EVALUATION OF MICROBIAL LOAD ON PUBLIC PHONES AND MOBILE DEVICE'S USED BY UNIVERSITY STUDENTS

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE IN MICROBIOLOGY

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DECLARATION

I hereby declare that this project has been written by me and is a record of my 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 acknowledged using references.

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…….

Date

CERTIFICATION

This is to certify that the content of this project entitled **"Evaluation Of Microbial Load On Public Phones And Mobile Device's Used By University Student's** was prepared and submitted by **UCHENDU, CHIOMA BLESSING** in partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN MICROBIOLOGY.** The original research work was carried out by her under my supervision and is hereby accepted.

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DEDICATION

This project is dedicated to God most high for his protection, love, wisdom, and knowledge, which he provided me with throughout the compilation of my research project. Also, to my loving parents and siblings for their undying love and care during this project.

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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 been reported to be a source of for many microorganisms,With the consent of the students,a total of 15 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. 15 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 opposite genders in the tertiary institution were contaminated with at least one or more bacterial isolates. It was noticed that *Staphylococcus aureus* (90%) were present in four samples and *Bacillus spp.* (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: Bacteria, *Bacillus spp.*, contamination, mobile phones, *Staphylococcus aureus*.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Mobile phone has become an integral part of human lives and their use in several environments is proliferating (Anjumn, 2018). Mobile phones mean different things to different people, but people have come to regard it as a crucial part of their lives because of their applicability in everyday living. In many countries mobile phones outnumber landline telephones. Most adults and many children now own mobile phones. At present, Asia has the fastest growth rate of cellular phone subscribers in the world. The number of cell phones used globally increased from less than one million to six billion from 2000-2012 (). It takes only a conversation with one or more people who recently lost their phones or have had to do without their phones for even two days to see how important the device has become.

The use of this mobile devices and public phones has not only increased in institutions of higher learning, but also other areas such as healthcare and industries. This increase has gained interest in evaluating their potential roles as a reservoir of pathogenic, opportunist bacteria, and also as a source of contamination to our foods and humans in these locations (Brady, 2018). The frequent use of mobile phones in schools such as the one in the current study raises the opportunity for cross-contamination, especially if no hygienic measures and safety practices are put in place by the users (Koscova *et al.,* 2018). Bacteria from sources such as human skin or hand, pets, bags, pockets, environment, and food particles can contaminate the surface of mobile phones. From these sources, the microorganisms can colonize the phone and potentially cause diseases that can range from mild to chronic (AlOmani *et al.,* 2020). When pathogens are present on the surface of a mobile phone, they could be transferred to the user's skin, other surfaces, or foods, where nutrients are available and can make their survival and growth happen (Adejoro *et al.,* 2019). Bacteria isolated from mobile phones are usually normal flora of the source of contamination, but these organisms may serve as mobile reservoirs of infection while facilitating the spread of the bacteria to other locations .

A mobile phone can harbor more microorganisms than a man's laboratory seat the sole of a shoe or the door handle. Among health care workers, It has been reported that medical devices like thermometers, stethoscopes and non- medical devices like computer, keyboards, files, books and mobile phones have an important role in the transmission and spread of microorganisms.it can be envisaged beyond doubt that mobile phones are potential reservoirs of pathogenic microorganisms and could lead to severe infections. Furthermore, the common exchanges and sharing of mobile phones between users may directly aid the spread of potentially pathogenic bacteria in the community (Olsen *et al*., 2020).

Several studies have been conducted to evaluate the bacteria population of student's mobile phones. In a survey by (Momani et al., 2019), high levels of bacteria on university student's mobile phones were reported. *Staphylococcus aureus* was the most common bacteria detected.

1.2 Statement of the Problem

A mobile phone is an electronic device for personal telecommunication. Due to the ease of using mobile phones and its added applications it is widely used by all kinds of people and so we generally overlook the health hazards associated with it. The aim of this research is to explore and assess the microbial load the possible microorganisms that could be found on mobile phones and public phones that has not being widely studied, to justify and validate claims made about its health hazards, and to identify a few of these microorganisms present, to also find a way to minimize how it is being transferred from person to person, thereby reducing the possible health hazards.

1.3 Aim and Objectives of the Study

This study aims to assess the microbial load of public phones and mobile devices used by students of the Mountain Top University (MTU) and also to identify possible microbes regularly associated with mobile devices and also their pathogenicity.

Based on this, the specific objectives of the present study are as follows

- To assess the microbial load of public phones and mobile devices of students.

- To identify pathogenic microbes associated with these devices and how to minimize the possible health hazard.

1.4 Justification Of The Study

Because of information on the destructive impacts of contaminations, the need to keep up with general wellbeing a broad review is done to explain the microbial foreign substances on mobile devices from university students as well as their anti-infection agent helplessness designs. Notwithstanding, it is normal to see many bacteria inside our devices, fomites and environments. Subsequently, this study is led to determine the microbial assessment on mobile devices, its effect on public health and treatment to any infections pertaining to the contaminants.

1.5 Significance Of The Study

This study will provide information of microbes associated with public and mobile devices and their possible health hazards.

1.6 Definition Of Terms

MICROBES: This is an organism of microscopic size, which may exist in its single-celled form or as a colony of cells.

PATHOGENS: Pathogens are Organisms causing disease to its host, with the severity of the disease symptoms referred to as virulence. They are organisms that cause diseases.

CULTURE MEDIUM: this is also known as a growth medium. It is a solid, liquid or semi-solid designed to support the growth of a population of microorganisms or cells via the process of cell proliferation.

CHAPTER TWO

LITERATURE REVIEW

2.1 Pathogens Associated With Mobile Devices

Today mobile phones have become indispensable accessories for professional and social life. Although they are usually stored in bags or pockets, mobile phones are handled frequently and held close to the face (Taher et al., 2019). A mobile phone is a device that can make and receive telephone calls over a radio link whilst moving around a wide geographic area (Akpan, 2017). Mobile smart phones are usually operated by a glass touch screen, with finger tapping. There is a great importance of the human hands for many aspects of personal, industrial, occupational and clinical hygiene (Mamurov et al., 2020).

In 2018 World Health Organization (WHO) described the electromagnetic radiation emitted from mobile phones and base stations as threat to lives as it damages the DNA producing sperm cells (Miller et al., 2019). Mobile phones have also been reported to be a reservoir for microorganisms (Olsen et al., 2022). Microorganisms can be transferred from a person to another or from inanimate objects to hands, and vice versa (Saini et al., 2019). A mobile phone can spread infectious diseases by its frequent contact with hands (Tajouri et al., 2021). There is much evidence that contaminated fomites or surfaces play a key role in the spread of bacterial infections.

The sources of infection can be divided into two main groups: exogenous and endogenous (Wasan et al., 2021). Endogenous infections occur when the infectious agent comes from the patient's own body, usually from his/her own normal flora. Endogenous sources of infections are particularly important when the person's own immunity against his/her normal flora becomes compromised (e.g. the bacterial flora at a surgical site). The exogenous infection, on the other hand, develops from bacteria outside the body, which is the case most of the time. To be more specific, exogenous sources of infections can be human, animal, or environmental in origin. Humans can be a source of infection in three cases: when they are clinically infected (symptomatic infection), when they are asymptomatically infected or when they are carriers. Air, mobiles, toys; hands of surgeons are exogenous source of infections (Wasan et al., 2021) Mobile

phones could be contaminated via a source such as human skin or hand, bag, phone pouch, pockets, environment and food particles. These sources are links through which microorganisms colonize the phone, thus causing diseases that range from mild to chronic (Horváth et al., 2020). The combination of constant handling and the heat generated by the phones create a prime breeding ground for all sorts of microorganisms. The human surface tissue is constantly in contact with environmental microorganisms and becomes readily colonized by certain microbial species (Yusha'u, 2021).

Hand washing is a process which removes soil and transient microorganisms off the hands. Hence, the simple process of hand washing has long been a mainstay of any control measure for reducing nosocomial infections (Lotfinejad et al., 2018). A well-practiced infection control plan that encompasses hand hygiene, environmental decontamination, surveillance and contact isolation is effective for the prevention of such nosocomial infections (Fuller, 2019). Unfortunately, despite the simplicity of hand washing procedure, studies continue to report unacceptably low hand washing compliance rates amongst health workers (Varghese et al., 2021). The constant handling of a mobile phone by a user makes it a breeding place for transmission of microorganisms as well as hospital-associated infections (Yousif et al ., 2020). The range of microorganisms can vary from one person to another and may have different hand flora from ordinary members of the public. The hands, thus, are permanently colonized with pathogenic flora acquired (Ataee et al., 2017). Some of this microorganisms can include *Staphylococcus aureus, Escherichia coli, Bacillus, Aspergillus spp and Rhizopus spp, klebsiella spp, pseudomonas spp.*

2.2 *Staphylococcus Aureus*

Staphylococcus aureus is a Gram-positive round shaped bacterium, a member of the *Bacillus* spp, and is a usual member of the micro biota of the body, frequently found in the upper respiratory tract and the skin. The natural habitat of *S. aureus* in humans is the skin and nasopharynx(Raineri et al., 2022). It can cause a wide variety of infections involving skin and soft tissues, endovascular sites and internal organs. *S. aureus* continues to be an important pathogen in the community and in hospitals, causing high morbidity and mortality (Gnanamani

et al, 2017). The organism can be disseminated from a superficial site via the bloodstream to internal organs where it can set up a metastatic focus of infection.

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. *S. aureus* also causes the economically important ruminant mastitis. Disease caused by *S. aureus* is in general due to two types of virulence determinants, cell surface associated proteins and extracellular protein toxins(Cheung et al., 2021). *S. 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 serve as adhesions to promote bacterial attachment and colonization. *S. aureus* can express several factors that combat host defenses. *S. 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 (MSA) containing 7.5% sodium chloride which allows halo tolerant staphylococci to grow. Otherwise bacteria can be streaked on Trytipcase Soy Agar (TSA), possibly incorporating erythrocytes.*Staphylococcus aureus* is classified into Domain: Bacteria,Phylum:Bacillota,Class:Bacilli,Order:Bacillales,Family:StaphylococcaceaeGenus:Staphylococcus Species:S.aureus.

2.2.1 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) (Alsaimary *et al.,* 2017). 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 (Fernandes Queiroga Moraes *et al*., 2021). 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).

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 (Ahmadi *et al.,* 2019). 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 (Rapisarda *et al*., 2019). *S. aureus* can be passed from person to person via direct touch or via fomites (Xiao *et al.,* 2019).

2.2.2 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 (Ondusko and Nolt, 2018).

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 (Raafat et al., 2019). Infectious endocarditis, sepsis, and toxic shock syndrome

all have Staphylococcal superantigens (TSST-1 or toxic shock syndrome toxin 1) as key virulence factors (Schlievert *et al.,* 2020).

2.2.3 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 (Oliveira et al., 2018). When antimicrobial therapy is required, the length and style of treatment are largely determined by the type of infection as well as other factors (Spauldiing *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 (Boswihi *et al.,* 2018). 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 value 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).

2.2.4 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 (Alexander *et al.,* 2018). 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 (Davies and Wales., 2019). 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 (Nasaj et al., 2020; 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) (Shariati *et al.,* 2020).

2.3 *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 (Hsiao *et al*., 2021). 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 μm long and 0.25–1.0 μm in diameter, with a cell volume of 0.6–0.7 μm^3 (Lopez *et al*., 2018). 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(Patridge et al., 2019), it also attaches and effaces to the microvilli of the intestines via an adhesion

2.3.1 Characteristics of *Escherichia coli*

E. *coli* is characterized by having Gram negative non -sporulating bacilli,indole production from tryptophan,not using citrate as a carbon source,and not producing acetone. In addition it ferments glucose and lactose with gas fermentation. Like all Gram-bacteria,the envelope of *E.coli* consist of three elements:the cytoplasmic membrane,the outer membrane,and between noth a periplasmic space constituted by peptidoglycan.This last structure gives the bacterium its shape and rigidity and allows it to withstand relatively high environmental osmotic pressure.

E.coli is a mesophilic bacterium its optimal development is in the environment of the body temperature 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.

2.3.2 Epidemology of *Escherichia coli*

Escherichia coli are gram-negative bacteria within the family Enterobacteriaceae that can harmlessly colonize the human gut or cause intestinal or extraintestinal infections, including severe invasive disease such as bacteremia and sepsis(Bonten et al., 2021). *E. coli* is the most common cause of bacteremia in high-income countries, exceeding other leading bacteremia-causing pathogens such as *Staphylococcus aureus* and *Streptococcus pneumoniae*, and is a leading cause of meningitis in neonates. Emerging multidrug E. coli strains are more challenging to treat and confer a higher risk of bacteremia and death (Holmes et al., 2021). Vaccines that can prevent invasive E. coli infections such as bacteremia are currently unavailable but are in clinical development . To inform the development and implementation of effective prevention strategies, a better understanding is needed of the current epidemiology of invasive *E. coli* infections.

2.3.3 Pathogenesis of *Escherichia.coli*

E.coli forms a part of normal intestinal flora of man and animal, great variety of strains of E. coli which include purely commensal strains as well as strains possessing virulence determinants that causes wide spectrum of diseases in all age groups of human beings and animals(Sarowska et al., 2019). The virulent strains of E.*coli* act as specific pathogens of the gut (enteritis) and of extra-intestinal sites.

2.3.4 Prevention and Treatment of *Escherichia coli* isolates

Intestinal disease can best be prevented by care in selection, preparation, and consumption of food and water. Maintenance of fluid and electrolyte balance is of primary importance in treatment (Peek et al., 2018). Antibiotics may shorten the duration of symptoms, but resistance is nevertheless widespread. Extra intestinal diseases require antibiotic treatment. Antibiotic sensitivity testing of isolates is necessary to determine the appropriate choice of drugs.

2.3.5 Antimicrobial Resistance of *Escherichia coli*

Multidrug resistance in *Escherichia coli* has become a worrying issue that is increasingly observed in human but also in veterinary medicine worldwide (Poirel et al., 2018). *E. coli* is intrinsically susceptible to almost all clinically relevant antimicrobial agents, but this bacterial

species has a great capacity to accumulate resistance genes, mostly through horizontal gene transfer.

2.4 *Pseudomonas Aeruginosa*

P. aeruginosa is a common organism in the soil and in water and it can also be found on plants and animals (Abd El-Ghany, 2021). It is an aerobic, saccharolytic, non-spore forming gram negative bacillus measuring 0.5 to 0.8μm by 1.5 to 3.0μm.5. Most strains of *P. aeruginosa* possess a single polar flagellum that is used for motility. *P. aeruginosa* commonly produces two soluble pigments: pyocyanin, which gives colonies a blue color and pyoverdine also known as the fluorescent pigment, which is a yellow green or yellow-brown pigment (Suer, 2021). When a strain of *P. aeruginosa* produces both pyoverdine and pyocyanin, the resulting colonies have a blue-green color. This organism may also produce other water- soluble pigments such as pyorubrin or pyomelanin which give colonies a red or brown color, respectively. On sheep blood agar plates, colonies of *P. aeruginosa* often display beta-hemolysis and a greenish metallic sheen due to their pigment production. No other species of gram-negative non-fermenters produce pyocyanin, making its presence helpful in identifying the organism (Umesh, 2020). One of the most recognizable signs of an unknown colony being *P. aeruginosa* is the characteristic fruity grape-like odor from the production of 2-amino acetophenone by the organism. Infections caused by *P. aeruginosa* have always been difficult to treat, but like other bacteria *P. aeruginosa* is becoming increasingly resistant to antimicrobial agents (Pachori et al., 2019).

2.4.1 Characteristics of *P.aeruginosa*

The growth of *P. aeruginosa* in solid agar media can occur between the temperature of 4°C and 44°C; however, the growth at the higher temperature is more prominent. It has a simple nutritional requirement and can grow in media containing acetate as a source of carbon and ammonium sulfate as the source of nitrogen (Gao et al., 2020). The colonies produced by *P. aeruginosa* are usually of two types; large and smooth colonies with flat edges and elevated centers resulting in fried-egg appearance, small, rough and convex type. The organisms isolated from clinical materials form large types of colonies, whereas those from natural sources form a small types of colonies (Pestrak et al., 2018). The large colonies might have silver-grey metallic shining patches at the edges. A third mucoid type of colony is also observed in isolates from respiratory and urinary tract

infections.*P. aeruginosa* colonies on agar media have a tendency to form localized swarming from the edge of the colony (Demirdjian, 2019). Besides, the colonies produce green and fluorescing pigments.Another important feature of these colonies is a fruity odor and the occurrence of metallic patches.*Pseudomonas aeruginosa* is an opportunistic pathogen that is associated with various mild and severe nosocomial infections in immunocompromised people. The entry, colonization, and infection by *P. aeruginosa* depend on a number of factors; termed virulence factors that assist the survival of the organisms and evasion of host defense (Sultan et al., 2021). Such virulence factors in *P. aeruginosa* are classified into different groups based on their involvement during infection. The three major types of virulence factors in *P. aeruginosa* include; factors involved in attachment and motility, factors involved in colonization, and factors involved in chronic infection (Liao et al., 2022).

2.4.2 Pathogenesis of *P.aeruginosa*

The pathogenesis of infections caused by *P. aeruginosa* is mediated by different virulence factors that facilitate the entry colonization and invasion of host tissue. *P. aeruginosa* is unique in its ability to cause severe invasive infections while evading the immune system defenses, resulting in persistent infections (Rossi et al., 2021). During the course of infection, subsequent tissue damage, invasion, and dissemination of *P. aeruginosa* are attributed to such virulence factors (Bobrov et al., 2022). The pathogenesis of infections caused by *P. aeruginosa* follows a particular path, ultimately leading to persistent invasive disease.

2.4.3 Bacterial Attachment and Colonization

The first step in the pathogenesis of disease caused by *P. aeruginosa* is the entry of the bacteria into the host. The route of entry might be different in different individuals, but in the case of nosocomial or acquired infections, the entry usually occurs through punctured skin and tissues (Nayek, 2019). The initial colonization of host tissue is brought about by factors like the flagellum and pili. The flagellum supports the movement of bacteria through the host body to reach the target sites (Qin et al., 2022). Once in the target sites, structures like the Type IV pili bind to the glycosphingolipid present on the host epithelial cells.

2.4.4 Invasion

The colonization of host tissue by *P. aeruginosa* is followed by the release of the Type III secretion system which delivers proteins directly from the cytoplasm of *P. aeruginosa* into the cytoplasm of

the host cells (Hardy et al., 2021). The system delivers cytotoxins among which, Exo S and Exo T are the most lethal ones. These are bifunctional cytotoxins that possess both Rho GTPase-activating protein and ADP ribosyltransferase activities. The toxins then inhibit phagocytosis by disrupting actin cytoskeleton rearrangement, focal adhesions, and signal transductions. Exo U is another toxin present in the secretion system that contributes directly to acute cytotoxicity towards epithelial cells and macrophages (Hardy et al. 2022).

Similarly, other toxins induce an adenylate cyclase activity that affects intracellular cAMP levels and cytoskeleton reorganization.

2.4.5 Biofilm formation

Resistance against antimicrobial agents is essential pathogenesis of infections caused by *P. aeruginosa*. A different component like the exopolysaccharide, rhamnolipid, pyoverdine, and proteinaceous surface appendages are involved in the process of biofilm formation(Thi et al., 2020). During biofilm formation, cell differentiation occurs, and oxygen and water-filled channels are formed to provide nutrition to the deep-rooted cells of the mature biofilm. Studies have shown that cells growing in biofilms have up to 1000 fold more resistance to antibodies than the free-swimming cells(Tuon et al., 2022).

2.4.6 Treatment of *P.aeruginosa*

The method of treatment employed by the clinicians depends on the severity of the infection. In the case of mild infections, courses of IV antibiotics are adequate enough for treatment; however, under deeper infections, surgical debridement might also be required. In patients with respiratory failure, pneumonia, sepsis, or other systemic infections, ICU admissions might be necessary. In addition to the broad-spectrum antibiotics, double pseudomonal coverage might also be needed.

Common antibiotics that are used as first-line therapy include carbapenems, cephalosporins, aminoglycosides, and fluoroquinolones.

2.4.7 Prevention of *P.aeruginosa*

Since most of the infections caused by *P. aeruginosa* are nosocomial infections, specific precautions can be taken in the hospital environment to prevent infections (Sikora and Zahra, 2022). The health personnel are advised to follow strict infection control precautions, strict adherence to hand hygiene and regular use of gowns and gloves are advised.

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), Eiosin Methylene Blue (E.M.B), MacConkey Agar (MAC), 1%Buffer peptone water (BPW), Nutrient agar (NA), Calcium chloride (CaCl2), Normal saline were used for this research.

3.1.1 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.

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 aluminium foil. The mixture was then heated so as to completely dissolve, then was sterilized by autoclaving for 15min at 121^{o}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.

3.1.2 Preparation of Calcium Chloride (CaCl2)

A weighing balance was used to weigh 1g of CaCl2, 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^{o}C, then removed from the autoclave and allowed to cool.

3.1.3 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 CaCl2 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^{o}C to 100^{o}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 cacl2

3.1.4 Collection of Samples

The samples were collected from the tabs of students in College of Basic and Applied Sciences(CBAS) in Mountain Top University, the screen and the mouth pieces 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. Each swabs were stored separately, the swabs were kept in a sterile container containing 5ml of Cary Blair Medium.Samples were collected and was transported to the laboratory within 30 minutes for processing.

3.1.5 Preparation of Swab Samples Collected

5ml of 1% Buffered Peptone Water (BPW) was transferred using pipette into empty test tubes, the samples were then placed in the test tubes and was incubated for 1 hour. After incubation 1ml of each sample was transferred using a pipette into test tubes for serial dilution in this order 10^{-1}, 10^{-2},10^{-3},10^{-4},10^{-5} . The samples were then cultured into various agars. Labeled MAC-10^{-2},10^{-4},10^{-5}, MBS-10^{-2},10^{-3}, NA-10^{-2},$10^{-4}10^{-5}$ and incubated for 24 hours. After which they were then

subcultured and streaked using NA and incubated for another 24 hours, before gram staining, biochemical test and microscopy were carried out.

3.2 Preparation of Culture Media

3.2.1 Nutrient Agar

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

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.

3.2.2 Macconkey Agar (Mac)

MacConkey agar is a selective and differential culture medium for bacteria, it is designed to selectively isolate Gram negative and enteric bacteria and differentiate them based on lactose fermentation. Lactose fermenters turn red or pink on MacConkey agar, and non fermenters do not change color.

Using a weighing balance 49.53g of dehydrated MAC was weighed and then placed into a conical flask with 1000ml 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.

3.2.3 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.

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,allow the medium to cool down,mix well before putting in a sterile petri dish. put a label on the medium,Place the plate at 2 to 8 degree 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.

3.2.4 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 aluminium foil.The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121 degree celsius for 15 mins 5ml of the 0.1% was then dispensed into various test tubes.

3.3 Biochemical Test

Catalase test, Simmon's Citrate test, Oxidase test, Dnase test

3.3.1 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, pastraw pipette, inoculating loop

Procedures for Catalase Test

Label the slides,Prepare a smear on the slide,Pipette the $H2O_2$(hydrogen peroxide) and place on the slide,and Observe for immediate bubbling.

3.3.2 Coagulase Test

Coagulase test is a valid means of identifying Staphylococcus aureus, provided that only a firm clot that does not move when the tube is tipped is considered a positive reaction. The widely promulgated interpretation that all degrees of clotting in coagulase plasma are positive identification of Staphylococcus aureus.

Procedures

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

3.4 Gram Staining

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

Procedures

Flood air dried, heat fixed smears of cells for 1 minute with crystal violet staining,Wash slide in a gentle and indirect stream of tap water for 2 seconds ,flood slide with the mordant; Gram's iodine wait for 1 minute, wash slide in a gentle and indirect stream of tap water for 2 seconds ,flood slide with decolorizing agent wait for 10-15 seconds or add a drop on slide until

decolorizing agent running from the slide ,flood slide again with a counter stain, safranin,wash slide in a gentle and indirect stream of tap water until no color appears in the effluent and then blot dry with absorbent paper.

3.5 Microscopy

Slides stained were observed under oil immersion (100x) using a bright field microscope.

3.6 Antibiotics Susceptibility Testing

Susceptibility testing is performed on each type of bacteria or fungi that may be relevant to the individual's treatment and whose susceptibility to treatment may not be known. Each pathogen is tested individually to determine the ability of antimicrobials to inhibit its growth. This can be measured directly by collecting the pathogen and the antibiotic together in a growing environment, such as nutrient media in a test tube or agar plate, to observe the effect of the antibiotic on the growth of the bacteria. Resistance can also be determined by detection of a gene that is known to cause resistance to specific antibiotics.

Antibiotic susceptibility test was done by preparationof 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 37ºc.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.

3.7 Molecular Characterization

3.7.1 DNA Exraction By Heating Block

DNA extraction was done by heating block to denature the proteins, extract DNA spots, inactivate enzymatical inhibitors reactions and increase the chemical reactions. Firstly, The Eppendorf tubes containing the samples were placed in the heating block, then was covered to prevent the cap from opening. The heating block was programed at 100c for 15mins, after the block was placed on ice to cool for 5mins. Afterwards it was then centrifuge at 7000g for 6mins and the DNA was extracted from the pellet into a new tube by taking 150µl of the supernatant and was carefully transferred into an already coded fresh Eppendorf tube.

3.7.2 Activation Of DNA Extraction

1ml of BHI was dispensed into 2ml of Eppendorf tube and autoclave, then 100ml of each isolate of *Staphylococcus spp* labelled accordingly from 1-5 were added to the Eppendorf tubes and incubated @37ºc. Each of the isolates in the Eppendorf tubes were centrifugated at 500g for 3mins, BHI supernatant was dispensed into waste leaving the pellet,then add distilled water (i.e. almost 500ml full) the vortex and centrifuge at 50g for 3mins. After, the supernatant was discarded and 200ml of nuclease free water/ injection water was added then vortexed. The samples are ready for DNA extraction.

3.7.3 Polymerase Chain Reaction and Gel Electrophoresis

The agarose was prepared using dry agarose powder, 1g of the agarose powder was then dissolved in 50ml of TAE buffer the mixture was then boiled until a clear solution was gotten 3ul of ethidium bromide was added to the mixture using a micropipette it was swirled and left to cool but not solidify, the content of the flask was then transferred into the gel cast with the

combs in place, after, it was left to solidify and the gel was gently removed and placed in an electrophoresis tank containing TAE buffer. 4ul of the PCR products were then pipetted into each well that was formed after removing the comb. 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.

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

No.	**Component**	**Initial concentration**	**Final concentration**	**Volume/rxn**
1	Master mix	5x	1x	2ul
2	16sf	20um	0.25um	0.125ul
3	16sr	20um	0.25um	0.125ul
4	DNA			2ul
5	dH_2O			5.75ul
6	Total			10ul

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Colony Counts

A total 216 isolates were obtained from mobile devices of students in Mountain Top University. About 50% of samples showed growth of at least one type of bacteria, out of the swab collected from CBAS students 95% were found positive. Mobile phones and laptops used by male students showed higher positivity rate (80%) than those used by female students (20%). The results of bacterial counts in (Table 4.1) showed there is a difference between both genders.

Table 4.1: Bacterial counts distribution

SAMPLES	GENDER	BACTERIAL COUNTS
S1	Female	26
S2	Female	29
S3	Male	53
S4	Male	95
S5	Female	13

4.1.1 Cultural Characteristics of the Isolates

The cultural characteristics of the isolates including their shape, size, surface, edge, opacity, texture, and elevation. The colors observed are orange and milky, the shape included are irregular and circular, the elevation includes raised, the size includes small and large, the surface included are smooth.

Table 4.2: shows the morphological characteristics of the isolates

SAMPLES	SHAPE	COLOR	SIZE	SURFACE	OPACITY	ELEVATION	EDGE	TEXTURE
S1	Irregular	Milky	Small	Smooth	Transparent	Raised	Entire	Viscoid
S2	Circular	Orange	Large	Smooth	Opaque	Raised	Entire	Viscoid
S3	Circular	Orange	Large	Smooth	Opaque	Raised	Entire	Viscoid
S4	Circular	Milky	Small	Smooth	Opaque	Raised	Entire	Viscoid
S5	Circular	Orange	Large	Smooth	Opaque	Raised	Entire	Viscoid

4.1.2 Morphological Characteristics of The Isolates

The morphological characterization of the isolates includes; Gram's staining, catalase, coagulase test. Both positive and negative reactions were observed. Table 4.3 shows the results of the reactions.

Table 4.3: Morphological characterization of the isolates

SAMPLES	GRAM STAIN	SHAPES	CATALASE	COAGULASE	BACTERIAL ISOLATES
S1	Positive(+)	Cocci	Positive (+)	Positive(+)	*Staphylococcus*
S2	Positive(+)	Cocci	Positive (+)	Positive(+)	*Staphylococcus*
S3	Positive(+)	Cocci	Positive (+)	Positive (+)	*Staphylococcus*
S4	Positive(+)	Bacilli	Positive (+)	Positive (+)	*Bacillus*
S5	Positive(+)	Cocci	Positive (+)	Positive (+)	*Staphylococcus*

4.1.3 Antibiotics Susceptibility Testing

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 4.1**. 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. Table 4.4 shows the susceptibility, intermediate or resistance of the isolates.

Table 4.4 Antibiotic susceptibility of staphylococcus isolates

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

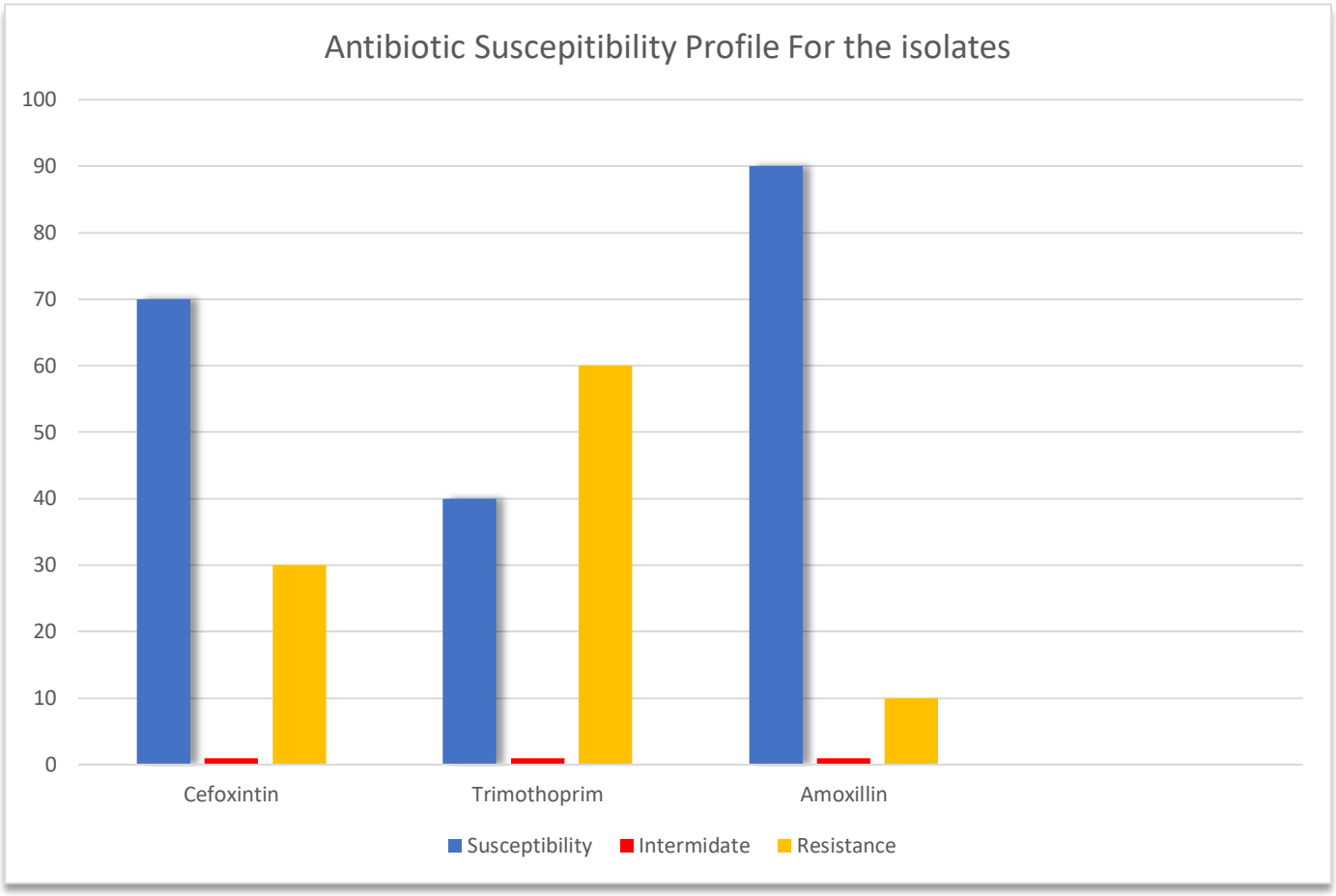

Figure 4.1: Antibiotics Susceptibility Testing for the isolates

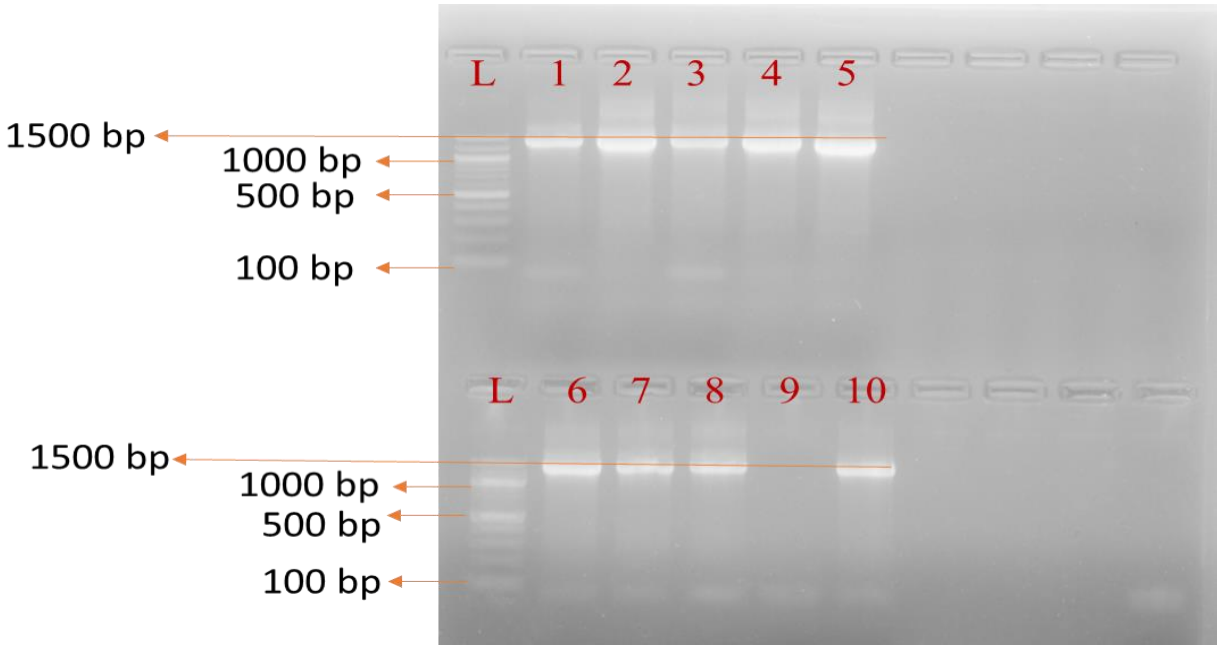

Plate 4.2: Illustrative agarose gel electrophoresis image of PCR amplified products of 16 rRNA strains. Lane L: molecular weight marker 100 bp (Bio-Mark). Lane 1-3: Bacillus spp. strain isolated from mobile phone samples. Lanes 4-10: S. aureus strain isolated from mobile phone samples.

DISCUSSION

4.2 Discussion

The use of public phones and mobile devices has become widely common among people around the world. Most of them are unaware or always forget cleaning their devices which can harbor different types of microbes. Isolation of bacterial agents from electronic devices such as mobile phones and personal digital assistants has shown these devices to be possible modes of transmission of nosocomial pathogens (Bures et al., 2018). In the study, only 95% of the total samples were positive for bacterial growth. It is observed that mobile devices and public phones used by students in Mountain Top University has to be properly kept clean especially those belonging to the male gender, proper hygiene should be observed always, later on mobile phones should be produced with protective material to prevent them from bacterial contamination. Furthermore it was seen that most of the samples were contaminated with *staphylococcus aureus,* this organism is well known to be very harmful especially when it comes in contact with the skin and other aeras of the body.The results of bacterial counts in (Table 4.1) showed the differences between both genders and also shows the rate of contaminants which occurs more in male students than that of female students 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 (table 4.2). 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 sp, 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. Table 4.4 shows the susceptibility, intermediate or resistance of the isolates.

CONCLUSION AND RECCOMENDATIONS

5.1 CONCLUSION

In this study, it is concluded that the mobile phone devices is a means of transmitting diseases because it represent a repository for many microorganisms, and this device is a necessary means for communication, it's advised that the device must be cleaned once a day especially if someone else uses it, Furthermore is advisable to follow hygiene habits to prevent germs such as handwashing, and not taking phones to contaminated places like bathrooms.

5.2 RECCOMENDATIONS

- Education campaigns of hygienic practices is highly recommended for the university students to increase their awareness about bacterial contamination of mobile phones and devices.
- Routine cleaning of mobile phones and their devices may be effective in reducing bacterial contamination.
- In the future, mobile phones could be produced with protective material against bacterial contamination.

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