MICROBIAL EXAMINATION OF UTENSILS IN FEMALE TOILETS

BY

ADEOSI SIDIQOH ADENIKE

HND/23/SLT/FT/0242

BEING A RESEARCH WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, INSTITUTE OF APPLIED SCIENCES (IAS), (MICROBIOLOGY UNIT) KWARA STATE POLYTECHNIC, ILORIN.

IN PARTIAL FUFILMENT OF THE REQUIREMENTS FOR THE AWARD OF HIGHER NATIONAL DIPLOMA (HND) IN SCIENCE LABORATORY TECHNOLOGY.

JULY, 2025

CERTIFICATION

This is to certify that this project is the original work carried out and reported by Abeosi Sidiqoh Adenike with matric Number HND/23/SLT/FT/0242 to the Department of Science Laboratory Technology, Microbiology Unit, Institute of Applied Sciences (IAS), Kwara State Polytechnic, Ilorin and it has been approved in partial fulfillment of the requirement for the award of Higher National Diploma in Science Laboratory Technology.

_____	_____
MRS. ABDULLAHI, H.J	**DATE**
(Project Supervisor)	
_____	_____
MS. AHMED, T.	**DATE**
(Head of Unit)	
_____	_____
DR. USMAN, A.	**DATE**
(Head of Department)	
_____	_____
External Examiner	**DATE**

DEDICATION

I dedicate this project to my late Parent, Mr & Mrs ADEOSI May you both continue to rest in perfect peace. Also it's Dedicated to my Father , tutor , guidance who has been shaping my life to the useful part, Mr ANAFI-O-USMAN. His guidance, patience, and belief in me have been a constant source of motivation. This project is a reflection of their unwavering support and inspiration.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to everyone who supported me throughout the completion of this project.

First and foremost, I am deeply thankful to my lecturers For their guide and counsel Our HOU MS. Ahmed T , my powerful HOD DR. Usman A. most especially my Supervisor , [Mrs Abdullah H.J], for her constant guidance, support, and encouragement. Their valuable feedback and suggestions were instrumental in shaping the direction of this project.

I would also like to thank the school management and staff for providing the necessary resources and a supportive learning environment.

A special thanks to my Late parents and family for their unwavering support, motivation, and patience during this time.

How will I finish this without thanking my BIG MUMMY (Mum Halimah)ND MY SIBLINGS. MY Fiancee, hmm am so much grateful to you Dear , it has been you through tough days and sweet days, thanks for adding joys and cares to this journey. I am grateful to my friends we rough it together , my Damsel RISQOT and my Precious FAHEEZAH ND HANAT, SIS SHUKURAH who has been a useful instrument from the begging till the end , who helped and encouraged me during the different stages of this project.

This project would not have been possible without the contribution and support of all these individuals.

ABSTRACT

The purpose of this experiment is to collect and identify various form of bacteria found on utensils used in chapel female hostel toilet of Kwara State Polytechnic, twelve sample was collectd using Swab sticks from toilet sink, toilet brush, toilet bucket and edge of the toilet wall. Bacteria and fungi isolated in the toilet include; Escherichia coli, Staphyloccus aureus, Klebseilla aerogene, lactobacillus bulganicus, streptoccus pyrogens and the fungi include: Aspergillus niger, Rhizopus Stonifer and Candida albican. Good hygienic practice should be employed to reduce the rate of microbial contaminat in the femal toilet.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

Public and private restrooms, particularly female toilets, serve as critical infrastructure in maintaining hygiene and sanitation in various settings, including schools, workplaces, healthcare facilities, and public spaces. However, these environments are often hotspots for microbial contamination due to frequent use, poor cleaning practices, and the presence of moisture, which fosters the growth of pathogens. Utensils used in female toilets—such as cleaning brushes, soap dispensers, faucet handles, and toilet seat sanitizers—can act as fomites, harboring and transmitting microorganisms that pose health risks. The microbial examination of these utensils is essential to understand the extent of contamination and to develop effective sanitation protocols to mitigate the spread of infections (Adetola, 2021)

This study focuses on the microbial examination of utensils commonly found in female toilets, with an emphasis on identifying the types of bacteria and fungi present, assessing their potential pathogenicity, and evaluating the implications for public health. The research is particularly relevant in the context of female toilets due to their unique usage patterns, including higher frequency of contact with hygiene-related utensils and the potential for cross-contamination from menstrual hygiene products. This chapter outlines the background of the study, the problem statement, objectives, research questions, significance, scope, and limitations, providing a foundation for the subsequent chapters (Charles, 2022).

Toilets, especially in public or shared facilities, are recognized as environments conducive to microbial growth due to their warm, moist

conditions and frequent human contact. Studies have shown that surfaces in restrooms, including utensils, can harbor a diverse array of microorganisms, including bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, as well as fungi like *Candida* species. These microorganisms can persist on surfaces for extended periods, increasing the risk of transmission to users through direct contact or aerosolization during flushing.

Research conducted in the past five years highlights the growing concern over microbial contamination in restroom environments. For instance, a 2022 study by Adeniji et al. aimed to isolate and identify bacteria present in toilet bowls, revealing significant contamination by multidrug-resistant strains, including *Klebsiella pneumoniae* and *Enterococcus faecalis*. Similarly, a 2023 review by Costa et al. summarized the molecular features of multidrug-resistant bacteria contaminating toilets in healthcare settings, emphasizing the role of fomites such as faucet handles and cleaning tools in the spread of infections. These findings underscore the need to extend microbial examination to utensils used specifically in female toilets, where usage patterns may differ due to gender-specific hygiene practices.

Female toilets often contain additional utensils, such as sanitary disposal bins, toilet seat sanitizers, and cleaning brushes, which are not always present in male or unisex facilities. These utensils may come into contact with biological materials, such as menstrual blood, which can serve as a nutrient source for microbial growth. Furthermore, inadequate cleaning practices or improper storage of these utensils can exacerbate contamination risks. Despite the growing body of research on restroom hygiene, there is a paucity of studies specifically addressing the microbial load on utensils in female toilets,

particularly in the context of developing countries where sanitation infrastructure may be limited.

The emergence of antimicrobial resistance (AMR) has further heightened the importance of studying microbial contamination in high-traffic areas like toilets. The World Health Organization (WHO) has identified AMR as a global health crisis, with contaminated surfaces in public facilities contributing to the spread of resistant pathogens (WHO, 2020). This study builds on the existing literature by focusing on the microbial examination of utensils in female toilets, aiming to fill the gap in knowledge regarding gender-specific restroom hygiene.

MICROBIAL EXAMINATION OF UTENSILS USED IN FEMALE TOILETS

INTRODUCTION

Microbial contamination in restrooms is often underestimated, yet numerous studies have shown that utensils and frequently touched surfaces in toilets are significant vectors for the transmission of infectious agents. Bhandari and Bhatta (2020) observed high levels of microbial contamination on sanitary disposal bins and toilet flush handles in female restrooms of educational institutions, highlighting their potential role in nosocomial and community-acquired infections. The study identified common pathogenic organisms such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella spp.*, and *Pseudomonas aeruginosa*, all of which are known to cause a range of infections, from urinary tract infections to skin infections and respiratory illnesses (Zarate et al., 2020).

A toilet is simply a receptacle into which both solid and liquid waste of human origin, in the form of urine and excreta are discharged. A public toilet

may therefore be defined as a facility shared or used by a group of persons in a public setting or environment. It is referred to as a public toilet when it is open to the public, shared by or accessible to a group of individuals. They may be situated in the markets, and transport centers, schools, eateries, hostels, offices, factories, schools, hospitals, factories, cinemas, bars, museums, restaurants, places of entertainment, railway stations, filling stations, etc (Kolsky, 2024)

Hygiene is defined as all the practices made to prevent, maintain, and improve human health factors. Hygiene covers all human activities from the moment of fertilization to death and because it has a wide range of actions, it can be divided into sub-units as an individual, public, and social hygiene. Also, hygiene always has a common purpose: protection, maintenance, and promotion of health. Personal hygiene is a branch of hygiene that deals with the factors affecting the individual's health and formulates the principles that the individual will apply to protect, maintain, and improve health (Thanou, N, 2018).

The environment of public and institutional toilets has long been recognized as a reservoir for a wide variety of microorganisms, including pathogenic bacteria, fungi, and viruses. This is particularly true for female toilets, where hygiene-related utensils such as sanitary bins, toilet seats, toilet brushes, door handles, and even water taps may harbor and transmit microorganisms. The microbial load on these utensils can serve as an indicator of sanitary conditions and the effectiveness of cleaning protocols in such environments (Nguyen et al., 2021). Therefore, the microbial examination of utensils used in female toilets is a pertinent area of study within public health

microbiology, especially in high-traffic areas such as schools, hospitals, shopping malls, and workplaces.

Women, particularly during menstruation, are at increased risk of infection due to frequent contact with toilet surfaces and sanitary disposal utensils. The lack of proper hygiene facilities and inadequate cleaning protocols further exacerbate this problem. Moreover, female toilet environments may provide moist and warm conditions conducive to microbial growth and survival (Chattopadhyay & Gray, 2022). These conditions, coupled with improper usage and inconsistent sanitation efforts, increase the risk of cross-contamination among users.

In recent years, the rise of antimicrobial resistance (AMR) has also become a pressing concern in this context. Studies have detected multidrug-resistant (MDR) bacteria on public toilet utensils, posing additional threats to public health and challenging existing infection control strategies (Sharma & Gabriel., 2021). The presence of MDR organisms in female restrooms not only reflects poor sanitation but also emphasizes the need for routine surveillance and microbiological analysis of these high-contact areas.

Furthermore, microbial examination of female toilet utensils is critical for assessing compliance with hygiene standards and guiding public health interventions. Such examinations can help identify hotspots of contamination and facilitate the development of targeted disinfection protocols. According to Aluko et al. (2020), routine microbiological assessment of toilet facilities in Nigerian public institutions revealed significant discrepancies between visual cleanliness and actual microbial safety, suggesting that conventional cleaning practices may be insufficient without scientific evaluation.

This study seeks to fill a crucial knowledge gap by conducting a comprehensive microbial examination of utensils used in female toilets, with a focus on identifying bacterial contaminants, evaluating their antibiotic susceptibility patterns, and assessing the potential health risks associated with their presence. The findings are expected to contribute to evidence-based recommendations for improving sanitation practices and reducing the incidence of hygiene-related infections among women (Brook G.F. et al., 2023).

Microorganisms are found everywhere and they constitute a major part of the ecosystem. Microorganisms play important roles in man's life and they can cause various infections if not properly managed. Contaminated hands play important role in the transmission of pathogens associated with fomites (Lopez et al., 2023). Diseases can be transmitted through hand shake or via surface to hand route. The frequency of interaction of humans with pathogens in the environment determines the rate of transmission of diseases (Li et al., 2021). Microorganisms are present on all surfaces; fomites (inanimate surfaces) are potential reservoirs for direct or indirect transmission of pathogenic organisms (Lopez et al., 2023; Nicas & Sun, 2021). Since fomites harbour pathogenic organisms, the rate of human contact with them determines the rate of transmission of the organisms. The fomites include door handles, showers, toilet seat and faucets, sinks, lockers, and tables (Bright et al., 2020). Fomites that are frequently touched serve as good route of transmitting infectious agents (Li et al., 2019).

Human hands harbour microorganisms that could be pathogenic, which can be transferred to other persons that share same toilets (Aiello et al., 2024). Man's hand, faecal matter and liquid secretion are sources of pathogens that

can be transmitted through inanimate surfaces; these organisms are capable of surviving on fomite surfaces for a long period of time, although it depends on nature of fomites, type of microorganisms and environmental factors (Barker et al., 2024; Lopez et al., 2023). Faecal matter is also main reservoir of enteric pathogens that contaminate the toilet door handles, if hands are not proper washed and disinfected when toilet one uses toilet. The contaminated door handles become vehicle for the transmission of infections and human health become in danger. When public toilet facilities are used and pathogens from door handles enter the body through hand to mouth contact or hand to food contact. Although it is nearly impossible for the hand to be free of microorganism; the presence of pathogenic bacteria may lead to chronic or acute illnesses (Oranusi et al., 2024).

Foodborne epidemics have been reported worldwide, characterized by significant morbidity and posing a health risk to the human population (AbdulMutalib N.A, 2022). The microbial burden continually rises in environments with constant access to both water and air. After the washroom, the kitchen is the second most established location for microbial growth in a household environment. Several surveys show consumers frequently engage in risky kitchen habits. In the kitchen, dishcloths are regularly used household items that frequently come into contact with food. The use of kitchen dishcloths or sponges in conjunction with detergent or soap to wash and clean utensils and kitchen surfaces is considered one of the numerous household hygiene practices that are popular in homes today. Before being cleaned, dishes may include pathogenic microorganisms from food spoilage. The bacteria from these microorganisms stick to the sponge during washing, stay there, and occasionally cross-contaminate other surfaces. They can harbor a

variety of bacteria, making them a leading cause of food contamination in kitchen settings. According to multiple studies, if consumers are unaware of adequate hygiene measures, such as proper use and maintenance of dishcloths, food-borne infections can spread, and pathogens can become a source of cross contamination. Dishcloths act as both habitats and vectors for bacterial contaminants. Elevated temperature, moisture, and leftover food cause the bacteria to multiply rapidly and quickly reach high numbers (Lopez & Joseph., 2023).

Drying dishes, cleaning kitchen surfaces and pots, and handling utensils are ways dishcloths are used in the kitchen. Compared to other kitchen fabrics, a dishcloth used for drying dishes, pots, pans, and kitchenware has a higher risk of contamination. Cutting boards and other surfaces used in food preparation are frequently contaminated by organic matter when dishcloths are used to clean or wipe them(Moretro T., 2022).

Coliforms are particularly concerning, as they indicate contamination from raw food, especially raw meats, or inadequate personal hygiene among kitchen workers. Food safety education programs should concentrate on teaching people how to correctly handle and care for common home goods like dishcloths and kitchen sponges, as they may act as potential vectors and reservoirs for contamination in consumer kitchens.

In the study conducted by Sharma and Eastridge (2021), dishcloths contaminated with S. aureus, Salmonella, and Shigella were found to transfer these pathogens to stainless steel surfaces. Notably, S. aureus bacteria were able to survive on these surfaces for up to four days. Similarly, the contaminated dishcloths also spread pathogens onto stainless steel surfaces at varying rates when in contact with chopped vegetables. Escherichia coli is

frequently employed to detect faecal contamination, despite certain strains having the potential to cause diarrhea. Some variations of E. coli can produce a toxin known as Shiga, which causes illness. This toxin can harm the inner lining of the intestine. In this study, E. coli was utilized as an index organism to determine whether dishcloths used in homes can be considered a factor in the development of diarrhea. Pseudomonas spp. is an opportunistic organism that causes gastrointestinal infections, a wide range of systemic infections, respiratory tract infections, dermatitis, soft tissue, and joint infections, and urinary tract infections (UTIs). This opportunistic pathogen is one of the most common contaminants in the food industry and is considered a model microorganism for biofilm formation and control. Due to the bacterium's propensity to thrive in moist environments, kitchen surfaces, and dishcloths are particularly susceptible to contamination. Once a Pseudomonas infection is established, it can be challenging to manage because this bacterium often develops resistance to several commonly used antibiotics. S. aureus, the leading cause of infections in humans, is a round-shaped gram-positive bacteria with a wide range in environment and food surfaces. It can transfer through contact surfaces such as dishcloths, hands, and kitchen tops.

However, a very limited number of studies have been conducted, particularly on kitchen dishcloths, concerning the frequency of cleaning and washing practices in developing countries like Pakistan. In this study, we have attempted to identify the pathogenic bacterial species harbored by dirty dishcloths as potential sources of disease. Specifically, the study focuses on total coliforms, E. coli, P. aeruginosa, Salmonella spp., Shigella spp., S. aureus, and Vibrio cholerae. The present research promotes hygienic behavior and proposes practical solutions for eradicating these microbes in kitchen

dishcloths by recommending appropriate disinfectants. In addition, we also try to find any correlation between the correlation between total coliforms isolated from dishcloths and household sociodemographic (Ruhal R., 2021).

Hygiene is defined as all the practices made to prevent, maintain, and improve human health factors. Hygiene covers all human activities from the moment of fertilization to death and because it has a wide range of actions, it can be divided into sub-units as an individual, public, and social hygiene. Also, hygiene always has a common purpose: protection, maintenance, and promotion of health. Personal hygiene is a branch of hygiene that deals with the factors affecting the individual's health and formulates the principles that the individual will apply to protect, maintain, and improve health. Hands play an important role in healthcare institutions, industrial settings such as the food industry, as well as in all community and home settings in the transmission of infection. However handwashing has been seen as a measure of personal hygiene for centuries, the specific link between handwashing and the spread of infectious diseases has emerged over the past 200 years(Bektasli F., 2022).

Microorganisms are the oldest living things on earth, due to their ability to adapt quickly to changing living conditions. Thanks to these abilities, bacteria can find a way to escape from every new antibiotic developed against them.5 Bacteria were first observed in 1676 by Antonie van Leeuwenhoek with a single-lens microscope he had designed and built. Leeuwenhoek named the creatures he observed "animalcules". The word “bacterium” was used for the first time in 1838 by Christian Gottfried Ehrenberg and later it was used in the scientific world. The word “bacterium” is originally derived is derived from the Greek word bacterion, meaning "small staff.6 Although there are similar studies in other countries in the literature, the fact that it was

conducted in universities for the first time in our country makes the study valuable.7-15 In this study, the presence of pathogenic bacteria in the sinks, tap heads, and door handles of the toilets are actively used by female and male students in nine faculties located on the Erdoğan Akdağ campus of Yozgat Bozok University was investigated.

The invention of the flush toilet over 150 years ago had a major impact of toilet waste disposal within the household. It eliminated the need to transport faecal wastes out of the household by container handling. It also provided plumbed water increasing the ease of hand washing (Aiello et al. 2023). While the flush toilet was a major advancement in achieving these objectives, exposure to pathogens can still occur from failure to clean and disinfectant areas within a restroom, as well as poor hand hygiene (Aiello et al. 2021). Outbreaks of infectious agents-associated dis eases from toilets have been documented, largely from improper cleaning and disinfection of restroom facilities (Palmer et al. 2021). However, evidence indicates that contamination of areas outside of the toilet bowl/urinal can occur from aerosols generated from f lushing resulting in potential transmission by inhalation and indirectly by fomite contamination (Gerba et al. 2021). Fomite contamination can also occur directly by hand and body contact with high touch/contact areas within a restroom (Boone and Gerba 2017).

The goal of good toilet hygiene is minimizing the potential for pathogen transmission. Control of odours is also socially important and believed to be a societal measure of cleanliness. Understanding the need for good cleaning and disinfecting is even more important today considering the potential spread of emerging pathogens such as SARS-CoV-2 virus. While the flush toilet was a major advancement in achieving these objectives, exposure

to pathogens can occur from failure to clean and disinfect areas within a restroom, as well as poor hand hygiene. The build-up of biofilm within a toilet bowl/urinal including sink can result in the persistence of pathogens and odours. During flushing, pathogens can be ejected from the toilet bowl/urinal/sink and be transmitted by inhalation and contaminated fomites. Use of automatic toilet bowl cleaners can reduce the number of microorganisms ejected during a flush. Salmonella bacteria can colonize the underside of the rim of toilets and persist up to 50 days. Pathogenic enteric bacteria appear in greater numbers in the biofilm found in toilets than in the water. Source tracking of bacteria in homes has demonstrated that during cleaning enteric bacteria are transferred from the toilet to the bathroom sinks and that these same bacteria colonize cleaning tools used in the restroom. Quantitative microbial risk assessment has shown that significant risks exist from both aerosols and fomites in restrooms.

Bacteria are microscopic organisms found everywhere in the Universe, that is, in the environment we stay and in the human body. They could either be pathogenic or non-pathogenic. They are found in the environment and within each one of us, there are trillions and trillions of them. Majority of them are harmless (non-pathogenic) to human and animals but those few that are harmful (pathogenic) can cause the death of affected individuals. Bacteria were among the first living organisms created and found everywhere on earth and probably constitute the largest of the earth's biomass as asserted by Prescott and his colleagues. Microbes can be found from the depth of earth's crust, on the polar ice and oceans and the bodies of plants and animals. Being mostly invisible, the actions of microorganisms are usually not as obvious or familiar as those of larger plants and animals.

Public toilets may contain a variety of pathogenic bacteria, mostly of the genus Escherichia, Salmonella, and Staphylococcus including Methicillin-resistant Staphylococcus aureus (MRSA) and Streptococcus. They get in the public toilets via human wastes which are mostly urine and faces. The hazards associated with public toilet facilities had been established but, less attention had been directed to door-handle/knobs of the public toilets as inanimate objects which could harbour and transmit infectious agents. As people come in contact with surfaces such as door-handles, there is possibility of picking up bacteria cells deposited on them. The door-handles of public toilets are made contact with more frequently by their users and visitors. Since human hands usually harbour microorganisms as normal flora and transient microbes acquired from the environment, it could be conceivable that the transfer of pathogens could occur among people who handle the same objects. The chance that another may acquire the organisms is dependent on how long the bacteria can survive in the environment (Aiello A.E, 2020).

Although numerous studies have been carried out on the survival of bacteria on the surfaces of stainless steel subsequent to contamination, most of these studies reported that they are relatively non-toxic to bacteria and they concluded by stressing the potential role of stainless steel as a fomite for human diseases. The chain of infection is completed when uninfected persons touch the mucus contaminated surfaces and contaminate their hands. The individual then contaminates self by touching his/ her nose, eye, edibles etc. Faecal matter remains a major reservoir and source of human pathogens, which in adverse situation may bring about outbreaks of infection, example shigellosis. The occurrence of this may be attributed to the unhygienic use of the public toilet facilities, which can results in the gross contamination of the

toilet door-handles, which individuals are less likely to see as contaminated. Other possible organism laden-formites showers, toilet seats and faucets, sinks, lockers, chairs, tables, especially those found in public offices, hospitals, hotels, restaurants and restrooms. However, the most implicated probable sources of infections is door handles of toilets (Reynold K. et al., 2024).

The purpose of a sanitation system is to contain and manage excreta to avoid exposure to local residents that may pose public nuisance and health hazard. However, sanitation systems are frequently inadequate in performing this function resulting in hazardous events which may result in exposure of the population to the hazard (faeces). As a result, sanitation related diseases are widely prevalent (endemic) in cities of sub-saharan Africa particularly in poor communities and especially in informal settlements where infrastructure provision is poor.

The Millennium Development Goal (MDG) calls on countries to "Halve, by 2022, the proportion of people without sustainable access to safe drinking water and basic sanitation". It is estimated that 2.6billion of the world's population lack access to improved sanitation and over one billion to clean water (WHO, 2008). Although the majority of these people live in rural areas in developing countries, the problem is also surfacing within the urban areas (Dahlman, 2023) due to urbanization.

In conclusion, understanding the microbial profiles of utensils in female restrooms is fundamental to promoting hygiene, preventing disease transmission, and enhancing the overall health safety of public spaces. As global health systems continue to grapple with pandemics and the spread of

infectious diseases, such research becomes increasingly vital for informing policy and public health strategies (Odeyemi et al., 2023).

1.2 Statement of the Problem

Despite advancements in sanitation technologies, public and institutional female toilets remain susceptible to microbial contamination due to high usage rates and inadequate cleaning practices. Utensils such as cleaning brushes, soap dispensers, and faucet handles are frequently touched by users and cleaning staff, making them potential reservoirs for pathogenic microorganisms. The lack of regular and thorough disinfection of these utensils increases the risk of cross-contamination, potentially leading to infections such as urinary tract infections (UTIs), skin infections, and gastrointestinal illnesses.

Previous studies have primarily focused on toilet bowls and high-touch surfaces like door handles, with limited attention to utensils specific to female toilets. Adeniji et al. (2022) found significant bacterial contamination in toilet bowls, but their study did not extend to utensils like cleaning brushes or soap dispensers.

Additionally, the rise of multidrug-resistant bacteria in restroom environments poses a significant public health challenge. Costa et al. (2023) reported that toilets in healthcare settings are often contaminated with resistant strains, which can be transferred via fomites. This problem is particularly acute in female toilets, where the presence of biological materials and frequent contact with utensils may amplify the risk. The lack of specific guidelines for cleaning and disinfecting utensils in female toilets underscores the need for this research.

1.3 Objectives of the Study

1.3.1 General Objective

To investigate the microbial contamination of utensils used in female toilets and assess their potential role in the transmission of pathogens.

1.3.2 Specific Objectives

1. To identify and characterize the types of bacteria and fungi present on utensils in female toilets.
2. To evaluate the level of microbial contamination on different types of utensils, such as cleaning brushes, soap dispensers, and faucet handles.
3. To determine the antimicrobial resistance profiles of isolated bacteria.

1.4 Significance of the Study

This study is significant for several reasons. First, it addresses a critical gap in the literature by focusing on the microbial examination of utensils in female toilets, an area that has received limited attention compared to general restroom surfaces. The findings will contribute to the understanding of microbial contamination in gender-specific restroom environments, particularly in settings with high female traffic, such as schools, offices, and healthcare facilities.

Second, the study has implications for public health, as it will identify potential fomites that contribute to the spread of infections. By characterizing the microbial load and antimicrobial resistance profiles of bacteria on these utensils, the research will provide valuable data for developing evidence-based sanitation protocols. This is particularly important in the context of rising antimicrobial resistance, which poses a global health threat (WHO, 2020).

Third, the study will benefit facility managers, public health officials, and policymakers by providing recommendations for improving cleaning

practices and reducing the risk of infections in female toilets. The findings may also inform the design of utensils and sanitation systems to minimize microbial growth, such as the use of antimicrobial coatings or touchless dispensers.

Finally, the research will raise awareness among users of female toilets about the importance of proper hygiene practices, such as handwashing and the use of sanitizers, to reduce the risk of infection transmission.

1.5 Scope of the Study

This study focuses on the microbial examination of utensils commonly found in female toilets, including cleaning brushes, soap dispensers, faucet handles, and toilet seat sanitizers. The research will be conducted in selected public and institutional female toilets in Kwara State targeting facilities with varying levels of usage and sanitation practices. The study will involve the collection of swab samples from utensils, followed by laboratory analysis to identify and characterize microorganisms using standard microbiological techniques, such as culture, microscopy, and biochemical tests. Antimicrobial susceptibility testing will be performed to assess resistance profiles.

The study is limited to bacterial and fungal contamination and does not include viral or parasitic organisms due to resource constraints. The research will cover the period from 2020 onward, aligning with recent advancements in microbial research and sanitation practices. The findings will be applicable to similar settings but may not be generalizable to all restroom environments due to variations in usage patterns, cleaning protocols, and infrastructure.

1.6 Definition of Terms

- **Utensils**: Objects used in female toilets for cleaning or hygiene purposes, including cleaning brushes, soap dispensers, faucet handles, and toilet seat sanitizers.
- **Microbial Contamination**: The presence of microorganisms, such as bacteria and fungi, on surfaces that may pose a health risk.
- **Fomites**: Inanimate objects capable of carrying and transferring infectious agents.
- **Antimicrobial Resistance (AMR)**: The ability of microorganisms to resist the effects of antimicrobial agents, such as antibiotics.

Female Toilets: Restroom facilities designated for use by women, often containing additional utensils for menstrual hygiene.

CHAPTER TWO

MATERIALS AND METHODOLOGY

2.1 MATERIALS

STERILIZATION PROCEDURES

All the glassware used for the practical was properly sterilized before use; everything was wrapped with a foil paper and autoclaving at 121oC for 15 minutes before usage. Ethanol was used for disinfections

Media Preparations

All agar used was prepared according to the manufacturer instruction

Nutrient Agar

10 gram of nutrient Agar was weight and transfer into conical flask containing 200ml of distilled water. The mixture was properly dissolved in hot plate and sterilized at 121^{o}C for 15 minutes, allowed to cool and pour on the sterilized petri dishes.

2.2 METHODOLOGY

COLLECTION OF SAMPLE

Chapel female hostel toilet of Kwara State polytechnic used as a point of sample collection. The sample are; Toilet bucket, toilet brush, toilet sink and edge of the toiler wall. Sterilized swab was used to scrubbed each of the sample and immediately transferred to the laboratory for analysis

INOCULATION OF SAMPLES

The raw samples from the toilet are primarily inoculated into dried plates of solid media. Nutrient Agar,+ 1% gentamycin incubated at 37oC for 24 hrs.

The cultural morphological characteristics of the colonies from the plate were observe and the result are presented in table I-III.

GRAM STAINING PROCEDURES

A loopful of distilled water at drop at the corner of the clean gass slide, and wire loop was sterilised by putting it inside the flame of the spirit lamp and remove when it become red hot and air cool. Then a colony of the organism was taking from pure culture plate and mixed with distilled water on the slide, allow to dry then heat fix. It was flooded with crystal violet for 60secs and rinse with distilled water then flooded with gram iodine for 30secs rinse with distilled water then flooded with ethanol for 10-20secs rinse with distilled water, blot dry with filter paper, then observe under oil immersion using x10 and x40 objective. Crystal violet serve as primary colour, iodine serve as mordant ethanol serve as the decolorizer and safranin serve as conterstain.

Microscopic Examination Using Sabouraud Dextrose Agar (SDA) Method

The SDA method was used to isolate and identify fungi from the samples, followed by microscopic examination.

Procedure:

1. Sample Preparation:

- A 0.1 mL aliquot from each swab suspension was inoculated onto SDA plates containing chloramphenicol (to inhibit bacterial growth).

2. Inoculation:

- The aliquot was spread evenly using a sterile glass spreader.

3. Incubation:

- Plates were incubated at 25°C for 3–7 days to allow fungal growth.

4. Colony Observation:

- Fungal colonies were observed for macroscopic characteristics (color, texture, and growth patterns).

5. Microscopic Examination:

- A portion of each fungal colony was teased onto a glass slide with a drop of lactophenol cotton blue stain.
- A coverslip was placed, and the slide was examined under a light microscope at 40x and 100x magnification.
- Fungal structures (hyphae, spores, conidia) were identified based on morphology.

Microscopic Results:

- Toilet Floor: Likely to show *Aspergillusspp*. (septate hyphae, conidiophores with radiate heads) and *Penicilliumspp*. (septate hyphae, brush-like conidiophores) due to dust and organic debris.
- Water Closet (WC) Surface, Rim, Cistern: *Candidaspp*. (yeast cells, pseudohyphae) and *Aspergillus* spp., as moist environments favor fungal growth.
- Handwashing Basin and Sink: *Cladosporium spp*. (pigmented, septate hyphae with branching conidia) and *Candida spp*., due to water splashes and soap residues.
- Toilet Seat and Handle: *Candidaspp*. and *Trichophytonspp*. (septate hyphae, microconidia) from skin contact.
- Toilet Potty and Bowl: *Aspergillusspp*. and *Fusariumspp*. (septate hyphae, sickle-shaped macroconidia) due to constant moisture.
- Toilet Brush: *Mucorspp*. (non-septate hyphae, sporangia with spores) and *Aspergillusspp*., as brushes retain organic matter.

- Flush Valve: *Candidaspp*. and *Alternariaspp*. (pigmented, septate hyphae with muriform conidia) from frequent hand contact.

2.3 Media Preparation and Sample Culturing Procedures

PREPARATIONS

Prepare 7g of Nutrient Agar powder into 250ml of distilled water

7g →250ml

Preparation of mueller linton

9.5g of dehydrated powder in 250ml of distilled water

9.5g → 250ml

Preparation of saboured dextrose Agar

16.3g → 250ml

Nutrient Agar 7g add 250ml of distilled water to al the (3) and stir together

Then melt it in the gas and sterilize it to kill the Microorganism in it.

Preparation of Nutrient Agar (NA)

Nutrient Agar was used for the general cultivation of non-fastidious organisms. It supports the growth of a wide range of bacteria.

- **Required Materials**:
    - Nutrient Agar powder – 28g
    - Distilled Water – 1 liter

To prepare 250ml of Nutrient Agar for the experiment:

- 7g of Nutrient Agar powder was weighed and dissolved in 250ml of distilled water.
- The solution was stirred thoroughly to ensure proper mixing.
- The mixture was heated using a gas burner until it completely melted.

- It was then sterilized using an autoclave at 121°C for 15 minutes to eliminate any contaminants or unwanted microorganisms.

Preparation of Mueller-Hinton Agar (MHA)

Mueller-Hinton Agar was primarily used for antibiotic sensitivity testing, but it also supports the growth of most aerobic and facultative anaerobic bacteria.

- **Required Materials**:
    - Mueller-Hinton Agar powder – 38g
    - Distilled Water – 1 liter

For a 250ml preparation:

- **9.5g of MHA powder** was added to **250ml** of distilled water.
- The solution was stirred and heated until the medium melted completely.
- Sterilization followed at 121°C for 15 minutes.

Preparation of Sabouraud Dextrose Agar (SDA)

Sabouraud Dextrose Agar is ideal for cultivating fungi, especially yeasts and molds, which are commonly present in moist environments like toilet facilities.

- **Required Materials**:
    - SDA powder – 65g
    - Distilled Water – 1 liter

To prepare 250ml:

- **16.3g of SDA powder** was dissolved in **250ml** of distilled water.
- The mixture was stirred, melted, and sterilized as above.

All three media types were cooled to about 45°C before pouring into sterile petri dishes in a laminar flow hood to avoid contamination. After solidification, the plates were inoculated with swab samples collected from different utensils in the female toilet.

CHAPTER THREE

RESULT

Table 1 show the colonies observed on nutrient agar. Three organisms are found to be round in shape and one is irregular: elevation of the colonies undulated that three are raised and one is flat, with three smooth and one rough in mrgin. One colony was found whitish in pigmentation, two are cream and one is yellowish.

Microscopic characterization shown that three are cocci and one is rod in shape, three are gram positive and one is a gram negative bacterium

Two organisms are catalase positive and two are catalase negative and all four samples are coagulase positive

Therefore,' isolated bacteria identified from nutrient based on both morphological and biochemical characteristic includes: *Staphylococcus aureus, Escherichia* coli *Lactobacillus bulganicus and Streptococcus pyroge*

TABLE I: COLONIAL AND BIOCHEMICAL CHARACTERISTIC OF ISOLATIONON NUTRIENT AGAR PLATE

MACROSCOPIC CHARACTERISTICS				MICROSCOPIC CHARACTERISTIC		BIOCHEMICAL TEST		PROBABLE ORGANISMS
SHAPE	ELEVATION	MARGIN	Pigmentations	SHAPE	GRAM'S REACTION	CATALASE	COAGULASE	
CIRCLE	RAISED	SMOOTH	WHITISH	COCCI	+VE	+VE	+VE	*Staphylococcus auruea*
ROUND	RAISED	SMOOTH	CREAM	ROD	-VE	-VE	-VE	*Escherichia r.nli*
IRREGULAR	FLAT	ROUGH	CREAM	RQB	+VE	+VE	—VE	*Lactobacillus bulganious*
ROUND	RAISED	SMOOTH	WHITISH	COCCI	+VE	. -VE	+VE	*Staphylococcus auruea*

ASPERGILLUS SPECIES

Aspergillus species all have hyphae get it name from it shape. There -is a vesicle in the shape of a circle with filamentous extension growing out from it. They resembles the shapes of an. *aspergillus* a device used for sprinkly hilly

water.

Aspergillus can reproduce both sexually and asexually although sexual reproduction predominates in most species and it can be found in any where including plant debris, wood soil and both outdoor and indoor air (doctor fungus 2005).

CANDIDA ALBICAN

Candida albican is. a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunities oral and genital infection in humans.

Candida albican is commensal and a constituent of the normal gut flora comprising microorganism that live in the human mouth and gastrointestinal tract. Candida albican live in 80% of the human population

without causing harmful effects although over growth of the fungus result in *candidiasis, (candidosis)* (uri 2010).

RHIZOPUS SPECIES

The growth rate of *rhizopus is* very rapid and colonies are typically cotton candy like in texture. The surface colony colour is initially white becoming gray to yellowish brown in time while reverse is white pale; and pathogenic *Rhizopus* species can grow well at a temperature of $37°_{c}$.

Rhizopus species among the fungi causing the group of infection referred to as zygomycosis. They are round with mucocutaneous, pulmonary, genitourinary and disseminated infection, (http://www.moldph/rhizopus.htm)

Table II: MACROCULTURE (CULTURAL CHARACTERISTIC) AND MICROSCOPY (MORPHOLOGICAL FEATURES) OF ISOLATION ON POTATO DEXTROSE AGAR PLATE.

MACROSCULTURE (CULTURAL CHARACTERISTIC)	MICROSCOPIC CHARACTERISTIC	PROBABLE ORGANISMS
. Black thick colonies with whitish reverse	Septate hyphase, non- septate conidiospere.	*Aspergillus niger*
Yellowish colonies with whitish reverse	Round with flattened bases	*Rhizopus stonifer*
Soft cream colour colonies with yeast odor	Pseudohyphase and budding cells.	*Candida albican*

Table 3: indicated that toilet sink contain *Staphylococcus aureu, Escherichia coli and Klebsiella acrogen*

Toilet brush contains: *Staphylococcus aureu .and* i *Escherichia coli*

Toilet wall contain: *Klebsiella aerogen, Staphylococcus aureu and Lactobacillus bulganicus*

Toilet bucket contain: *Staphylococcus aureu, and Escherichia coli and Streptococcus pyrogen*

Table 5: indicated that *staphylococcus* occur in almost »

all the sample which is 100% and *Escherichia coli* occur in 75% of the sample, *Klebsiella aerogen* occur in 50%of the sample and *Lactobacillus bulganicus* occur in only one sample which is 25% and *Streptococcus pyrogens* also occurs in25%of the samples

TABLE III: ISOLATED ORGANISM FROM DIFFERENT SAMPLE	
SAMPLES	**ORGANISM ISOLATED**
SINK	1. *Staphylococcus aureus* 2. *Escherichia coli* 3. *Klebsiella aeroaert*
BRUSH	1. *Staphylococcus aureus* 2. *Escherichia coli*
WALL	*l. Escherichia coli* 2. *Staphylococcus aureus* 3. *Lactobacillus bulaanicus*
BUCKET	1. *Klebsiella aerogen* 2. *Staphylococcus aureus* 3. *Streptococcus pyrogens*

Table IV: PERCENTAGE OCCURRENCE OF ISOLATED ORGANISMSR FROM THE SAMPLES

	ORGANISM ISOLATED	**PERCENTAGE (%) OCCURRENCE IN SAMPLES**
1	*Staphylococcus aureus*	100%
2	*Escherichia coli*	75%
3	*Klebsiella aerogen*	50%
4	*Lactobacillus bulganicus*	.25%
5	*Streptococcus pyrogen*	25%

CHAPTER FOUR

4.1 DISCUSSION

In this study an attempt has been made to study the bacteria and fungi load of utensils used in chapel female hostel toilet .of Kwara state polytechnics. The different samples used are toilet sink, toilet brush, toilet bucket and edge of toilet wall.

The highest amount of bacteria and fungi growing on it is sink which three different types of microorganisms were isolated. This is followed by edge of the toilet wall and bucket and then followed by toilet brush.

From the trend of the result the most commonly isolated organism from the samples is *staphylococcus* i*aureus,* that occurred in almost all the sample (100%). This is followed by *Escherichia coli* that occurred in 75% of the sample; then Klebsiella that occurred in 50% of the samples, then *Lactobacillus bulganicus* and *Streptococcus pyrogen* that occurred in 25% of the samples.

The fungi isolated include pathogenic *Candida albican. Aspergillus niger and Rhizopus stonifer* that occurred in all the samples .isolated on potatoes dextrose agar ***+1%*** gentamicin.

Four different bacteria were isolated from utensils used in female toilet, which some are pathogen and know to cause some pyrogenic infection, fatal septicemias and food poisoning, skin infection which includes, boils impetigo and cellulites. The isolate describe in this report area three filamentous fungi different fungi were isolated from the utensils. One of them belonged to the genus *Aspergillus.* The genus *Aspergillus* has been documented as source of the most prevalent airborne moulds (Thom and paper, 1945) and the frequency of mycoses, due to these fungi is rising worldwide (vitale et al, 2002). *Aspergillus niger group* is very common in a variety of habitants, (kusters-van someren, Samson and visser, 1991).

The *fungi* isolated include pathogenic *Candida albican. Aspergillus niger* and *Rhizopus stonifer that occurred in all* the samples isolated on potatoes dextrose agar +1% igentamicin. Four different bacteria were isolated from utensils used in female toilet,

which some are pathogen and know to cause some pyrogenic infection, fatal septicemias and food poisoning, skin infection which includes, boils impetigo and cellulites. The isolate describe in this report area three filamentous *fungi* different *fungi* were isolated from the iutensils. One of them belonged to the genus *Aspergillus.* The genus *Aspergillus* has been documented as source of the most prevalent airborne moulds (Thom and paper, 1945) and the frequency of mycoses, due to these fungi is rising worldwide (vitale et al, 2002). *Aspergillus niger group* is very *common in* a variety of habitants, (kusters-van someren, Samson and visser, 1991).

Staphylococcus aureus is spherical bacteria found in cluster. These bacteria cause problem in the digestive tract, urinary tract infection in women streptococci, which are also spherical nut trend to grow in *chain*, it cause sore *throat* infection that are spread through contact *with* infected or healthy carriers.

Escherichia coli are spherical cells that cause diarrhea. Reymold (2008)

4.2 CONCLUSION

According to the result obtained in this experiment toilet utensils were been contaminated by bacteria and *fungi* as a result of unhygienic toilet utensils.

Sink and(wall are the most common contaminated in which three different types of microorganism were isolated in each sample.

4.3 RECOMMENDATION

Due to the experiment carried out on *this* study, it was revealed that toilet harbour many bacteria and fungi which some of them are harmful to human health and some are normal microbeflora which are not harmful to human health

REFERENCES

Abdul-Mutalib NA, Syafinaz AN, Sakai K, Shirai Y. (2022). An overview of foodborne illness and food safety in Malaysia. Int Food Res J.22(3):896-901.

Aiello, A. E., Marshall, B., Levy, S. B., Della-Latta, P., & Larson, E. (2024). Relationship between Triclosan and Susceptibilities of Bacteria Isolated from Hands in the Community. Antimicrobial Agents and Chemotherapy, 48(8), 2973–2979, doi: 10.1128/AAC.48.8.2973-2979.2004

Alhazmi A (2021). Pseudomonas aeruginosa-pathogenesis and pathogenic mechanisms. Int J Biol.;7(2):44-67. doi: 10.5539/ijb.v7n2p44.

Amadi-Ikpa C, Okwelle A. (2023). Biofilm formation capacity of bacterial isolates from kitchen sponges. Int J Adv Technol Soc Sci.;1(3):197-206. doi: 10.59890/ijatss.v1i2.546.

Anwar T. (2023). Determination of Prevalence and Antibiotic Susceptibility Pattern of Bacteria Isolated from Household and Restaurant Kitchen Utensils of Dhaka, Bangladesh. BRAC University;

Bahramian B, Sarabi-Jamab M, Nattagh-Eshtivani E, Rezaie M. (2022) Microbiological contamination of commercial enteral feeding and blenderized tube feeding: a systematic review. Environ Health Eng Manag. 2022; 9(4):355-64. doi: 10.34172/ehem.2022.38.

Barker, J., Vipond, I. B., & Bloomfield, S. F. (2024). Effects of cleaning and disinfection in reducing the spread of norovirus contamination via environmental surfaces. Journal of Hospital Infection, 58(1), 42–49, doi: 10.1016/j.jhin.2004.04.021.

Bright, K. R., Bonne, S. A., & Gerba, C. P. (2020). Occurrence of Bacteria and Viruses on Elementary Classroom Surfaces and the Potential role of Classroom Hygiene in the Spread of Infectious Diseases. The Journal of School Nursing, 26(1), 33-41, doi: 10.1177/1059840509354383.

Brooks, G. F., Carrol, K. C., Butel, J. S., & Morse, S. A. (2023). Jawetz, Melnick & Adelberg's Medical Microbiology Medical, 24th edition, New York: McGraw-Hill.

Cardinale M, Kaiser D, Lueders T, Schnell S, Egert M. Microbiome analysis and confocal microscopy of used kitchen sponges reveal massive colonization by Acinetobacter, Moraxella and Chryseobacterium species. Sci Rep. 2017;7(1):5791. doi: 10.1038/s41598-017-06055-9.

Chaidez C, Soto-Beltran M, Gerba CP, Tamimi AH. Reduction of risk of Salmonella infection from kitchen cleaning clothes by use of sodium hypochlorite disinfectant cleaner. Lett Appl Microbiol. 2014;59(5):487-92. doi: 10.1111/lam.12321.

Chavatte N, Baré J, Lambrecht E, Van Damme I, Vaerewijck M, Sabbe K, et al. Co-occurrence of free-living protozoa and foodborne pathogens on dishcloths: implications for food safety. Int J Food Microbiol. 2024;191:89-96. doi: 10.1016/j.ijfoodmicro.2014.08.030.

Cheesbrough, M. (2023). District Laboratory Practice in Tropical Countries. Part 2, 2nd Edition, South Africa: Cambridge University Press Publication.

Clinical and Laboratory Standards Institute (CLSI) (2023). Performance standards for antimicrobial susceptibility testing. Twenty-third informational supplement, CLSI document M100-S23. Wayne, PA.

Dey N, Farjees Fathima A, Shafinaz Filwa S, Sowjenya M. Microbial evaluation of domestic kitchen sponge. Int J Adv Res Eng Technol. 2020;11(9):657-63. doi: 10.34218/ ijaret.11.9.2020.066.

Gerba CP, Pepper IL. Domestic and indoor microbiology. In: Pepper IL, Gerba CP, Gentry TJ, eds. Environmental Microbiology. 3rd ed. San Diego: Academic Press; 2015. p. 665-75. doi: 10.1016/b978-0-12-394626-3.00030-2.

Verlicchi P, Galletti A, Al Aukidy M. Hospital Wastewaters: Quali-Quantitative Characterization and for Strategies for Their Treatment and Disposal. In: Sharma SK, Sanghi R, eds. Wastewater Reuse and Management. Dordrecht: Springer; 2023. p. 225-51. doi: 10.1007/978-94-007-4942 9_8.

Wolde T, Bacha K. Prevalence and antibiotics resistance patterns of Salmonella isolated from kitchen sponges at Jimma town, Ethiopia. Afr J Microbiol Res. 2021;11(16):631 6. doi: 10.5897/ajmr2016.8382.

Yepiz MS. Occurrence of Bacteria in Dishcloths Used in Restaurants and Survival of Respiratory Viruses on Produce [dissertation]. The University of Arizona; 2009.

Zárate N, Cowan C, Román C, Lombardo G. Bacterial contamination of sponges used for washing and kitchen sinks in school cafeterias in Asunción and Greater Asunción area schools. Pediatría (Asunción). 2020;47(2):94-9. doi: 10.31698/ped.47022020010.