

**ASSESSMENT OF MICROBIAL LOAD OF PUBLIC PHONES AND MOBILE  
DEVICES USED BY UNIVERSITY STUDENTS**

**BY**

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**A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES,  
COLLEGE OF BASIC AND APPLIED SCIENCES, MOUNTAIN TOP UNIVERSITY,  
MAKOGLI, IBAFO, OGUN  
STATE, NIGERIA**

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF  
BACHEOR OF SCIENCE (B.Sc.) IN MICROBIOLOGY**

**SEPTEMBER, 2022**

**DECLARATION**

I hereby declare that this project has been written by me and is a record of my own research work. It has not been presented in any previous application for a higher degree of this or any other university. All citations and sources of information are clearly acknowledged by means of reference.

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.....

**Date**

## CERTIFICATION

This is to certify that this research project titled “**ASSESSMENT OF MICROBIAL LOAD ON PUBLIC PHONES AND MOBILE DEVICES OF UNIVERSITY STUDENTS**” was carried out by **SOLOMON BUSOLA** with matriculation number **18010101012**. This project meets the requirement governing the award of **Bachelor of Science (B.Sc.)** degree in **Microbiology** from the Department of Biological sciences of Mountain Top University, Ogun State, Nigeria and is approved for its contribution to knowledge and literacy presentation.

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Head of Department

## **DEDICATION**

I dedicate this project to the Almighty God, who has been my strength and provider. Also to my parents Mr. and Mrs. SOLOMON for their constant prayers, advise and financial support.

## **ACKNOWLEDGEMENTS**

All glory to God Almighty for his unending love and faithfulness throughout my journey in Mountain Top University. I am sincerely grateful for the Grace to start well and finish this degree program successfully.

My profound gratitude goes to my wonderful and most loving parent, Mr. and Mrs. Solomon, my lovely brother and sister for their endless moral, financial and prayer supports.

I also appreciate the Head of Department, Biological Sciences Dr. (Mrs) C. I. Ayolabi.

Special thanks to my wonderful supervisors Dr. O. I. Ogunsuyi and Dr. G. B. Akanni whose constructive criticism and mentorship and guidance have helped in putting me on the right path during my research work.

Finally, my course mates and friends who have made this journey memorable and worthwhile especially Uchendu Chioma, Odozi Blessing, Shokunbi Oluwaseyi, Achigbue Anthony and Sekoni Praise. God bless you all.

I also appreciate the lab attendant and technologist Mrs M. A. Osagie and Miss. Adegbala.

## TABLE OF CONTENTS

Title page.....	i
Declaration.....	ii
Certification.....	iii
Dedication.....	iv
Acknowledgements.....	v
CHAPTER ONE: INTRODUCTION.....	1
1.1    Background to the study.....	1
1.2    Statement of the problem.....	2
1.3    Justification of the study.....	2
1.4    Aim and objectives.....	2
CHAPTER TWO: LITERATURE REVIEW.....	4
2.1    Literature Review.....	4
2.2    Microbial contamination and mobile phones.....	5
2.3    Pathogens associated with mobile devices.....	7
2.4 <i>Staphylococcus aureus</i> .....	7
2.4.1    Characteristics of <i>Staphylococcus aureus</i> .....	8
2.4.2    Epidemiology of <i>Staphylococcus aureus</i> .....	10
2.4.3    Pathogenesis of <i>Staphylococcus aureus</i> .....	10
2.4.4    Treatment of <i>Staphylococcus aureus</i> Infections.....	11
2.4.5    Antimicrobial Resistance of <i>Staphylococcus aureus</i> .....	11
2.5 <i>Escherichia coli</i> .....	12
2.5.1    Characteristics of <i>Escherichia coli</i> .....	12
2.5.2    Epidemology of <i>Escherichia coli</i> .....	13
2.5.3    Pathogenesis of <i>Escherichia coli</i> .....	13
2.5.4    Prevention and Treatment of <i>Escherichia coli</i> isolates.....	13
2.5.5    Antimicrobial Resistance of <i>Escherichia coli</i> .....	14
2.6 <i>Pseudomonas aeruginosa</i> .....	14
2.6.1    Characteristics of <i>P.aeruginosa</i> .....	15
2.6.2    Pathogenesis of <i>P.aeruginosa</i> .....	15
2.6.3    Biofilm formation.....	16
2.6.4    Treatment of <i>P.aeruginosa</i> .....	16
CHAPTER THREE: METHODS AND MATERIALS.....	17

3.1	Materials needed for swab collection.....	17
3.2	Sample collection and dispatch.....	17
3.2.1	Enumeration of bacterial load.....	17
3.2.2	Media and reagents.....	17
3.2.3	Preparation of culture media.....	18
3.2.4	Preparation of calcium chloride.....	18
3.2.5	Preparation of Cary Blair medium.....	18
3.2.6	Nutrient agar.....	18
3.2.7	Preparation.....	18
3.2.8	MacConkey agar.....	19
3.2.9	Preparation.....	19
3.2.10	Mannitol bile salt agar.....	19
3.2.11	preparation.....	19
3.2.12	Buffered peptone water.....	19
3.2.13	Preparation.....	20
3.2.14	Brain heart infusion broth.....	20
3.3	Biochemical test.....	20
3.3.1	Gram's staining.....	20
3.3.2	Procedures.....	20
3.4	Catalase test.....	21
3.4.1	Procedures.....	21
3.5	Coagulase test.....	21
3.5.1	Procedures.....	21
3.6	Oxidase test.....	21
3.7	Antibiotic susceptibility test.....	22
3.8	Molecular identification.....	22
3.9	DNA extraction.....	22
3.9.1	Activation of DNA extraction.....	23
3.9.2	DNA extraction by heating block.....	23
3.10	Polymerase chain reaction (PCR).....	23
3.11	Agarose gel electrophoresis.....	24
	CHAPTER FOUR:RESULTS.....	25
	RESULTS AND DISCUSSION.....	25

4.1	Colony counts.....	25
4.2	Morphological characteristics of the isolates.....	25
4.3	Biochemical characteristics of the isolates.....	25
4.4	Antibiotic susceptibility test of the isolate.....	25
4.5	Polymerase chain reaction (PCR).....	25
4.6	Discussion.....	32
CHAPTER FIVE:		
5.1	Conclusion.....	33
5.2	Recommendation.....	33
REFERENCES.....		34



## LIST OF TABLES

<b>Table 4.1:</b> Bacterial Distribution Counts.....	26
<b>Table 4.2</b> Morphological Characteristics of Isolates.....	27
<b>Table 4.3</b> Biochemical Characterization of the Isolate.....	28
<b>Table 4.4</b> Antibiotic Susceptibility of Staphylococcus Isolates.....	29

## LIST OF FIGURES

<b>Figure 2:</b> Antibiotics Susceptibility Test For the Isolate.....	30
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## ABSTRACT

Mobile phones are used in our daily lives. It has become a widely spread device in today's life. These devices are carried into different places like; toilet, kitchen, event centers, hospitals, markets etc., which are loaded with harmful microorganisms that can lead to sickness or illness of the human body. Mobile phones and other devices have also been reported to be a reservoir for many microorganisms. The aim of this study was to assess microbial load on public phones and mobile devices among university students. With the consent of the students, 15 swab samples were collected from male and female student's devices in different colleges in a tertiary institution, and were analyzed using standard microbiological techniques and molecular techniques such as Biochemical tests, Antibiotic Susceptibility Testing, DNA extraction and PCR. Fifteen swab samples were collected out of which five (75%) swab sample tested positive for different type of bacteria. Microbial analysis showed that the mobile devices were contaminated with different types of bacteria. The mobile devices of male students (80%) were found to be more contaminated than that of female students (20%). *Staphylococcus aureus* was found to be the most dominant bacteria found from the mobile devices. In conclusion, the study results had shown that mobile devices from both male and female in the tertiary institution were contaminated with at least one or more bacterial isolates. It was observed that *Staphylococcus aureus* (90%) were present in four samples and *bacillus* (10%) present in a sample. Therefore, people should endeavor to wash their hands, avoid taking their phones into the toilet cause these bacteria hang around the toilet area and keep a proper hygiene.

**Keywords:** *Staphylococcus aureus*, mobile devices, contamination, percentage.

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background to the study

Our daily lives involve the use of mobile phones. It is now a commonly used technology in today's society. Mobile phones and other gadgets provide extra assistance for a variety of functions, including message sending and receiving, internet access, and the sharing of films and photographs. These items are brought into a variety of locations, including restrooms, kitchens, event venues, hospitals, and markets, all of which are teeming with pathogenic bacteria that can cause human illness (El Manama et al., 2015). These elements, along with the heat produced by cell phones, contribute to the dangerous quantities of microorganisms that are present there. The immediate health dangers associated with using germ-ridden mobile devices are evident when one considers the everyday contact that a cell phone has with the face, mouth, ears, and hands. All age groups may now access mobile phones thanks to technological advancements, particularly in terms of their processing power, which has made them a need in daily life. It has also developed into a substitute or chance for these bacteria as a storage facility.

According to estimates from 2017, 71% of Nigeria's population used mobile phones as their primary form of communication (Gillwald et al., 2018). These microorganisms can be transmitted via direct or indirect physical contact between people or via inanimate things like televisions, furniture, game pads, etc. Cell phones with buttons and keyboards and other personal mobile phones in general have been found to be even more conducive to bacterial contamination, even while indirect contamination from person to person has decreased with the drop in the usage of public payphones (Lee et al., 2013). Gram-positive and Gram-negative bacteria have both been found in hand-to-mouth transmission during casual activities, according to Rusin and his colleagues. This suggests that mobile devices could act as channels for the spread of illnesses like diarrhoea, pneumonia, boils, and abscesses (Rusin et al., 2002).

Our mobile phones are difficult to clean, in contrast to our hands, which can be easily cleaned. Even we hardly ever try to clean them. Therefore, a variety of germs are carried by these gadgets. In hospitals, patients, visitors, and healthcare professionals frequently use cellphones. In addition, since it is unknown whether phone accessories have the potential to spread bacteria, tourists who visit low-income nations with inadequate access to drinkable water and sanitary facilities run the danger of getting sick (Brady et al., 2006). These microorganisms are located on fomites and,

according to studies, can live for a very long time depending on the environment. Ya'aba and colleagues 2020. These bacteria cells are part of the bacteria that live on our bodies whether they do so naturally or as a result of an infection. They attach to mobile phone surfaces easily and may even form colonies. Our phones and other mobile gadgets have surfaces that are difficult to clean, which allows bacteria to move there and thrive. Once on our hands or faces, the germs can contaminate any area of the body that has a scratch or an open wound (Kawakib I.AI-Zubaidy, 2019). E. coli, Staphylococcus aureus, Streptococcus, and other bacteria are connected to mobile phones. The most well-known fecal coliform bacterium is E. coli, and it is well-known since people carry their mobile devices when using the restroom these days.

## **1.2 Statement of the problem**

An electronic gadget for personal telecommunication is a mobile phone. We frequently ignore the health risks linked with mobile phones because of how simple they are to use and the additional applications they offer. The purpose of this study is to investigate and evaluate the microbial load of potential microorganisms that may be present on mobile phones and public phones but have not been extensively studied, to support and validate claims made about its potential health risks, and to identify a few of these microorganisms present. It also aims to find a way to reduce how these microorganisms are spread from person to person, thereby lowering the potential health risks.

## **1.3 Justification of the study**

A thorough investigation is conducted to explain the microbiological foreign substances on mobile devices from university students as well as their anti-infection agent helplessness designs due to knowledge of the damaging effects of contaminations and the need to maintain general wellbeing. Nevertheless, it is common to find numerous microorganisms in our furnishings, settings, and technology. The purpose of this study is to ascertain the microbiological evaluation of mobile devices, their impact on public health, and the methods for treating any infections caused by pollutants.

## **1.4 Aim and objectives**

The aim is to access microbial load on mobile phones and mobile devices among university students.

The objectives of this study are as follows;

- To collect samples from mobile phones, laptops and gadgets.

- To determine the level of bacterial load on mobile phones and to identify both gram positive and gram-negative bacteria on mobile phones.

## CHAPTER TWO

### LITERATURE REVIEW

In order to provide a better communications network, the global system for mobile telephony was founded in Europe in 1982. In India, mobile phones were first used in 1995. India had 287 million mobile phone customers as of 2008, making up 85% of all telecommunications consumers in that country (Kapdi et al., 2008). Even though they are constantly handled and kept in bags, pockets, and close to the face, mobile devices have evolved into essential accessories for people in today's environment. Touch screens in contemporary phones are used to operate them. Human hands are very important for many areas of personal, industrial, clinical, and occupational hygiene. Numerous germs have been discovered to be stored on mobile phones and other gadgets. These microbes can be spread from one person to another, from inanimate things to our hands, or even the other way around (Brandy, 2007). By often coming into contact with hands, a cell phone might transmit contagious diseases. Fomites that have been contaminated are crucial in the transmission of bacterial diseases.

Mobile phone usage has increased among the general population in Nigeria, and it is widespread in some environments where the percentage of germs present is probably high, such as in hospitals, animal slaughterhouses, and restrooms. In order to find out whether mobile phones might contribute to the spread of bacterial diseases and to suggest potential controls or preventive measures that could be implemented to avoid this likely source of infection, a study was done. In this investigation, bacterial agents were discovered to be present on 62 percent of the 400 mobile phones from all study groups. Electronic gadgets including handheld computers and personal digital assistants have been demonstrated to be potential sources of nosocomial infection transmission after bacterial organisms from these devices were isolated (Bures et al., 2000).

Exogenous and endogenous infections are the two primary categories of bacterial infections. (Jumaa, 2019). When the infectious agent originates from the patient's own body, typically from his or her own normal flora, endogenous infections take place. When a person's own immunity against his or her normal flora is weakened (as after surgery), endogenous causes of infections are especially crucial (Sunganya and Sumanthy, 2020).

The exogenous infection, on the other hand, typically arises from bacteria that are found outside of the body. Exogenous sources of infections can originate from humans, animals, or the

environment, to be more precise. Humans can spread infections in three different ways: when they are carriers, when they are asymptotically infected, or when they are clinically infected (symptomatic infection). Exogenous sources of infections include air, mobile devices, toys, and the hands of surgeons (Ilusanya et al., 2018). Soil and stray bacteria are removed from the hands during hand washing.

Consequently, the straightforward act of washing one's hands has long been a cornerstone of any control technique for lowering nosocomial infections (Ilusanya et al., 2018). Such nosocomial infections can be prevented with a well-executed infection control strategy that includes hand cleanliness, environmental cleansing, surveillance, and contact isolation (Jumaa, 2005). Unfortunately, studies continue to indicate unacceptable low hand washing compliance rates among health workers, despite the ease with which the procedure is carried out (Jumaa, 2005). A user's frequent handling of a mobile phone makes it a breeding ground for the spread of bacteria and illnesses linked to hospitals (Kilic et al., 2019). People can have diverse hand flora and various ranges of microorganisms than regular members of the public. Consequently, acquired pathogenic flora is permanently colonized on the hands (Kilic et al., 2019). These microbes may include *Klebsiella* spp., *pseudomonas* spp., *Staphylococcus aureus*, *Escherichia coli*, *Micrococcus*, *Enterococcus*, *Aspergillus* spp., and *Rhizopus* spp.

## **2.1 Microbial contamination and mobile phones**

The most significant of these concerns is that there are no precise, legally required standards for the handling of hospital-contact mobile phones. The use of mobile phones, which is common throughout the day and into the night as well as every day, increases the possibility that different germs will spread to different departments of health care workers and to people outside of hospitals (Parhizgari et al., 2013). Since we touch our phones more than 100 times per day, various microbes from our skin may be transferred to them and vice versa as a result of their use. Mobile phones also provide a crucial means of life collaboration. Moreover, we frequently expose our mobile devices to germ-filled surfaces, which increases the risk of microbial migration to our mobile devices (Jeske et al., 2007; Akinyemi et al., 2009). In many locations, particularly in the hospital sections, there are multiple users on a single mobile phone. Due to the humidity and ideal body temperature of the body, particularly the inner portion of hand palms, this may also constantly result in the transmission of bacteria between medical facilities, particularly those individuals associated to dermis.



As they come into contact with several body parts, including the mouth, ear, and fingers of different people working in various environments, mobile phones serve as a reservoir for numerous microbial infections. There are numerous other factors that contribute to the greater promotion of these germs' growth, such as the protection provided by our pouches, handbags, and snug pockets, which increase the possibility of microbial multiplication. The proliferation of microorganisms on mobile devices is facilitated by the overheating of mobile devices (Tagoe et al., 2011). Mobile phones are rarely cleaned, and these problems are related to their hygiene in particular as it relates to phone gadgets (Hadir EL-Kady, 2017).

In many research, it is stated that regular hand washing and hand disinfection by health professionals and other people will help to avoid the colonization of mobile phones by microorganisms. Our "fingerprints" and the microorganisms on our mobile devices are made up of about 80% of the common germs, according to an experimental study carried out in the United States of America (USA) (Meadow et al, 2014). Antimicrobial medications are frequently used again and again to treat infections caused by bacterial pathogens. Antimicrobial medication resistance is linked to nosocomial infections, which will lead to major cases of community health issues. Since infections caused by drug-resistant bacteria are now widely known and drug resistance contributes significantly to the rising costs of healthcare, many pathogens have evolved to be resistant to a variety of medications, Additionally, this is a side effect of prolonged hospital stays, which necessitates the use of other, less expensive medications (Bodena et al, 2019). Numerous research have shown that gram positive bacteria such as coagulase negative Staphylococci, Staphylococcus aureus, Micrococcus spp., and spore-forming Bacillus spp. were the most often isolated pathogens from mobile phones. Other gram-negative bacteria include Escherichia coli, Proteus species, Pseudomonas aeruginosa, Klebsiella species, and Acinetobacter species, among others. additionally serving as a haven for bacteria-causing diseases. Despite the possibility of pathogens contaminating the surfaces of regularly used smartphones, factors determining the duration of infectious infection transmission, such as the ability of germs to survive on inanimate surfaces and materials, Lack of environmental disinfection of commonly used objects and/or bad hand hygiene among people have a significant influence in the spread of many human diseases. Studies have shown that the survival of clinically relevant microbial pathogens on non-living surfaces depends on the surface and the characteristics of microorganisms. Microorganisms are determined by environmental factors

such as temperature, humidity, the presence of organic substances, and the ability to form biofilms. For instance, the most common strains of *S. aureus*, such as methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA), can live in the environment for at least seven days and possibly up to a year. The time frame has been estimated to be between 9 and 12 days and 72 hours for bacteria that have colonized on surfaces made of stainless steel and/or plastic, respectively. According to their respective survival times in the environment, *Escherichia coli*, *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Proteus* spp., *Klebsiella* spp., and *Klebsiella* spp. can remain infectious for up to 1.5 hours, 16 months, a year, 1-2 days, and more than 30 months. The risk of transmitting fungal and viral infectious illnesses is influenced by the persistence of yeasts and clinically relevant viruses on dry surfaces. Human coronaviruses (MERS-CoV), according to reports, can survive on inanimate surfaces and stay contagious for up to 9 days at room temperature and for a shorter period at higher temperatures. While both viruses are spread via contaminated airborne droplets, the duration for influenza viruses has been given as 4 weeks. Due to ineffective use of disinfectants, infections with respiratory pathogens such as respiratory syncytial virus and rhinoviruses, which tend to occur mostly in the winter and spread quickly, can survive for up to 6 hours and 7 days, respectively. Molds can persist in household soil for many months, which makes them related to contamination of settings, tools, and things (Kramer and Assadian, 2014; Russotto et al, 2017).

## **2.2 Pathogens associated with mobile devices**

- *Staphylococcus aureus*
- *Escherichia coli*
- *Streptococcus*
- *Pseudomonas*

## **2.3 *Staphylococcus aureus***

*Staphylococcus aureus* is a gram-positive bacterium forming irregular clusters of cocci, round shaped bacterium. *S. aureus* are a widespread in nature, though they are mainly found on the skin, fomites, mucous membranes of mammals and birds, but can cause infection under certain circumstances. It is more pathogenic than other common members of genus such as *S. epidermidis* and *Saprophyticus*. Major sites of infection in hospital patients are surgical wounds and indwelling medical devices. In latter the bacteria may colonize the implanted device causing local damage or it can disseminate. In addition, food poisoning can occur after ingestion of food

contaminated with enterotoxins. *Staph. aureus* also causes the economically important ruminant mastitis.

Disease caused by *Staph. aureus* is in general due to two types of virulence determinants, cell surface associated proteins and extracellular protein toxins. *Staph. aureus* expresses a multiplicity of cell surface associated and extracellular proteins which have the potential to contribute to pathogenesis. It can express several surface-located proteins which bind to components of the extracellular matrix and to components of blood clots and damaged tissues. These probably serves as adhesins to promote bacterial attachment and colonization. *Staph. aureus* can express several factors that combat host defenses. *Staphylococcus aureus* forms glistening, smooth, entire, raised, translucent colonies that often have a golden pigment. Specimens likely to be contaminated with other microorganisms can be plated on mannitol salt agar containing 7.5% sodium chloride which allows halotolerant staphylococci to grow. Otherwise, bacteria can be streaked on trypticase soy agar, possibly incorporating erythrocytes. The taxonomy is based on 16s rRNA sequences, and most of the staphylococcal species fall into 11 clusters: *S. aureus* group :*S. argenteus*, *S. aureus*, *S. schweitzeri*, *S. simiae* etc.

### **2.3.1 Characteristics of *Staphylococcus aureus***

*Staphylococcus aureus* is a Gram-positive (purple by Gram stain) bacteria that is cocci-shaped and organized in clusters that are described as "grape-like" (Taylor and Unakal, 2021). These organisms may grow in medium containing up to 10% salt, and colonies are frequently golden or yellow (aureus means golden or yellow) (Habib *et al.*, 2015). These organisms can develop aerobically or anaerobically (facultatively), and at temperatures ranging from 18 to 40 degrees Celsius (Hussien *et al.*, 2020). *S. aureus* are catalase positive (all pathogenic *Staphylococcus* species), coagulase positive (to distinguish *Staphylococcus aureus* from other *Staphylococcus* species), novobiocin sensitive (to distinguish from *Staphylococcus saprophyticus*), and mannitol fermentation positive are typical biochemical identification tests (Adetutu *et al.*, 2017). Scanning electron microscopy reveals cells that are about spherical in shape and have a smooth surface, the cells' diameters range from 0.5 to 1.0 M (Li *et al.*, 2016). Cells with robust cell walls, distinctive cytoplasmic membranes, and amorphous cytoplasm are visible under transmission electron microscopy (Rohde, 2019).

*Staphylococcus aureus* requires thiamine, nicotic acid, inorganic salts, and amino acids as nitrogen sources (Omotani *et al.*, 2017). It requires B vitamins (nicotic acid, thiamine), amino

acids, and inorganic salts for growth, while cysteine, valine, glutamic acid, arginine, and tyrosine do not aid in growth but are a key source of enterotoxin synthesis (Chen, 2018; Kehiller, 2019). *Staphylococcus aureus* has an extraordinarily long lifespan, it develops in a wide temperature range because it is Mesophilic; 7- 47.8°C (optional 35°C); pH: 4.5- 9.3 (opt. 7.0-7.5). Low levels of water activity (aw) of 0.83 (optional>0.99) (Landgraf and Destro, 2013; Nunes and Caldas, 2017; Lu *et al.*, 2020). *Staphylococcus aureus* can exist in a latent state for years if growth conditions (such as temperature or nutrition availability) are not favorable (essentially, being inactive and lying-in wait for a good time to begin growing (Michailova *et al.*, 2007).

Exoproteins produced by *S. aureus* contribute to the bacteria's capacity to colonize and cause illness in mammalian hosts (Kong *et al.*, 2016; Rudra and Boyd, 2020). A set of enzymes and cytotoxins secreted by nearly all strains comprises four hemolysins (alpha, beta, gamma, and delta), nucleases, proteases, lipases, hyaluronidase, and collagenase (Hassan *et al.*, 2012; Tam and Torres, 2019). These proteins' primary role could be to transform local host tissues into nutrients needed for bacterial growth (Ciborowski and Jeljaszewicz, 2018). Toxic shock syndrome toxin-1 (TSST-1), staphylococcal enterotoxins (SEA, (SEB, SECn, SED, SEE, SEG, SEH, and SEI), exfoliative toxins (ETA and ETB), and leucocidin are among the extra exoproteins produced by some strains (Bernardo *et al.*, 2002).

*S. aureus* is found in the environment as well as in normal human flora, and is found on the skin and mucous membranes (most commonly the nasal area) of most healthy people (Taylor and Unakal, 2021). *S. aureus* does not generally cause illness on healthy skin; but, if these bacteria reach the circulation or internal tissues, they can cause a number of potentially dangerous infections (Hussain *et al.*, 2018). Direct contact is generally used for transmission of staphylococcal infections (Tenover and Gorwitz, 2006). However, certain illnesses are spread through different means (Taylor and Unakal, 2021).

*S. aureus* food colonization has long been linked to a kind of gastroenteritis characterized clinically by emesis with or without diarrhea (Castro *et al.*, 2016). This illness is known as staphylococcal food poisoning (SFP) and is caused by the consumption of one or more preformed staphylococcal enterotoxins (SEs) on food contaminated with *S. aureus* (Fetsch and Johler, 2018). Systemic toxicity symptoms such as fever and hypotension are uncommon in SFP instances (Pereira *et al.*, 2021). Furthermore, SFP is a self-limiting illness that usually cures within 24 to 48 hours of start (Argaw and Addis, 2015). The prevalence of SFP is unknown,

however it is most likely the most common cause of food poisoning in the United States (Sergelidis and Angelidis, 2017). It is unknown whether humans develop long-term immunity to SFP (Argaw and Addis, 2015). Antibodies to a single SE, however, would not necessarily provide immunity to SFP because several SEs are capable of causing illness (Reddy *et al.*, 2017). Antibodies generated against one SE may give cross-protection against another SE in some cases, because these two SEs share antibody binding epitopes, heterologous antibodies to SEB may provide cross-protection against staphylococcal enterotoxin C (SEC) (Augustyniak *et al.*, 2017).

### **2.3.2 Epidemiology of *Staphylococcus aureus***

*Staphylococcus aureus* including drug-resistant strains such as Methicillin-resistant *Staphylococcus aureus* (MRSA) is present on the skin and mucous membranes, and humans are the primary reservoir for these organisms (Taylor and Unakal, 2021). It is estimated that between 20 and 80% of people have *Staphylococcus aureus* in their anterior nares (Brown *et al.*, 2014). Some populations, including as health care professionals, those who use needles on a frequent basis (such as diabetics and IV drug users), hospitalized patients, and immunocompromised people, have higher rates of *S. aureus* colonization (Tenover and Gorwitz, 2006). *S. aureus* can be passed from person to person via direct touch or via fomites (Xiao *et al.*, 2019).

### **2.3.3 Pathogenesis of *Staphylococcus aureus***

*S. aureus* is the causative agent of many human infections, including bacteremia, infective endocarditis, skin and soft tissue infections (e.g., impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, and others), osteomyelitis, septic arthritis, prosthetic device infections, pulmonary infections (e.g., pneumonia and empyema) and gastroenteritis (David and Daum, 2017). These bacteria can induce invasive infections and/or toxin-mediated illnesses depending on the strains involved and the site of infection (Taylor and Unakal, 2021). The pathophysiology of *S. aureus* infection differs widely (Taponen and Pyörälä, 2009).

Antiphagocytic capsule synthesis, sequestering of host antibodies or antigen masking by Protein A, biofilm formation, intracellular survival, and preventing leukocyte chemotaxis are all mechanisms for evading the host immune response (Taylor and Unakal, 2021). In infectious endocarditis, bacterial cell wall-associated proteins such as fibrinogen-binding proteins, clumping factors, and teichoic acids mediate bacterial attachment to extracellular matrix proteins and fibronectin (Heilmann, 2011). Infectious endocarditis, sepsis, and toxic shock syndrome all

have Staphylococcal superantigens (TSST-1 or toxic shock syndrome toxin 1) as key virulence factors (Kulhankova *et al.*, 2014).

Pneumonia infections are linked to the development of PVL (Panton-Valentine leukocidin), Protein A, and alpha-hemolysin by bacteria, and they're more likely after an influenza virus infection or a Cystic Fibrosis diagnosis (Radke, 2021). Infections of prosthetic devices are frequently mediated by *S. aureus* strains' ability to build biofilms and communicate utilizing quorum sensing in a bacterial cell density-dependent way (Taylor and Unakal, 2021).

#### **2.3.4 Treatment of *Staphylococcus aureus* Infections**

*S. aureus* infections are treated differently depending on the type of infection and whether or not drug-resistant strains are present (Jones, 2008). When antimicrobial therapy is required, the length and style of treatment are largely determined by the type of infection as well as other factors (Spaulding *et al.*, 2018). If the isolates are sensitive (MSSA, or methicillin sensitive *S. aureus* strains), penicillin is the drug of choice, and vancomycin is the drug of choice for MRSA strains (Dibah *et al.*, 2014). Alternative therapy may be required in addition to antimicrobial therapy in some circumstances. For toxin-mediated sickness, for example, fluid replacement is frequently required, as is the removal of foreign devices for prosthetic valve endocarditis or catheter-associated infections (Taylor and Unakal, 2021). MRSA infections are becoming a significant disease in both hospital and community settings since many MRSA strains are resistant to various antibiotics (Lee *et al.*, 2018).

#### **2.3.5 Antimicrobial Resistance of *Staphylococcus aureus***

Beta-lactamase, a serine protease that hydrolyzes the beta-lactam ring, deactivates penicillin. All penicillinase-resistant penicillins and cephalosporins are resistant to methicillin resistance (Altshuler *et al.*, 2019). The presence of the *mec* gene, which encodes penicillin-binding protein 2a, is required for resistance (Jousselin *et al.*, 2015). Although many methicillin-resistant strains appear to be descended from a small number of clones, others appear to be multiclonal in origin, implying horizontal *mec* DNA transfer (Raphael *et al.*, 2017). Other staphylococcal genes, such as *bla* (for -lactamase) and *fem* (for factors required for methicillin resistance), influence resistance expression (Lee *et al.*, 2018). Resistance to methicillin is frequently diverse, with the fraction of a bacterial population expressing the resistance phenotype varying according to environmental factors (Brauner *et al.*, 2016).

There is growing concern regarding the advent of vancomycin-resistant *S. aureus* strains (Yousefi *et al.*, 2017). Vancomycin resistance has been identified in clinical isolates of *S. haemolyticus*, a coagulase-negative species (Akinwunmi and Lamikanra, 2010; Al-Tamimi *et al.*, 2020). The vancomycin resistance gene from an enterococcal plasmid has been transmitted to *S. aureus* in vitro by conjugation (Haaber *et al.*, 2017). Four case reports (one from Japan and three from the United States) describe the isolation of clinical strains with intermediate vancomycin sensitivity (minimum inhibitory concentration, 8 g per milliliter) (Sieradzki *et al.*, 2003).

## **2.4 *Escherichia coli***

*Escherichia coli* also known as *E. coli* is a negative Gram-positive anaerobic rod-shaped coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms. Most of *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts and are occasionally responsible for food contamination incidents that prompt product recalls. The cells in *E. coli* are typically rod-shaped, and are about 2.0  $\mu\text{m}$  long and 0.25–1.0  $\mu\text{m}$  in diameter, with a cell volume of 0.6–0.7  $\mu\text{m}^3$ . Antibiotics can effectively treat *E.*

*coli* infections outside the digestive tract and most intestinal infections but are not used to treat intestinal infections by one strain of these bacteria. The flagella which allow the bacteria to swim have a peritrichous arrangement. It also attaches and effaces to the microvilli of the intestines via an adhesion molecule known as intimin. *E. coli* is often referred to as the best or most-studied free-living organism. More than 700 *E. coli* serotypes have been identified. The bacteria's "O" and "H" antigens, as well as their flagella, distinguish the different serotypes. The *E. coli* strains responsible for the numerous reports of contaminated foods and beverages produce Shiga toxin, so named because the toxin is nearly identical to that produced by *Shigella dysenteriae* type 1. *E. coli* O157:H7 is the most well-known and notorious *E. coli* bacteria that produces Shiga toxin. *E. coli* is one of the microorganisms found in mobile phones, and the agar used to grow *E. coli* is MacConkey agar, which is pink red in color.

### **2.4.1 Characteristics of *Escherichia coli***

*E. coli* is distinguished by Gram negative non-sporulating bacilli, indole production from tryptophan, the absence of citrate as a carbon source, and the absence of acetone production. It also uses gas fermentation to ferment glucose and lactose. The envelope of *E. coli*, like that of all Gram-bacteria, consists of three components: the cytoplasmic membrane, the outer membrane,

and a periplasmic space formed by peptidoglycan between the two. This final structure gives the bacterium its shape and rigidity, allowing it to withstand relatively high osmotic pressure in the environment.

*E. coli* is a mesophilic bacterium that thrives in the body temperature environment of warm-blooded animals (35-43C). The limit temperature of growth is around 7C which indicates that an effective control of the cold chain in the food industries is essential to prevent the growth of *E.coli* in food.

#### **2.4.2 Epidemiology of *Escherichia coli***

*Escherichia coli* are gram-negative bacteria in the *Enterobacteriaceae* family that can colonize the human gut harmlessly or cause intestinal or extraintestinal infections, including severe invasive disease such as bacteremia and sepsis. *E. coli* is the most common cause of bacteremia in high-income countries, outnumbering other pathogens that cause bacteremia, such as *Staphylococcus aureus* and *Streptococcus pneumoniae*, and is a leading cause of neonatal meningitis. Emerging multidrug-resistant *E. coli* strains are more difficult to treat and carry a higher risk of bacteremia and death. Vaccines to prevent invasive *E. coli* infections, such as bacteremia, are currently unavailable but are being developed in clinical trials. To inform the development and implementation of effective prevention strategies, a better understanding is needed of the current epidemiology of invasive *E. coli* infections.

#### **2.4.3 Pathogenesis of *Escherichia coli***

*E. coli* is part of the normal intestinal flora of humans and animals. There are many strains of *E. coli*, including purely commensal strains as well as strains with virulence determinants that cause a wide range of diseases in all age groups of humans and animals. *E. coli* virulent strains are specific pathogens of the gut (enteritis) and extra-intestinal sites.

#### **2.4.4 Prevention and Treatment of *Escherichia coli* isolates**

Intestinal disease is best avoided by exercising caution when selecting, preparing, and consuming food and water. The preservation of fluid and electrolyte balance is critical in treatment. Although antibiotics can reduce the duration of symptoms, resistance is still widespread.



Antibiotics are required for extraintestinal diseases. Antibiotic sensitivity testing of isolates is required to determine the best drug to use.

#### **2.4.5 Antimicrobial Resistance of *Escherichia coli***

Multidrug resistance in *Escherichia coli* has become a major concern in both human and veterinary medicine around the world. Although *E. coli* is intrinsically susceptible to almost all clinically relevant antimicrobial agents, this bacterial species has a high capacity for resistance gene accumulation, primarily through horizontal gene transfer. The acquisition of genes coding for extended-spectrum  $\beta$ -lactamases (conferring resistance to broad-spectrum cephalosporins), carbapenems (conferring resistance to carbapenems), 16S rRNA methylases (conferring pan-resistance to aminoglycosides), and plasmid-mediated quinolone resistance (PMQR) genes are the most problematic mechanisms in *E. coli* (conferring resistance to fluoroquinolones, and genes conferring resistance to polymyxins). Although the spread of carbapenems genes has been well documented in humans but less so in animals, colistin resistance in *E. coli* appears to be more closely related to the use of colistin in veterinary medicine on a global scale. Other resistance traits' cross-transfer between the human and animal sectors is still debatable, despite genomic research indicating that extended-spectrum  $\beta$ -lactamase producers found in animals are distinct from those found in humans. Furthermore, *E. coli* of animal origin frequently exhibit resistance to other, mostly older, antimicrobial agents such as tetracyclines, phenicol, sulfonamides, trimethoprim, and fosfomycin. Plasmids, particularly multi-resistance plasmids, but also other mobile genetic elements such as transposons and gene cassettes in class 1 and class 2 integrons, appear to play an important role in resistance gene dissemination. It is worth noting that cos election and persistence of resistances to critically important antimicrobial agents in human medicine also occur as a result of widespread use of antimicrobial agents in veterinary medicine, such as tetracyclines or sulfonamides, as long as all of those determinants are located on the same genetic elements.

#### **2.5 *Pseudomonas aeruginosa***

*P. aeruginosa* is a common organism in soil and water, as well as on plants and animals. It is a gram negative, aerobic, saccharolytic, non-spore forming bacillus measuring 0.5 to 0.8 $\mu$ m by 1.5 to 3.0 $\mu$ m. Most *P. aeruginosa* strains have a single polar flagellum that is used for motility. *Pseudomonas aeruginosa* frequently produces two soluble pigments: pyocyanin, which gives

colonies a blue color, and pyoverdine, also known as the fluorescent pigment and a yellow green or yellow-brown pigment (Nicholas, 2020). When a strain of *P. aeruginosa* produces both pyoverdine and pyocyanin, the colonies produced are blue-green. Other water-soluble pigments produced by this organism include pyorubrin and pyomelanin, which give colonies a red or brown color, respectively. *P. aeruginosa* colonies on sheep blood agar plates frequently exhibit beta-hemolysis and a greenish metallic sheen due to pigment production. Because no other gram-negative nonfermenters produce pyocyanin, its presence aids in identifying the organism. The characteristic fruity grape-like odor produced by the organism from the production of 2-aminoacetophenone is one of the most recognizable signs of an unknown colony of *P. aeruginosa*. *P. aeruginosa* infections have always been difficult to treat, but as with other bacteria, *P. aeruginosa* is becoming increasingly resistant to antimicrobial agents. To make matters worse, multidrug resistant *P. aeruginosa* has been identified, which is resistant to three or more antimicrobial agents and accounted for nearly 30% of all isolates obtained from nursing home and ICU patients in one study. Eosin Methylene Blue (E.M.B) culture medium is used to test the growth of microorganisms in *P. aeruginosa*.

### **2.5.1 Characteristics of *P. Aeruginosa***

*P. aeruginosa* can grow in solid agar media at temperatures ranging from 4°C to 44°C; however, growth at higher temperatures is more pronounced. It has simple nutritional requirements and can grow in media containing acetate as a carbon source and ammonium sulfate as a nitrogen source. *P. aeruginosa* colonies are typically of two types: large and smooth colonies with flat edges and elevated centers, resulting in a fried-egg appearance, and small, rough and convex colonies. Organisms isolated from clinical materials form large colonies, whereas those isolated from natural sources form small colonies. The edges of large colonies may have silver-grey metallic shining patches. In isolates from respiratory and urinary tract infections, a third mucoid type of colony is also found. On agar media, *P. aeruginosa* colonies have a tendency to form localized swarming from the colony's edge. Furthermore, the colonies generate green and fluorescing pigments. Another distinguishing feature of these colonies is their fruity odor and the presence of metallic patches. *Pseudomonas aeruginosa* is an opportunistic pathogen linked to a variety of mild to severe nosocomial infections in immunocompromised patients. *P. aeruginosa* entry, colonization, and infection are dependent on a number of factors known as virulence factors, which aid in organism survival and evasion of host defense. *P. aeruginosa* virulence factors are classified into different groups based on their involvement during infection. *P. aeruginosa* virulence factors are classified

into three categories: attachment and motility factors, colonization factors, and chronic infection factors.

### **2.5.2 Pathogenesis of *P. aeruginosa***

Pathogenesis of *P. aeruginosa* infections is mediated by various virulence factors that facilitate entry, colonization, and invasion of host tissue. *Pseudomonas aeruginosa* is unique in its ability to cause severe invasive infections while evading immune system defenses, resulting in chronic infections. Such virulence factors are attributed to subsequent tissue damage, invasion, and dissemination of *P. aeruginosa* during the course of infection. The pathogenesis of *P. aeruginosa* infections follows a specific pattern, eventually leading to persistent invasive disease.

### **2.5.3 Biofilm formation**

Resistance to antimicrobial agents is critical in the pathogenesis of *P. aeruginosa* infections. Exopolysaccharide, rhamnolipid, pyoverdine, and proteinaceous surface appendages are all involved in the biofilm formation process. Cell differentiation occurs during biofilm formation, and oxygen and water-filled channels are formed to provide nutrition to the mature biofilm's deep-rooted cells. Cells growing in biofilms are up to 1000 times more resistant to antibodies than free-swimming cells, according to studies.

### **2.5.4 Treatment of *P. aeruginosa***

The clinicians' treatment method is determined by the severity of the infection. Courses of IV antibiotics are sufficient for treatment of mild infections; however, surgical debridement may be required for deeper infections. ICU admissions may be required in patients suffering from respiratory failure, pneumonia, sepsis, or other systemic infections. Along with broad-spectrum antibiotics, double pseudomonal coverage may be required.

Carbapenems, cephalosporins, aminoglycosides, and fluoroquinolones are common antibiotics used as first-line therapy. Longer exposure to medicinal therapy may be required in the case of systemic infections. Infections caused by medical devices such as catheters are treated by removing the devices.

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.1 Materials Needed for Swab Collection**

Incubator (set at 37°C), Bunsen burner, oven, inoculating loop, distilled water, Magnetic stirrer, weighing balance, Transport swab sticks, Petri dishes, Cary-Blair medium and freshly prepared Mannitol Salt Agar (MSA), MacConkey Agar (MAC), 1% Buffer peptone water (BPW), Nutrient agar (NA), Calcium chloride (CaCl<sub>2</sub>), Normal saline were used for this research.

#### **3.2 Sample Collection and Dispatch**

The samples were collected from both male and female students of mountain top university. The screen and the mouth piece of the phone were sampled by a sterile cotton wool swab. The swab was moistened with a transport medium (Cary Blair Medium) just for rubbing the swab on the surface and back of the mobile phone. The swab was kept in a sterile container containing 5ml of Cary Blair Medium. A total of 15 samples were collected and was transported to the laboratory within 30 minutes for processing.

##### **3.2.1 Enumeration of Bacterial Load**

A total of 15 samples were collected from both male and female students of CHMS using a swab stick. Mannitol bile salt agar was poured into 15 petri dishes and a smear was made on the agar using the sampled swab stick, then a sterilized inoculating loop was used to make a Streak on the agar and was incubated at 37° c for 18-24 hours. After 24hours of incubation period, it was then sub cultured into Nutrient agar (N.A) plates using a sterile inoculating loop and then incubate also for 24 hours. Perform gram staining, biochemical test and microscopy

##### **3.2.2 Media and Reagents**

- Nutrient agar (N.A), Eosin Methylene Blue (E.M.B), Mannitol Bile Salt agar (M.B.S), MacConkey Agar, Buffer peptone water (B.P.W)1%, Calcium Chloride, Cary Blair Medium.

### **3.2.3 Preparation of Culture Media**

### **3.2.4 Preparation of Calcium Chloride**

A weighing balance was used to weigh 1g of CaCl<sub>2</sub>, which was poured into a 150ml conical flask with 100ml of distilled water and properly labeled. The solution was mixed thoroughly, then autoclaved for 15 minutes at 121°C, then removed from the autoclave and allowed to cool.

### **3.2.5 Preparation of Cary Blair Medium**

A magnetic stirrer was put into the conical flask, a weighing balance was then used to weigh 6.3g of Cary-Blair Medium and was added into a conical flask, 495.5ml of distilled water was added to it, the solution was then heated. 4.5ml of CaCl<sub>2</sub> was transferred using a pipette into the medium and was then allowed to mix properly. The solution was then placed into a water bath and was heated from 97°C to 100°C for about 1 hour. 7ml of Cary-Blair Medium was transferred into the transport swab using a pipette. The transport swab containing the medium was then placed into the water bath for 15 minutes. It was then allowed to cool and solidify.

6.3g - 495.5ml

+

4.5ml of CaCl<sub>2</sub>

### **3.2.6 Nutrient Agar**

Nutrient agar was prepared according to the manufacturer's instruction for isolation and detection of total count of mesophilic organism.

### **3.2.7 Preparation**

A weighing balance was used to weigh 28g of powdered NA, which was then placed into a conical flask with 1000ml of distilled water and was thoroughly mixed. The conical flask is then closed in cotton wool that is wrapped in aluminum foil. The mixture was then cooked in a water bath until the powder was completely dissolved and a homogenous mixture formed. It was then autoclaved for 15 minutes at 121°C, the medium was then allowed to cool to a range of 45-50°C and poured aseptically into sterile Petri-dishes and left to set. The medium appears opalescent and is light amber in color.

### **3.2.8 MacConkey Agar**

MacConkey agar is a selective and differential bacteria culture medium that is used to isolate Gram negative and enteric bacteria and differentiate them based on lactose fermentation. Lactose fermenters turn red or pink on McConkey agar, whereas nonfermenters do not.

### **3.2.9 Preparation**

Using a weighing balance, 49.53g of dehydrated MAC was weighed and then thoroughly mixed in a conical flask with 1000ml of distilled water. The conical flask is then wrapped in aluminum foil and wrapped in cotton wool. The mixture was then cooked in a water bath until the powder was completely dissolved and the mixture became homogeneous. After autoclaving for 15 minutes at 121°C, the medium was allowed to cool to a temperature range of 45-50°C before being poured aseptically into sterile Petri-dishes and allowed to set.

### **3.2.10 Mannitol Bile Salt Agar**

Mannitol salt agar test is used to isolate and identify the presence of *Staphylococcus aureus* in a clinical specimen, which makes it both a selective, differential, and indicator medium.

### **3.2.11 Preparation**

Prepare the medium as directed by the manufacturer. It is best to use a ready to use dehydrated powder (the one readily available on most suppliers of culture media). The medium has a concentration of 11.1 grams in every 100 ml of distilled water. Sterilize through autoclaving at a temperature of 121 degree Celsius for 15 minutes and allow the medium to cool down. It was mixed well before putting in a sterile petri dish. Put a label on the medium. It was plated at 2 to 8 degrees Celsius in a plastic bag in order to prevent loss of moisture. The medium can last for a few weeks provided no abnormalities in the medium's appearance. Do not use the medium if there are any signs of abnormalities as they could indicate a possible contamination, alteration, and deterioration. The pH of the medium ranges between 7.3 and 7.7 at a room temperature.

### **3.2.12 Buffered Peptone Water**

Peptone water is a microbiological growth medium made up of sodium chloride and peptic digested animal tissue. The medium is rich in tryptophan and has a pH of 7.20.2 at 25 °C. Peptone water can also be utilized as a primary enrichment medium for bacteria growth because it is a nonselective broth medium.

### **3.2.13 Preparation**

BPW was prepared by dissolving the dehydrated medium in 1000 ml of distilled water to make up 0.1% and 1% peptone water based on manufacturer's instructions in a conical flask and was mixed thoroughly. The conical flask was then sealed with a cotton wool wrapped in aluminum foil. The mixture was then heated so as to completely dissolve, then was sterilized by autoclaving for 15min at 121°C. 9ml of the 0.1% was then dispensed into various test tubes for serial dilution. 22ml of the 1% was dispensed into a conical flask and stored appropriately for later use.

### **3.2.14 Brain Heart Infusion Broth**

Brain heart infusion broth is a general-purpose liquid medium for the culture and maintenance of a wide range of fastidious and non- fastidious microorganisms, including aerobic and anaerobic bacteria, yeast and molds from a variety of clinical and non -clinical specimens.

#### **Preparation**

- The dehydrated medium is dissolved in 1 liter of distilled water based on manufacturer's instructions in a conical flask and was mixed thoroughly. The conical flask is then closed with cotton wool that is wrapped in aluminum foil.
- The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15 mins
- 5ml of the 0.1% was then dispensed into various test tubes.

### **3.3 Biochemical Test**

Biochemical reactions were performed to identify the isolated bacteria and several commercial systems for identifying bacteria are available. These commercial biochemical tests were conducted as follows, (Cheesbrough,2000). The tests include; Gram staining, catalase test, coagulase test, oxidase test, antibiotic susceptibility test.

#### **3.3.1 Gram's Staining**

Reagents: Crystal violet, Iodine, Alcohol (95%), Safranin, 3% Hydrogen peroxide.

#### **3.3.2 Procedures**

A smear of the isolate was made on a clean, dry slide using a sterile normal saline and was allowed to air-dry. The smear was then fixed by a Bunsen flame and was stained by the gram

technique as follows; The smear was covered with crystal violet stain for 60 seconds. After, the stain was rapidly washed off with distilled water, all the water was tipped out and then Lugol's iodine was added for 60 seconds. The iodine was washed off with distilled water Then, the smear was decolorized rapidly just for few seconds with acetone (alcohol) and was washed immediately with distilled water. After the smear was covered with neutral red stain for 2 minutes. The stain was washed off with clean water, the slide was placed in a draining rack for the smear to air-dry. The smear was examined microscopically, first with 40x objective to check the staining to see the distribution of the material. Then with oil immersion objective to look for bacteria cells.

### **3.4 Catalase Test**

This test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci from non-catalase bacteria such as streptococci.

Materials: Glass slide, hydrogen peroxide, past raw pipette, inoculating loop

#### **3.4.1 Procedures**

Regardless of the sample, the glass slide was labeled and placed on a rack. A smear was made on the slide, then a drop of hydrogen peroxide solution was immersed in it and bubbles were observed.

### **3.5 Coagulase Test**

The coagulase test is a reliable method for detecting *Staphylococcus aureus*, as long as a firm clot that does not move when the tube is tipped is considered a positive reaction. The widely accepted interpretation that all degrees of clotting in coagulase plasma indicate the presence of *Staphylococcus aureus*.

#### **3.5.1 Procedures**

A sterile inoculating loop was used to make a thick suspension of bacteria on the slide, and then a loopful of plasma was added to the smear and saline drop and gently mixed together. Within 10-15 seconds, there was immediate coarse clumping of the mixture.



### 3.6 Oxidase Test

The pure culture was smeared on filter paper with a few drops of oxidase reagent, and the results were examined. In less than 10 seconds, oxididase positive cultures produced a purple tint. Oxidase-negative cultures produced no purple color (Olutiola et al., 2000).

### 3.7 Antibiotic Susceptibility Test

Susceptibility testing is performed on all bacteria and fungi that may be relevant to the individual's treatment but whose susceptibility to treatment is unknown. Each pathogen is tested individually to see if antimicrobials can inhibit its growth. This can be measured directly by combining the pathogen and the antibiotic in a growing environment, such as nutrient media in a test tube or agar plate, and observing the antibiotic's effect on bacterial growth. Resistance can also be determined by detecting a gene that is known to cause antibiotic resistance.

Antibiotic susceptibility test was done by preparing a Mueller Hinton agar plate. Using a sterile inoculating loop to pick a colony from the sub cultured plate and was added into a sterile saline, the organisms were diluted to obtain a turbidity equivalent to 0.5 McFarland test standard. Both the diluted organisms and the 0.5 McFarland test standard was placed in a spectrometer to measure the turbidity. After 15 minutes of dilution, a sterile swab was dipped into the inoculum and lifted up gently to reduce excess flow of the suspension because if your swab is to wet your agar surface will not dry correctly and the antimicrobial agents in the disk will diffuse through the wet surface and not into the agar. Then the agar was streaked with the swab in 60degrees to obtain an even inoculum, cover for 3-5minutes and allow to dry. The disk was placed using a sterile swab to press it on the agar plate and was turned upside down letting the disk to face up and was incubated for 16-24hours at 37° c. The following antibiotics were used; oxacillin (3 µg cefoxitin), 20/10µg amoxicillin/clavulanic acid, 10µg gentamycin, 30µg ceftazidime, 30µg vancomycin, 5µg Levofloxacin, 10µg ampicillin, 30µg tetracycline, 1.25/ 23.75µg trimethoprim-sulfamethoxazole and 15µg erythromycin. Zone of inhibitions was determined by measuring the size of clear zones and compared to the CLSI guidelines. The reporting was done by indicating Resistant, Intermediate or Sensitive. *S. aureus* was used as positive control organism. After the incubation the disk was examined to know the zone of inhibition of the antibiotic susceptibility of the organisms.

### **3.8 Molecular Identification**

#### **3.9 Dna Extraction**

DNA extraction is a method used to purify DNA by using physical or chemical method from a sample separating DNA from cell membranes, proteins, and other cellular components.

##### **3.9.1 Activation Of Dna Extraction**

1ml of BHI was dispensed into a 2ml Eppendorf tube and autoclaved, then 100ml of each *Staphylococcus* spp isolate labeled 1-5 was added to the Eppendorf tubes and incubated at 37°C. Each isolate in the Eppendorf tubes was centrifuged at 500g for 3 minutes, the BHI supernatant was dispensed into the waste leaving the pellet, and then distilled water (almost 500ml full) was added to the vortex and centrifuged at 500g for 3 minutes. After discarding the supernatant, 200ml of nuclease-free water/injection water was added and vortexed. The samples have been prepared for DNA extraction.

##### **3.9.2 DNA Extraction by Heating Block**

Heating the block denatured the proteins, extracted DNA spots, inactivated enzymatic inhibitor reactions, and increased the chemical reactions. The Eppendorf tubes containing the samples were first placed in the heating block and then covered to prevent the cap from opening. The heating block was set to 100 degrees Celsius for 15 minutes before being placed on ice for 5 minutes to cool. After that, it was centrifuged at 7000g for 6 minutes, and the DNA was extracted from the pellet into a new tube by carefully transferring 150 $\mu$ l of the supernatant into an already coded fresh Eppendorf tube.

#### **3.10 Polymerase Chain Reaction (PCR)**

Table 3.10 lists the components of the PCR used to identify *S. aureus*. After preparing the PCR cocktail, it was transferred to a PCR tube and placed in the thermocycler. An initial denaturation step of 5 minutes at 95°C was followed by 35 cycles of 95°C for 2 minutes, 42°C for 30 seconds, and 72°C for 4 minutes, followed by a final elongation step of 10 minutes at 72°C. Negative control reactions were also provided. For the negative controls, the template DNA was replaced with sterile water. The PCR products were confirmed using electrophoresis, and they were visualized under UV light using a Gel Documentation system.

**Table 3.0:** PCR reaction components used for 16s rRNA amplification

<b>No.</b>	<b>Component</b>	<b>Initial concentration</b>	<b>Final concentration</b>	<b>Volume/rxn</b>
<b>1</b>	Master mix	5x	1x	2ul
<b>2</b>	16sf	20um	0.25um	0.125ul
<b>3</b>	16sr	20um	0.25um	0.125ul
<b>4</b>	DNA			2ul
<b>5</b>	dH <sub>2</sub> O			5.75ul
<b>6</b>	Total			10ul

### **3.11 Agarose Gel Electrophoresis**

Dry agarose powder was used to make the agarose, which was then dissolved in 50ml of TAE buffer and boiled until a clear solution was obtained. Using a micropipette, 3ul of ethidium bromide was added to the mixture, which was swirled and left to cool but not solidify. The contents of the flask were then transferred into the gel cast with the combs in place, which was then left to solidify before being gently removed and placed in an electrophoresis tank containing TAE buffer. After removing the comb, 4ul of the PCR products were pipetted into each well formed. The tank was connected to the power pack and left to run till it gets to one-third of the gel and then it was turned off and the gel was viewed under the UV transilluminator.

## CHAPTER FOUR

### RESULTS AND DISCUSSION

#### 4.1 Colony Counts

Five (5) morphologically different bacteria were isolated from both male and female in college of humanities and management sciences. Table 4.1 shows the colony count in each of the swab cultures plated for sample 1, 2, 3, 4, 5 after the end of 24 hours incubation period, the colony counts for sample 1 to 5 were taken respectively.

#### 4.2 Morphological Characteristics of the Isolates

The morphological characteristics of the isolates including their shape, size, surface, edge, opacity, texture, and elevation. The color observed are orange, the shape included are circular, the elevation includes umbonate and raised, the size includes small and large, the surface included are smooth and glistening. Table 4.2 shows the morphological characteristics of the isolates.

#### 4.3 Biochemical Characteristics of the Isolates

The biochemical characterization of the isolates includes; Gram's staining, catalase, coagulase test, antibiotic susceptibility test. Both positive and negative reactions were observed. Table 4.3 shows the results of the reactions and different bacterial isolated from the mobile phones.

#### 4.4 Antibiotic Susceptibility Test of the Isolate

Antibiotic susceptibility testing was performed on all the 10 isolates. Shows the proportion of isolates, classified as susceptible, intermediate or resistant to the antibiotics that were tested and these results are presented in **Figure 1**.

#### 4.5 Polymerase Chain Reaction (PCR)

The representative visualized result of gel electrophoresis for the detection of 16 rRNA gene from isolates obtained from game-meat using the PCR technique (Figure 4.4). *Bacillus* (n=10) and *S. aureus* (n=20) isolates were randomly selected from the 362 isolates each for the gene confirmation. The gene was then confirmed in 63.3% and 73.3% of *Bacillus* and *S. aureus* isolates, respectively which indicates

**Table 4.1:** Bacterial Distribution Counts

<b>SAMPLES</b>	<b>GENDER</b>	<b>BACTERIAL COUNTS</b>
<b>1</b>	Male	68
<b>2</b>	Male	86
<b>3</b>	Female	22
<b>4</b>	Male	105
<b>5</b>	Female	81

**Table 4.2** Morphological Characteristics of Isolates

<b>SAMPLES</b>	<b>SHAPE</b>	<b>COLOUR</b>	<b>SIZE</b>	<b>SURFACE</b>	<b>OPACITY</b>	<b>ELEVATI ON</b>	<b>EDGE</b>	<b>TEXTURE</b>
<b>CHMS1</b>	Circular	Orange	Large	Smooth	Translucent	Umbonate	Entire	Viscoid
<b>CHMS2</b>	Circular	Orange	Small	Smooth	Translucent	Raised	Entire	Viscoid
<b>CHMS3</b>	Circular	Orange	Large	Smooth	Translucent	Raised	Entire	Viscoid
<b>CHMS4</b>	Circular	Orange	Large	Glistering	Opaque	Raised	Entire	Viscoid
<b>CHMS5</b>	Circular	Orange	Large	Dull	Opaque	Umbonate	Entire	Viscoid

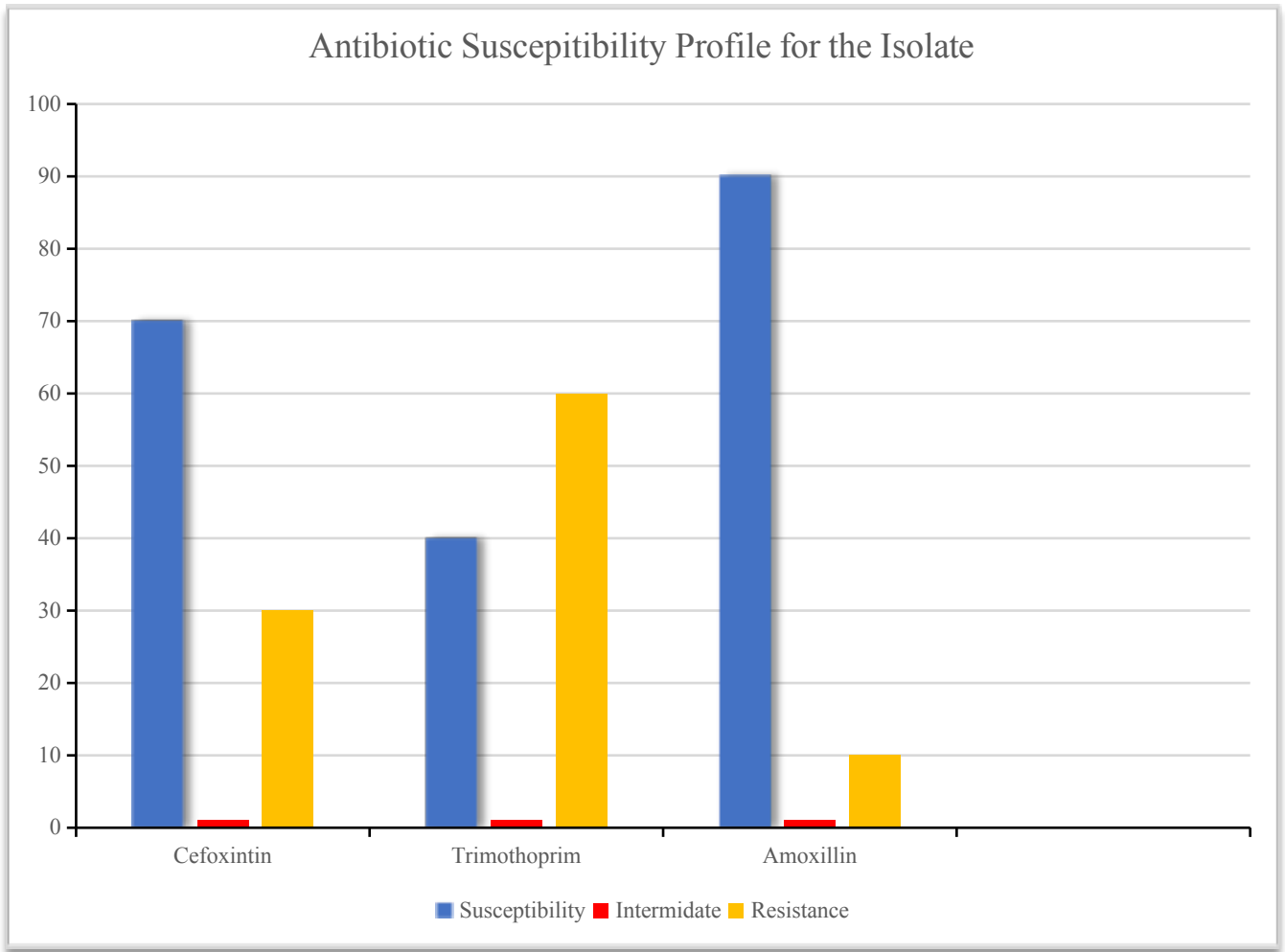
**Table 4.3** Biochemical Characterization of the Isolate

<b>SAMPLES</b>	<b>GRAM STAINING</b>	<b>SHAPE</b>	<b>CATALASE</b>	<b>COAGULASE</b>	<b>BACTERIAL ISOLATES</b>
<b>CHMS1</b>	+	Bacilli	+	+	<i>Bacillus</i>
<b>CHMS2</b>	+	Cocci	+	+	<i>Staphylococcus</i>
<b>CHMS3</b>	-	Cocci	+	+	<i>Staphylococcus</i>
<b>CHMS4</b>	+	Cocci	+	+	<i>Staphylococcus</i>
<b>CHMS5</b>	+	Cocci	+	+	<i>Staphylococcus</i>

**Table 4.4** Antibiotic Susceptibility of Staphylococcus Isolates

<b>ANTIBIOTICS</b>	<b>SUSCEPTIBILITY</b>	<b>RESISTANT</b>
<b>Cefoxitin</b>	7(70%)	3(30%)
<b>Trimethoprim/sulfamethoxazole</b>	4(40%)	6(60%)
<b>Amox/clav</b>	9(90%)	1(10%)
<b>Others</b>	Sensitive	





**Figure 1:** Antibiotics Susceptibility Test for the Isolate

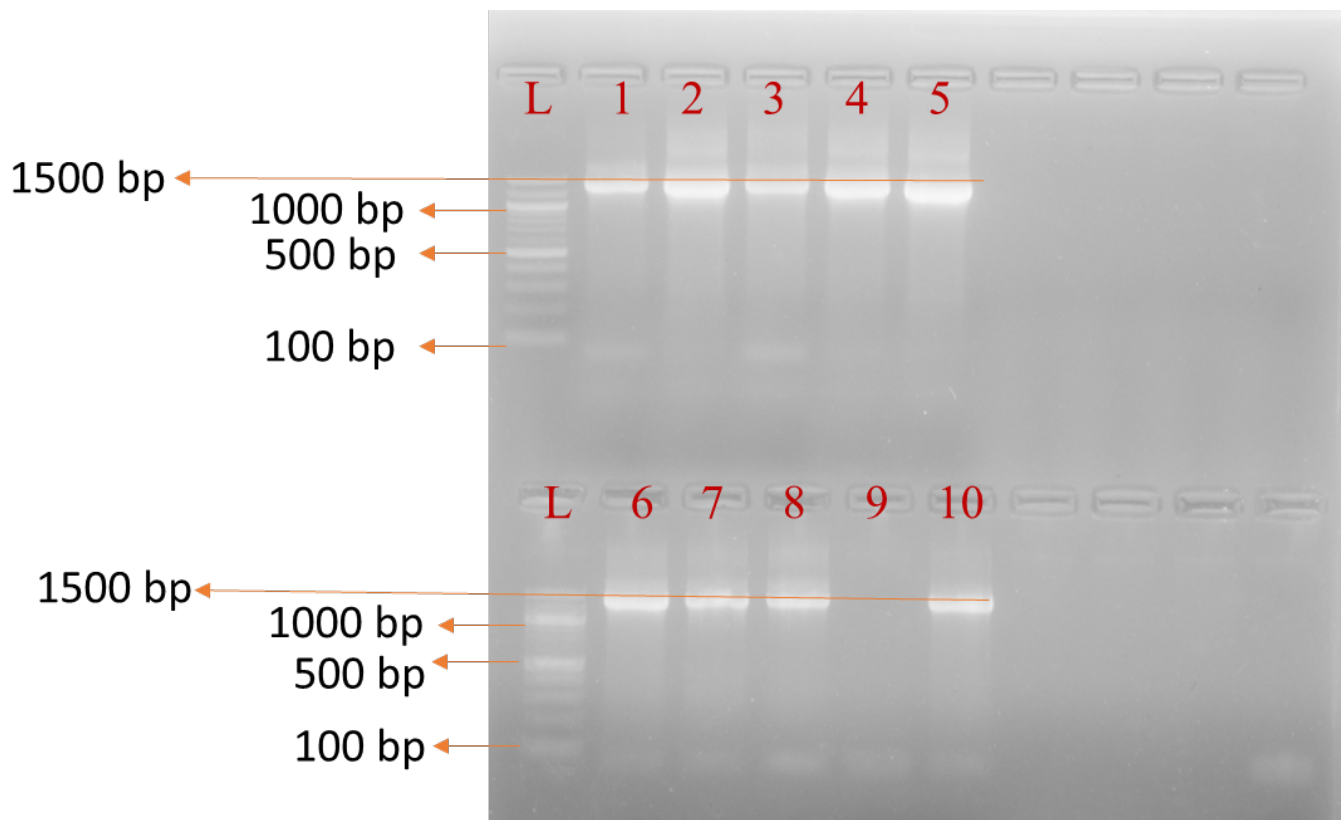


Figure 4.4: Agarose gel electrophoresis image of PCR amplified products of 16 rRNA strains. Lane L: molecular weight marker 100 bp (Bio-Mark). Lane 1-3: *Bacillus* spp. strain isolated from mobile phone samples. Lanes 4-10: *S. aureus* strain isolated from mobile phone samples.

## 4.6 DISCUSSION

A total of 362 isolates were obtained from 5 successful mobile phones out of the 15 samples collected from both male and female at college of humanities and management sciences, Mountain Top University, Ogun State, Nigeria. About 70% of sample showed growth of at least one type of bacteria, out of the swab collected from CHMS students 96% were found positive. Mobile phones and laptops used by male students showed higher positivity rate (70%) than those used by female students (30%), because males are more exposed to bacteria in terms of playing football, lack of personal hygiene. A similar study shows that the incidence of IUG (Islamic University Gaza) male's mobile phones contamination (79%) is much higher than that with the female counterparts (52%), with males also reporting higher count range of bacteria. These results are consistent with findings in an Iraqi study, which showed that the rate of bacterial contamination of personal mobile phones for males was 85%, compared with 80% for females (Auhim, 2013). In another study, A Nigerian study detected higher rate of bacterial contamination of mobile phones (Nwankwo et al., 2014). According to our study *Staphylococcus aureus* and *bacillus* were detected from the mobile phones. In a previous study research conducted in Peru by Loyola *et al* (2016) among mobile phones of health care workers working in intensive care unit reported that *E. coli* (55.9%), *Enterobacter* spp. (18.8%) and *K. pneumoniae* (30.8%) was found to be ESBL (extended spectrum beta lactamase) producers which might be associated with poor hygienic practices of handling mobile phones. The isolation of enteropathogenic bacteria including *Staphylococcus spp*, *bacillus sp*, *pseudomonas sp* from surface of handling mobile phones as demonstrated in this study is an indication of unhygienic practices, poor handling and sharing among multiple users (Yusha'u *et al.*,2010). There were 3 isolates (30%) which were resistant to Cefoxitin and 7 isolates (70%) were sensitive. The highest level of resistance was to trimethoprim/sulfamethoxazole, with 6 isolates (60%) exhibiting complete resistance. Only 4% of the isolates were sensitive to trimethoprim/sulfamethoxazole 9 isolates (90%) were susceptible to amox/clav and 1% were resistant. All the isolates were sensitive to Tetracycline, Erythromycin, Levofloxacin, Gentamycin, Ampicillin and Vancomycin.

## CHAPTER FIVE

### CONCLUSION AND RECOMMENDATION

#### 5.1 Conclusion

Through improper handling like carrying of mobile devices into places like the toilet, kitchen and unkept places, it was observed that this study was able to demonstrate how the ability of mobile phones served as a source of the transmission of variety of pathogens like bacteria e.g., Staphylococcus, Urinary Tract Infection (UTI), fungi, viruses that can invade the body and cause diseases leading to severe serious health implications like malaria, fever, flu, and diarrhea.

#### 5.2 Recommendation

There should be public awareness programs regarding hand hygiene, regular disinfection of mobile phones, discouraging the use of mobile phones in toilets and using antibiotics should be created especially among youths.

Mobile phone users are advised to use screen guards and regularly replace them to reduce microbial contamination.

Also, regular cleaning of mobile phones with wet wipes and frequent washing of hands should be encouraged to reduce any transmission of diseases.

Lastly, mobile phone companies are advised to use antimicrobial surfaces technologies that inhibit or kill bacteria transmitted through direct contact.

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