CHARACTERIZATION OF *Staphylococcus aureus* ISOLATED FROM DOOR HANDLES IN NEW DANIEL HALL, MOUNTAIN TOP UNIVERSITY

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DECLARATION

I hereby declare that this project under the supervision of Dr. (Mrs.) E.D. OLALEYE is a product of my own research work. Information derived from various sources has been properly recognized in the text and a list of references has been provided. This project has not been previously presented anywhere for the award of any degree or certificate.

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DATE

CERTIFICATION

This is to certify that the content of this project entitled '**CHARACTERIZATION OF *Staphylococcus aureus* ISOLATED FROM DOOR HANDLES IN NEW DANIEL HALL MOUNTAIN TOP UNIVERSITY'** was prepared and submitted by **ABIDOYE, ISRAEL OLUWAGBEMIGA** with matriculation number **18010101021** in partial fulfillment of the requirement for the degree **of BACHELOR OF SCIENCE (B.Sc.) IN MICROBIOLOGY**. The original research work was carried out by him under my supervision and is hereby accepted.

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DR (Mrs.) E.D. OLALEYE
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DR (Mrs.) C.I. AYOLABI
(Head of department)

DEDICATION

I dedicate this to the almighty God for the successful completion and I also dedicate it to my parents (MR. and MRS. ABIDOYE) who have supported me spiritually, physically, and financially.

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My utmost gratitude goes to the Almighty God, who in his infinite mercies inspired the conception of this project and also made it possible to be a great success.

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TABLE OF CONTENTS

LIST OF TABLES

PAGES

LIST OF FIGURES

ABSTRACT

Staphylococcus aureus is a commensal bacterium and a major human pathogen that causes a wide variety of clinical manifestations. Infections are common both in the community as well as hospital settings and treatment remain challenging to manage due to the emergence of multi-drug resistant strains. An additional challenging feature of the *Staphylococcus aureus* bacterium is its rapid dissemination to humans and through door handles. This research was done in order to investigate the incidence and prevalence of *Staphylococcus aureus* on door handles and the antibiotic susceptibility profile of the organism to a few popular antibiotics in New Daniel Hall, Mountain Top University. A total of 30 door swab samples were obtained, 15 from wing A and 15 from wing B. These were cultured on mannitol salt agar and identified using appropriate protocols. A total of 12 (40%) isolates were identified as *Staphylococcus aureus* as 7 (46.6%) was isolated from wing A and 5 (33.3%) was isolated from wing B. They were all subjected to antibiotic susceptibility test using the Kirby Bauer antibiotic disk susceptibility method on Mueller Hinton agar. An examination of the susceptibility to antibiotics showed Gentamicin, Erythromycin and Cefotaxime were the most powerful of all the 10 antibiotics used against the *Staphylococcus aureus* isolated. It was discovered that 12 (40%) of the multidrug resistant *S. aureus* isolates produced beta lactamase enzymes that are linked to resistance to beta lactam drugs. High resistance was evident in the isolates. to Cotrimoxazole (75%) and Tetracycline (75%), making them the least effective antibiotic to the isolates. The presence of multi -drug resistant *S. aureus* in this study emphasizes the need to formulate hygiene measures to prevent possible dissemination of *S. aureus* and other transmissible pathogens to students and staffs in the schools.

Keywords: Bacteria, Door Handles, Antibiotics Susceptibility, Beta-lactamase, Kirby Bauer antibiotic dis

CHAPTER ONE

1 INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Microorganisms are present everywhere and play a significant role in the ecology. They can exist freely or as parasites in these conditions. Sometimes they exist as temporary contaminants in hands or fomites, where they pose a serious health danger by spreading infections that can be acquired in the community or in hospitals. Some bacteria have been discovered to have a connection with environmental surfaces like door handles, toilet surfaces etc. *Staphylococcus aureus* a common micro-organism is a major and leading cause of human bacterial infections due to it wide range of habitat. It became a public health concern when scientist discovered it is easily transmitted either from in animate object or animals (Taylor & Unakal, 2021). Certain harmful bacteria found in the environment can spread more easily to human hands due to poor personal hygiene (Browne *et al.,* 2008). According to studies, environmental surfaces that are frequently touched 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. 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. Infections and/or diseases gotten by contact with environmental surfaces are common cold and sores, conjunctivitis, giardiasis, diarrhea, impetigo, meningitis, pneumonia etc. These diseases are caused by a myriad of bacterial organisms. (Krautkramer *et al.,* 2021).

Staphylococcus aureus is a gram-positive bacterium that is mainly coagulase positive and catalase positive. This gram-positive coccus is differentiated from other *staphylococcus* species due to it ability to coagulate. The bacterium has a variety of habitat including environmental surfaces like classroom and toilet door handles, tissue and blood stream., in nasal nares of domestic animals like cats, dogs and on human body surfaces as a part of normal microflora (Otto, 2014). A significant human pathogen that causes a wide

variety of clinical infections is *staphylococcus aureus.* It is the leading cause of endocarditis as wells as skin and soft tissue, osteoarticular, pleuropulmonary and device related infection (Peton & Le Loir, 2014). Infections with *Staphylococcus aureus* are frequent in both community-acquired settings and hospital-acquired settings. It is both a commensal bacterium and a human pathogen. *S. aureus* colonizes about 30% of the population of people. (Tong *et al*., 2015). Little is known about the reservoirs or contexts through which community members are exposed to *S. aureus,* with the exception of breakouts in high-risk settings. While various studies conducted in hospitals have shown the role of *S. aureus* nasal colonization as a risk factor for subsequent infection with the colonizing strain, the role of nasal colonization in the community is less well established. To date community-based studies have yielded conflicting results on the role of *S. aureus* nasal colonization as a risk element for infection in the future (Fluit, 2012). Moreover, community-based research has not consistently examined the relationship between potential *S. aureus* risk factors and severe skin infection, and instead frequently depends on *S. aureus* nasal colonization as a stand-in for infection. Incomplete or inadequate hygiene practices and a high incidence of *S. aureus* are the two main environmental risk factors that contribute to S. aureus transmission in the hospital setting. Two different lines of investigation are established if these factors are taken into account in the context of the community. The first topic deals with hygiene-related issues and the kinds of social contexts—like the home—where hygienic practices may help spread *S. aureus*. The presence of *S. aureus* in a specific population or environment that might serve as an identifiable reservoir is the subject of the second question. (Tong *et al.,* 2015).

A variety of virulence factors that the Gram-positive bacteria possesses help the host become infected. The organism's capacity to develop resistance to several antibiotic classes is well documented. The bacterium was a significant nosocomial pathogen at first, and then epidemiologically separate clones started to appear in the population. S. aureus expresses a variety of virulence factors that promote tissue adhesion and aid in the establishment of infection, tissue invasion and evasion from host immune response (Kobayashi *et al.,*2015) *S. aureus* is a tough pathogen to treat due to its capacity to develop resistance to a variety of antibiotic classes. Beta-lactam antibiotics are the first line treatment for staphylococcal infections. For many years, vancomycin was the antibiotic of choice, but the advent of resistance put a question mark on its usefulness.

The emergence of antibiotic resistance has emerged as a major global problem, *and S. aureus* has developed resistance due to the selection pressure anti-microbials place on it. The resistance is mediated by chromosomes or plasmids, and transduction, transformation, and conjugation are blamed for it. (Guillet *et al.*, 2013).

1.2 JUSTIFICATION FOR THE STUDY

Staphylococcus aureus, a commensal bacterium, is present on the skin. and also be transmitted by the hands. However, this bacterium can also be pathogenic when it comes in contact with the blood stream, organs of the body, making it a threat to public health. It is established that *Staphylococcus aureus* produces multiple virulence factors in abundance and is the causative agent of diseases like endocarditis, septic arthritis and toxic shock syndrome. Unfortunately, it has also acquired resistance, overtime, to different antibiotic classes making *S. aureus* infection treatment difficult. This is a significant worry as fomites (particularly door handles) can also be a major vector in dispersion of this bacteria. Therefore, there is a need to carry out studies on the prevalence and profiling of antibiotic sensitivity of this organism in areas where spread is highly likely, such as students' hostels as will be done in this study.

1.3 AIM OF THE STUDY

The study is aimed at investigating the incidence and prevalence of *Staphylococcus aureus* on door handles in the New Daniel Hall in Mountain Top University Ogun state. The study further aims to determine the antibiotic sensitivity profiles of the isolated strain of S. aureus from the door knobs.

1.4 OBJECTIVES OF THE STUDY

- To detect the presence of *Staphylococcus aureus* on door knobs
- To determine the susceptibility profile of the isolates to selected antibiotics.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 *Staphylococcus aureus*

Microorganisms belonging to the *Staphylococcus* species are Gram-positive, non-motile, and do not generate spores. The most virulent strain of this species, S. aureus, is capable of inflicting a variety of illnesses both in the general population and in medical facilities (Otto, 2019). According to taxonomy, *Staphylococcus aureus* is a member of the genus Staphylococcus of the bacterial family Staphylococcaceae, which also includes the five genera Macrococcus, Nosocomiioccus, Salinicoccus, and Jeotgalicoccus Gomella. Currently, 53 different *staphylococci* species have been identified. S. aureus, S. epidermidis, and S. saprophyticus are the staphylococci most frequently linked to human infection. Human infection may potentially be linked to other staphylococcus species (PHE, 2020).

2.2 CHARACTERISTIC, ISOLATION & IDENTIFICATION OF *Staphylococcus aureus*

After examining a direct gram stain, staphylococci are initially assumed to be present. By strewing material from the specimen into either blood agar or brain heart infusion agar, the organism is isolated. The halo-tolerant staphylococci can thrive on specimens that are contaminated with other bacteria when they are cultivated on mannitol salt agar with 7.5% sodium chloride (NACL) (Anetter *et al*., 2010). The staphylococcal genome consists of a circular chromosome (of about 2800 bp), prophages, plasmids, and transposons. On the first chromosome and in the extrachromosomal components are the genes that control virulence and antibiotic resistance. These genes are spread via extrachromosomal elements between staphylococcal strains, species, or other gram-positive bacterial species. Peptidoglycan makes up 50% of the *staphylococcal* cell wall by weight (Wendy *et al*., 2010). The N-acetylglucosamine and N-acetylmuramic acid polysaccharide subunits of peptidoglycan alternate with 1,4-b connections. A Gram stain of the colony and tests for catalase and coagulase production are ideal because they enable fast identification of the *S. aureus* that is coagulase-positive. The manufacture of thermostable deoxyribonuclease is another excellent method for testing for S aureus. It may be

validated by looking for clumping with immunoglobulin G and fibrinogen-coated latex particles that bind protein A and clumping factor, respectively, on the bacterial cell surface. Commercial suppliers have these available (e.g., Staphaurex). The most recent latex test (Pastaurex) includes monoclonal antibodies to serotype 5 and 8 capsular polysaccharides to lower the amount of false negatives. (Some recent clinical isolates of S aureus fail to produce coagulase or clumping factor, making identification challenging.) (Dawn & Devlynne, 2018) The identification of S epidermidis and other *Staphylococcus* species is now done with the aid of commercial biotype identification kits, such as API Staph Ident, API Staph-Trac, Vitek GPI Card, and Microscan Pos Combo. Premade strips with test substrates make up these (Medical Microbiology, 4th edition 1996).

2.2.1 Microscopic Morphology

The spherical *Staphylococcus* aureus organisms are known as cocci and are gram-positive (gram stain gives them a purple color). When examined under a light microscope following the gram stain, they frequently form clusters that resemble a bunch of grapes as opposed to *streptococci*, which form chains. The cells' diameter varies from 0.5 to 10 m. Despite occasionally seeming to appear in short chains, they can also be seen in pairs. Cocci's structure aids in separating *staphylococci* from *streptococci* (Holland & Fowler, 2014).

2.2.2 General Cultural and Biochemical Characteristics

An aerobic and anaerobic (Facultative) bacterium, *Staphylococcus aureus* grows at temperatures ranging from 18 C to 40 C. These may thrive on conditions with up to 10% salt, and the colonies are frequently golden or yellow (aureus means golden or yellow). It's always known as the "golden staph" (Arumugam et al., 2017). On nutrient agar media, it can also form a white colony; the organism produces carotenoids, which give the colonies their characteristic yellow color. Because *Staphylococcus aureus* produces all four types of hemolysins, it regularly hemolyzes on blood agar (alpha, beta, gamma and delta). The organism can thrive on mannitol-salt agar medium with 7.5% sodium chloride since it is salt tolerant (Jenul & Horswill, 2019). All strains of *Staphylococcus aureus* are catalase-positive, non-motile, and sporing. Under aerobic circumstances, they expand quickly and prodigiously. They show on blood agar as glossy, uniform, elevated,

translucent colonies that frequently have a golden tint. After a 24-hour incubation period, the colonies are 2-3 mm in diameter, and many of the strains are -hemolytic. After three days of incubation, colonies can grow up to 6 to 8 mm in diameter (Smith & Wardyn, 2015).

2.2.2.1 Catalase

A typical enzyme called catalase catalyzes the conversion of hydrogen peroxide into water and oxygen. The catalase test is crucial for differentiating between *staphylococci* that generate bubbles when exposed to hydrogen peroxide, which indicates positive *staphylococci*, and *streptococci* that are catalase negative (Smith, 2011). Agar slants or broth cultures are flooded with several drops of 3% hydrogen peroxide to conduct the test. At once, catalase positive culture bubble.

2.2.2.2 Coagulase

The *staphylococcus aureus* has this enzyme, which causes coagulation. A protein enzyme called coagulase is necessary for the transformation of fibrinogen into fibrin. It separates *staphylococcus aureus* from *staphylococcus saprophyticus* and *staphylococcus* epidermidis (Anetter *et al*., 2010).

2.3 EPIDEMIOLOGY OF *Staphylococcus aureus* INFECTION

Staphylococcus aureus, a common bacterium that colonizes the upper respiratory, gastrointestinal, and urogenital tracts of 20% to 30% of humans and serves as a long-term carrier, is a significant contributor to hospital- and community-acquired infections. *Staphylococcus aureus* is still a dangerous infection for humans more than a century after it was first described. Up to 50% of adults are thought to be colonized, and 15% of people have staphylococcus aureus in their anterior nares permanently (Tadayuki *et al*., 2010). Hospitalized patients, frequent needle users, immunocompromised patients, and health care personnel typically have higher rates of *staphylococcus aureus* colonization (up to 80%). (Tong *et al*., 2015). *Staphylococcus aureus* can be spread directly by contact with a fomite or from one person to another. The majority of nosocomial infections are contracted by contact with an infected patient or through the hands of a healthcare worker who has already been colonized by *staphylococci* from their own reservoir. Despite

advancements and improvements in the infection's treatment, there is still a high rate of morbidity and mortality in both hospitals and the general population (Rasigade, Dumitrescu & Lina 2014). Since the emergence of CA-MRSA (community-associated methicillin-resistant staphylococcus aureus), which is now a colonizing strain resistant to both methicillin and vancomycin, the clinical epidemiology of staphylococcus aureus infection has changed (Chuang *et al*., 2013).

In the past 20 years, there has been an increase in both the number of community- and hospital-acquired *staphylococcal* infections. This pattern coincides with the rise in intravascular device usage. According to data from the National Nosocomial Infections Surveillance system of the Centers for Disease Control and Prevention, *S. aureus* was the most frequent cause of nosocomial cases of pneumonia and surgical-wound infections between 1990 and 1992, and the second most frequent cause (behind coagulase-negative staphylococci) of nosocomial bloodstream infections (CDC) (Franklin 1998). The advent of vancomycin resistant *staphylococcus aureus*, which was found in Michigan in 2002, has made the treatment of *staphylococcus aureus* infection more challenging. Vancomycin has emerged as the preferred medication for the management of severe MRSA infections since the 1980s. There are currently just four vancomycin-resistant *Staphylococcus aureus* (VRSA) isolates from the USA (Chang et al., 2009). A van gene-mediated VRSA from Asia has not yet been reported, with the exception of vancomycin-intermediate *S. aureus* (VISA) in Japan and Korea (CDC, 2005). (Kim et al., 2004). Van gene-negative VRSA has recently been discovered by Tiwari & Sen (2006). Skin, heart-valve, blood, and bone infections are only a few of the suppurative disorders in which *Staphylococcus* can infect humans (Morse, 1980). Penicillin resistance affects more than 90% of *Staphylococcus* strains, with increased resistance to methicillin, aminoglycosides, macrolides, and lincosamide following (Chambers, 2001). (Dickgiesser & Kreiswirth 2009). Due to this antibiotic resistance, vancomycin has historically considered a last-resort treatment. This study only included incidence rates for community-acquired SAB, despite the fact that the overall incidence of SAB (*Staphylococcus aureus* bacteremia) from 2004 to 2010 in northeast Thailand was 2.5 per 100,000 person-years. This low reported incidence may potentially have been caused by insufficient case ascertainment. In comparison, the frequencies of SAB were 27 per 100,000 person-years in Kilifi, Kenya,

and 48 per 100,000 person-years in the Manhica District, Mozambique, among children under the age of 15.

2.4 PATHOGENESIS OF *Staphylococcus aureus*

Staphylococcus aureus infection is one of the most prevalent bacterial infections in people. Infections in humans such impetigo, gastroenteritis, endocarditis, furuncles, scalded skin syndrome, soft tissue infections, urinary tract infections, etc. are also caused by it (Tong et al., 2015). *S. aureus* clinical infections can be classified into two based on origin of infection which are

1. Hospital infection
2. Community infection

Clinical infection symptoms, antibiotic susceptibility, and the genetic make-up of the staphylococcus aureus strain causing the infection are all different between these two types. Staphylococcus aureus has been a nosocomial pathogen (a pathogen seen in hospitals) for a very long time. It has a very high rate of death and morbidity. According to a recent discovery, staphylococcus aureus infections in the community are increasing and have a resistant variety, which is a serious threat to public health (Fluit, 2012). Depending on the strain present at the infection site, these bacteria can cause invasion and toxin-mediated illness.

Depending on the strain present at the infection site, these bacteria can cause invasion and toxin-mediated illness. Depending on the infection type that *S. aureus* causes, different pathogenic physiologies exist. It has a few virulence-enhancing enzymes, such as hyaluronidase, protease, lipase, and nuclease, which facilitate bacterial penetration (Kobayashi *et al*., 2015).

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 established its pathogenicity throughout time by expressing a large number of extracellular and cell surface-associated proteins that have the potential to be virulence factors. For the majority of the illnesses brought on by *staphylococcus aureus*, the pathogenesis is complex. This makes it more difficult to ascertain the precise function of any particular factor and more complicated (Arumugam *et al*., 2017). Although there are commonalities in the expression of particular components and strains obtained from distinct disorders, demonstrating their relevance in pathogenesis *Staphylococcus aureus* pathogenesis increased dramatically as a result of the introduction of various resistant strains. The virulence of staphylococcus has increased as a result of its capacity to acquire various resistance genes. *Staphylococcus aureus* from hospitals and the general public have developed genes for resistance to various antibiotics. (Holland *et al*., 2014). In addition, *Staphylococcus aureus* can be categorized according to its antibiotic resistance, such as HA-MRSA (hospital-acquired methicillin-resistant S. aureus) and CA-MRSA (community-acquired methicillin-resistant *S. aureus*). The infections caused by *Staphylococcus aureus* can range from minor skin issues to fatal conditions. They proliferate in food and produce toxins that are harmful to humans (Scott *et al.*, 2015).

2.4.1 Virulence Factors

The virulence factors exhibited by *Staphylococcus aureus* are numerous and include toxins (leukocidins and hemolysins), immunological evasive surface factors (capsule and protein A), and tissue invasion-promoting enzymes (hyaluronidase) (Arumugam *et al*., 2017). Because successful lineages frequently differ from their ancestors at several loci, it can be difficult to assess the success of strains from dominant clonal complexes. S. aureus has a variety of virulence factors at its disposal. These elements allow the organism to be successful as a pathogen that causes a variety of diseases in both humans and animals (Powers & Wardenburg, 2014). Virulence factors aid in tissue invasion, sepsis, inducing toxin-mediated syndromes, and breaking down the host immunological shield when attached to host cells. *S. aureus* is able to stay in the bloodstream, seed deep tissues, and create secondary foci of infection because to its capacity to up-regulate virulence factors in response to stressful stimuli (such as the host immune response or circulating

antibiotics) (Lacey *et al*., 2016) *S. aureus* strains are adept in attaching to and colonizing the skin and mucosa of the nares, gaining access to the bloodstream, dodging host immune reactions, forming protective biofilms, and becoming resistant to a number of medications. Because of this, *S. aureus* is a very successful and becoming more and more clinically significant gram-positive pathogen, despite the availability of numerous medicines with activity against wild-type strains. (Zecconi & Scali, 2013)

2.4.1.1 Adhesion

The pathogen must enter the host and connect to host cells or tissues in order to start an infection. *S. aureus* has the ability to up-regulate a number of virulence factors, allowing it to attach to and colonize the surfaces of implanted devices or prostheses, injured skin, and nares, as well as to cause significant bloodstream infections. This is accomplished by using a polymer on the surface of *S. aureus* called teichoic acid (Foste *et al*., 2014).

Microbiological Surface Elements Cell surface proteins called Recognizing Adhesive Matrix Molecules (MSCRAMM) interact with host molecules such collagen, fibronectin, and fibrinogen to promote tissue adhesion. *S aureus* cells produce proteins on their surface that aid in host protein attachment, including as laminin and fibronectin, which are found in the extracellular matrix.

2.4.1.2 Invasion

By releasing exfoliative toxins, hemolysins (including alpha-hemolysin [alpha toxin], which creates pores in skin cell membranes), and different tissue-destructing enzymes, *Staphylococcus aureus* can compromise the skin barrier. When the immune system is weak, there is a physical integument rupture, and/or there is localized inflammation, invasion may be initiated (Foster *et al*., 2014).

1. Proteases

2. lipases,

3. Nucleases

4. Hyaluronatelyase,

5. Phospholipase C,

6. Metalloproteases (elastase),

7. Staphylokinase

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

2.4.1.3 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 (Otto, 2014). A variety of enterotoxins, which are strong gastrointestinal exotoxins, are produced by *Staphylococcus aureus*. *Staphylococcal* food poisoning is an intoxication that happens after eating food that has enough enterotoxins already in it (Oliviera *et al*., 2018).

2.4.1.4 Antibiotic resistance

Antibiotic strains of *S. aureus* have been resistant to methicillin, vancomycin, cephalosporins, penicillin, and linezolid. Penicillin-resistant *S. aureus* strains have the mec gene, which encodes penicillin binding protein 2a, and the fem gene, which confers resistance to methicillin, penicillinase-resistant penicillins, and cephalosporins. *S. aureus* counteracts the effects of penicillin by generating b-lactamase (Shenoy *et al*., 2019). While reduced vancomycin susceptibility in vancomycin-intermediate and heteroresistant vancomycin-intermediate *S. aureus* has been linked to a different mechanism: mutations in structural or regulatory genes associated with the accessory gene regulator pathway, true vancomycin resistance in S. aureus appears to depend on acquisition of the vanA gene (Yang *et al*., 2018).

2.5 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 (Broker *et al*., 2016). 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 molecules, vascular cell adhesion molecule 1 (CD106), and intercellular adhesion molecule 1 (CD54), which likely contribute to this process (Rose *et al*., 2012). Despite having a deficiency in leukocyte migration that increases mortality, genetically altered mice lacking intercellular adhesion molecule 1 also experience less severe *staphylococcal* infections than normal mice, possibly as a result of less leukocyte-mediated damage (Parcina *et al*., 2013). In vitro, phagocytosis is made easier by the presence of an opsonizing antibody that is directed against a capsule, peptidoglycan, or complement. Since the titre of anti-staphylococcal antibodies is not associated with immunity to infection, with the exception of toxic shock syndrome, where the presence of antitoxic shock syndrome toxin 1 is protective, the function of antibodies in vivo is less certain. Which *staphylococcal* components can cause protection from recurrent infection is unknown at this time (Franklin &Lowry, 2016).

2.6 CLINICAL MANIFESTATION OF *Staphylococcus aureus* INFECTION

Staphylococcus aureus is notorious for causing boils, furuncles, styes, impetigo and other superficial skin infections in humans. It may also cause more serious infections, particularly in persons debilitated by chronic illness, traumatic injury, burns, or immunosuppression. These infections include pneumonia, deep abscesses, osteomyelitis, endocarditis, phlebitis, mastitis, and meningitis, and are often associated with hospitalized patients rather than healthy individuals in the community (Taylor& Unakal, 2021). *S aureus* is a common cause of infections associated with indwelling devices such as joint prostheses, cardiovascular devices, and artificial heart valves. *Staphylococcus aureus*

infections can range from minor skin problems to life-threatening illnesses. For example, endocarditis, a serious infection of the inner lining of your heart (endocardium) can be caused by *S. aureus* bacteria. Signs and symptoms of staph infections vary widely, depending on the location and severity of the infection (Tong *et al.,* 2015).

2.6.1 Skin infections

The following skin conditions are brought on by S. aureus infections: boils, impetigo, cellulitis, and staphylococcal scalded skin syndrome.

2.6.2 Food poisoning

One of the most typical causes of food poisoning is Staphylococcus aureus. Food is a breeding ground for microorganisms that create poisons that can make you sick. Within hours of consuming a contaminated food, symptoms typically manifest swiftly. Additionally, symptoms may only endure for a half-day before disappearing entirely.

2.6.3 Bacteremia

Bacteremia, sometimes referred to as bloodstream infection, develops when Staphylococcus aureus germs get into the bloodstream. The symptoms of bacteremia include fever and low blood pressure. Infections that affect internal organs, such as the brain (meningitis), heart (endocarditis), or lungs (pneumonia), bones and muscles, as well as medically implanted devices like artificial joints or cardiac pacemakers, can be brought on by bacteria that can travel to deep inside your body.

2.6.4 Toxic shock syndrome

Toxins produced by *Staphylococcus aureus* are the cause of this potentially fatal illness. Certain kinds of tampons, skin wounds, and surgery have all been connected to the illness. High temperature, nausea, and vomiting, as well as muscle aches, are typical abrupt symptoms.

2.6.5 Septic arthritis

An infection with *Staphylococcus aureus* is frequently the cause of septic arthritis. The bacteria frequently target the fingers or toes, shoulders, hips, and knees. Infected artificial

joints are another possibility. Fever, severe joint discomfort, and joint swelling are a few possible signs and symptoms.

2.6.6 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. *S. aureus* endocarditis is distinguished from less virulent pathogen-caused endocarditis 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 (Asgiersson *et al*., 2018).

2.7 ANTIMICROBIAL DRUGS

Staphylococcus aureus has demonstrated a remarkable capacity for adaptation ever since the first use of penicillin. Within a short period of their debut, new medications have developed resistance. Some strains are currently immune to the majority of common antibiotics. The lack of novel antibiotics that appear to be in development is concerning. Any new innovations have only altered already-available medications (Foster 2012). The pharmaceutical industry initially screened synthetic chemicals and natural materials for antimicrobial activity in order to find antimicrobial medications. Then, it was looked into how something works.

In order to find the next generation of antibiotics, new strategies are being used. Based on understanding of bacterial physiology and metabolism, potential targets are found, such as enzymes involved in a crucial process (for example, cell division). Then, screening techniques are created to find target molecule inhibitors. Additionally, specialized inhibitors can be created with a thorough understanding of the target molecule's chemical structure (Arumugam *et al*., 2017).

Drugs used to treat staphylococcus aureus infections are categorized according to their chemical makeup;

- Beta-lactams (Penicillin and its derivatives)
- Glycopeptides e.g. (Vancomycin)

- Quinolones (Gemifloxacin)
- Oxazolidinone (Linezolid and Tedizolid)
- Aminoglycoside
- Tetracycline

2.7.1 Beta-lactams

The beta-lactam drugs are classified based on the presence of beta-lactam rings in their structure. As a result of this, the following drugs have been classified as a beta-lactam.

- Penicillin
- Monobactam
- Cephalosporin
- Carbapenem
- Aztreonam

2.7.1.1 Penicillin

Penicillin G, the first beta-lactamase antibiotic, was created in 1928 by Alexander Fleming, and it was used as a chemotherapeutic agent on people in 1941. Depending on the experimental circumstances, penicillin has bacteriostatic or bactericidal effects. Until 99% of the organisms are eliminated, the number of organisms declines steadily. Penicillin-class medications function by subtly rupturing bacterial cell walls. They accomplish this by directly interacting with peptidoglycans, which are crucial for the structural integrity of bacterial cells (Bud, 2007). Peptidoglycans build a mesh-like structure surrounding the plasma membrane of bacterial cells, strengthening the cell walls and obstructing the entry of external fluids and particles. Small holes appear in a

bacterium's cell walls as the cells split during growth. The walls are then rebuilt by filling these holes with newly generated peptidoglycans. The protein struts that hold the peptidoglycans together are blocked by penicillins (Shenoy *et al*., 2019). As a result, the bacterium is unable to seal the openings in its cell walls. Water rushes through the perforations into the cell and the bacteria explodes because the water content in the surrounding fluid is higher than that inside the bacterium (Sschito, 2006).

2.7.1.2 Methicillin

The first semi-synthetic penicillinase-resistant penicillin was methicillin. Due to the high prevalence of interstitial nephritis linked to its use, it has been taken off the market in the United States. It is given intravenously or intramuscularly to treat gram-positive aerobic cocci. Methicillin is active against numerous penicillinase-producing strains of Staphylococcus aureus and is resistant to most staphylococcal penicillinases (Sharon, 2007). Methicillin works by preventing the bacterial cells from being made. It prevents the cross-linking of the linear peptidoglycan polymer chains, which constitute a significant portion of the gram-positive bacteria's cell wall. It binds to PBP to accomplish this (Yang et al., 2018).

2.7.1.3 Oxacillin

Oxacillin is a penicillin-class narrow-spectrum beta-lactam antibiotic created by Beecham. It was granted a patent in 1960 and given medical use authorization in 1962. A penicillinase-resistant beta-lactam is oxacillin. It shares similarities with methicillin and has taken its position in clinical settings. Nafcillin, cloxacillin, dicloxacillin, and flucloxacillin are further related substances (Hryniewicz & Garbacz, 2017). It is frequently used clinically in the US to treat Staphylococcus aureus that is penicillin-resistant since it is resistant to penicillinase enzymes like those produced by that organism. Oxacillin, on the other hand, covalently binds to penicillin-binding proteins, which are enzymes involved in the formation of the bacterial cell wall, after the discovery and widespread usage of both methicillin and oxacillin. This binding contact prevents the transpeptidation reaction from occurring and prevents the production of peptidoglycan, a crucial part of the cell wall. It is believed that oxacillin and other penicillins destroy

actively growing bacteria by cell autolysis by weakening the bacterial cell wall (Jenkins *et al*., 2017).

2.7.1.4 Cephalosporin

The fungus Acremonium, formerly known as Cephalosporium, is the source of the antibiotic class known as cephalosporins. Cephalosporins can be used to prevent infections and treat them when they are brought on by bacteria that are responsive to this specific class of antibiotic. Gram-positive bacteria like Staphylococcus and Streptococcus are the main targets of first-generation cephalosporins. They are consequently mostly utilized for treating skin and soft tissue infections as well as preventing surgical infections developed while in the hospital (Arumugham, *et al*., 2019). Due to the distinct -lactam antibiotic structure, the antibiotic may be utilized for people who are allergic to penicillin. The medicine can be removed from the body through the urine. Similar to penicillin, cephalosporin works by inhibiting the penicillin-sensitive enzymes (transpeptidases, carboxypeptidases) that are in charge of creating the final, rigid, three-dimensional structure of the bacteria cell wall. This prevents the formation of bacteria cell walls made of peptidoglycans (Purnima, 2017). Cephalexin, second-generation cefuroxime, and third-generation cefotaxime are examples of first generation cephalosporins.

2.7.2 Glycopeptides (Vancomycin)

A glycopeptide antibiotic called vancomycin is prescribed to treat several bacterial infections. Methicillin-resistant Staphylococcus aureus complex skin infections, bloodstream infections, endocarditis, bone and joint infections, and meningitis are all indicated to be treated with it intravenously (Marsot *et al*., 2012). The ideal dose can be determined by measuring blood levels. Gram-positive bacterial infections that are serious, life-threatening, and resistant to other antibiotics can be treated with vancomycin. Vancomycin works by preventing Gram-positive bacteria from properly synthesizing cell walls (Patel *et al*., 2021).

2.7.3 Quinolones

Levofloxacin is an example of a third-generation quinolone. Moxifloxacin and Gemifloxacin are examples of a fourth-generation quinolone. Both showed better and

increased action against gram positive bacteria. Quinolones prevent bacterial topoisomerases (topoisomerase IV and DNA Gyrase), which are necessary for releasing DNA supercoiling and separating concatenated DNA strands, from doing their antibacterial work (Scott *et al*., 2012). In S. aureus, quinolone resistance develops gradually as a result of point mutations, primarily in the GrlA subunit of topoisomerase IV and the GyrA subunit of Gyrase. S. aureus can develop quinolone resistance through the development of Nor A efflux pumps, which is another route.

2.7.4 Aminoglycoside (Gentamicin)

The antibiotic class known as aminoglycosides includes gentamicin. It acts by preventing bacterial development. Gentamicin is a bactericidal antibiotic that inhibits protein synthesis by binding to the 30S subunit of bacterial ribosomes. It is well acknowledged that the main mechanism of action involves impairing the ribosome's capacity to distinguish between appropriate transfer RNA and messenger RNA connections (Randjelovic *et al.,* 2017).

2.7.5 Oxazolidinone (Linezolid)

The oxazolidinone class of drugs includes the antibiotic linezolid, which is used to treat infections brought on by Gram-positive bacteria that are resistant to other antibiotics. Linezolid functions as a protein synthesis inhibitor by reducing bacterial protein synthesis. This either halts bacterial growth or causes bacterial death (Ruiz *et al*., 2012). Although many antibiotics function in a similar manner, linezolid's precise mode of action appears to be distinct in that it prevents protein creation from beginning in the first place rather than at a later stage. Bacterial resistance to linezolid has remained minimal as of 2014. The drug class known as oxazolidinone includes linezolid (Kang *et al*., 2020). Midway through the 1990s, linezolid was found, and it was given the go-ahead for commercial use in 2000. Linezolid should not be used against bacteria that are sensitive to medications with a narrower spectrum of activity, such as penicillins and cephalosporins. Its major usage is the treatment of severe infections brought on by aerobic Gram-positive bacteria that are resistant to other antibiotics. Linezolid has been referred to as a "reserve antibiotic"—a medication that should only be taken as a last resort in

cases of potentially incurable infection—in both the public press and scholarly research (Kelesidis *et al*., 2020).

2.8 *Staphylococcus aureus* RESISTANCE TO ANTIMICROBIAL DRUGS

The primary method of staphylococcal infection treatment is beta-lactam medicines. The emergence of drug resistance to antibiotics has emerged as a major global problem, and S. aureus is particularly susceptible due to the selection pressure of antimicrobials. The transduction and conjugation processes are blamed for the resistance, which is mediated by the chromosome or plasmid (Tenover *et al.,* 2010). *S. aureus* nonosocomial strains frequently exhibit broad antibiotic resistance. It is true that bacteria have been described that are resistant to all clinically helpful medications, including the glycopeptides vancomycin. Some enterococci have been found to have plasmid-associated vancomycin resistance, and the resistance determinant has been transmitted from enterococci to *S. aureus* in the lab and may occur naturally. Aside from that, *S. aureus* exhibits resistance to antiseptics and disinfectants such quaternary ammonium compounds, which may help it survive in a hospital setting (Gulzar *et al*., 2018).

S. aureus has responded to the introduction of new medications since the dawn of the antibiotic era by quickly developing resistance through a variety of genetic pathways, including;

- The insertion of transposons or other forms of DNA into the chromosome to acquire extrachromosomal plasmids or additional genetic data; and
- by mutations in chromosomal genes
- There are essentially four mechanisms of resistance to antibiotics in bacteria:
- enzymatic inactivation of the drug,
- alterations to the drug target to prevent binding,
- accelerated drug efflux to prevent toxic concentrations accumulating in the cell

- a bypass mechanism that expresses a different drug-resistant form of the target (Harkins *et al*., 2017)

2.8.1 Penicillin Resistance

The antibiotic worked wonders against *staphylococcal* infections and Gram positive organisms. However, after a year of penicillin's clinical usage, the first reports of *S. aureus* strains that were resistant to the drug surfaced. Such penicillin-resistant isolates carried the plasmid gene blaZ, which encoded the penicillinase beta-lactamase enzyme (Lucitra 2013). Penicillin's beta-lactam ring can be broken by the enzyme, rendering the antibiotic inactive. First wave of resistance describes the emergence and spread of penicillinase-mediated resistance in *S. aureus*. In the 1960s, this grew to alarming levels before going global. By the late 1960s, around 80% of *S. aureus* isolates from hospitals and the general public had developed penicillin resistance. More than 90% of staphylococcal isolates, regardless of origin, generated penicillinase enzyme by the early 2000s. In 2016 (Arumugam *et al*.), The organism's blaZ gene promotes resistance to -lactam antibiotics, including penicillin and its derivatives (Oslen *et al.,* 2006).

2.8.2 Quinolones Resistance

In *S. aureus*, quinolone resistance develops gradually as a result of point mutations, primarily in the GrlA subunit of topoisomerase IV and the GyrA subunit of Gyrase. *S. aureus* can develop quinolone resistance through the development of Nor A efflux pumps, which is another route. Even though the mechanism of resistance and the genes that encode it are completely different from one another, methicillin resistance and quinolone resistance in *S. aureus* are primarily related. This might be because quinolones are used more frequently in nosocomial settings. where there is a high frequency of HA-MRSA and quinolone resistance. (Chandrashekhar and Tracey, 2022).

2.8.3 Methicillin Resistance

Methicillin, a penicillinase-stale semisynthetic penicillin, was discovered to combat staphylococcus aureus penicillinase resistance. It was first used in clinics in 1961, and that same year reports of strains that were resistant to methicillin were made. The first reports of methicillin-resistant staphylococcus aureus (MRSA) strains appeared in the

early 1960s, not long after the drug's launch. Following the initial report, MRSA clones quickly spread throughout the world, but only in hospitals. The second wave of beta-lactam resistance in *S. aureus* is what is being discussed here. High morbidity and mortality rates as well as an increase in healthcare costs were caused by the rise in MRSA infections in hospitals. With the first reports of MRSA infections in the community in the early 1990s, the third wave of beta-lactam resistance in *S. aureus* emerged. Hospital acquired methicillin-resistant staphylococcus aureus (HA-MRSA) and Community acquired resistant staphylococcus aureus (CA-MRSA) were defined as strains that were phenotypically and genetically distinct from MRSA isolates from hospital settings (Arumugam *et al*., 2016). A low-affinity penicillin-binding protein (PBP2a) encoded by the mobile genetic element mecA and found on the s*taphylococcal* cassette chromosome mec (SCCmec) is the main mediator of methicillin resistance (Scott *et al*., 2012).

2.8.4 Vancomycin Resistance

Based on the clinical laboratory standard institute's definition of the vancomycin susceptibility breakpoint (Vancomycin MIC of 8mg/L), *S. aureus* strains referred to as hVISA and VISA are not regarded as resistant (CLSI). These strains lack the vanA or vanB type of genes that provide vancomycin resistance, unlike VRE. The first report of a *S. aureus* strain with a vancomycin MIC of greater than 128 mg/L was published in 2002. The strain was resistant to methicillin and possessed the vanA gene, which conferred high levels of vancomycin resistance. Following this study, isolated *S. aureus* strains appeared infrequent (Kos *et al*., 2012). Vancomycin and other glycopeptides work to kill bacteria by preventing the production of the *S. aureus* cell wall. Vancomycin resistance is currently assumed to occur due to cell wall thickening and, possibly, the transfer of genetic material. Vancomycin works by forming an irreversible bond with the terminal D-alanyl-D-alanine of bacterial cell wall precursors, limiting the manufacture of cell walls by going after the sites involved (Appelbaum 2006). Changes in peptidoglycan production are hypothesized to be the cause of resistance in VISA strains. The D-alanyl-D-alanine residues in peptidoglycan are produced in greater amounts by VISA strains. Vancomycin molecules are effectively sequestered by these residues, which attach to them and prevent them from reaching their intended bacterial target (Scott *et al*., 2012).

Additionally, the vancomycin-bound cell walls that have just undergone alteration hinder the movement of drug molecules even more. Vancomycin MICs of 4 mg/L or even 2 mg/L may not accurately reflect the clinical susceptibility of some *S. aureus* strains. Given this, it could be appropriate for the CDC and CLSI to lower their guideline for the amount of vancomycin to use in screening plates (Harkins *et al*., 2017).

2.9 HUMAN HANDS AS A MAJOR TRANSMITTER OF MICRO-ORGANISM TO ENVIRONMENTAL SURFACES

People who regularly come into contact with dirty surfaces can transfer harmful bacteria to their hands. Disease can be spread through contaminated hands, both to oneself and to others. According to Price (1992), bacteria isolated from the hands might be classified as either resident or transient. The bacteria that live under the stratum corneum's surface cells and on the skin's surface make up the resident flora (resident microbiota). The predominant species is *Staphylococcus* epidermidis, and oxacillin resistance is extremely high. *S. hominis* and other coagulase-negative *staphylococci* are among the additional resident bacteria, followed by coryneform bacteria. Two primary defense mechanisms of the local flora are microbial conflict and competition for resources in the ecosystem. In general, resident flora is less likely to be linked to illnesses, although it can still spread pathogens to non-intact skin, sterile bodily cavities, and the eyes.

Routine hand hygiene is better able to get rid of transitory flora (transient microbiota), which colonizes the outer layers of the skin. Although transient microorganisms seldom multiply on the skin, they occasionally do so in order to live and reproduce. They are the organisms most frequently connected with HCAIs and are frequently acquired by HCWs (Healthcare Workers) through close contact with patients or contaminated surfaces near the patient (Health care associated infections). Transient flora can spread due to the species present, the quantity of microorganisms on the surface, and the wetness of the skin. Some HCWs may develop a persistent pathogenic flora colonization on their hands, including S. aureus. Through contaminated hands, pathogens can spread amongst each other. The kind of organism, the source and destination surfaces, the moisture content, and the size of the inoculum are all factors that affect the transfer of microorganisms from one surface to another and the rates of cross-contamination.

2.10 FOMITES (DOOR HANDLES) AS VECTORS OF TRANSMISSION OF INFECTIOUS DISEASE

Fomites are inanimate objects that can passively facilitate the transfer of infectious pathogens (such as bacteria, viruses, and parasites) between hosts by harboring such agents. Infectious disease transmission may also involve a vector. You can have mechanical or biological vectors. A mechanical vector passively transmits an infectious pathogen that it has picked up on its exterior. One of the main methods of spreading infectious diseases is contact transmission, which includes fomite-mediated transmission as a subset. As an illustration, a healthcare professional caring for a patient who has *staphylococcus aureus* infection without using safe handling techniques may accidently contaminate door knobs or wear gloves, putting other workers at risk of contracting the infection (WHO, 2009). Another illustration is a person who has *S. aureus* infection, which causes issues with the lungs. This person may eventually cough, allowing a droplet to fall onto the hand that he or she would continue to use to turn doorknobs (Stephens *et al*., 2019).

CHAPTER THREE

MATERIALS AND METHOD

3.1 STUDY AREA

The study area was New Daniel Hall of Mountain Top University which is located in Km-12, Lagos -Ibadan Expressway, Ogun State. The hall is a large boy's hostel accommodating up to 1,000 students with over 60 sixty rooms. *Staphylococcus aureus* is also skin-surface commensal microorganisms making it possible for it to be transmitted easily with the help of fomites like door handles. An area with such a large population might at a spreading risk of the organism which can also be pathogenic.

3.2 COLLECTION OF SAMPLES

Samples were obtained from door handles/knobs of the room of the boys' hostel (New Daniel Hall) 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 laboratory for microbiology of Mountaintop University aseptically for identification and microbial analysis within 1-2 hours of sampling.

3.3 REAGENTS AND EQUIPMENT USED

Materials used include Petri -dishes, beakers, flasks, Bijou bottles, Eppendorf tubes, micropipette (with their tips), test tubes (with their racks), spatula, Glass slide, measuring cylinder, immersion oil, gram staining kit, gloves, glass spreader, Conical flask, and filter paper.

The equipment used are; Autoclave, weighing balance, distiller, Microscope, swash bottles, water bath (set at 50°C and 100°C), oven, incubator (set at 37°C), and Bunsen burner, inoculating loop.

3.4 STERILIZATION

To ensure aseptic conditions of working environment and materials, proper sterilization was practiced at every necessary phase of the bench work. Work bench area were also sterilized with 70% ethanol solution applied by the use of cotton balls, and also through the usage of Bunsen burner to keep the air around the work area sterile and aseptic. Eppendorf tubes, micro pipette tips and test tubes were sterilized in the autoclave at 121°C for 15 minutes, while petri-dishes, beakers, flasks, scotch bottles and McCartney bottles were sterilized in the oven at 160°C for 1 hour.

3.5 PREPARATION OF CULTURE MEDIA

Selective media and differential were employed to improve viability and isolation for the isolation and characterization 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 can distinguish or classify microorganisms based on the variety of their appearance and patterns of growth and morphology.

3.5.1 Mannitol Salt Agar

Any bacterial species other than the halotolerant *Staphylococcus* species cannot grow on mannitol salt agar, which is a differential and selective medium with a high sodium chloride concentration (Collee *et al.,* 2010).

- The dehydrated medium was dissolved in the appropriate amount of distilled water i.e., 111g of Mannitol salt agar in 1000 ml distilled water based on manufacturer's instructions (Ritcher) in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil.
- The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15 minutes.

- The medium was then permitted to cool to a range of 45-50°C and poured aseptically into sterile petri dishes and left to solidify. The medium is red- phenol in color.

3.5.2 Nutrient agar

Nutrient agar was prepared according to the manufacturer's instruction (Ritcher) for isolation and. subculture from mannitol salt agar to obtain a pure culture for the biochemical test.

- The dehydrated medium was dissolved in the appropriate volume of distilled water i.e., 28g of Nutrient agar in 1000 ml distilled water based on the manufacturers instruction's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil.
- The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15 minutes.
- The medium was then allowed to cool to a range of 45-50°C and poured aseptically into sterile petri dishes and left to solidify. The medium appears opalescent and is light amber in color.

3.5.3 Mueller Hinton agar

Mueller Hinton agar a general-purpose medium was prepared according to the manufacturer's instruction (Ritcher) for isolation and performing antibiotics susceptibility test.

- The dehydrated medium was dissolved in the appropriate volume of distilled water i.e., 38g of Mueller-Hinton agar in 1000 ml distilled water based on the manufacturers instruction's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil.
- The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15 minutes.

- The medium was then allowed to cool to a range of 45-50°C and poured aseptically into sterile petri dishes and left to solidify. The medium appears opalescent and is light amber in color.

3.6 SAMPLE PREPARATION

The sample swabs were shaken vigorously so as to displace the microorganisms into the nutrient broth and were incubated for 18-24hrs at 35°C. After which it was plated into Mannitol salt agar media and plates incubated at 35°C for 24 h. The high concentration of sodium chloride in selective media of Mannitol salt agar does not allow the growth of any other bacteria species on the agar media except that of Staphylococcus species, which is indicated by a change in the color of red-phenol to a golden or yellowish coloration. Mannitol Salt Agar is used for the selective isolation and enumeration of *Staphylococcus aureus* from clinical and nonclinical materials. Only Staphylococci grow on agar media containing 7.5% sodium chloride. Addition of 7.5% sodium chloride to phenol red mannitol agar results in an improved medium for the isolation of plasma coagulating staphylococci. The 7.5% concentration of sodium chloride results in the partial or complete inhibition of bacterial organisms other than staphylococci. Mannitol fermentation, as indicated by a change in the phenol red indicator, aids in the differentiation of staphylococcal species. The identity of the isolates was confirmed by standard laboratory methods which included colony morphology, gram staining, catalase test and coagulase test.

3.7 SUB CULTURING

The isolated bacterial colonies were sub cultured to be purified and to obtain a single, pure culture from a mixed culture. The bacterial isolates transferred or sub cultured were those that were distinguished based on their colony morphology, shape, color, elevation, and other physical characteristics. Colonies with distinct morphological traits are placed onto newly prepared petri dishes with nutrient agar. The loop for inoculation was used to take a loopful of the desired isolate (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). The streaking method process is used to transport the isolate-containing loop to the new petri plate for subculturing.

3.8 BIOCHEMICAL TEST

These examinations are performed to ensure accurate identification and characterization. The criteria in Bergey's Manual of Determinative Bacteriology were used to base everything on the biochemical properties of *Staphylococcus aureus*. Gram staining, coagulase, and catalase tests are a few of the biochemical procedures used to identify and characterize the isolates.

3.8.1 Gram Staining

This was done to differentiate the bacterial isolates based on their staining properties based on the function of the properties of their cell wall structure. Gram staining involves the use of dyes to enhance the visibility of bacterial isolates and to differentiate them based on their morphology. 24 The ability of the bacteria to retain the color of crystal violet after being treated with alcohol, makes it Gram-positive, while those that lose the color of the crystal violet but retain the color of the counter stain (safranin) are Gram – negative. A smear was prepared by transferring aseptically a loopful of the bacterial isolate to be stained onto a sterile slide containing about one to two drops of water (loopful) and mixed. The smear was then heat fixed by passing it through the flame of the Bunsen burner multiple times. The slide was then flood with crystal violet and left for 1 minute), then rinse with running water, after which iodine was added to the slide (to act as a mordant), followed by decolorization with 70% alcohol and then rinsed with water. The counterstain, safranin was then added to the slide for 30 seconds. The slide was rinsed with water and then dried with blotting paper. The stained slide is observed under the microscope after the application of oil immersion. *S. aureus* are *gram* positive cocci appearing purple and occurring singly, in pairs, tetrads or in irregular clusters

3.8.2 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 (H2O) into water (H2O) and oxygen (O2).

$$2H_2O \rightarrow 2H_2O + O_2$$

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.

3.8.3 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; The bacterial cell wall-bound coagulase (clumping factor) interacts with fibrinogen directly. 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 involves the activation of plasma coagulase-reacting factor (CRP), which is a modified or derived thrombin molecule, to from a coagulase-CRP complex. This complex in turn reacts with fibrinogen to produce the fibrin clot.

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.

3.9 PRESERVATION OF CULTURES

In 2 ml of sterile Brain Heart Infusion broth, two or three colonies of the isolates were diluted to 2 Mac Farland standard. A BHI broth with 15% glycerin and one ml of the isolate were placed in an Eppendorf tube. Following an even mixing, the Eppendorf tubes were stored at -85°C in an extremely low freezer.

3.10 ANTIBIOTIC SUSCEPTIBILITY TEST

Antimicrobial susceptibility testing was done by use of Kirby Bauer disk diffusion method. The antibiotics used in this study include Vancomycin (VAN) 30 µg, Cotrimoxazole (COT) 25 µg. Erythromycin (ERY) 5 µg, Cefuroxime (CRX) 10 µg, Gentamicin (GEN) 10 µg, Ciprofloxacin (CIP) 5 µg, Cefotaxime (CTX) 10 µg, Cephalexin (CEX) 1.5 µg, Meropenem (MEM) 10 µg, Augmentin (AUG) 30 µg, Tetracycline (TET) 30 µg. Five colonies of the organism were emulsified in 5 mls of sterile normal saline and mixed well; the turbidity was compared to 0.5 Mac Farland standard A sterile inoculating loop was used to inoculate the 18-24 hours old bacterial culture into 5mL normal saline. Sterile swab sticks were then used to spread the suspension into already prepared Muller Hilton plates. The disc was placed on the inoculated agar plates using sterile plates using sterile forceps and incubated at 35±2ºC for 18-24 hours after which the zone of inhibition for each antibiotic was measured using a meter rule in millimeter (mm) and interpreted with Clinical Laboratory Standards Institute (CLSI) 2020 guide lines. The reporting was done by indicating Resistant, Intermediate or Sensitive.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

Out of the 30 door handles obtained (15 samples from wing A, 15 samples from wing B), A total of 12 (40%) *Staphylococcus aureus* isolates were discovered and identified from door handles in New Daniel Hall in Mountain top university. The isolation frequency based on different locations in the study area showed that wing A has the highest prevalence while B wing have the least prevalence of the *S. aureus* isolates.

The isolates were identified morphologically to have an entire margin, convex elevation, small size, round shape and an opaque transparency as shown in Table 4.3.

The isolates also were identified with biochemical test and results are shown in Table 4.2 Most of the isolates fermented mannitol.

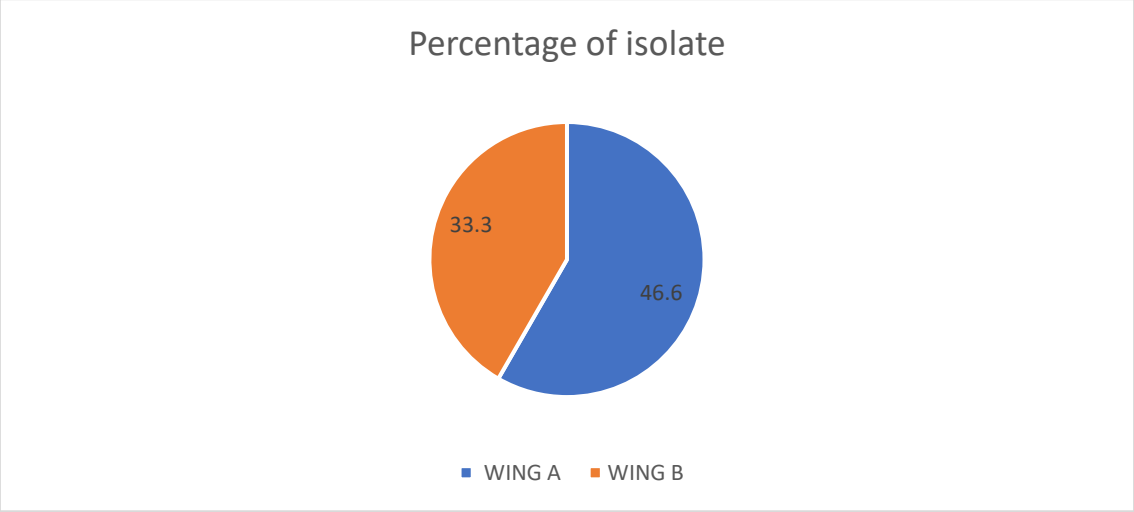

Figure 4.1: Percentage of isolate

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

S/No	Wing	No of samples	No of isolates (S. aureus) (%)
1	A	15	7 (46.6%)
2	B	15	5 (33.3%)

TABLE 4.2: Morphological characteristics of the colonies selected on mannitol salt agar.

Isolate code	Margin	Surface	Mannitol fermentation	Elevation	Color (MSA)	Transparency
NDA 101	Entire	Smooth	NO	Convex	Golden yellow	Opaque
NDA 103	Entire	Smooth	NO	Convex	Red	Opaque
NDA 105	Entire	Smooth	YES	Convex	Golden yellow	Opaque
NDA 107	Entire	Smooth	YES	Convex	Golden yellow	Opaque
NDA 108	Entire	Smooth	YES	Convex	Golden yellow	Opaque
NDA 109	Entire	Smooth	YES	Convex	Golden yellow	Opaque
NDA 201	Entire	Smooth	YES	Convex	Golden yellow	Opaque
NDA 102	Entire	Smooth	YES	Convex	Golden yellow	Opaque
NDA 104	Entire	Smooth	NO	Convex	Red	Opaque
NDA 202	Entire	Smooth	YES	Convex	Golden yellow	Opaque
NDA 106	Entire	Smooth	NO	Convex	Red	Opaque
NDA 203	Entire	Smooth	NO	Convex	Red	Opaque
NDA 204	Entire	Smooth	NO	Convex	Red	Opaque
NDA 205	Entire	Smooth	NO	Convex	Red	Opaque
NDB 202	Entire	Smooth	YES	Convex	Golden yellow	Opaque
NDB 204	Entire	Smooth	YES	Convex	Golden yellow	Opaque
NDB 105	Entire	Smooth	NO	Convex	Red	Opaque
NDB 106	Entire	Smooth	NO	Convex	Red	Opaque
NDB 206	Entire	Smooth	YES	Convex	Golden yellow	Opaque
NDB 208	Entire	Smooth	NO	Convex	Red	Opaque
NDB 205	Entire	Smooth	YES	Convex	Golden yellow	Opaque
NDB 107	Entire	Smooth	NO	Convex	Red	Opaque
NDB 102	Entire	Smooth	NO	Convex	Red	Opaque
NDB 104	Entire	Smooth	NO	Convex	Red	Opaque
NDB 108	Entire	Smooth	NO	Convex	Red	Opaque
NDB 109	Entire	Smooth	NO	Convex	Red	Opaque
NDB 103	Entire	Smooth	YES	Convex	Golden yellow	Opaque
NDB 101	Entire	Smooth	NO	Convex	Red	Opaque
NDB 203	Entire	Smooth	NO	Convex	Red	Opaque

Table 4.3: Biochemical characteristics of the isolates

Isolate Code	Shape	Gram	Catalase	Coagulase
NDA 101	Cocci	+	+	-
NDA 103	Rod	+	+	-
NDA 105	Cocci	+	+	+
NDA 107	Cocci	+	+	+
NDA 108	Cocci	+	+	+
NDA 109	Cocci	+	+	+
NDA 201	Cocci	+	+	+
NDA 102	Cocci	+	+	+
NDA 104	Rod	+	+	-
NDA 202	Cocci	+	+	+
NDB 102	Rod	+	-	-
NDB 104	Rod	+	+	-
NDB 108	Rod	+	+	-
NDB 109	Rod	+	-	-
NDB 103	Cocci	+	+	+
NDB 202	Cocci	+	+	+
NDB 208	Rod	+	-	-
NDB 204	Cocci	+	+	+
NDB 205	Cocci	+	+	+
NDB 206	Cocci	+	+	+
NDB 101	Rod	+	-	+
NDB 203	Rod	+	+	-
NDB 105	Rod	+	-	
NDB 106	Rod	+	-	-
NDB 107	Rod	+	+	-
NDA 106	Rod	+	-	-
NDA 203	Rod	+	-	-
NDA 204	Rod	+	+	-
NDA 205	Rod	+	-	-
NDA 206	Cocci	+	+	-

Key

Positive is represented as +

Negative is represented as -

Antibiotic susceptibility test for *S. aureus* isolates from door handles

Antibiotic susceptibility test was carried out on all the 12 *S. aureus* isolate which was obtained from door handles using the Kirby Bauer antibiotic disk susceptibility method. Interpretation of observed zones of inhibition was based on the CLSI 2020 guidelines (Table 4.4). The results showed that 4 (33.33%) isolates were resistant to both Cefotaxime, Erythromycin and Gentamycin (Table 4.5). A moderate resistance to Augmentin (41.66 %), Cefuroxime (41.66 %), Meropenem (41.66%). The result showed that 6 (50%) isolates were resistant to Cephalexin, Ciprofloxacin (Table 4.5). The isolates showed a higher resistance to Cotrimoxazole (75 %) and Tetracycline (75 %).

TABLE 4.4: 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)

TABLE 4.5: Antibiotics resistance pattern of the isolates

Isolate code	COT	ERY	CRX	CEX	CIP	AUG	CTX	TET	GEN	MEM	Phenotypic resistance of isolates
NDA 105	R	S	R	S	S	S	S	R	S	S	COT-CRX-TET
NDA 107	R	S	R	S	R	S	R	R	R	S	COT-CRX-CIP-AUG-TET-CEX
NDA 108	I	I	R	R	S	S	S	R	S	S	COT-CRX-CRX-AUG-TET
NDA 109	R	R	S	S	S	S	S	S	R	S	COT-ERY-CEX
NDA 201	R	I	S	R	R	R	R	R	S	R	COT-CRX-CIP-CTX-AUG-TET-MEM
NDA 102	R	R	R	S	R	S	S	I	R	S	COT-ERY-GEN-CEX-CIP
NDA 202	R	I	S	R	S	R	S	R	R	S	COT-CRX-CIP-CTX-TET
NDB 103	I	R	I	S	R	S	S	R	S	S	ERY-CIP-TET
NDB 202	R	S	S	R	I	S	S	R	S	R	COT-CRX-TET-MEM
NDB 204	I	R	R	S	I	R	S	I	S	R	GEN -ERY-CTX-AUG-MEM
NDB 205	R	S	S	R	R	R	R	R	S	R	COT-CRX-CIP-CTX-AUG-TET-MEM
NDB 206	R	S	S	R	R	R	R	R	S	R	COT-CRX-CIP-CTX-AUG-TET-MEM
% Resistance	**75**	**33.33**	**41.66**	**50**	**50**	**41.66**	**33.33**	**75**	**33.33**	**41.66**	

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, , Cephalexin (CEX) 1.5 µg, Meropenem (MEM) 10 µg, Augmentin (AUG) 30 µg, Tetracycline (TET) 30 µg.

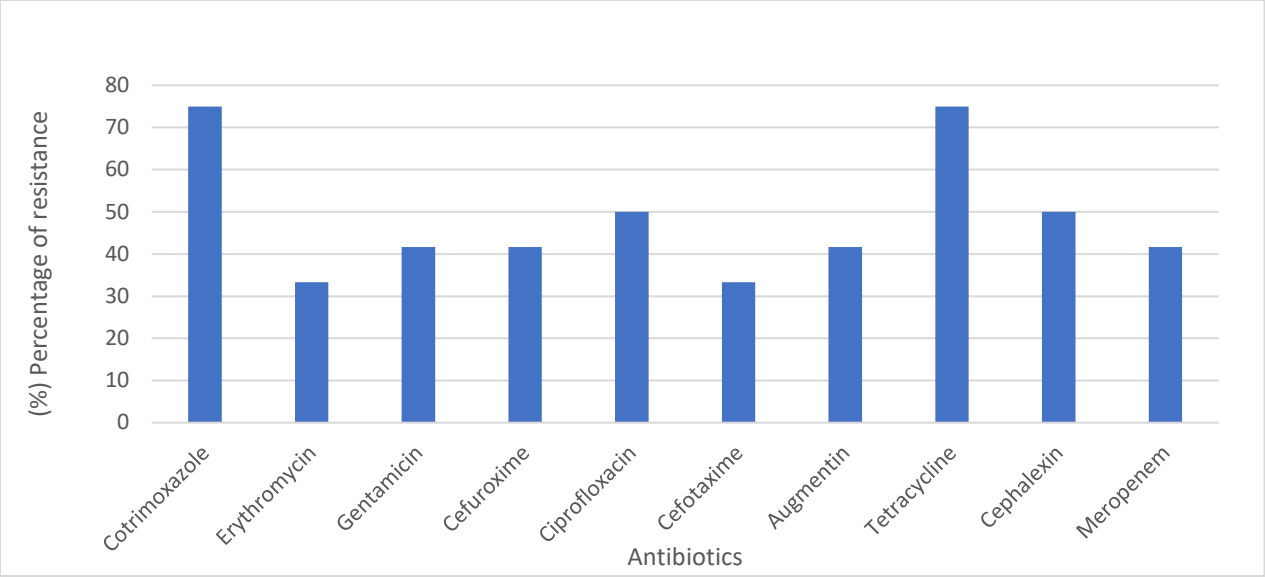

Figure 4.2: Resistance of *Staphylococcus aureus* to antibiotics.

4.2 DISCUSSION

Staphylococcus aureus was isolated from door handles in New Daniel Hall (A male hostel) in Mountain Top University. *S. aureus* is the leading cause of bacterial infections, causing a wide range of diseases such as bacteremia, wound infection, septicemia, and pneumonia; but when it has not acquired the resistant gene (just the normal *S. aureus*), it is an important human-friendly microorganism which is often found as commensal on body surface but its transfer to other parts of the human body may lead to a wide variety of infections (David and Daum, 2010).

A prevalence of 40% for *S. aureus* was recorded in this study in which 7 isolates were confirmed from wing A and 5 isolates were confirmed from wing B making a total of 12 isolates. From this study, *S. aureus* was isolated from 12 (40%) of the 30 door handle swab samples. The prevalence rate in this study is lower compared to the one reported by Akinrotoye *et al.,* 2019; they isolated *S. aureus* from 42 (50.60%) out of the 300 door handle swab samples that were obtained from secondary schools in Abeokuta and its environs.

Results from the antimicrobial susceptibility test, using the disk diffusion method showed 9 (75%) of the *S. aureus* isolates to be resistant to Cotrimoxazole; this was also the case with Tetracycline (Figure 4.2). However, only 6 (50%) isolates, each, were resistant to Ciprofloxacin and Cephalexin while 5 (41.66%) isolates, each, were resistant to Meropenem, Augmentin, Cefuroxime. The lowest rate of resistance was seen to Erythromycin, Gentamycin and Cefotaxime as only 4 (33.3%) isolates, each, were resistant to them.

All 12 (100%) *S. aureus* isolates were seen to be multi-resistant as they were resistant to more than one of the 10 antibiotics used in this study (Table 4.5).

Isolates NDA201 and NDA 206 were seen to be the most resistant in this study as they were resistant to 7 of the 10 antibiotics used in this study (Table 4.5). Based on this resistance profile; *S. aureus* was shown to be multi-drug resistant. It is shown in this study that Wing A had more isolates and also the two isolates (NDA 201 &206) that showed most resistance was also from there.

This study also shows that some of the isolates were beta- lactamase produces as resistance were shown to some cephalosporin class of antibiotics (Cefuroxime (41.66%), Cefotaxime (33.3%), Cephalexin (50%)). Most of the isolates were resistant to tetracycline and cotrimoxazole due to the over use of the drug making most organism develop mechanism of resistance and acquiring resistance against them. Gentamycin was more effective to *staphylococcus aureus* in this study and it is similar to the study done by Owaku *et al.*,2018 where gentamycin proved to be the most effective antibiotic against *Staphylococcus aureus* isolated from door handles in Nasarawa state university.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The result of this study indicates the prevalence rate of *S. aureus* in New Daniel Hall to be slightly high. It is clear that *S. aureus* is a threat to public health of both students and staff within the school community, The study has revealed that more people need to be sensitized about hand hygiene it is, therefore, necessary to put in place control measures that may be important to stop the rapid transmission amidst the population. The study has revealed that more people need to be sensitized about hand hygiene.

The occurrence of multi-drug resistant *Staphylococcus aureus* in the environment is a threat to the population therefore hygiene measures such as hand washing should be encouraged in New Daniel Hall and other hostel in it environ to prevent the spread of *Staphylococcus aureus* amongst students and staffs.

5.3 RECOMMENDATION

In order to prevent the emergence of the spread of the multi-drug resistance *Staphylococcus aureus,* the following measure are recommended.

i. Proper washing of hands

ii. Avoid indiscriminate use of antibiotics

iii. Usage of Hand sanitizers

iv. Maintain proper hygiene in the hostel

v. Proper cleaning of door handles periodically

All these recommend processes are essential to curb the increasing thread of antibiotic resistance in Staphylococcus aureus.

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