CHARACTERIZATION OF *staphylococcus aureus* ISOLATED FROM DOOR HANDLES IN THE COLLEGE OF HUMANITIES, MANAGEMENT AND SOCIAL SCIENCES, MOUNTAIN TOP UNIVERSITY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES, MOUNTAIN TOP UNIVERSITY, MAKOGI, IBAFO, OGUN

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DECLARATION

I hereby declare that this project report written under the supervision of Dr. ENIOLA DOLAPO OLALEYE is a product of my research work. Information derived from various sources has been duly acknowledged in the text and a list of references provided. This research project report has not been previously presented anywhere for the award of any degree or certificate.

_____

UMOH HARVEY PAUL   Date

CERTIFICATION

This is to certify that this research project titled "**CHARACTERIZATION OF** *staphylococcus aureus* **ISOLATED FROM DOOR HANDLEIN THE COLLEGE OF HUMANITIES, MANAGEMENT AND SOCIAL SCIENCES, MOUNTAIN TOP UNIVERSITY**" was carried out by **UMOH, HARVEY PAUL**, with matriculation number **18010101042**. 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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Dr. ENIOLA DOLAPO OLALEYE   DATE

    (PROJECT SUPERVISOR)

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(Ag. 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. Paul Umoh for their constant prayers, advise and unending support.

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ABSTRACT

Staphylococcus aureus is a gram-positive organism that causes diseases and infections in humans, in order to ascertain the prevalence of *Staphylococcus aureus* on door handles and the organism sensitivity to commonly used antibiotics in the College of Humanities Management and Social Sciences, Mountain Top University, A study was conducted. A total of 30 swab samples were collected from door handles, 10 each from restrooms, office doors, and lecture halls of the College of Humanities Management and Social Sciences at Mountain Top University. Using a combination of morphological characteristics on agar and biochemical tests, the samples were cultivated and *S. aureus* identified. *Staphylococcus aureus* was isolated from a total of 16 (53%) of the 30 samples collected. Results from the susceptibility testing showed that Tetracycline was the most effective against the *S. aureus* isolates (14 isolates were seen to be susceptible). Findings from this study demonstrate that *Staphylococcus aureus*, which is capable of infecting people, is present on the door handles. Serious health issues may result from these microbes. Therefore, maintaining excellent personal hygiene practices like washing your hands frequently will help to lower the risk of microbial transmission.

KEYWORDS: door handles, antibiotic resistance, *staphylococcus aureu*s.

CHAPTER ONE
INTRODUCTION

1.1   Background of study

A problem for world health has been the spread of infectious diseases by hand contact. Gram positive and negative bacteria are notably known to infect surfaces that come into touch with people, such as door handles, tables, chairs, windows, etc (Itah *et al.,* 2004). Since most countries lack statistics, it is difficult to estimate how much infectious diseases contribute to morbidity and mortality, and they continue to be a major cause of death globally (Barbosa *et al.,* 2011). Common cold and sores, conjunctivitis, giardiasis, diarrhea, impetigo, meningitis, pneumonia, and other illnesses can be contracted through contact with environmental surfaces. Numerous bacterial species are responsible for these disorders. in 2012 (Samant *et al.*).The primary source of microbe transmission to environmental surfaces has been identified as human hands. Hands frequently serve as vectors that spread disease-causing organisms including bacteria and viruses from person to person through direct contact or indirectly through surfaces, according to (Curtis *et al.,* 2003) and (Fewtrell *et al.,* 2007). Some of these dangerous microorganisms prevalent in the environment can be transmitted to human hands through poor personal hygiene (Mensah *et al.,* 2002).

According to studies, environmental surfaces that are frequently handled by humans have a higher bacterial load than toilet seats and bathroom floors. This consequence might be caused by the cumulative contamination of door knobs brought on by unsanitary environments (Augustino *et al.,* 2014). The traditional practice of hand washing, which was the first line of defense in halting the transmission of disease, has been ignored and has to be enthusiastically adopted by families, schools, and healthcare workers. But many people appear to simply run water over their hands without soap, and some people don't even wash their hands after using the restroom (Barker *et al.,* 2000) *Staphylococcus aureus* is a significant human and animal pathogen that causes both community-acquired illnesses and infections associated

with health care. infection (Shen *et al.,* 2013). (Shen *et al.,* 2013). Because of its pathogenicity, it is a pathogen of considerable concern (Giannini *et al.,* 2009). Its capacity to produce a wide variety of fatal diseases and its capacity to adjust to various environmental conditions (Reynolds *et al.,* 2005).

The most often isolated pathogen for pneumonia, urinary tract infections, skin and soft tissue infections, and infections of the bones and joints has been discovered to be *Staphylococcus aureus* (Klein *et al.,* 2013). One of the most frequently cited potential sources of these *S. aureus* infections is door handles (Nworie, *et al.,* 2012).

Over the past 100 years, *Staphylococcus aureus* has been identified as a significant contributor to human disease. Numerous infections are known to be caused by it. These diseases range in severity from mild skin infections to debilitating septicemia and endocarditis (Howden *et al.,* 2010). *S. aureus* strains had a death rate of more than 75% prior to the development of antibiotics (Van *et al.,* 2012). Penicillin first treated *S. aureus* infections successfully, but then the drug's resistance started to spread (Atkinson and Lorian, 1984). Penicillin resistance now affects more than 90% of staphylococcus strains, with methicillin, aminoglycosides, macrolides, and lincosamide following closely behind (Chambers, 2001). (Levin *et al.,* 2005). Vancomycin, a glycopeptide antibiotic, was first made available in 1958. Therefore, vancomycin has been the most effective treatment for severe staphylococcal infections, which are becoming more prevalent internationally (Hiramatsu *et al.,* 1997). Vancomycin resistance in *S. aureus* was not thought to be a likely issue for many years. As a result, initial reports of vancomycin resistance in clinical *S. aureus* isolates from Japan in 1997 led to serious worry (Hiramatsu *et al.,* 1997). VRSA strains have been isolated from other nations outside of Japan, demonstrating that the issue is a worldwide one (Trakulsomboon *et al.,* 2001). There are two types of vancomycin resistance in staphylococci. Van genes cause the first sort of resistance (Perichon and Courvalin, 2009). The second kind results in thicker and more disordered cell walls due to enhanced autolytic activity and cell wall synthesis (Hanaki *et al.,* 1998).

A significant source of the spread of infectious diseases is posed by nematodes that are in regular contact with people or the environments of pathogenic organisms (Osterholm *et al.,* 1995). The fomites include the door handles for the amenities, showers, toilets, and hand lockers, particularly those found in public buildings, hospitals, hotels, and restaurants (Bright *et al.,* 2010). In addition to regular human contact, which is one

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method of disease transmission, the main factor in the development of community-acquired illnesses is termites (Presscott *et al.,* 1993).

Bacteria and fungus pollute our bodies, homes, places of employment, and the entire environment. Microorganisms are present everywhere. Fortunately, only 1500 of the many billions of bacteria that exist can be harmful to our health and cause various illnesses like pneumonia or skin infections (Eltablawy and Elhinfnawi, 2009). Every ecosystem is largely made up of microorganisms. They exist either freely or as parasites in various habitats (Sleigh and Timbury, 1998). Microorganisms can spread from person to person and from place to place through the hands. The presence of pathogenic germs can cause chronic or acute sickness even though it is practically impossible for the hand to be germ-free (Oranusi *et al.,* 2013).

The regular flora of the human body as well as transient bacteria that are touched from the environment are both present on human hands (Lindberg *et al.,* 2004). Students frequently have access to service offices in a university setting for a variety of objectives. Given that the door handles are not regularly cleaned and sanitized, there is a high potential for the spread of pathogenic bacteria. Despite the fact that it is widely acknowledged that the risk of infection among the general public is lower than that of hospital patients (Scott *et al.,* 1982). A significant public health worry is the rise in the frequency of epidemic breakouts of some diseases and the speed at which they are spreading from one community to another (Nworie *et al.,* 2012).

1.2   Statement of problem

Antibiotic resistance has become a major threat to human health around the world. Due to the ease of spread of antibacterial resistance among bacteria as well as the widespread use of door handles, which are fomites that facilitate the spread of bacteria, bacteria spread in the environment. Unfortunately, a good number of people believe that microbes are only present in research laboratories or in hospitals and clinics and thus, have a misleading feeling of security outside healthcare facilities. This is due to the lack of knowledge about where bacteria/disease causing organisms can be found. Thus, there is a need for studies that will educate people on the incidence and prevalence of disease-causing organisms in the community.

1.3   Aim and Objectives of the study

The aim of this research is to determine the presence of *staphylococcus aureus* on door handles and how widely spread it is the College of Humanities Management and Social Science in Mountain Top University. The study further aims to determine the antibiotic resistance profile of *S aureus* isolates, as they are potential public health threat in Mountain Top University.

Based on this, the specific objectives of the present study are as follows:
- To isolate *Staphylococcus aureus* bacteria on the door handles.
- To identify and characterize *Staphylococcus aureus*
- To determine the susceptibility test profile of the isolate.

1.4   Scope of the study

To achieve the objectives, the following procedures were taken
- Swabbing of door handles
- Preparation of culture media
- Inoculation/streaking of swabs on culture plates
- Incubation of streaked plates.

1.5   Justification of the study

This study will provide information about *Staphylococcus aureus* associated with door handles as they spread public threat to human health.

CHAPTER TWO
LITERATURE REVIEW

2.1 The ubiquitous nature of microorganisms

Everywhere you look, there are microorganisms that are important to every environment. In these settings, they either live freely or as parasites (Sleigh and Timbury, 1998). Sometimes they may be temporary pollutants on surfaces or on people's hands, which pose major health risks as potential sources of both community- and hospital-acquired illnesses (Pittet *et al.,* 1999). The increase in the frequency of epidemic breakouts of particular diseases and the rate at which they are moving quickly from one community to another are serious issues in terms of public health (Scott *et al.,* 1982; Galtelli *et al.,* 2006). The annual increase in food poisoning cases, where household outbreaks are a major contributing factor, necessitates an assessment of the likely causes and sources of infection, even though it is generally agreed that the infection risk in the general public is lower than that associated with hospital patients (Scott *et al.,* 1982). Along with regular human contact, which is one way that diseases spread, mosquitoes are the primary cause and vector of community-acquired infections (Prescott *et al.,* 1993; Li *et al.,* 2009) When formites come into touch with people or settings that naturally harbor harmful organisms, they play a significant role in the transmission of infectious diseases (Osterholm *et al.,* 1995). In particular those seen in public buildings, hospitals, hotels, and restrooms, these formites include door handles for conveniences, sinks, chairs, tables, toilet seats, faucets, and lockers (Bright *et al.,* 2010). One of the most commonly stated potential sources of infections is bathroom and toilet door handles (Reynolds, 2005).

People frequently bring their own microbial flora and other organisms from other places into public toilets and restrooms, placing them on door handles or knobs when they enter and exit the facility (Goldhammer *et al.,* 2006).

However, the frequency of site contamination and exposure, the level of pathogen excreted by the host, the likelihood of transmitting the infectious agent to a susceptible person, the virulence of the organism, the immunocompetence of the people in contact,

and the practice of control measures like the use of disinfectants and personal hygiene determine the risk of disease transmission through formites (Reynolds, 2005).

Unfortunately, water systems are rarely present in public restrooms in markets and parks, even in Nigeria, and even if they are. Because they don't have much time to wash their hands after using these facilities, users can bring pathogens with them (Giannini *et al.,* 2009).

Cholera outbreaks and CAMRSA (methicillin-resistant *Staphylococcus aureus*) outbreaks in the community could result as a result (Giannini *et al.,* 2009). Numerous pathogens, such as *Staphylococcus aureus*, *Klebsiella species*, *Bacillus species*, *Escherichia coli*, *Proteus mirabilis*, and *Proteus vulgaris*, as well as *Enterococcus feacalis*, have been connected to the contamination of door knobs in hospitals (Nworie *et al.,* 2012; Onwubiko and Chinyeaka, 2015). *Staphylococcus aureus*, *Pseudomonas species*, and *E coli* were often isolated. using doorknobs (Augustine *et al.,* 2017). In a recent investigation, *E coli* was found to be the second most prevalent bacterial isolate from door handles (Frank, 2017). If *E coli* is discovered on pediatric unit door knobs, newborn infections could become serious.

2.2 *Staphylococcus aureus*

The broad group of facultative anaerobic Staphylococci can colonize and infect both people and animals. They are Gram-positive (G+ve) bacteria. They are responsible for a wide range of infectious illnesses, including abscesses, toxic shock syndrome, endocarditis, minor skin infections, and food poisoning (Foster, 2004). *Staphylococcus aureus* infections continue to be a substantial contributor to community and hospital-acquired infections in spite of extensive attempts to control them (Foster, 2004). According to Foster (2004) and Randrianirina et al. (2007), this genus is home to two principal pathogens: *S. aureus* and *S. epidermidis*, an opportunistic pathogen. *S. aureus* is mostly found on moist skin in the anterior nares, axillae, and perineum, where it is carried by a small percentage of humans asymptomatically. In contrast, *S. epidermidis* is found across the entire cutaneous ecosystem of humans, where it coexists in harmony with its host. However, staphylococci can enter the body and spread infection if the cutaneous organ system is broken by trauma, needles, or direct medical device implantation (Foster, 2004, Randrianirina *et al.,* 2007). *S. epidermidis* is mainly linked to

infections brought on by external objects (Randrianirina *et al.,* 2007). The far more virulent bacteria *S. aureus* frequently causes acute pyogenic infections (Randrianirina *et al.,* 2007). The anterior nares and other moist parts of human skin serve as a reservoir for these bacteria (Henderson, 2006). People who have nasal colonization also momentarily carry the bacterium on their hands, which causes transmission from one person to another. *S. aureus* is most commonly spread nosocomially via healthcare personnel' hands (Henderson, 2006). Patients with diabetes, those undergoing haemodialysis or peritoneal dialysis, injecting drug users, and those with disorders of skin integrity have higher rates of staphylococcal colonization and infection (e.g. burns, indwelling lines, etc.) (2006) Henderson. Human immunodeficiency virus carriers are more likely to become colonized by and infected with *Staphylococcus spp* (Henderson, 2006). Major pathogens in hospital environments include staphylococci and enterococci that cause post-surgical infections. They are troublesome bacteria because they thrive in settings where antibiotics are frequently used and have developed to become resistant to a variety of medications (Dixon, 2002). In some clinical wards, meticillin-resistant *S. epidermidis* [MRSE] and MRSA infection rates are 40% and 50%, respectively, for staphylococci. In hospital acquired infections of the blood, these bacteria are linked to a high death rate of 25% to 63%. Vancomycin-resistant enterococci (VRE) accounted for up to 12% of nosocomial infections in the developed world in the late 1990s (Carbon, 2000). Effective staphylococcal infection treatment and control have become extremely challenging due to the advent of MRSA, MRSE, and *S. aureus* that exhibits tolerance to and resistance to vancomycin (VISA/VRSA) (Carbon, 2000). A significant human pathogen that causes a variety of clinical infections is *Staphylococcus aureus*. Along with osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections, it is the main cause of endocarditis. Both a commensal bacteria and a human pathogen, *Staphylococcus aureus* infections are frequent in both community-acquired settings and hospital-acquired settings. *S. aureus* colonizes about 30% of the population of people (Steven et al., 2005).

2.2.1 Taxonomy of *Staphylococcus aureus*

According to taxonomy, *Staphylococcus aureus* belongs to the wider bacterial family Staphylococcaceae, which also consists of the five additional genera Macrococcus, Nosocomiioccus, Salinicoccus, and Gomella. The three staphylococci that cause human infections most frequently are *S. S. aureus S. epidermidis* and saprophyticus. There are

53 known staphylococci species as of right now. Additional staphylococcus species connected to human infection may exist.

2.2.2 Characteristic, Isolation & Identification of *Staphylococcus aureus*

Peptidoglycan makes up 50% of the staphylococcal cell wall's weight. N-acetylglucosamine and N-acetylmuramic acid with 1, 4-b links make up the alternating polysaccharide subunits that make up peptididoglycan. The colony should ideally be stained with a Gram stain, and tests should be run to see if catalase and coagulase are produced. This will help identify the coagulase-positive *S aureus* fast. The ability of *S aureus* to produce thermostable deoxyribonuclease is another very helpful assay. By examining colonies for agglutination with latex particles coated with immunoglobulin G and fibrinogen, which bind protein A and the clumping factor, respectively, on the bacterial cell surface, *S aureus* can be identified. Commercial providers offer these (e.g., Staphaurex). In order to decrease the amount of false negative results, the most recent latex test (Pastaurex) contains monoclonal antibodies to serotype 5 and 8 capsular polysaccharides. It can be challenging to identify some recent clinical isolates of *S aureus* because they don't produce coagulase or clumping factor. Today, commercial biotype identification kits like API Staph Ident, API Staph-Trac, Vitek GPI Card, and Microscan Pos Combo are used to identify *S epidermidis* and other species of Staphylococcus. These consist of test substrate-filled prefabricated strips (Medical Microbiology 4th edition 1996).

2.2.3 Microscopic Morphology

The circular Cocci, or gram-positive bacteria, are what *Staphylococcus aureus* is (gram stain gives them a purple color). In contrast to streptococci, which form chains when observed under a light microscope following the gram stain, they typically form clusters that resemble a bunch of grapes. The cells have sizes between 0.5 and 10 meters. They sometimes prefer to show up in short chains, but they can also be found in pairs. The form of the cocci helps distinguish staphylococci from streptococci.

2.2.4 General cultural and biochemical characteristics

Staphylococcus aureus, an aerobic and anaerobic (Facultative) bacteria, can grow between 18 and 40 degrees Celsius. Colonies can develop on surfaces with up to 10% salt and are often golden or yellow in color (aureus means golden or yellow). People always

call it the golden staph. It can also grow as a white colony on nutrient agar media; the organism's synthesis of carotenoids gives the colonies their yellow color. *Staphylococcus aureus* typically hemolyzes on blood agar due to the synthesis of the four distinct hemolysins (alpha, beta, gamma, and delta). The bacterium is salt tolerant and may thrive on mannitol-salt agar medium that includes 7.5% sodium chloride. Catalase is present and *Staphylococcus aureus* is not motile or sporing. They grow rapidly and in large numbers when there are aerobic conditions. They appear as glossy, regular, elevated, translucent colonies with a tendency to have a golden tint on blood agar. The colonies have a diameter of 2 to 3 mm after a 24-hour incubation period, and several of the strains are hemolytic. The diameter of colonies may reach 6 to 8 mm after three days of incubation.

2.2.5 Epidemiology of *Staphylococcus aureus* infection

Different populations have varying rates of *S. aureus* nasal carriage. The average rate of *S. aureus* carriage in the general population is 37% (with a range of 19–55%) (Kluytmans *et al.,* 1997), but in some subpopulations, such as those with insulin-dependent diabetes mellitus, dialysis patients, intravenous drug users, people with the human immunodeficiency virus, and people with S. aureus skin infections, the rate is noticeably higher. For instance, up to 100% of people with atopic dermatitis have microbial colonization. There are three categories of *S. aureus* carriers: persistent carriers (20%), who are thought to constantly carry the bacterium; intermittent carriers (60%), who occasionally carry the bacterium; and non-carriers (20%), who are thought to never carry the bacterium (Kluytmans *et al.,* 1997). Children's colonization rates have been reported to be significantly higher than adults' when populations of different ages are compared (Armstrong-Esther, 1976; Cunliffe, 1949; (Melles *et al.,* 2004) Noble *et al.,* 1967) Wertheim *et al.,* 2005), and in some studies a difference between genders has been observed, both in the rate of colonization (Mernelius *et al.,* 2013) (Olsen *et al.,* 2013) (Sangvik et al., 2011). There are variations in prevalence among different nations. In a recent European study, there was significant variance in the nasal carriage rates, with Sweden having the highest rates (29%) and Hungary having the lowest (12%). (den Heijer et al., 2013). According to a Norwegian survey, Norway's rate (29%) is the same as that of the general Swedish population (Olsen *et al.,* 2013). In one Canadian study, the reported incidence of invasive *S. aureus* infections was 28.4 cases per 100,000 people, and infections were more prevalent in men and people over 65. (Laupland *et al.,* 2003).

S. aureus infections affected 0.8% of hospital inpatients in the United States; these patients had a longer hospital stay, greater medical bills, and a higher chance of death than inpatients who did not have *S. aureus* infections (Noskin *et al.,* 2005). Methicillin-sensitive *S. aureus* (MSSA) bacteraemia rates grew steadily in Europe between 2002 and 2008 (12125-15266) (Kraker *et al.,* 2012).

2.2.6 Pathogenesis of *Staphylococcus aureus*

One of the most common bacterial infections in people is *staphylococcus aureus.* Additionally, it contributes to a number of human illnesses, such as impetigo, gastroenteritis, urinary tract infections, and soft tissue infections. Based on where the infection originated, *Staphylococcus aureus* clinical infections can be divided into two groups:

 1. Hospital infection

2. Community infection

These two varieties are distinct from one another in terms of the clinical illness symptoms, antibiotic susceptibility, and genetic makeup of the *staphylococcus aureus* strain producing the infection. *Staphylococcus aureus* has been around for a very long period as a nosocomial pathogen (pathogen found in hospitals). In nosocomial settings, it has a very high death and morbidity rate. The health of the general populace is seriously threatened by the recent discovery that community staphylococcus aureus infections are growing and have a resistant variation. These bacteria can also result in toxin-mediated illness, depending on the strain that is present at the infection site. *S.aureus* can produce a variety of infections with various pathogenic physiologies. Nuclease, lipase, protease, and hyaluronidase are a few of the enzymes it has that increase its virulence factors and aid bacterial penetration. *Staphylococcus aureus* process of infections deals with five stages which are

1. Colonization

2. Local infection

3. Systemic dissemination

4. Metastatic infections

5. Toxicosis

Staphylococcus aureus has over time shown that it is harmful through the expression of various extracellular and cell surface-associated proteins that have the potential to be virulence factors. The majority of *Staphylococcus aureus*-related illnesses have a complex etiology. Because of this, figuring out the specific function of any given element is more challenging and confusing. Nevertheless, there are similarities between strains obtained from specific disorders and the expression of specific proteins, highlighting the role these factors play in pathogenesis. The development of several resistant strains significantly accelerated *staphylococcus aureus* pathogenesis. The staphylococcus' ability to acquire different resistance genes has boosted the organism's pathogenicity. Both Staphylococcus aureus infections in hospitals and infections in the general public have genes that make them resistant to antibiotics. According to their antibiotic resistance, HA-MRSA (hospital acquired methicillin resistant *S. aureus*), CA-MRSA, and other varieties of *Staphylococcus aureus* can be divided (community acquired methicillin resistant *S. aureus*). *Staphylococcus aureus* can cause everything from simple skin problems to life-threatening disease. They develop in food and exude poisons that might be harmful.

2.2.7 *Staphylococcus aureus* colonization and infection

Staphylococcus aureus is widely found on the skin and in the noses of many healthy persons. They usually don't bother people or only result in small skin infections. But when staphylococcus aureus infiltrates the body to the level of the tissue, blood, joints, lungs, heart, and bones and gains access to the cells, it can be lethal. To transmit infection, the organism needs to be able to enter the host and attach to the surface or tissues of the host. The exceptional aggressiveness of the illness is due to *Staphylococcus aureus*, a commensal that colonizes the nares, axillae, vagina, pharynx, or injured skin surfaces. Staphylococci can penetrate surrounding tissues or the bloodstream and infect people when the skin or mucosal barrier is broken. Whether an infection is controlled or spreads depends on how *S. aureus* virulence factors interact with host defensive mechanisms. On the biology of staphylococci colonization of the nares, the primary staphylococci reservoir, there remains unfinished study. Mucin appears to be the primary host surface

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that gets colonized due to interactions between staphylococcal protein and mucin carbohydrate. It is unknown what use some staphylococcal adhesins, secretory IgA, or other commensals serve.

2.2.8 Virulence factors expressed by *Staphylococcus aureus*

Staphylococcus aureus is a bacterium that produces a wide range of virulence factors, including toxins (leukocidins and hemolysins), immune-evading surface elements (capsule and protein A), and enzymes that promote tissue invasion (hyaluronidase). It can be challenging to evaluate the viability of strains from dominant clonal complexes because successful lineages frequently diverge from their ancestors at numerous loci. There are numerous virulence factors present in the bacterium *Staphylococcus aureus*. These components aid the organism's ability to function as a pathogen that can sicken both people and animals with a range of diseases. Virulence factors interfere with the host immune system by adhering to host cells and causing tissue invasion, sepsis, and toxin-mediated disorders. *Staphylococcus aureus* has the ability to up-regulate virulence factors in response to stressful stimuli, which allows it to stay in the bloodstream, seed deep tissues, and produce secondary foci of infection (such as the host immune response or circulating antibiotics). *S. aureus* strains are skilled in clinging to and colonizing skin and nares mucosa, gaining entry to the circulation, avoiding host immune responses, forming protective biofilms, and developing antibiotic resistance. Because of this, despite the availability of multiple medicines with efficacy against wild-type strains, *Staphylococcus aureus* is a very successful and steadily growing clinically significant gram-positive bacterium.

2.2.8.1 Adhesion

The pathogen must enter the host and connect to host cells or tissues in order to start an infection. *Staphylococcus aureus* has the ability to up-regulate a number of virulence factors, allowing it to cling to and colonize the surfaces of implanted devices or prostheses, the nares, and injured skin. It can also cause significant bloodstream infections. For this, a polymer on the surface of *Staphylococcus aureus* called teichoic acid is crucial. Microbiological Surface Elements A cell surface protein called Recognizing Adhesive Matrix Molecule (MSCRAMM) interacts with host molecules like collagen, fibronectin, and fibrinogen to make tissue attachment easier. *Staphylococcus*

aureus cells produce proteins on their surface that facilitate adhesion to host extracellular matrix proteins such laminin and fibronectin. Fibronectin can be found on the surfaces of epithelial and endothelial cells as well as in blood clots. Additionally, the majority of strains produce the clumping factor, a fibrinogen/fibrin binding protein that facilitates adhesion to blood clots and injured tissue. The majority of *Staphylococcus aureus* strains produce fibronectin and fibrinogen-binding proteins. *S aureus* can attach to the surface of cultivated human endothelial cells and then become ingested by a mechanism akin to phagocytosis. It is unclear if attachment uses a brand-new receptor or a well-known surface protein of *S aureus*. Some scientists believe that *S aureus* can start endocarditis by adhering to the healthy endothelium. Others believe that shock, even of a very small type, is necessary to encourage bacterial adhesion (Medical Microbiology 8th 2014).

2.2.8.2 Invasion

Staphylococcus aureus can weaken the skin barrier by exuding tissue-destructing enzymes, hemolysins (including alpha-hemolysin [alpha toxin], which opens pores in skin cell membranes), and exfoliative toxins. Invasion may be initiated by a compromised immune system, a physical integument tear, or localized inflammation.

Which includes: Proteases, Lipases, Nucleases, Hyaluronidase, Phospholipase C, Metalloproteases, (elastase), Staphylokinase.

These extracellular enzymes damage tissue, which facilitates bacterial invasion of tissues.

2.2.8.3 Toxicoses

Multiple forms of protein toxins that *Staphylococcus aureus* can express are likely to blame for the symptoms seen during infections. Some can cause hemolysis by damaging the erythrocytes' membranes, but this is unlikely to be important in living things. Leukocytes are damaged by the leucocidin's membrane damage but it is not hemolytic. Enterotoxins and TSST-1 produce toxic shock, while systemic release of -toxin causes septic shock. A variety of enterotoxins, which are strong gastrointestinal exotoxins, are produced by *Staphylococcus aureus*. The ingestion of foods containing an adequate quantity of enterotoxins leads in the intoxication known as staphylococcal food poisoning.

One of the toxins that *staphylococcus aureus* introduces into the body and produces toxic shock syndrome is called toxic shock syndrome toxin-1 (TSST-1). Enterotoxins, which come in six different serotypes (A, B, C, D, E, and G), and toxic shock syndrome toxin are two different forms of toxins that *Staphylococcus aureus* can express that have super antigen action (TSST-1). When consumed, enterotoxins produce vomiting and diarrhea and are the cause of staphylococcal food poisoning. Enterotoxins can cause toxic shock syndrome (TSS) when they are expressed systemically; in fact, enterotoxins B and C are responsible for 50% of non-menstrual TSS. TSST-1 lacks emetic function and has a very tenuous relationship to enterotoxins.

All cases of menstrual TSS, including 75% of all TSS, are caused by TSST-1. Any staphylococcal infection can result in TSS if an enterotoxin or TSST-1 is produced systemically and the host is deficient in the necessary neutralizing antibodies. Serine proteases known as exfoliative toxins A and B identify and break down desmosomal proteins in the skin. Staphylococcal-scalded skin syndrome, a disorder that primarily affects infants, is brought on by ETs. Neonatals with the scalded skin condition, which includes extensive blistering and epidermal loss, are affected by the epidermolytic (exfoliative) toxin ET.

The toxin comes in two antigenically different forms, ETA and ETB. These toxins appear to possess protease action. The three most crucial amino acids in the protease's active region are conserved, and both toxins share a sequence resemblance with the serine protease of *S aureus*. Additionally, totally switching the serine active site to a glycine (Franklin and Lowy 2016).

2.2.8.4 Biofilms

In order to form slimy biofilms on medical devices, healthy or damaged heart valves, and injured skin, *Staphylococcus aureus* quorum sensing may influence gene expression. Lack of oxygen and nutrition causes bacteria to go into a non-growing stage, which makes them more resistant to some antibiotics. When adherent and in the stationary phase, *S aureus* small-colony variations in particular display nearly 100% resistance to antimicrobial agents. The biofilm matrix protects against immune cells and may hinder the deep penetration of some drugs.

2.2.8.5 Avoidance of host defence (evasion)

By secreting anti-opsonizing proteins (such chemotaxis inhibitory protein), *Staphylococcus aureus* evades the host immune response by preventing neutrophils from phagocytosing it. Additionally antiphagocytic in nature is surface protein A from *Staphylococcus aureus*. Additionally, *S aureus* expresses superantigens and secretes leukotoxins that lyse leukocytes, such as Panton-Valentine leukocidin (such as enterotoxins and toxic shock syndrome toxin 1), which cause intense, polyclonal stimulation and growth of T cells with the T cell receptor Vb specificity, which is followed by the deletion or repression of these T cells to an anergic state, disrupting the normal immune response. *Staphylococcus aureus* expresses a wide range of substances that may interfere with host defensive processes. There isn't enough evidence to be certain that these elements cause virulence, though.

2.2.8.6 Capsular polysaccharide

Most clinical isolates of *Staphylococcus aureus* express a serotype 5 or 8 surface polysaccharide. The name "microcapsule" was given to this structure because, unlike the numerous capsules of other bacteria, it can only be observed by electron microscopy following antibody labeling. *Staphylococcus aureus* isolated from infections expresses large quantities of polysaccharide during laboratory culturing but quickly depletes them. The purpose of the capsule is unclear. Though in vitro tests could only demonstrate this in the absence of complement, it might impede phagocytosis. The production of polysaccharides, however, may have hindered colonization of injured heart valves by concealing adhesins, according to study contrasting wild-type and a mutant strain with a faulty capsule in an endocarditis model.

2.2.8.7 Protein A

Surface protein of *Staphylococcus aureus* Through the Fc region, protein A binds immunoglobulin G molecules. This non-immune process causes bacteria to bind serum IgG molecules incorrectly. This ought to prevent opsonization and phagocytosis from occurring. Research on *Staphylococcus aureus* mutants in infection models suggests that protein A improves pathogenicity, whereas in vitro investigations have demonstrated that mutants missing protein A are better phagocytized.

2.2.8.8 Leukocidin

Staphylococcus aureus has a toxin that only affects polymorphonuclear leukocytes. Because the phagocytosis defense against staphylococcal infection is so important, leukocidin ought to be a virulence factor. Leukocidins are a kind of cytotoxin produced by some bacteria (Staphylococcus). It belongs to the group of toxins that generate pores. The pore formation model is iterative. First, an integrin or a certain protein-containing receptor on the surface of the host cell is recognized by the "S" subunit of the cytotoxin. Following the recruitment of a second "F" subunit, the S subunit dimerizes with the second subunit on the surface of the host cell. Oligomerization follows dimerization. The oligomers, which are made up of alternate S and F subunits, eventually underwent a substantial structural shift and formed a beta-barrel that pierced the lipid bilayer of the host cell. 2014 [Alonzo *et al*.]. Leukocidins kill ("-cide") leukocytes, hence their name. Leukocidins aim to suppress both innate and adaptive immune responses by targeting phagocytes, natural killer cells, dendritic cells, and T lymphocytes (Alonzo *et al*., 2014; Alonzo *et al*., 2012). Leukocidins are categorized as bacterial invasive substances. Enzymatic secretions called invasivens are used by bacteria to enter the host tissue to which they are connected. Despite being comparable to exotoxins, invasins differ from them in two ways: their activities are typically more localized, and they operate through far less specific processes. Panton-Valentine leukocidin is one kind.

2.2.8.9 Hemolysins

Lipids and proteins known as hemolysins or haemolysins damage the cell membrane of red blood cells to produce lysis. Many hemolysins produced by pathogens may not significantly destroy red blood cells during infection, despite the fact that the lytic action of some hemolysins obtained from microbes on red blood cells may be of enormous importance for food intake. However, hemolysins frequently have the ability to lyse red blood cells in a lab setting. Some hemolysins are lipid biosurfactants, however the majority are protein-based substances. (2005) [Stipcevic *et al.]*

2.3 Antibiotic resistance in *Staphylococcus aureus*

When it comes to acquiring antimicrobial resistance genes from other organisms, *Staphylococcus aureus* is a very tolerant bacterial species (Zetola *et al.,* 2005). It has developed resistance to every antimicrobial agent used in clinical practice over the past 50 years. The fact that the first penicillin-resistant strain of Staphylococcus aureus clinical isolates was discovered in 1942, immediately following the introduction of penicillin to clinical use, serves as the best example of the adaptability of *Staphylococcus. aureus* (Zetola *et al*., 2005). *Staphylococcus aureus* and *S epidermidis* are increasingly prone to several antibiotic resistances. Multiple resistance is a sign of methicillin resistance. In hospitals, Methicillin-resistant *Staphylococcus aureus* (MRSA) outbreaks can become epidemic.

2.4 Immunologic response to *Staphylococcus aureus* infection

Abscess development is the usual pathological feature of staphylococcal illness. The main host defense against S. aureus infection is provided by leukocytes. Leukocyte migration to the infection site is caused by the controlled production of adhesion molecules on endothelial cells. Both bacteria and tissue-based macrophages are involved in this cytokine-mediated mechanism. Following infection, cytokines are initially detectable in arteries before spreading into tissues when inflammatory cells move toward the infection sites. Endothelial cells infected with S. aureus also express MHC class I, vascular cell adhesion molecule 1 (CD106), and intercellular adhesion molecule 1 (CD54), all of which are likely involved in this process. Intercellular adhesion molecule 1 (ICAM-1)-deficient mice show a deficiency in leukocyte migration that increases mortality, but they also have less severe staphylococcal infections than normal mice, probably because leukocyte-mediated damage is reduced. In vitro, phagocytosis is made easier by the presence of opsonizing antibodies that are directed against the capsule, peptidoglycan, or complement. With the exception of toxic shock syndrome, where the presence of anti-toxic shock syndrome toxin 1 is protective, the titer of anti-staphylococcal antibodies is not connected with protection from infection, the significance of antibody in vivo is less certain. Which staphylococcal components can cause protection from recurrent infection is unknown at this time (Franklin and Lowry 2016).

2.5 Clinical manifestation of *Staphylococcus aureus* infection

Skin infections like cellulitis, boils [furuncles], carbuncles, impetigo, etc., soft-tissue infections like abscesses, respiratory infections like pneumonia and sinusitis, bone and joint infections like osteomyelitis and septic arthritis, and endovascular infections are all commonly caused by *Staphylococcus aureus* (e.g. endocarditis, vascular graft infections, etc.). Bacteremia, endocarditis, metastatic infections, sepsis, and staphylococcal toxic shock syndrome are among the severe infections brought on by *Staphylococcus aureus* (Cookson and Leaper 2009).

2.5.1 Skin infections

Skin infections caused by staph bacteria include:

1.   **Boils:** The most frequent staph infection kind is boils. A pus-filled pocket that develops in an oil gland or hair follicle is known as this. The skin around the affected area typically becomes red and swollen.

If a boil splits open, pus will likely begin to pour from it. Boils most typically develop in the groin, buttocks, and underarms.

2.   **Impetigo:** This infectious, frequently painful rash may be caused by staph bacteria. Impetigo is characterized by large blisters that may ooze fluid and develop a crust with a honey-colored crust.

3.   **Cellulitis:** Cellulitis is a skin illness that affects the deeper skin layers. It causes swelling and redness on the skin's surface. In addition, sores or discharge-filled areas could develop.

4.   **Staphylococcal scalded skin syndrome:** Toxins produced by the staph bacteria can cause staphylococcal scalded skin syndrome. A fever, rash, and occasionally blisters are symptoms of this illness, which typically affects newborns and young children. When the blisters burst, the top layer of skin is removed. It leaves a red, unfinished surface that resembles a burn.

2.5.2 Food poisoning

Staph bacterium is one of the most typical causes of food poisoning. Food contains bacteria that multiply and release toxins that can be harmful to your health. In the majority

of instances, symptoms start to show up soon after eating infected food. In most cases, symptoms disappear after a half-day.

A staph infection acquired through food typically doesn't cause a fever. The following are warning signs and symptoms of this particular staph infection:

• Vomiting and diarrhea, dehydration, low blood pressure, and other symptoms.

2.5.3 Bacteremia
Staph bacteria produce bacteremia, often known as a bloodstream infection, when they enter the bloodstream. Low blood pressure and a fever are two signs of bacteremia. The germs can enter your body deeply and infect:

• Internal organs, including your brain (meningitis), heart (endocarditis), or lungs (pneumonia)

• The bones and muscles

• Surgical implants, such as pacemakers for the heart or artificial joints

2.5.4 Toxic shock syndrome
This potentially lethal illness is brought on by some staph bacterium strains that emit toxins. Injuries to the skin, certain kinds of tampons, and surgery have all been connected to the illness. A high temperature, nausea, vomiting, a rash that looks like sunburn on your hands and soles, confusion, muscle aches, diarrhea, and stomach pain are typical symptoms that appear abruptly.

2.5.5 Septic arthritis
Septic arthritis is usually brought on by staph infections. The shoulders, hips, knees, and fingers or toes are typically the targets of the bacterium. Another issue is infected artificial joints. Among the warning indicators and symptoms are:

• Joint edema

 • Excruciating pain in the affected joint

• Fever

2.5.6 Endocarditis

Patients who use intravenous drugs, those who are elderly, those who have prosthetic valves, and hospitalized patients all experience it. The early symptoms in all four groups could only be a temperature and a general malaise, making a diagnosis challenging. *Staphylococcus aureus* endocarditis is distinguished from endocarditis brought on by less virulent bacteria by its quick start, high fever, frequent involvement of healthy heart valves, and lack of outward signs of the illness at the time of initial presentation.

Intravascular catheters are the most common source of bacterial inoculation, and *Staphylococcus aureus* is one of the most prevalent pathogens in nosocomial and prosthetic-valve endocarditis. Nosocomial endocarditis has a mortality incidence of 40 to 56 percent regardless of the infection, and the percentage is significantly greater when the pathogen is Staphylococcus aureus. 91 In many of these instances, additional illnesses or the use of medications mask the diagnosis. Prosthetic-valve endocarditis, particularly in the early postoperative period, is frequently fulminant and is defined by the development of valvular insufficiency and cardiac abscesses (Fang *et al.,* 2015).

2.6 The reservoir

Staphylococcus aureus is one of the strongest pathogenic bacterial species. These bacteria tolerate high salt concentrations and a variety of harsh environmental conditions, such as heat, desiccation, and relative cold (West Virginia Department of Health and Human Resources 2007). These characteristics significantly increase this species' capacity to infect humans with illnesses that could be fatal.

2.7 Mode of transmission

The anterior nares are the primary site of *Staphylococcus aureus* colonization; 20–30% of the general population carries coagulase-positive staphylococci in their noses, mostly exclusively *Staphylococcus aureus* isolates (Henderson,2006). At least one-third of *Staphylococcus aureus* infections are brought on by auto-infections (Cookson and Leaper 2009). People who have a draining lesion or any other purulent discharge are more likely to carry the infection and spread it than healthy individuals (Henderson, 2006). The involvement of contaminants as prevalent sources of this bacterium's epidemic spread (West Virginia Department of Health and Human Resources 2007). Contact with a person who either has a purulent lesion or is an asymptomatic (often nasal) carrier of a pathogenic

strain results in transmission. Perineal carriage of *Staphylococcus aureus* is a key source of this pathogen in an operating room environment. Some carriers spread infections more successfully than others (Henderson, 2006). Hands are the most significant vectors for spreading *Staphylococcus aureus* infection; the importance of contaminated objects in the spread of the bacteria has been overemphasized. Although it is uncommon, airborne transmission of the associated viral respiratory disease has been recorded in babies (Henderson, 2006).

2.8 Pathogens associated with door handles

As frequent isolates from door handles, *Staphylococcus aureus, Pseudomonas species*, and *E coli* were discovered [Augustine *et al*., 2017].

2.8.1 *Escherichia coli*

Escherichia coli, sometimes referred to as *E. coli*, is a rod-shaped, gram-negative, anaerobic coliform bacteria that is typically found in the lower intestine of warm-blooded animals. The majority of *E. coli* strains are non-lethal, but some serotypes have the potential to seriously injure their hosts and occasionally result in food contamination episodes that force manufacturers to recall their products. *E. coli* cells are typically rod-shaped, measuring around 2.0 mm in length, 0.25 mm to 1.0 mm in diameter, and having a cell volume of 0.6 – 0.7 mm3. Although most intestinal infections caused by *E. coli* can be successfully treated with antibiotics, only one strain of these bacteria can cause intestinal infections that must be treated with antibiotics. The bacteria can swim thanks to the peritrichous arrangement of their flagella. Through an adhesion molecule called intrimin, it also adheres and effaces to the intestine's microvilli. One of the greatest or most extensively researched free-living organisms is *E. coli*. There are more than 700 different *E. coli* serotypes known. Different serotypes of bacteria are identified by their flagella and "O" and "H" antigens. The *E. coli* that produce Shiga toxin—so named because it is nearly identical to that produced by Shigella dysenteria type 1—are the ones that are in charge of the frequent instances of tainted foods and beverages. *E. coli* O157:H7 is the most well-known and infamous strain of *E. coli* that produces Shiga toxin. One of the microorganisms discovered in cell phones is *E. coli*, and macconkey agar, which is pink and red in color, is used to grow *E. coli*.

2.8.2 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a typical organism that can be found on plants, animals, as well as in soil and water. It is a gram-negative, aerobic, saccharolytic, non-spore-forming bacillus that measures 0.5 to 0.8 by 1.5 to 3.0 mm. *Pseudomonas aeruginosa* strains typically have a single polar flagellum that is employed for movement. Pyocyanin, which gives colonies a blue hue, and pyoverdine, also known as the fluorescent pigment, which is a yellow-green or yellow-brown pigment, are the two soluble pigments that *Pseudomonas aeruginosa* frequently generates (Nicholas, 2020). The colonies that develop from a strain of *Pseudomonas aeruginosa* producing both pyoverdine and pyocyanin are blue-green in hue. Other water-soluble pigments that this organism may make include pyorubrin and pyomelanin, which give colonies their distinct red and brown colors, respectively. Pseudomonas aeruginosa colonies frequently exhibit beta-hemolysis and a greenish metallic sheen on sheep blood agar plates as a result of their pigment synthesis. The presence of pyocyanin aids in the identification of the bacterium because no other species of gram-negative non-fermenters produce it. One of the easiest ways to tell if a colony is *Pseudomonas aeruginosa* is by its distinctive fruity, grape-like odor, which is caused by the organism's synthesis of 2-amino acetophenone. *Pseudomonas aeruginosa* infections have long been challenging to treat, but like other bacteria, it is evolving a greater resistance to antimicrobials. To make matters worse, Pseudomonas aeruginosa that is multidrug resistant, or resistant to three or more antimicrobial drugs, has been discovered. In one study, it accounted for over 30% of all isolates taken from patients in nursing homes and intensive care units. Eosin Methylene Blue (EMB), a culture medium, is used to test for the growth of bacteria in *Pseudomonas aeruginosa*.

2.9 Antibiotics used in treatment of *Staphylococcus aureus*

Doctors regularly recommend cefazolin, nafcillin, oxacillin, vancomycin, daptomycin, and linezolid to treat staphylococcus infections. For severe staphylococcus infections, vancomycin may be required.

2.9.1 Beta-lactams

Beta-lactam antibiotics have the ability to attach to these PBP enzymes, and by doing so, they prevent the formation of peptidoglycan, which causes lysis and cell death

(Heesemann, 1993). The most well-known members of the beta-lactam class are Carbapenems, Monobactams, Cephalosporins, and Penicillins.

2.9.2 Penicillin

Alexander Fleming originally discovered and reported penicillin in 1929; it was later discovered to be one of numerous antibacterial substances known as the penicillins. (2000) McGeer et al. Certain bacterial strains manufacture penicillins, which help antibiotics pass through the outer layer of these bacteria's cell walls. With two weapons at their disposal, they can more effectively combat Gram-negative bacteria. Particularly, some penicillins, like Augmentin, are created in conjunction with non-antibiotic substances that can impede the function of the bacterial penicillinase enzyme. In reality, augmentin is a medication made of the antibiotic amoxicillin and the non-antibiotic clavulanic acid. Even among bacteria that produce penicillinase, clavulanic acid can inhibit the beta-lactamase enzyme, extending the antibacterial activity of the amoxicillin component of Augmentin (Poirel *et al.,* 2005).

2.9.3 Tetracyclines

Benjamin Duggar found tetracycline in a soil bacterium of the genus Streptomyces in 1945. (Sanchez *et al.,* 2004). Chlorotetracycline was this class's original member (Aureomycin). Members of this class are identified by the suffix "-cycline" and have four (4) hydrocarbon rings . In the past, this class of antibiotics was divided into generations based on how they were synthesized. The first generation refers to those produced through biosynthesis. Tetracycline, chlortetecycline, oxytetracycline, and demeclocycline are all members. Due to the fact that they are semi-synthesis derivatives, members including Doxycycline, Lymecycline, Meclocycline, Methacycline, Minocycline, and Rolitetracycline are categorized as Second Generation. Third generation drugs are those made from total synthesis, such as tigecycline (Fuoco, 2012).

2.9.4 Aminoglycosides

Streptomycin, initially identified in 1943, was the first medication in this class of antibiotics to be found (Mahajan and Balachandran, 2012). Against Mycobacterium tuberculosis, the cause of tuberculosis in humans, streptomycin has been widely utilized. The aminoglycosides are mixtures of glycosidic linkages connecting typically three amino sugars. They are derived from Actimomycetes found in soil. The antibacterial spectrum of aminoglycosides is very extensive. They work well against aerobic Gram-

negative rods and some Gram-positive bacteria by attaching to one of the ribosomal subunits and preventing the synthesis of proteins in bacteria (Peterson, 2008).

2.9.5 Vancomycin

Streptomyces orientalis, an actinomycete bacteria identified from soil samples in India and Indonesia, produces the glycopeptide antibiotic vancomycin (Castellano *et al.,* 2008). A tricyclic glycopeptide with a molecular mass of approximately 1500 Da, it is intricate and unusual (Vila MMDC et al., 2008). The chemical structure is $C_{66}H_{75}Cl_2N_9O_{24} \cdot HCl$. According to Vila MMDC et al. (2008), vancomycin hydrochloride has the following structural formula: Other glycopeptide antibiotics are teicoplanin and daptomycin. Vancomycin and Teicoplanin are restricted to treating infections brought on by G+ve bacteria like Staphylococci, Streptococci, and Enterococci because they are unable to penetrate the pores of G-ve outer membranes (Boneca and Chiosis 2003).

2.9.5.1 Vancomycin: mode of action

Cell wall synthesis of vancomycin

According to the location of the reaction, the synthesis of the bacterial cell wall can be divided into three stages (Scheffers and Pinho 2005). A sophisticated macromolecule called peptididoglycan creates a robust structural network to shield cells from their surroundings. Disaccharide units repeat in peptididoglycan. The disaccharide's two sugar subunits, NAG (N-acetylglucosamine) and NAM (N-acetylmuramic acid). The cytosol produces the uridine diphosphate (UDP) derivatives that are the NAG and NAM sugars. The carrier UDP $C_{66}H_{75}Cl_2N_9O_{24}$–22 aids in bringing the sugar across the membrane. A pentapeptide chain is added to the UDP-NAM complex to change it, and it is subsequently joined to the second carrier molecule bactoprenol (C55-isoprenyl

pyrophosphate). When the NAM binds, the bactoprenol carrier also receives a second phosphate group from the UDP, which is converted into UMP, or uridine monophosphate.

The ability to transfer the carrier across the membrane depends on this phosphorylation event. The complete NAG-NAM-pentapeptide unit is then linked to an UDP-NAG sugar and carried across the membrane by the bactoprenol carrier.

The bactoprenol loses one inorganic phosphate and is recycled to the interior of the cell to start a new cycle as the component is added to the expanding peptidoglycan chain. The peptide chains of the monomers are cross-linked to the sugar chains already present in the peptidoglycan superstructure in the final step of peptidoglycan synthesis to enhance the whole structure and create a robust network. To demonstrate the addition of another subunit, the entire sequence is repeated once again. Everything that has happened up to this point has happened inside or near the cell membrane.

The transport of units from the inside of the cell via the cell membrane to the exterior side of the membrane depends on the connection of cell wall precursor units with the phospholipids. Vancomycin and teicoplanin are two glycopeptides that prevent bacteria from using the bactoprenol lipid intermediates (C55-isoprenyl pyrophosphate) to make cell walls. Lipid II is made up of a pyrophosphate (PP)-unde caprenyl lipid tail that serves as the carrier for the movement of the peptidoglycan moiety from the cytoplasm to the extra-cellular domain and a peptidoglycan head group, MurNAc-(pentapeptide)-GlcNAc, which serves as the fundamental component of the cell wall. The finished unit's attachment to the accepter molecule and separation from the membrane-bound phospholipids are two of vancomycin's main effects. 2005 (Scheffers and Pinho).

2.9.6 Naficillin
A semi-synthetic antibiotic related to penicillin, naficillin is a beta-lactam with a specific spectrum. With the exception of infections brought on by MRSA, beta-lactamase-resistant penicillin is recommended for treating Staphylococcal infections brought on by strains that are resistant to other penicillins.

Mode of action

On microorganisms that are susceptible to penicillin, nafcillin has a bactericidal action during the active phase of bacterial cell wall production. It stops the construction of the bacterial cell wall by forming covalent connections with penicillin-binding proteins, which are necessary for the final phase of transpeptidation (Sakoulas *et al.,*2014)

2.9.7 Cefazolin

A broad-spectrum cephalosporin antibiotic called cefazolin is typically used to treat moderate to severe bacterial infections of the lungs, bones, joints, stomach, blood, and heart valve in addition to skin infections. It is effective in treating infections brought on by gram-positive streptococci and staphylococci.

Mode of action

In vitro tests reveal that cephalosporins' reduction of cell wall synthesis is what gives them their power to destroy bacteria. By interacting with certain penicillin-binding proteins (PBPs) within the bacterial cell wall, it hinders the third and final stage of bacterial cell wall production. Bacterial cell wall autolytic enzymes such autolysins then assist cell lysis (Sinan *et al*.,2006)

2.9.8 Meticillin

Based on the range of activity of the relevant antibiotic, the penicillin family has been classified into subgroups. The first penicillin family member to be used clinically to treat infections was benzylpenicillin (penicillin G) (Miller, 2002). Penicillinase-producing staphylococci first appeared shortly after penicillin G was developed, rendering natural penicillins ineffective against these bacteria (Miller, 2002). Penicillinase-resistant penicillins, also referred to as anti-staphylococcal penicillin, were created as a result of this. The first individual in this category was meticillin (Conley and Johnston 2003). The drugs nafcillin, oxacillin, cloxacillin, and dicloxacillin are also included in this group. In contrast to *S. aureus* that produces penicillinase, they are particularly powerful against germs that are sensitive to penicillin G. Gram-negative bacteria cannot be treated with anti-staphylococcal penicillins (Conley and Johnston 2003)

Meticillin: Mode of action

All -lactam antibiotics, such as penicillins and cephalosporins, prevent the production of the 18-layer bacterial cell wall. These antibiotics initially attach to penicillin-binding proteins (PBPs) found in a cell's cytoplasmic membrane. They primarily hinder normal peptidoglycan structure development and peptidoglycan chain cross-linking in the cell wall (Hiramatsu, 2001). About 30 bacterial enzymes are used during the three stages of biosynthesis of the bacterial cell wall. Precursor formation, the initial phase, occurs within the cytoplasm. The final step in the cytoplasmic synthesis of cell wall monomer is the addition of D-Ala-D-Ala to the developing precursor (Scheffers and Pinho 2005). Prior racemization of L-Ala and a condensation reaction facilitated by D-Ala-D-Ala synthetase are required for the synthesis of this dipeptide. The enzymes racamase and synthetase are both competitively inhibited by D-cycloserine, a structural homologue of D-alanine. UDP-acetylglucosamine and UDP-acetylmuramyl pentapeptide are joined to create a lengthy polymer during the second stage of peptidoglycan production (Scheffers and Pinho 2005). The cross-linkage is completed during the third and last stage, which entails incorporating the monomer into the developing peptidoglycan polymer. In order to achieve this, a transpeptidation event takes place outside of the cell membrane. They are membrane-bound transpeptidases. In S. aureus, the pentaglycine bridge's terminal glycine residue is connected to the pentapeptide's fourth residue (D-alanine), releasing the fifth residue—also D-alanine—into the environment. The antibiotics -lactam and glycopeptide block the final stage of peptidoglycan production (Scheffers and Pinho 2005).

2.10 Prevention and control of *Staphylococcus aureus* infections

Hospitalized individuals have been associated with infections caused by *Staphylococcus aureus*. The results of tests for the identification and detection of microorganisms within bacterial colonies serve as the foundation for its diagnosis. *Staphylococcus aureus* infections can be avoided and treated by:

- Keeping your hands clean; maintaining sterile conditions during medical procedures using antimicrobial medications as needed.
- Appropriate environmental management techniques, such as routine air, water, and surface monitoring.
- Thorough cleaning and disinfection of tools and surroundings.
- When necessary, isolate patients in clinical settings.
- Careful observation of at-risk individuals and groups.

2.11 Treatment of *Staphylococcus aureus* infections

Different antimicrobial drugs react differently to *Staphylococcus aureus*. Due to the formation of beta-lactamase (Penicillinase) or changes in the nature of penicillin binding proteins, 90% of strains isolated from patients or carriers are resistant to penicillin (PBPs). Infections brought on by strains of *Staphylococcus aureus* that produce -lactamases were treated with -lactam clavulanic acid (such as co-amoxiclav). While vancomycin, teicoplanin, and mupirocin are used to treat methicillin-resistant bacteria, oxacillin, cloxacillin, and nafcillin are effective against methicillin-sensitive -lactamase-producing strains (Murray *et al.,* 2003). Vancomycin is an alternative to trimethoprim/sulfamethoxazole. Trimethoprim-sulfamethoxazole is effective against staphylococcal infections that are susceptible to or resistant to methicillin. Patients with vancomycin sensitivity may utilize trimethoprim-sulfamethoxazole. To eliminate resistant *Staphylococcus aureus* disseminated by nasal carriers in an outbreak of nosocomial infections, trimethoprim is used in conjunction with rifampin (Omar *et al.,* 2014).

CHAPTER THREE
MATERIAL AND METHODS

3.1 Study area

The study area was The College of Humanities Management and Social Sciences of Mountain Top University which is located in Km-12, Lagos -Ibadan Expressway, Ogun State.

3.2 Collection of samples

Samples were obtained from door handles/knobs of the offices (College of Humanities Management and Social Sciences) in Mountain Top University. Door handles were swabbed using a sterile, cotton-tipped applicator (swab stick) moistened with normal saline. After the use of swab sticks on the door handles, each swab stick was labeled properly according to the rooms they were gotten from. The swab stick was then transported to the Microbiology laboratory of Mountain top University aseptically for identification and microbial analysis within 1-2 hours of sampling.

3.3 Materials used

Materials used include include petri-dishes, beakers, swab sticks, conical flasks, measuring cylinders, markers, 70% ethanol, marCartney bottles, Eppendorf tubes, cotton wool, test tubes (with their racks), glass slides.

3.4 Reagents and equipment used

Equipment used: Autoclave, weighing balance, distiller, wash bottles, water bath (set at 100°C), incubator (set at 37°C), Bunsen burner, oven, inoculating loop.

3.5 Media used for isolation of *Staphylococcus aureus*

Nutrient Agar, Nutrient Broth, Mannitol Salt Agar (MSA), Brain Hearth Infusion Broth (BHI), Normal Saline, Distilled water, Ethanol, Crystal violet, Iodine, Alcohol (95%), Safranin, 3% Hydrogen peroxide, blood plasma.

3.6 Sterilization

Every critical step of the bench work required thorough sanitation in order to maintain an aseptic working environment and supplies. To maintain the air surrounding the work area sterile and aseptic, the work bench area was additionally sterilized using a 70% ethanol solution applied with cotton balls. While petri dishes, beakers, flasks, scotch bottles, and McCartney bottles were sterilized in the oven at 160°C for an hour, Eppendorf tubes, micro pipette tips, and test tubes were sterilized in the autoclave at 121°C for 15 minutes.

3.7 Preparation of culture media

Selective media and differential were employed for the improvement of viability and isolation of *Staphylococcus aureus* isolates. Due to the way that these ingredients alter the metabolic systems of microorganisms, selective media contain sugars, salts, antibiotics, and dyes that only the chosen microorganism can utilize. These ingredients may also be the only sources of carbon or nitrogen, which inhibits the growth of other undesirable or screened out microorganisms as a result of their inability to grow. Additionally, differential media are those that have the capacity to distinguish or classify microorganisms according to their various morphology, growth, and appearance patterns.

3.7.1 Normal saline

It is a crystalloid fluid, normal saline. It is, by definition, an aqueous solution containing hydrophilic molecules and electrolytes. It can have different concentrations; the two that are being discussed here are 0.9% and 0.45%.

Preparation

In accordance with the manufacturer's recommendations, 0.9g of NaCl was dissolved in 100ml of distilled water and carefully mixed. The conical flask is then sealed with a piece of cotton wool that has been wrapped in aluminum foil. To fully dissolve the powder, the mixture was heated for a time. It was then autoclaved at 121°C for 15 minutes to sanitize

it. The concoction was then poured into several MarCartney bottles and set aside for later use, totaling 7ml.

3.7.2 Nutrient broth

Nutritional broth is nutrient agar without agar powder, which serves as a solidifier. They continue to be liquid at room temperature and are typically used to replenish microbial stocks. They are typically used to cultivate precise organisms.

Preparation

1L of distilled water should contain 13g of nutritional broth powder. Completely combine and dissolve them. Place them in the remaining containers (eg. conical flask). Use an autoclave to sterilize for 15 minutes at 121 °C.

3.7.3 Brain heart infusion (BHI)

A wide variety of fastidious and non-fastidious microorganisms, including aerobic and anaerobic bacteria, yeast, and molds, from a variety of clinical and non-clinical materials, can be cultured and maintained in Brain Heart Infusion (BHI) broth, a general-purpose liquid medium.

3.7.4 Mannitol salt agar:

Only the halotolerant Staphylococcus species can grow on mannitol salt agar, which is a selective medium and differential with a high concentration of sodium chloride. (Collee et al., 2010)

Preparation

According to the manufacturer's instructions (Ritcher), the dehydrated medium was dissolved in the right amount of distilled water—111g of Mannitol Salt Agar in 1000 ml distilled water—and carefully mixed in a conical flask. The conical flask is then sealed with cotton wool that has been wrapped in aluminum foil. After the mixture had been heated for a while to completely dissolve the powder, it was autoclaved at 121°C for 15 minutes to sterilize it. The medium was then aseptically put into sterile petri dishes and let to solidify after being allowed to cool to a temperature of 45 to 50 °C. The medium is colored red-phenol.

3.7.5 Nutrient agar: To obtain a pure culture for the biochemical test, nutrient agar was prepared per the manufacturer's instructions (Ritcher) for isolation and subculture from mannitol salt agar.

Preparation

According to the manufacturer's instructions, the dehydrated medium was properly mixed after being dissolved in the right amount of distilled water, which was 28g of Nutrient agar in 1000 ml of distilled water. Next, cotton wool coated in aluminum foil is used to seal the conical flask. To completely dissolve the powder, the mixture was heated for a time. It was then autoclaved at 121°C for 15 minutes to sanitize it. After allowing the medium to cool to a temperature between 45 and 50°C, it was aseptically placed into sterile petri dishes and given time to set. Light amber in color, the medium has an opalescent appearance.

3.7.6 Mueller hinton agar: According to the manufacturer's instructions (Ritcher), Mueller Hinton agar, a general-purpose medium, was made for isolation and conducting antibiotic susceptibility tests.

Preparation

According to the manufacturer's instructions, the dehydrated medium was dissolved in the right amount of distilled water—38g of Mueller-Hinton agar in 1000 ml distilled water—and carefully mixed in a conical flask. After that, cotton wool that has been wrapped in aluminum foil is used to seal the conical flask. The mixture was heated for a while to completely dissolve the powder, and it was then autoclaved at 121°C for 15 minutes to sanitize it. The medium was then aseptically put into sterile petri dishes and let to solidify after being allowed to cool to a temperature of 45 to 50 °C. The medium is light amber in hue and seems opalescent.

3.8 *Staphylococcus* species isolation

In the College of Basic and Applied Sciences at Mountain Top University, 30 sterile swab sticks were moistened by being soaked in ordinary saline before being used to swab 30 distinct door knobs and sent to the lab.

3.8.1 Primary enrichment

To suspend the microorganisms in the buffered normal saline solution, the bacteria from 20 swab sticks were carefully mixed in the lab. Using the streaking plate method, the suspension was inoculated onto Mannitol Salt Agar media and incubated at 35°C for 18 to 24 hours. For the selective isolation and counting of staphylococcus aureus from clinical and non-clinical sources, mannitol salt agar is used. On agar media containing 7.5% sodium chloride, only staphylococcus aureus grows. The isolation of plasma coagulating staphylococci is improved when 7.5% sodium chloride is added to phenol red mannitol salt agar. The distinction of staphylococcal species is aided by mannitol fermentation, as seen by a shift in the phenol red indicator. Gram staining, catalase, and coagulase tests were used in the normal laboratory procedures to validate the isolates' identities.

3.8.2 Secondary enrichment

The remaining ten swab sticks containing the samples were placed in Mar Cartney vials containing 7 ml of nutrient broth and incubated for 24 hours at 37 °C. 24 hours after incubation. The colonies on the plate were sub cultured using nutrient broth on freshly made Mannitol Salt Agar.

3.8.3 Sample preparation

After giving the sample swabs a vigorous shake to move the microbes into the nutrient broth, they were incubated for 18 to 24 hours at 35°C. It was then plated onto Mannitol Salt Agar media, and the plates were incubated for 24 hours at 35°C. *Staphylococcus* species are the only bacteria species that can grow on Mannitol salt agar selective medium due to the high sodium chloride content. This is shown by a shift in the color of red-phenol to a golden or yellowish hue, which denotes the presence of *Staphylococcus* species. *Staphylococcus aureus* is selectively isolated and counted from clinical and nonclinical sources using mannitol salt agar. On agar media containing 7.5% sodium chloride, only Staphylococci develop. The isolation of plasma coagulating staphylococci is improved when 7.5% sodium chloride is added to phenol red mannitol agar. Bacteria other than staphylococci are partially or completely inhibited by the 7.5% sodium chloride concentration. The distinction of staphylococcal species is aided by mannitol fermentation, as seen by a shift in the phenol red indicator. Standard laboratory techniques

including as colony morphology, gram staining, catalase test, and coagulase test were used to validate the isolates' identities.

3.8.4 Sub culturing

Bacterial isolates were moved or sub-cultured based on their colony morphology, shape, color, elevation, and other physical properties in order to purify and obtain pure cultures of the isolated bacterial colonies from a mixed culture to a fresh and single culture. Colonies with distinct morphological traits are placed onto newly prepared petri dishes with nutrient agar. Using the inoculating loop, a loopful of the chosen isolate was collected (the inoculating loop is heated using the Bunsen burner and allowed to cool for like 5 seconds before taking the loop from the original mixed culture and streaked onto the new petri-dish). For sub culturing, the streaking method approach is used to move the isolate-containing loop to the new petri plate.

3.9 Biochemical test for *Staphylococcus aureus*

To ensure accurate identification and characterization, these tests are performed. This was all done using the criteria from Bergey's Manual of Determinative Bacteriology and the biochemical traits of *Staphylococcus aureus*. Gram staining, coagulase, and catalase tests are among the biochemical procedures used for the isolates' identification and characterization.

3.9.1 Gram staining

This was done to distinguish the bacterial isolates based on the function of the characteristics of their cell wall structure and their staining qualities. In order to make bacterial isolates more visible and to distinguish them based on their morphology, Gram staining uses dyes. 24 Gram-positive bacteria are those that retain their crystal violet color after being exposed to alcohol, whereas Gram-negative bacteria lose their crystal violet hue but keep their counter stain (safranin) color. A smear was made by aseptically adding one to two drops of water to a sterile slide before adding a loopful of the bacterial isolate to be stained. The smear was then heat-fixed by repeatedly putting it in the Bunsen burner's flame. The slide was then flooded with crystal violet and allowed to sit for one minute. It was then rinsed with running water, adding iodine (to serve as a mordant), decolored with 70% alcohol, and then rinsed with water. The slide was then treated for

30 seconds with the counterstain, safranin. The slide was wetted, dried with blotting paper, and then rinsed with water. After applying oil immersion, the stained slide is examined under a microscope. Gram-positive cocci known as *S. aureus* appear purple and can be seen alone, in pairs, tetrads, or in sporadic clusters.

3.9.2 Catalase test

This is additionally used to distinguish between bacteria that have an enzyme (catalase). It is typical of aerobic organisms for this enzyme to catalyze the breakdown of hydrogen peroxide (H_2O) into water (H_2O) and oxygen (O_2).

$$2H_2O \rightarrow 2H_2O + O_2$$
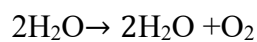
Drops of 3% hydrogen peroxide, the bacterium, and a slide with a smeared bacterial isolate were applied, and the ensuing reaction was watched. Catalase positivity indicated the presence of the enzyme catalase, whereas catalase negativity indicated the absence of the enzyme. Catalase is present in *Staphylococcus*, Micrococcus, and *Rothia species*. Catalase is not produced by the *S. aureus* subspecies *S. anaerobius* and *S. saccharolyticus*.

3.9.3 Coagulase test

The coagulase test allows *Staphylococcus aureus* to be distinguished from other staphylococci. Both bound and free coagulase are produced by *S. aureus*.

Bound coagulase (clumping factor) interacts with fibrinogen immediately after being attached to the bacterial cell wall. When a bacterial suspension and plasma are combined, the result is an alternation of fibrinogen that precipitates on the staphylococcal cell. Coagulase-reacting factor is not necessary for this.

Free coagulase includes the formation of a coagulase-CRP complex, which activates plasma coagulase-reacting factor (CRP), a modified or derived thrombin molecule. The fibrin clot is created when this complex interacts with fibrinogen. On a glass slide, an inoculating loop was used to combine a suspension of an isolate colony with a drop of human plasma. The presence of plasma will induce the bacterial cells to clot if there is bound coagulase present in the bacterial cells. The clumping will happen because the

adhesion, which makes the cells bind to fibrinogen in the plasma and causes them to cluster together visibly on the microscope slide, is the clumping factor.

3.10 Preservation of cultures

The isolates were diluted to a MacFarland standard of 2 using two milliliters of sterile brain heart infusion broth and two or three colonies. A BHI broth with 15% glycerine-containing Eppendorf tube was filled with one milliliter of the isolate. After being evenly mixed, the Eppendorf tubes were maintained in an extremely low freezer at -85°C.

3.11 Antibiotic susceptibility test

The Kirby Bauer disk diffusion method was used to test the susceptibility of various microbes. The antibiotics utilized in this trial are Cotrimoxazole (COT) 25 mg and Vancomycin (VAN) 30 mg. Cefuroxime (CRX) 10 mg, Gentamicin (GEN) 10 mg, Ciprofloxacin (CIP) 5 mg, Ampicillin (AMP) 10 mg, Cephalexin (CEX) 1.5 mg, Meropenem (MEM) 10 mg, Augmentin (AUG) 30 mg, and Tetracycline (TET) 30 mg. Five colonies of the organism were emulsified in 5 ml of sterile normal saline and thoroughly mixed. The turbidity was compared to the 0.5 Mac Farland standard. The 18–24-hour-old bacterial culture was inoculated into 5mL of ordinary saline using a sterile inoculating loop. The suspension was then distributed into already prepared Muller Hilton plates using sterile swab sticks. For each antibiotic, the zone of inhibition was measured using a meter rule in millimeters (mm) and interpreted in accordance with Clinical Laboratory Standards Institute (CLSI) 2020 guidelines after the disc was placed on the inoculated agar plates using sterile forceps and incubated at 35°C for 18–24 hours. Resistant, Intermediate, or Sensitive were used as reporting categories.

CHAPTER FOUR
RESULTS

4.1 Isolation of *staphylococcus aureus*

A total of 16 (53%) *Staphylococcus aureus* isolates were found and identified from door handles in Mountain Top University's College of Humanities, Management, and Social Sciences out of the 30 door handles collected (15 samples from First Floor, 15 samples from Ground Floor). First floor has the highest prevalence of *S. aureus* isolates, while ground floor has the lowest prevalence, according to the isolation frequency based on various sites in the research area.

The isolates were identified morphologically to have an entire margin, convex elevation, small size, round shape and an opaque transparency as shown in **Table 4.2**.

The isolates also were identified with biochemical test and results are shown in **Table 4.3** Most of the isolates fermented mannitol.

TABLE 4.1 Number of samples and percentage of isolated *Staphylococcus aureus*

S/No	Floor	No of samples	No of isolates (S. aureus) (%)
1	First	15	10 (63%)
2	Ground	15	6 (38%)
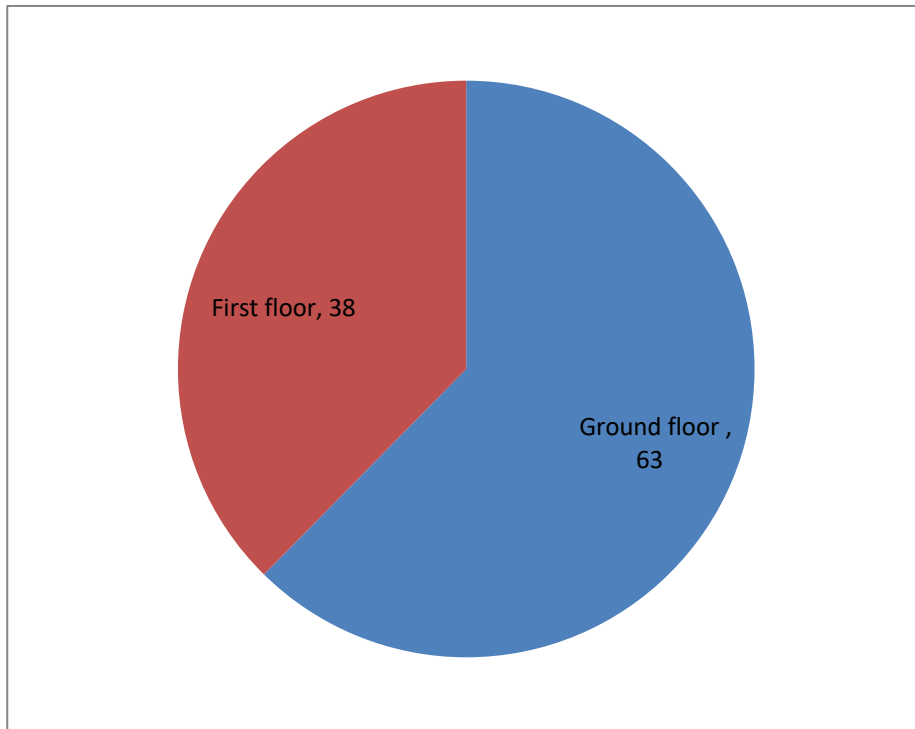
Figure4.1:   Percentage of isolate

4.2 Morphological characteristics of the isolates on mannitol salt agar

Inoculating a sample in MSA resulted in the selection of one colony, which was then sub cultured into nutritional agar to create a pure culture. Every sample colony was picked or chosen because it possessed the following characteristics: an entire margin, convex elevation, tiny size, round form, and opaque transparency. (as shown in **Table 4.2**). The vast majority of the isolates fermented mannitol.

Table 4.2Morphological characteristics of the isolates on Mannitol Salt Agar

Isolate code	Shape	Margin	Surface	Mannitol fermentation	Elevation	Color (MSA)	Transparency
BIG LT5A	Cocci	Entire	Smooth	NO	Convex	Red	Opaque
BIG LTB CHMS	Cocci	Entire	Smooth	NO	Convex	Golden yellow	Opaque
LANG LAB	Rod	Entire	Smooth	NO	Convex	Red	Opaque
BIG LT4A CHMS	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
NEWS ROOM	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
LIBRARY	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
SRC COUNCIL	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
RADIO STATION	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
PHOTO STUDIO	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
SMALL LT4	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
SMALL LT 3	Rod	Entire	Smooth	NO	Convex	Red	Opaque
TEL STUDIO	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
DEAN OFFICE	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
SMALL LT5	Rod	Entire	Smooth	NO	Convex	Red	Opaque
SRC EXE	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
GRACE OFFICE	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
POST OFFICE	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
SEC PHIL	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
MALE TOILET	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
ROOM 97	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
HOD MASS	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
ROOM 95	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
FEMALE T	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
ROOM 82	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
HOD PHIL	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
PRAD LAB	Rod	Entire	Smooth	NO	Convex	Red	Opaque
CONFRENCE ROOM	Rod	Entire	Smooth	NO	Convex	Red	Opaque
SMALL LT 1	Cocci	Entire	Smooth	YES	Convex	Golden yellow	Opaque
SMALL LT 2	Rod	Entire	Smooth	NO	Convex	Red	Opaque
BIG LT 4B	Rod	Entire	Smooth	NO	Convex	Red	Opaque

4.3 Biochemical test of the isolates on mannitol salt agar (MSA)

Table 4.3: Biochemical test of the isolates on mannitol salt agar

Isolate Code	Shape	Gram	Catalase	Coagulase
BIG LT5A	Cocci	+	+	-
BIG LTB CHMS	Cocci	+	+	-
LANG LAB CHMS	Rod	+	+	-
BIG LT4A CHMS	Cocci	+	+	+
NEWS ROOM CHMS	Cocci	+	+	+
LIBRARY	Cocci	+	+	+
SRC COUNCIL	Cocci	+	+	+
RADIO STATION	Cocci	+	+	+
PHOTO STUDIO	Cocci	+	+	+
SMALL LT4	Cocci	+	+	+
SMALL LT 3	Rod	+	+	+
TEL STUDIO	Cocci	+	+	+
DEAN OFFICE	Cocci	+	+	+
SMALL LT5	Rod	+	+	-
SRC EXE	Cocci	+	+	+
GRACE OFFICE	Cocci	+	+	+
POST OFFICE	Cocci	+	+	+
SEC PHIL	Cocci	+	+	+

MALE TOILET	Cocci	+	+	+
ROOM 97	Cocci	+	+	+
HOD MASS	Cocci	+	+	+
ROOM 95	Cocci	+	+	+
FEMALE TOILET	Cocci	+	+	+
ROOM 82	Cocci	+	+	+
HOD PHIL	Cocci	+	+	+
PRAD LAB	Rod	+	+	-
CONFERENCE ROOM	Rod	+	-	-
SMALL LT 1	Cocci	+	-	-
SMALL LT 2	Rod	+	+	-
BIG LT 4B	Rod	+	-	-

KEY

Positive is represented as +

Negative is represented as –

4.4 Antibiotic Susceptibility profile of Staphylococcus aureus isolates from door handles

Antibiotic susceptibility test was carried out on all the 16 *S. aureus* isolated from the fomites which show the proportion of *S. aureus* isolates which are considered to be susceptible, intermediate or resistant to the various antibiotics as shown in **Table 4.4** All *S. aureus* tested showed various level of resistance to Augmentin (50%), Gentamicin (18.75 %), Cefuroxime (50%), Ciprofloxacin (62.5%), Cotrimoxazole (75 %), Erythromycin (25 %), Cefotaxime (25 %), Tetracycline (81.25 %), Cephalexin (62.5%) and Meropenem (50%).

TABLE 4.4: CLSI Guideline for Interpretation of zone of inhibition for selected antibiotics to *Staphylococcus aureus*

Antibiotics	Sensitive	Intermediate	Resistant
Vancomycin (VAN)	-	-	-
Cotrimoxazole (COT)	≥16	11-15	≤10
Erythromycin (ERY)	≥23	14-22	≤13
Gentamicin (GEN)	≥15	13-14	≤12
Cefuroxime (CRX)	≥22	-	≤21
Ciprofloxacin (CIP)	≥21	16-20	≤15
Cefotaxime (CTX)	≥22	-	≤21
Augmentin (AUG)	≥22	-	≤21
Tetracycline (TET)	≥16	11-15	≤10
Cephalexin (CEX)	≥22	-	≤21
Meropenem (MEM)	≥22	-	≤21

(CLSI, 2020)

4.5 Antibiotics resistance pattern of the isolates

TABLE 4.5 Antibiotics resistance pattern of the isolates

Isolate code	COT	ERY	GEN	CRX	CIP	CTX	AUG	TET	CEX	MEM	Phenotypic resistance of isolates
GRACE OFFICE	R	S	S	S	R	R	S	R	R	S	COT-CIP-CTX-TET-CEX
POST OFFICE	R	S	S	R	R	S	R	R	R	R	COT-CRX-CIP-AUG-TET-CEX-MEM
SEC PHIL	I	S	S	S	S	S	S	R	S	S	TET
MALE TOILET	R	R	R	R	R	R	R	R	R	R	COT-ERY-GEN-CRX-CIP-CTX-AUG-TET-CEX-MEM
ROOM 97	R	R	R	R	R	I	R	R	R	R	COT-ERY-GEN-CRX-CIP-AUG-TET-CEX-MEM
HOD MASS COM	R	S	S	R	R	S	R	R	R	R	COT-CRX-CIP-AUG-TET-CEX-MEM
ROOM 95	R	I	S	S	R	S	S	R	R	S	COT-CIP-TET-CEX
FEMALE TOILET	R	R	R	R	R	R	R	R	R	R	COT-ERY-GEN-CRX-CIP-CTX-

	VAN	COT	ERY	CRX	GEN	CIP	AMP	CEX	MEM	AUG	Resistance Pattern
											AUG-TET-CEX-MEM
ROOM 82	R	S	S	R	R	S	R	R	R	R	COT-CRX-CIP-AUG-TET-CEX-MEM
HOD PHIL	I	R	I	S	I	R	S	I	I	S	ERY-CTX
LIBRARY	R	S	S	R	R	S	R	R	R	R	COT-CRX-CIP-AUG-TET-CEX-MEM
DEAN OFFICE	R	S	S	S	S	S	S	R	S	S	COT-TET
SMALL LT4	I	S	S	S	S	S	S	I	S	S	
RADIO STATION	R	I	S	R	R	S	R	R	R	R	COT-CRX-CIP-AUG-TET-CEX-MEM
TELEVISION STUDIO	I	S	S	S	S	S	S	I	S	S	
SRC EXE	R	S	S	S	S	S	S	R	S	S	COT-TET
% Resistance	75	25	18.75	50	62.5	25	50	81.25	62.5	50	

KEY: S= Sensitive I= Intermediate R= Resistant

Vancomycin (VAN) 30 µg, Cotrimoxazole (COT) 25 µg. Erythromycin (ERY) 5 µg, Cefuroxime (CRX) 10 µg, Gentamicin(GEN) 10 µg, Ciprofloxacin (CIP) 5 µg, Ampicillin (AMP) 10 µg, Cephalexin (CEX) 1.5 µg, Meropenem (MEM) 10 µg, Augmentin (AUG) 30 µg, Tetracycline (TET) 30 µg

Figure4.5: Resistance of the isolates to antibiotics
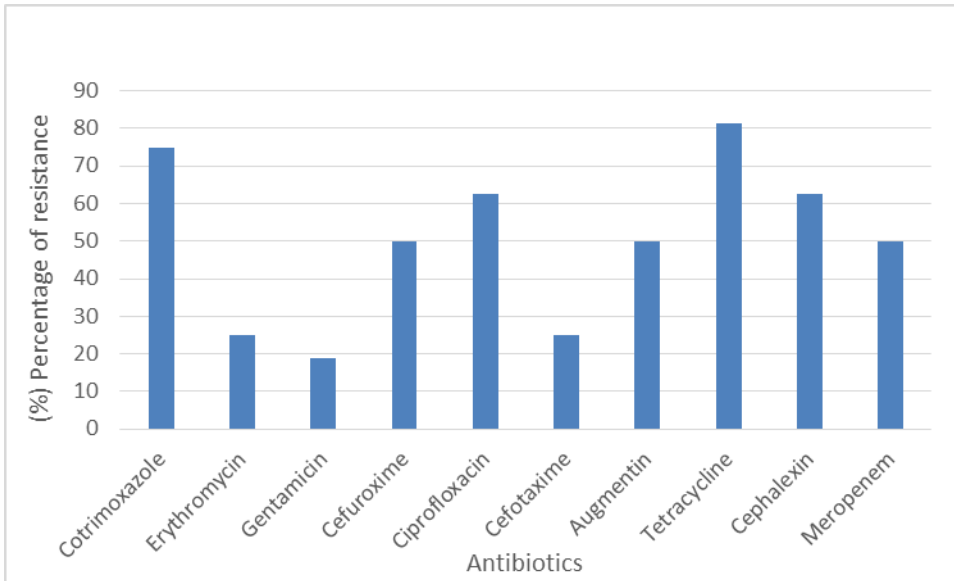
CHAPTER FIVE
DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

In the College of Humanities, Management, and Social Sciences at Mountain Top University, *Staphylococcus aureus* was found on door handles. The aim of this study was to identify it, measure its prevalence, and determine how responsive it was to routinely prescribed antibiotics.

This study found a prevalence of 53% for *S. aureus*, with a total of 16 isolates after confirmation of 11 first-floor isolates and 6 ground-floor isolates. Of the 30 door handle swab samples used in this study, *S. aureus* was recovered from 16 (or 53%) of them. When compared to the prevalence rate reported by Nworie *et al*. (2012) in Abuja Metropolis, Nigeria, they found that 156 (86.7%) out of 180 door handle swabs tested were obtained from door handles of toilets.

According to the results of the disk diffusion antimicrobial susceptibility test, 13 (75%) of the *S. aureus* isolates were resistant to cotrimoxazole, while 14 (81.25%) were resistant to tetracycline. However, only 8 (50%) isolates were resistant to Meropenem, Augmentin, and Cefuroxime, whereas 10 (62.5%) isolates were resistant to Ciprofloxacin and Cephalexin. While just four (or 25%) *S. aureus* isolates were cefotaxime and erythromycin resistant. Only 3 (18.25%) *S. aureus* isolates were found to be resistant to Gentamycin, which had the lowest percentage of resistance.

Due to their resistance to more than one of the 10 antibiotics utilized in this study, all 16 (100%) *S. aureus* isolates were determined to be multi-resistant (Table 4.5). In this investigation, isolates from the male and female restrooms were shown to be the most resistant since they were resistant to every one of the 10 antibiotics utilized (Table 4.5). This resistance profile indicated that *S. aureus* was multi-drug resistant. This investigation demonstrated that the first level had a higher number of isolates as well as the two isolates (the male and female restrooms) that displayed the greatest resistance.

5.2 Conclusion

This investigation on *Staphylococcus aureus* from door handles at Mountain Top University's College of Humanities, Management, and Social Sciences has demonstrated how door handles can act as a reservoir and a vector for the spread of pathogens during an epidemic.

Additionally, it offers Ciprofloxacin, Cotrimoxazole, Cephalexin, Oxacillin, and Tetracycline as the top antibiotics for *Staphylococcus aureus* infections linked to door handles.

In order to stop the spread of bacterial resistance, it also supports the need to encourage good hygiene habits and adherence to antibiotic therapy.

5.3 Recommendations

It is essential to use self-disinfecting door knobs, such as copper, especially in universities because they play a significant part in the transmission of many illnesses. Regular surface cleaning and disinfection is also strongly encouraged since it reduces the risk of the spread of these potentially pathogenic organisms.

More research is advised in order to confirm the findings of the current study by focusing on the peak period of movement that will minimize bacterial contamination and using various culture media.

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