors 4(28.6%) while *Penicillium* species occurred highest with bathroom floors 3(21.4%). *Mucor* species had highest occurrence with bathroom floors 5(35.7%) while *Candida* species had the highest occurrence with flush knobs 3(33.3%).

Table 8: Frequency and percentage occurrence of the fungal isolates from the sanitary facilities used in the referral hospital (FETHA 2)

Bacterial isolates	**Sanitary facilities/Occurrence of fungi**								
	BF	**TS**	**DH**	**FK**	**TC**	**WHB**	**TK**	**Total**	**%**
Rhizopus spp	1(7.1)	2(66.7)	5(41.7)	0(0)	0(0)	2(50.0)	0(0)	10	20.8
Aspergillus spp	4(28.6)	0(0)	3(25.0)	2(22.2)	3(100.0)	0(0)	0(0)	12	25.0
Penicillium spp	3(21.4)	0(0)	1(8.3)	0(0)	0(0)	0(0)	1(33.3)	5	10.4
Mucor spp	5(35.7)	0(0)	3(25.0)	4(44.4)	0(0)	2(50.0)	2(66.7)	16	33.3
Candida spp	1(7.1)	1(33.3)	0(0)	3(33.3)	0(0)	0(0)	0(0)	5	10.4
Total	**14(100.0)**	**3(100.0)**	**12(100.0)**	**9(100.0)**	**3(100.0)**	**4(100.0)**	**3(100.0)**	**48**	**100.0**

Key: BF = Bathroom floors TS = Toilet seats DH = Door handles FK = Flush knobs TC = Toilet covers

WHB = Wash hand basins TK = Toilet knobs % = Percentage

4.1.9 *In vitro* pathogenicity of the isolated microorganisms

Table 9 shows the *in vitro* pathogenicity of the isolated microorganisms from the different sanitary facilities used in the two referral hospitals. Out of 24 isolates of *Staphylococcus* species, 9(37.5%) produced beta while 7(29.2%) produced gamma haemolysis. Out 16 isolates of *Streptococcus* species recovered, 2(33.3%) produced alpha while 2(33.3%) produced beta haemolysis. Out of 16 isolates of *Aspergillus* species recovered, 5(31.2%) produced alpha while 3(18.7%) produced beta haemolysis.

Table 9: ***In vitro*** **pathogenicity of the isolated microorganisms**

Microbial isolates	**Pathogenicity test**		
	Alpha zones	**Beta zones**	**Gamma zones**
Klebsiella species	0	0	0
Pseudomonas species	0	0	0
Staphylococcus species	0	9(37.5)	7(29.2)
Bacillus species	0	0	0
Streptococcus species	2(33.3)	2(33.3)	0
Proteus species	0	0	0
Micrococcus species	0	0	0
Enterobacter species	0	0	0
Escherichia species	0	0	0
Aspergillus species	5(31.2)	3(18.7)	0
Rhizopus species	0	0	0
Candida species	0	0	0
Mucor species	0	0	0
Penicillium species	0	0	0

Key: 0 = No haemolysis

4.1.10 Antimicrobial susceptibility patterns of the microorganisms isolated from the referral hospital sanitary facilities

Table 10 shows the antimicrobial susceptibility patterns of the microorganisms isolated from the referral hospital sanitary facilities. Zones of inhibition recorded with the antibiotics against the bacterial isolates ranged from 12mm to 28mm with tarivid antibiotics, 10mm to 26mm with riflacine antibiotics, 18mm to 32mm with ciprofloxacin antibiotics, 20mm to 30mm with augmentin antibiotics, 14mm to 26mm with gentamycin antibiotics, 14mm to 26mm with streptomycin antibiotics, 20mm to 34mm with ceporex antibiotics, 22mm to 36mm with ceftazidime antibiotics, 18mm to 30mm with cefepime antibiotics and 22mm to 34mm with cefuroxime antibiotics. *Bacillus, Escherichia*, *Streptococcus,* and *Micrococcus* species were resistant to some of the antibiotics used in this study. *Staphylococcus* and *Pseudomonas* species showed higher susceptibility compared to other bacterial isolates.

For the antifungal activities, fluconazole and ketoconazole produced zones of inhibition ranging from 20mm to 28mm. *Aspergillus* species and *Rhizopus* species showed resistance to fluconazole antifungal drug. *Candida* species was resistant to fluconazole and ketoconazole but susceptible to nystatin antifungal drugs.

Table 10: Antimicrobial susceptibility patterns of the microorganisms isolated from the referral hospital sanitary facilities

Microbial isolates	Antimicrobial agents/Zones of inhibition (mm)												
	Antibiotics										Antifungal drugs		
	TV	**RF**	**CPX**	**AU**	**CN**	**S**	**CP**	**CFT**	**CEF**	**CFU**	**KT**	**FN**	**NYS**
Bacteria													
Klebsiella species	20	16	24	20	18	16	20	26	28	24	-	-	-
Escherichia species	-	18	18	28	26	22	24	22	32	22	-	-	-
Pseudomonas species	18	-	22	20	20	24	20	26	22	30	-	-	-
Staphylococcus species	28	26	32	30	26	20	34	30	34	34	-	-	-
Bacillus species	12	24	18	-	-	14	-	22	18	22	-	-	-
Streptococcus species	-	-	20	22	18	24	20	28	20	30	-	-	-
Enterobacter species	22	16	18	20	22	18	26	30	26	28	-	-	-
Micrococcus species	-	10	18	28	-	14	-	22	18	26	-	-	-
Proteus species	16	-	24	28	26	16	24	36	22	32	-	-	-
Fungi													
Aspergillus species	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhizopus species	-	-	-	-	-	-	-	-	-	-	-	-	-
Candida species	-	-	-	-	-	-	-	-	-	-	-	-	28
Mucor species	-	-	-	-	-	-	-	-	-	-	24	26	-
Penicillium species	-	-	-	-	-	-	-	-	-	-	22	24	-

Key: - = No zone of inhibition	mm = Millimeter	CPX = Ciporfloxacin	CN = Gentamycin
S = Streptomycin	RD = Rifampicin	AU = Augmentin	TV = Tarivid
RF = Riflacine	S = Streptomycin	CP = Ceporex	CEF = Cefepime
CFT = Ceftazidime	CFU = Cefuroxime	FLU = Fluconazole	KETO = Ketoconazole
CLSI guidelines	R = Resistant (0 – 12 mm)	S = Susceptible (16mm and above)	

4.2 DISCUSSION

4.2.1 Availability and nature of the sanitary facilities in the Referral Hospitals

The study's results showed that borehole water was the major source of water in the hospitals. The borehole water was connected to different taps that served the patients. Flush toilets were the major toilets used in the study area. Most of the toilet facilities were classified as basic. Most were dedicated to staff and sex-separated.

Odjegba et al. (2021) reported on water, sanitation, and hygiene in healthcare centres in parts of Southwestern Nigeria was conducted. Sixty-one PHCs in urban and rural areas were selected using a stratified random sampling technique. Their results showed that boreholes and hand-dug wells are the most prevalent water source type, and flush toilets and pit latrines are the major types of toilet facilities used. All but two PHCs engaged in handwashing practices. Water quality analysis results showed that chloride, nitrate, and turbidity were within the WHO drinking-water standards. Poor water quality and sanitation practices could expose health staff and patients to healthcare-associated infections.

Onyedibe et al. (2020) in their study on assessment of hand hygiene facilities and staff compliance in a large tertiary health care facility in northern Nigeria: a cross sectional study reported that 72% of the units had no poster or written policy on HH; 87% did not have alcohol-based hand rubs; 98% had at least one handwash sink; 28% had flowing tap water all day while 72% utilized cup and bucket; and 58% had no hand drying facilities. A total of 406 HH opportunities were observed among 175 HCWs. The overall compliance was 31%, ranging from 18% among ward attendants to 82% among medical students. Based on WHO “5 moments” for HH, average compliance was 21% before patient contact, 23%

before aseptic procedure, 63% after body fluid exposure risk, 41% after patient contact and 40% after contact with patients' surrounding. Being a medical student was independently associated with high HH compliance, adjusted odds ratio: 13.87 (1.70–112.88).

4.2.2 Microbial load of the sanitary facilities in the referral hospitals

Table 2 shows the microbial load of the sanitary facilities available in the referral hospitals. Toilet seats had the highest total viable bacterial count followed by bathroom floors and toilet covers. Bathroom floors had the highest coliform count followed by flush knobs of the sanitary facilities. Highest fungal count was recorded with door handles followed by toilet covers. There were high total viable bacterial counts recorded compared to coliform and fungal counts. Sanitary facilities with high contact and usage showed higher microbial load.

Cobrado et al. (2017) in their study reported that high-touch surfaces in hospitals were responsible for hospital-acquired infections (HAIs) during admission in the hospital. Their results showed that patient care items and environmental surfaces frequently touched play an important role in the chain of transmission. Tania and Marese (2015) in their study on surface microbial contamination in hospitals showed that toilet door handles were the most heavily contaminated (7.97±0.68) colony forming units [cfu]/cm^2) and exceeded proposed standards on 74% of occasions. Petrifilms detected statistically higher cfu from bedside lockers. Nwankwo and Afuruobi (2015) reported that Gram positive bacteria were the major bacterial contaminants associated with door handles in a tertiary institution in Umuahia, Abia State, Nigeria. The results of this study are in line with their report.

4.2.3 Cultural morphology and biochemical characteristics of the bacterial isolates associated with the sanitary facilities in the referral hospital

Table 3 shows the cultural morphology and biochemical characteristics of the bacterial isolates associated with the sanitary facilities in the referral hospital. A total nine (9) bacteria comprising five (5) Gram negative and four (4) Gram positive bacteria were isolated from the sanitary facilities. The Gram bacteria were; *Pseudomonas, Escherichia, Klebsiella, Enterobacter* and *Proteus* species. The Gram positive bacteria were; *Bacillus, Micrococcus, Streptococcus* and *Staphylococcus* species.

Maori et al. (2011) reported the isolation of *Staphylococcus* species, *Candida* species, *Escherichia coli, Citrobacter* species, *Klebsiella* species, *Proteus* species and *Salmonella* species from toilet door handles in secondary schools in Bokkos L.G.A., Jos, Plateau State, Nigeria. Nworie et al. (2012) isolated organisms such as; *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Enterobacter* species, *Citrobacter* species, *Pseudomonas aeruginosa* and *Proteus* species from door handles/ knobs in selected public conveniences in Abuja Metropolis, Nigeria.

Staphylococcus species which had the highest occurrence may be due to the fact that it is a major component of the normal flora of the skin and nostrils, which probably explain its high prevalence as a contaminant, as it can easily be discharged by several human activities. This observation is in agreement with the findings of other researchers (Nworie et al., 2012; Ducel et al., 2002; Brooks et al., 2007).

A high percentage of *Bacillus* species was isolated from this research and its predominance could be explained by the fact that *Bacillus* species are ubiquitous in nature with their spores able to resist environmental changes, withstand dry heat and certain

chemical disinfectants for moderate periods. This is also in agreement with the research carried out by Brooks et al. (2007) who reported that *Bacillus* species was found to be among the predominant organism that was isolated from door handles.

Table 4 shows the result of the frequency and percentage occurrence of the bacterial isolates from the toilet door handles. From the result, *Staphylococcus* species 8(24.2%) was the highest occurring bacteria followed by *Escherichia* species and *Klebsiella* species 5(15.2%). From Table 4.5, *Candida* species was the only fungal isolates from the office and door handles.

Other researchers have reported the isolation of similar bacterial isolates from door handles of offices, toilets and other surface. Maori et al. (2011) in their study the prevalence of bacterial organisms on toilet door handles in secondary schools in Bokkos L.G.A., Jos, Plateau State, Nigeria isolated *Staphylococcus* species (43.3%), *Candida* species (10%), *Escherichia coli* (16.7%), *Citrobacter* species (1.7%), *Klebsiella* species (20%), *Proteus* species (6.7%) and *Salmonella* species (1.7%) from the door handles. Nworie et al. (2012) investigated bacterial contamination of door handles/ knobs in selected public conveniences in Abuja Metropolis, Nigeria. The isolated organisms were *Staphylococcus aureus* (30.1%), *Klebsiella pneumoniae* (25.7%), *Escherichia coli* (1%), *Enterobacter* species (11.2%), *Citrobacter* species (7.1%), *Pseudomonas aeruginosa* (5.9%), and *Proteus* species (4.5%).

Lorina et al. (2015) carried out a research on potential pathogenic bacteria contaminants of shared utility devices in a university setting at Al-Hofuf, Saudi Arabia. The bacterial contaminants found were *Staphylococcus aureus* (4.02%), *Staphylococcus haemolyticus* (18.59%), *Staphylococcus epidermidis* (21.10%), other *Staphylococcus* species (51.76%),

Enterococcus faecalis (2.01%), *Enterococcus* species (1.51%), *Klebsiella pneumoniae* (0.50%), *Streptococcus sanguins* (0.50%), *Pseudomonas aeruginosa* (14.03%), *Pseudomonas stutzeri* (3.5%), *Pseudomonas luteola* (10.53%), and *Pantoea* species (72%).

In a study by Maryam et al. (2014) aimed at characterization and determination of antibiotic susceptibility pattern of bacteria isolated from some fomites (tables, chairs, pens, stethoscopes, uniforms, doorknobs and IVF stands) in a teaching hospital in northern Nigeria, *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* species (13.0%) were the major contaminants of the surfaces.

Similarly, Lorina et al. (2015) isolated *Staphylococcus aureus* (4.02%), *Staphylococcus haemolyticus*, *Staphylococcus epidermidis*, other *Staphylococcus* species, *Enterococcus faecalis*, *Enterococcus* species, *Klebsiella pneumoniae* and *Streptococcus sanguins*, *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Pseudomonas luteola* and *Pantoea* species in shared utility devices in a university setting at Al-Hofuf, Saudi Arabia.

4.2.4 Cultural morphology and microscopic characteristics of the fungal isolates from the sanitary facilities used in the referral hospitals

This shows the cultural morphology and microscopic characteristics of the fungal isolates from the sanitary facilities used in the referral hospitals. A total of five (5) fungal isolates comprising one (1) yeast and four (4) molds were isolated. They were; *Candida* species (yeast), *Rhizopus, Aspergillus, Penicillium* and *Mucor* species.

Augustino et al. (2014) reported frequency of; *Staphylococcus aureus* (25%), *E. coli* (36.7%), *Pseudomonas aeruginosa* (13.3%), *Streptocoocus pyogenes* (6.7%), *Proteus*

mirabilis (6.7%) and *Klebsiella pneumoniae* (11.6%). The results indicated that surfaces routinely touched with hands had the highest bacterial contamination compared to restroom floor and toilet seats. Opere et al. (2013) reported frequency of *Bacillus* species (4.35%), *Staphylococcus aureus* (34.72%), *Staphylococcus epidermidis* (34.72%), *Micrococcus* species (4.35%), *Pseudomonas* species (8.70%), *Enterococcus faecalis* (4.35%), *Salmonella typhi* (4.35%) and *Shigella dysenteriae* (4.35%) from environmental surfaces in public toilets. Samples were collected from door handles, tap handles and flush handles of a public toilet. Comparing the bacterial isolates reported in this study, high microbial isolates were recorded in this study. This could be as a result of the study area, number of samples used and type of hospital studied.

Staphylococcus species which had the highest occurrence may be due to the fact that it is a major component of the normal flora of the skin and nostrils, which probably explain its high prevalence as a contaminant, as it can easily be discharged by several human activities. This observation is in agreement with the findings of other researchers (Nworie et al., 2012). A high percentage of *Bacillus* species was isolated from this research and its predominance could be explained by the fact that *Bacillus* species are ubiquitous in nature with their spores able to resist environmental changes, withstand dry heat and certain chemical disinfectants for moderate periods. This is also in agreement with the research carried out by Brooks et al. (2007) who reported that *Bacillus* species was found to be among the predominant organism that was isolated from door handles.

4.2.5 Frequency and percentage occurrence of the fungal isolates from the sanitary facilities used in the referral hospital (FETHA 1)

This shows the frequency and percentage occurrence of the fungal isolates from the sanitary facilities used in the referral hospitals (FETHA 1). *Mucor* species 18(27.3%) had the highest occurrence followed by *Rhizopus* species 16(24.2%) and *Aspergillus* species 16(24.2%). Bathroom floors had the highest fungal isolates followed by door handles while tap knobs had the least fungal isolates.

Rhizopus species occurred highest with door handles 4(30.8%) and wash hand basins 4(57.1%), *Aspergillus* species occurred highest with bathroom floors 6(30.0%) while *Penicillium* species 5(25.0%) occurred highest with bathroom floors. *Mucor* species had highest occurrence with door handles 5(38.5%) while *Candida* species had the highest occurrence with toilet seats 3(50.0%). The organisms isolated from these sanitary facilities have been known to cause one or more diseases that are mild and could be sometimes serious, examples are but not limited to pimple, impetigo, scalded skin syndrome, pneumonia, meningitis, osteomyeelitis, rhinoscleroma, kidney failure, septicemia etc.

4.2.6 *In vitro* pathogenicity of the isolated microorganisms

This shows the *in vitro* pathogenicity of the isolated microorganisms from the different sanitary facilities used in the two referral hospitals. *Staphylococcus* species produced beta and gamma haemolysis, *Streptococcus* species produced alpha and beta haemolysis while *Aspergillus* species produced alpha and beta haemolysis. Haemolysis is the breakdown or destruction of red blood cells so that the contained oxygen-carrying pigment haemoglobin is freed into the surrounding medium. Haemolysis is considered a virulence factor.

4.2.7 Antimicrobial susceptibility patterns of the microorganisms isolated from the referral hospital sanitary facilities

This shows the antimicrobial susceptibility patterns of the microorganisms isolated from the referral hospital sanitary facilities. Zones of inhibition recorded with the antibiotics against the bacterial isolates ranged from 12mm to 36mm with *Bacillus, Escherichia, Streptococcus,* and *Micrococcus* species were resistant to some of the antibiotics used in this study. *Staphylococcus* and *Pseudomonas* species showed higher susceptibility compared to other bacterial isolates. For the antifungal activities, fluconazole and ketoconazole produced zones of inhibition ranging from 20mm to 28mm. *Aspergillus* species and *Rhizopus* species showed resistance to fluconazole antifungal drug. *Candida* species was resistant to fluconazole and ketoconazole but susceptible to nystatin antifungal drugs.

Lorina et al. (2015) reported multidrug resistant organisms with potential pathogenic bacteria contaminants of shared utility devices in a university setting at Al-Hofuf, Saudi Arabia. Similarly, Augustino et al. (2014) reported that *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Streptocoocus pyogenes*, *Proteus mirabilis* and *Klebsiella pneumoniae* from different surfaces including toilet seats, toilet bowls, door handles in and out of the restrooms, faucet handles, toilet flush handles and the restroom floors. Results of antibiotic susceptibility testing indicated that all bacterial isolates were resistant and intermediate resistant to at least one antibiotic.

Cladiou et al. (2023) reported one *K. pneumoniae* ST147 cluster encoding NDM- and OXA-48 carbapenemases and one VIM-encoding *P. aeruginosa* ST823 cluster with hospital sanitary facilities on wards. Similarly, Kiros et al. (2021) reported the prevalence

of high ampicillin-resistant *K. pneumoniae* 80% (95% CI: 78, 92) followed by *Citrobacter* species 78% (95% CI: 57, 83) from hospital facilities and equipment in Ethiopia.

Opere et al. (2013) reported that bacteria isolated from environmental surfaces in public toilets such as *Salmonella typhi* had the highest level of multidrug resistance, showing resistance to all eight antibiotics tested. *Bacillus* species and *Pseudomonas* species showed susceptibility to five of the eight antibiotics. Plasmid profile analysis of the isolates revealed the absence of plasmids.

Antibiotic resistance is a major global concern, a complex public health issue and is accelerated by improper use of antibiotics as well as a growing population and increased networking and travelling. Antibiotic resistance in bacteria has been reported to acquired through the uptake of resistance genes by bacterial conjugation or other horizontal transmission pathways, spontaneous mutation of genes, upregulation of efflux pumps or intrinsic resistance genes, which subsequently allow the spread of resistant clones by vertical propagation (Ferri et al., 2017; Serwecinska, 2020).

4.2.9 Public Health Implications of the Isolated Microorganisms

In this study, different microorganisms were isolated and tested for their antimicrobial susceptibility pattern. Among these organisms is *Pseudomonas aeruginosa. P. aeruginosa* is increasingly recognized for the ability of certain hospital populations to cause nosocomial infection outbreaks with significant morbidity and mortality. Both, *K. pneumoniae* and *P. aeruginosa*, form biofilms in toilet bowls, particularly behind the flushing rim of the toilet, and establish themselves in hospital water systems, which allow the pathogens to persist and potentially spread out of the toilet each time it is flushed

(Walker et al., 2014; Abney et al., 2021; Migliorini et al., 2022). *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are two of the most important opportunistic and nosocomial pathogens worldwide and are known for the ability to produce biofilms to escape treatment with antibiotics (Boltelho et al., 2019).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The findings from this study have shown that pathogenic microorganisms are present in most of the sanitary facilities available for in-and out-patients in two (2) referral hospitals at Abakaliki, Ebonyi State. The microbial load of the sanitary facilities sampled showed a high microbial load on bathroom floors, door handles, and toilet seats. Most of the isolated microorganisms were resistant to some antimicrobial agents such as antibiotics and antifungal drugs. The presence of these microorganisms from these sanitary facilities indicates that sanitary facilities in hospitals could be vehicles for hospital-acquired infections.

5.2 RECOMMENDATIONS

1. The findings of this study suggest the need for improved sanitary conditions among the cleaners and regular cleaning/disinfection of sanitary conveniences in referral hospitals.

2. Hand sanitizers or spray disinfectants should be available in all hospitals' conveniences to ensure adequate hand disinfection after using these sanitary facilities.

3. Routine surface disinfection of toilets, door handles and other sanitary facilities should be practised as this can prevent the rate of contamination of these surfaces.

4. The provision of safe, secure, and accessible water sources and toilet facilities in hospitals is necessary to ensure the effective cleaning of the sanitary facilities.

5. There should be adequate training of hospital cleaners on the proper utilization of disinfectants in the cleaning of sanitary facilities in the hospitals.

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APPENDICES

APPENDIX A: Checklist for Assessment of Hospital Sanitary Facilities

Tick appropriately in the box below

Water source type

- Borehole
- Hand dug well
- Rain water
- River

Water source service type

- Basic service
- Limited service
- No service

Toilet facility type

- Flush toilets
- Pit toilets
- Mobile toilets

Toilet facility service type

- Basic service
- Limited service
- No service

Hand wash stations

- Yes
- No

Hand wash facility service type

- Basic service
- Limited service
- No service

APPENDIX B: LIST OF ABBREVIATIONS

ANC: Antenatal care

CI: Confidence interval

CFU: Colony forming unit

CPA: Complementary package of activities

DHS: Department of Hospital Services

DPHI: Department of Planning and Health Information

EBSUTH: Ebonyi State University Teaching Hospital

FETHA: Federal Teaching Hospital, Abakaliki

HC: Health centre

HCF: Health care facility

H-EQIP: Health Equity and Quality Improvement Project

IPC: Infection prevention and control

JMP: Joint Monitoring Programme for water supply and sanitation

MM: Millimeter

MO: Ministry of Health

MPA: Minimum package of activities

NECHR: National Ethics Committee for Health Research

NIPH: National Institute of Public Health

OD: Operational district

OPD: Outpatient department

PHD: Provincial Health Department

RG: Royal Government of Cambodia

RH: Referral hospitals

SDGs: Sustainable Development Goals

UHC: Universal health coverage

WASH: Water, sanitation and hygiene

WHO: World Health Organization

APPENDIX C: OPERATIONAL DEFINITIONS OF TERMS

Antimicrobial resistance: This is a condition whereby bacteria, viruses, fungi and parasites change over time and no longer respond to medicines, making infections harder to treat and increasing the risk of disease spread, severe illness and death.

Fomites: These are inanimate objects that become colonized with microbes and serve as potential intermediaries for transmission to/from humans.

Hospital: A hospital is a healthcare facility where the diagnosis and treatment of patients are carried out with the help of specialized health science and auxiliary healthcare staff and medical equipment (Ezekiel, 2018).

Hospital-acquired infection: This is a nosocomial infection that is typically not present or might be incubating at the time of admission.

Microorganisms: These are organisms of microscopic size, which may exist in their single-celled form or as colonies of cells.

Nosocomial infection: This is referred to as healthcare-associated infections acquired while receiving health care that was not present during the time of admission.

Pathogen: A pathogen is any organism or agent that can produce disease. It may be referred to as an infectious agent.

Pathogenicity: This is the potential disease-causing capacity of pathogens involving a combination of infectivity (pathogen's ability to infect hosts) and virulence (severity of host disease).

Referral hospital: This is a hospital that has sufficient resources to receive emergency or non-emergency patient transfers and referrals from critical access hospitals (CAHs).

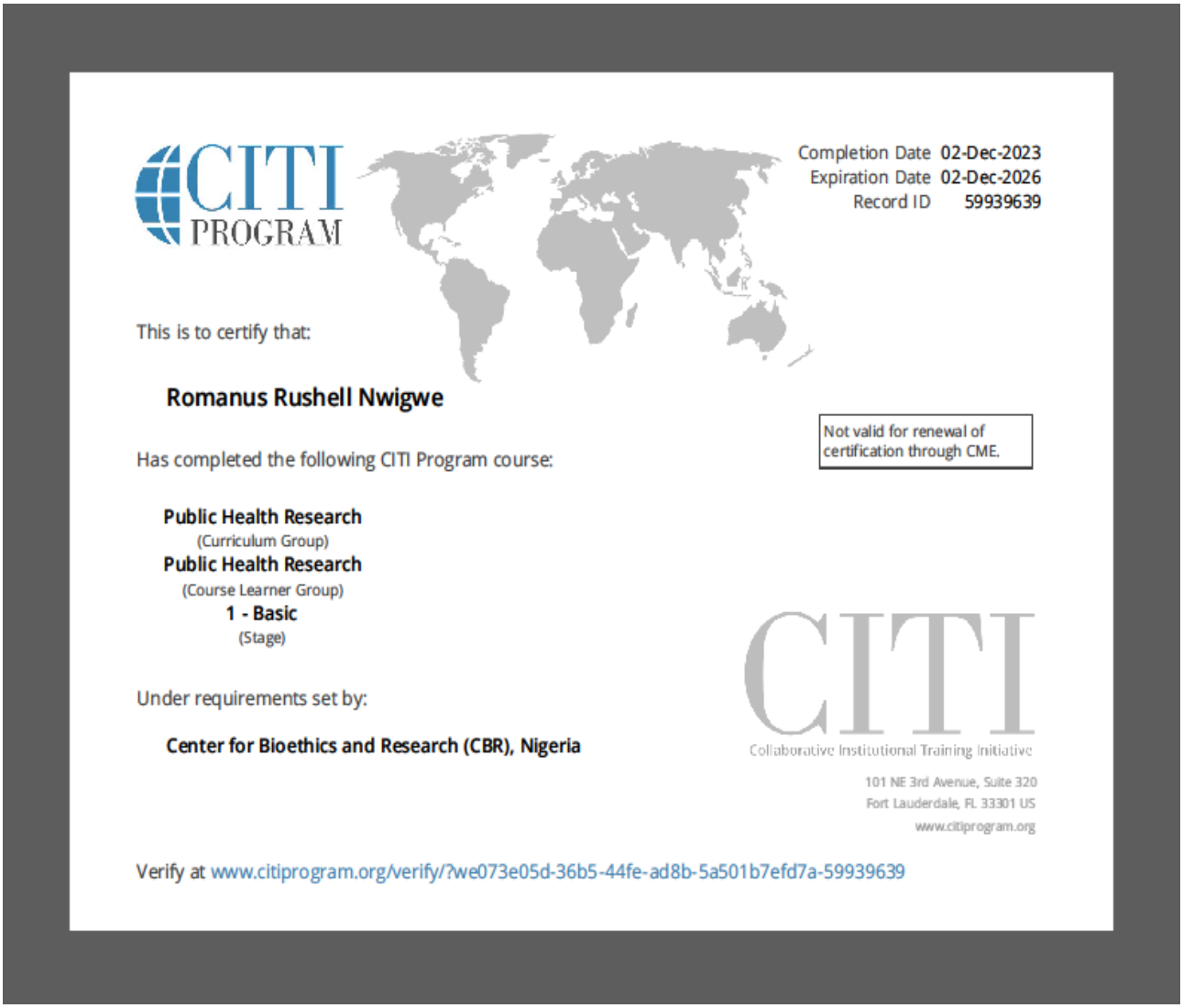
Reservoir: The reservoir of an infectious agent is the habit in which the agent normally lives, grows and multiplies.

Sanitary: This is the condition that affects hygiene and health, especially the supply of sewage facilities and clean drinking water.

Sanitation: This refers to public health conditions related to clean drinking water and treatment and disposal of human excreta and sewage.

APPENDIX D: PICTURES OF RESEARCH STUDENT DURING SAMPLE COLLECTION

APPENDIX E: ETHICAL CLEARANCE



CITI PROGRAM

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Has completed the following CITI Program course:

Public Health Research
(Curriculum Group)
Public Health Research
(Course Learner Group)
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(Stage)

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Group 5 - Students
(Course Learner Group)
1 - Basic Course
(Stage)

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(Curriculum Group)
NIGERIAN NATIONAL CODE FOR HEALTH RESEARCH ETHICS
(Course Learner Group)
1 - Stage 1
(Stage)

Under requirements set by:

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