PREVALENCE OF DRUG-RESISTANT *STAPHYLOCOCCUS AUREUS* IN NASAL SWABS ISOLATED FROM STUDENTS OF CALEB UNIVERSITY

BY

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DECLARATION

I, Ikuepamitan Kolawole Michael, hereby declare that the project work entitled "**Prevalence of Drug-Resistant *Staphylococcus aureus* In Nasal Swabs Isolated from Students of Caleb University"** is a record of an original work done by me, as a result of my research effort carried out in the Department of Biological Sciences and Biotechnology, Caleb University Imota, Lagos.

_____

Student's Signature and Date

CERTIFICATION

This is to certify that this project work titled prevalence of drug-resistant *Staphylococcus aureus* in nasal swabs isolated from students of Caleb University was carried out by Ikuepamitan Kolawole Michael with matric number 18/5050 in the Department of Biological Sciences and Biotechnology, College of Pure and applied sciences, Caleb University Imota, Lagos Nigeria.

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(Head of Department) Signature/Date

EXTERNAL EXAMINER _____

Signature/Date

DEDICATION

First and foremost, I dedicate this project to the almighty God, my creator and source of wisdom, knowledge, and insight. Special thanks to my devoted parents, Mr. and Mrs. Ikuepamitan, who have always been supportive of me in all aspects of my life, also to my Industrial Training supervisor Mrs. Igbonna Nkeiruka who played a significant role in the project's success.

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My appreciation goes to God, who has provided all I've needed to finish my endeavor, as well as his blessings on my entire university.

My gratitude also extends to my parents, who laid the groundwork for my education by giving it their best. My parents, Mr. and Mrs. Ikuepamitan, have provided all possible and even more vital things to ensure that I reach this level, and I will be eternally thankful to them.

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ABSTRACT

Staphylococcus spp are gram positive cocci that belong to the family *micrococcacae*. It is an organism. Thus is found everywhere, *Staphylococcus aureus* is a well-known organism that is becoming resistant to the most commonly used antibiotics, MRSA is an organism that is a threat to human and the community. The nasal cavity is a large nasal passage used for inhaling air from the nostril into the body. This study was done to determine the prevalence of nasal isolates from students of Caleb University, identify *Staphylococcus aureus* isolates and to determine the antibiotics susceptibility pattern of the isolates to examined prevalence of drug resistant *Staphylococcus aureus* in the nasal swabs isolates from students of Caleb University. 100 nasal swab samples were collected from 25 male and 25 female students respectively 50 nasal swab sample each from the male and female students. Cultural and biochemical tests were carried out on nasal swabs and the antimicrobial susceptibility testing was done using disc diffusion method. Multidrug resistant *Staphylococcus aureus* was found, the antibiotics with demonstrated multidrug resistance were amoxillin-clavalanate, cefotaxime, ceftriaxone, cefoxitin, imipenum, tetracycline, erythromycin, ciprofloxacin, gentamacin. The nose can serve as a means of transmitting bacterial infection, because it is depository for microorganism. After the use of toilet alcohol based hand sanitizer should be used before Deeping the hand in the nose

CHAPTER ONE

1.1 Background of Study

The *Staphylococcus* genus are gram-positive cocci and belong to the family *Micrococcaceae* (Taylor and Unakal, 2021). *Staphylococci* are typically categorized as coagulase-positive staphylococci (*Staphylococcus aureus*) and coagulase-negative staphylococci (*Staphylococcus epidermidis*) (Ondusko and Nolt, 2018; Taylor and Unakal, 2021). Both pathogens could lead to nosocomial infections, and biofilm formation further complicates clinical management (Ondusko and Nolt, 2018). Biofilm formation evolves in three steps, starting with nonspecific adherence of individual cells to the materials, followed by growth and biofilm formation, and ending with detachment of surface bacteria (Jenul and Horswill, 2019). In *Staphylococcus epidermidis*, biofilm formation is associated with the production of polysaccharide intercellular adhesion (PIA), and raise opsonic antibodies against PIA could be promising for the elimination of colonizing and biofilm-forming *Staphylococcus epidermidis (*Lee *et al*., 2018*).* Frequently, *Staphylococcus aureus* is resistant to methicillin (methicillin-resistant *Staphylococcus aureus* [MRSA]) and almost all β-lactam drugs (in up to 50% of hospital isolates) (Taylor and Unakal, 2021). MRSA are important bacteria in nosocomial infection and are listed by the World Health Organization as major bacteria in urgent need of new antibiotics (Turner *et al*., 2019).

Staphylococcus aureus is a well-known organism that is becoming increasingly resistant to the most commonly used antimicrobial agents (Lowdy 1998).

Nasal *Staphylococcus aureus* has been linked to soft tissue infections in the community (Foster, 2017) and hospital infections such as bacteremia (Guo *et al*; 2020). Antimicrobial drug resistance in *Staphylococcus aureus*, particularly multidrug-resistant strains, is a serious global concern (Shariat *et al;* 2020) that places a significant burden on health-care facilities (Yernigan *et al;* 2020) Most community and hospital-associated infections have been attributed to *Staphylococcus aureus* from the anterior nares, and recent investigations have revealed that nasal carriage is a source of bacteremia (Turner *et al;* 2019). Nasal carriage removal has been linked to a decrease in the incidence of *Staphylococcus aureus* infection (Shariat *et al;* 2020).

1.2 Statement of Problem

The frequency of incidence of infections caused by Drug-Resistant *Staphylococcus aureus* strains continues to grow in school settings worldwide. Schoolmates represent the risky groups for Drug-Resistant *Staphylococcus aureus* infections. It is known that students stay in close proximities, and share material such as pens and phones, which are potential fomites. Additionally, students suffering from skin and other soft tissue infections may experience a lot of discomfort, which may interfere with learning processes. Majority of the previous studies have focused on Drug-Resistant *Staphylococcus aureus* colonization among primary school children (Kejela and Bacha, 2013). Although a few studies have focused on college students or students participating in particular sporting activities, such as, football and athletics, (Jiménez-Truque *et al*., 2017), no study has been done in Nigeria to determine the prevalence of Drug-Resistant *Staphylococcus aureus* among University students. As such, the prevalence of Drug-Resistant *Staphylococcus aureus* among university students in Nigeria remains unknown.

Prevalence reports are crucial towards implementation of strategies that can help in preventing the spread of Drug-Resistant *Staphylococcus aureus* among high risk groups, and perhaps prevent outbreaks of Drug-Resistant *Staphylococcus aureus* infections.

1.3 Justification of Study

In Nigeria, no population based study had been carried out on nasal carriage rate, antibiotic susceptibility pattern, and associated risk factors of *Staphylococcus aureus* and MRSA among students particularly in Caleb University. Thus, this study is intended to assess and fill the information gap of the current nasal carriage rate, antibiotic susceptibility pattern, and associated risk factors of *Staphylococcus aureus* and MRSA among Caleb University students. Also to indicate the prevention and control measures in general, it will also be used as preliminary information for future studies.

1.4 Aim

This study aims to examine the prevalence of drug-resistant *Staphylococcus aureus* in nasal swabs isolated from students of Caleb University.

1.5 Specific Objective

i. To determine the prevalence of nasal isolates from students of Caleb University

ii. To determine the cultural and biochemical characteristic of *Staphylococcus aureus* isolates from students of Caleb university

iii. To determine the antibiotic susceptility pattern of the isolated *Staphylococcus aureus*

iv. To identify drug-resistance *Staphylococcus aureus* in nasal swab isolated from *Staphylococcus aureus*

v. To access the relationship between the colonization rate of drug-resistance MRSA to socio-demographic characteristics.

CHAPTER TWO

LITERATURE REVIEW

2.1 Description of the Nasal Cavity

The nasal cavity is a large, air-filled space above and behind the nose in the middle of the face. The nasal septum divides the cavity into two cavities (Rodriguez *et al.,* 2017), also known as fossae (López *et al.,* 2016). Each cavity is the continuation of one of the two nostrils. The nasal cavity is the uppermost part of the respiratory system and provides the nasal passage for inhaled air from the nostrils to the nasopharynx and rest of the respiratory tract (Kim *et al*., 2017).

2.2 Classification of nasal cavity

The nasal cavity is divided into two segments: the respiratory segment and the olfactory segment.

The respiratory segment comprises most of each nasal cavity, and is lined with ciliated pseudostratified columnar epithelium (also called respiratory epithelium). The conchae, or turbinates, are located in this region. The turbinates have a very vascularized lamina propria (erectile tissue) allowing the venous plexuses of their mucosa to engorge with blood, restricting airflow and causing air to be directed to the other side of the nose, which acts in concert by shunting blood out of its turbinates. This cycle occurs approximately every two and a half hours.

The olfactory segment is lined with a specialized type of pseudostratified columnar epithelium, known as olfactory epithelium, which contains receptors for the sense of the smell. This segment is located in and beneath the mucosa of the roof of each nasal cavity and the medial side of each middle turbinate. Histological sections appear yellowish-brown due to the presence of lipofuscin

pigments. Olfactory mucosal cell types include bipolar neurons, supporting (sustentacular) cells, basal cells, and Bowman's glands. The axons of the bipolar neurons form the olfactory nerve (cranial nerve I) which enters the brain through the cribriform plate. Bowman's glands are serous glands in the lamina propria, whose secretions trap and dissolve odoriferous substances.

2.3 The Nasal Cavity

The nasal channel runs from the nose's entrance (nostril or anterior nares) to the nasopharynx at the rear (top of the back of the throat). The nasal turbinate divides the nasal cavity into the inferior, middle, and superior meatus (Sahin-yilmaz and Naslenor, 2011). Filtering, warming, and humidifying breathed air are just a few of the psychological activities performed by upper respiratory tracts. The nasal cavity acts as a physical transition between the external environment and the lower respiratory and gastrointestinal tract (de Steenhuis, 2015). Other functions of the nasal cavity include 01 factory sensing and immunological tracks, including immediate pathogen detection such as sensing of bacteria lactone by taste receptor (de Steenhuis, 2015).

2.4 NORMAL FLORAL OF THE NASAL CAVITY

Commensal bacteria in the human nasal cavity compete for limited space and nutrients with opportunistic pathogens, and can even create poisonous substances that directly inhibit or kill competing microbes. Some microbes, such as *Staphylococcus aureus*, can be both commensal and versatile opportunistic pathogens (otto, 2010).

According to research into the bacteria communities in the nasal cavity, the nose is home to opportunistic pathogen that can spread to other parts of the respiratory tract, resulting in allergic rhinitis, chronic *rhinosinusitis*, acute otitis media, and asthma. *Staphylococcus aureus*,

Staphylococcus epidermidis alpha, and Y-*Streptococcus*, as well as *Propionibacterium* acnes and aerobic diphtheroid, are common bacteria found in the nasal cavity (Lee *et al*., 2017). From healthy nasal cavities, potential sinus pathogens have been isolated. *Streptococcus pneumoniae*, *Haemophilus influenzae, Moraxella catarrhalis*, *Streptococcus* pathogen, *peptostreptoccus* spp., and *prevotella* spp. are among the bacteria in this group (Mutua *et al*., 2017).

Children's nasal floral differs from that of adults, according to studies. *Moraxella* (*moraxellaceae*, *proteobacteria*), *Streptococcus*, *Haemophilus* (*pasteurellaceae*), *Staphylococcus*, and *cyanobacterium* are among the bacteria that live in the cavity of infants (*cyanobacterial*, *actinobacteria*). Individuals' mature microbial floral differs as well. Health state has also been demonstrated to alter nasal microbial flora in studies (Gressler *et al*., 2018).

2.5 NON BACTERIAL FLORA OF THE NASAL CAVITY

Aside from bacteria and viruses, the nasal cavity is home to a distinct *archaea* population with a wide range of species. *Archaea* are microorganisms that are biologically separate from bacteria. They're also important parts of the human microbiome since they live in the gastrointestinal system, the mouth cavity, the skin, and other places (Mahnert *et al*; 2018).

The *archaea* community of the nasal cavity is similar to the *archaeomes* of the skin and the gastrointestinal tract in that it is dominated by skin-associated *thaumarchaeota* (*Nitrososphaera*) and also methanogenic *Euryarchaeota* (*methanesphaera methanobrevibacter*), which are common in gastrointestinal tract *archaeomes*. Among other bodily areas with significant *archaea* 16Sr RNA gene concentration, the nasal cavity was discovered to be an *archaea* host soft (Pausan *et al;* 2018).

2.6 EFFECT OF SMOKING ON MICROBIAL FLORA OF THE NASAL CAVITY

Cigarette smoke comes into direct contact with the nasal surface, affecting the normal functions of the nasal cavity directly by oxygen deprivation, antibacterial activity, and other mechanisms.

According to Mason (2006), the toxic chemical affects the immunological response agent pathogen by disrupting effective mucociliary clearance in the lower and upper respiratory tracts. Cigarette smoke also aids bacteria attachment to airway epithelial cells by promoting the formation of robust, reversible biofilms by involving bacteria fimbria protein film synthesis.

The creation of this biofilm may help bacteria stay in the nasal cavity despite their resistance (Bagaitkar *et al;* 2010 Holstein *et al*; 2011). Schenck *et al.*, 2016, Kulkarn *et al.*, 2012, and McEachern *et al*., 2015 suggested a direct shift of bacterial infection and carriage pathway, as it has previously been demonstrated that *Staphylococcus auteurs* invasion and biofilm formation increased following cigarette exposure. Cigarette smoking has been found to deplete normal commensal airway bacteria and enmesh H. *Influenzae*, M. *Catarrhalis*, *campylobacter* spp, *Streptococcus*, *Pneumonia*, and *Streptococcus Pyogenes*. In general, smokers' upper respiratory tracks are more diversified than non-smokers', but they are less robust over time.

2.7 Epidemiology of *Staphylococcus aureus* Infections

Staphylococcus aureus (including drug-resistant strains such as MRSA) are found on the skin and mucous membranes, and humans are the major reservoir for these organisms (Pynnonen *et al*., 2011; López *et al*., 2016). It is estimated that up to half of all adults are colonized, and approximately 15% of the population persistently carry *Staphylococcus aureus* in the anterior nares. Some populations tend to have higher rates of *Staphylococcus aureus* colonization (up to

80%), such as health care workers, persons who use needles on a regular basis (*i.e.*, diabetics and intravenous (IV) drug users), hospitalized patients, and immuno-compromised individuals. *Staphylococcus aureus* can be transmitted person-to-person by direct contact or by fomites (Lee *et al*., 2018; Sakr *et al.*, 2018).

The highest prevalence of drug-resistance *Staphylococcus aureus* infections was recorded from classrooms where the number of students was more than 60 pupils. Additionally, drug-resistance *Staphylococcus aureus* nasal carriage was also highest among students who came from families of five to six individuals. A different study, that was almost similar to this established that drug-resistance *Staphylococcus aureus* carriage was more prevalent in students between ages 10 to 19 years. The prevalence of drug-resistance *Staphylococcus aureus* infections was higher in females compared to their male counterparts (Rijal *et al*., 2008). Studies conducted by (Yildirim *et al*., 2007) also revealed the same prevalence of drug-resistance *Staphylococcus aureus* infections among male and female students. From the information presented above, very few researchers have focused on MRSA infections among students.

Various studies have been conducted on MRSA infections among students in different educational institutions including high schools and tertiary institutions. These studies have revealed the burden of drug-resistance *Staphylococcus aureus* carriage among students. In a study conducted by (Lear *et al*., 2011), it was revealed that outbreaks of drug-resistance *Staphylococcus aureus* infections have been documented among athletics, football, rugby, and soccer players in schools. In athletic settings, for instance, multiple retrospective studies have assessed the risk of drug-resistance *Staphylococcus aureus* colonization and infection with differing study outcomes (Huijsdens *et al*., 2006; Kejela and Bacha, 2013). An outbreak occurred in one of the soccer teams in Netherlands where 11 members of the team were affected. The two

of the affected members lived in the same room in the college hostel (Huijsdens *et al*., 2006). In another American school football club, a retrospective analysis of a drug-resistance *Staphylococcus aureus* outbreak confirmed 11 players out of 107 players suffered from drug-resistance *Staphylococcus aureus* infections during a match season. Surprisingly, out of the 99 players that were tested, only eight players tested positive for drug-resistance *Staphylococcus aureus* colonization. Researchers in this study noted that only these eight players had their swabs collected after they had taken antibiotic medication and the number of colonized may have been more (Nguyen *et al*., 2005).This study was unique in that it identified highly resistant strains among people who had been subjected to antibiotic treatment. In another study, (Kejela and Bacha, 2013), conducted a study that focused on the drug-resistance *Staphylococcus aureus* colonization among school going children. The results in this study indicated that high nasal carriage of drug-resistance *Staphylococcus aureus* was attributed to major risk factors including sex, age, number of children per classroom, and previous hospitalization.

2.8 Mechanisms of Resistances of Drug-Resistant *Staphylococcus aureus*

The genetic determinant of resistance to β-lactam antibiotics is *mec*A gene (Fuda *et al*., 2004).The *mec*A gene lies in the SCC*mec* resistance island and is present in about 95% of isolates of *Staphylococcus aureus* displaying the phenotype of methicillin resistance (Wielders *et al*., 2002). Resistance to β-lactam has been shown not be native to *Staphylococcus aureus*, but rather, it has been acquired via the *mec*A gene for more than 40 years. The *mec*A gene encodes a protein known as penicillin binding protein (PBP), which is normally designated as PBP2a. *Staphylococcus aureus* usually produces four PBPs namely, PBP1, PBP2 PBP3 and PBP4 that are anchored on the cytoplasmic membrane (Navratna *et al.,* 2010). Penicillin-binding proteins

function in assembly and regulation of the stages of synthesis of the bacterial cell wall. Whereas the four PBPs are susceptible to alteration by β-lactam antibiotics resulting in death of bacterial cell, PBB2a is refractory to the action of all presently used β-lactam antibiotics. The PBP2a has the ability of taking over the functions of the four staphylococcal PBPs during exposure to β-lactam antibiotics (Kondo *et al*., 2007).

The *mec*A gene is known to be carried on a peculiar type of mobile genetically element inserted into the staphylococcal chromosome, known as the staphylococcal cassette chromosome *mec* (SCC*mec*) elements (Katayama *et al*., 2000). The SCC*mec* elements share four characteristics. First, they carry the *mec* gene complex, which consists of methicillin-resistance determinant gene (*mec*A) as well as its regulatory genes and insertion sequences; second, they carry the *ccr* gene complex responsible for mobility of the element and its associated sequences; third, they have distinct directly repeated nucleotide sequences and inverted complementary sequences at each end; and fourth, they integrate into the 3′ end of an open reading frame (ORF), *orfX*. Despite the similarities identified above, structures of SCCmec elements are also divergent (Kondo *et al*., 2007).

2.9 Pathogenesis of Drug-Resistant *Staphylococcus aureus*

Staphylococcus aureus are one the most common bacterial infections in humans and are the causative agents of multiple human infections, including bacteremia, infective endocarditis, skin and soft tissue infections (e.g., impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, and others), osteomyelitis, septic arthritis, prosthetic device infections, pulmonary infections (e.g., pneumonia and empyema), gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections (Verhoeven *et al*., 2018). Depending on the strains involved and the

site of infection, these bacteria can cause invasive infections and/or toxin-mediated diseases (Sakr *et al*., 2019). The pathophysiology varies greatly depending on the type of *Staphylococcus aureus* infection (Iwase *et al.*, 2010). Mechanisms for evasion of the host immune response include the production of an antiphagocytic capsule, sequestering of host antibodies or antigen masking by Protein A, biofilm formation, intracellular survival, and blocking chemotaxis of leukocytes (Pynnonen *et al*., 2011). Binding of the bacteria to extracellular matrix proteins and fibronectin in infectious endocarditis is mediated by bacterial cell wall-associated proteins such as fibrinogen-binding proteins, clumping factors, and teichoic acids (Liu *et al*., 2015). Also, Staphylococcal super antigens (TSST-1 or toxic shock syndrome toxin 1) are important virulence factors in infectious endocarditis, sepsis, as well as toxic shock syndrome (Verhoeven *et al*., 2018). Pneumonia infections are associated with the bacterial production of PVL (Panton-Valentine leukocidin), Protein A, and alpha-hemolysin, and infections are more common following influenza virus infection as well as a diagnosis of Cystic Fibrosis. Prosthetic device infections are often mediated by the ability of *Staphylococcus aureus* strains to form biofilms as well as communicate using quorum sensing in a bacterial cell density-dependent manner (Sakr *et al*., 2019).

2.10 Mechanisms of colonization of Drug-Resistant *Staphylococcus aureus*

Exposure of humans to *Staphylococcus aureus* occurs on a frequent basis and colonization occurs either for short or long periods through the stages of human life. The primary reservoir of *Staphylococcus aureus* is the anterior nares. Extra-nasal colonization occurs in sites like throat, skin, vagina, perineum and the gastrointestinal tract (Brown *et al.*, 2014). In a study conducted by Miller *et al*. (2012), it was reported that limiting the sampling site to the nasal cavity in regards to determination of whether a person is colonized with *Staphylococcus aureus* at a single

point may result in missing approximately 50% of individuals colonized with *Staphylococcus aureus* elsewhere. Even so, it appears that the nasal cavity site is, often, the source of inoculation for other sites through transfer by contact (Brown *et al.*, 2014). Moreover, the greater the load of *Staphylococcus aureus* in the nares, the higher the probability of colonization of other body sites. The colonization may also tend to be persistent in the presence of greater bacterial loads (Miller *et al*., 2012). Nasal carriers of *Staphylococcus aureus* can be classified into two categories. These include non-persistent carriers and persistent carriers (van Belkum *et al*., 2009). It is estimated that 20% of people are colonized persistently with a comparatively high load of *Staphylococcus aureus*, and the remaining proportion is either never colonized or is colonized intermittently with low loads of *Staphylococcus aureus* (Brown *et al*., 2014).

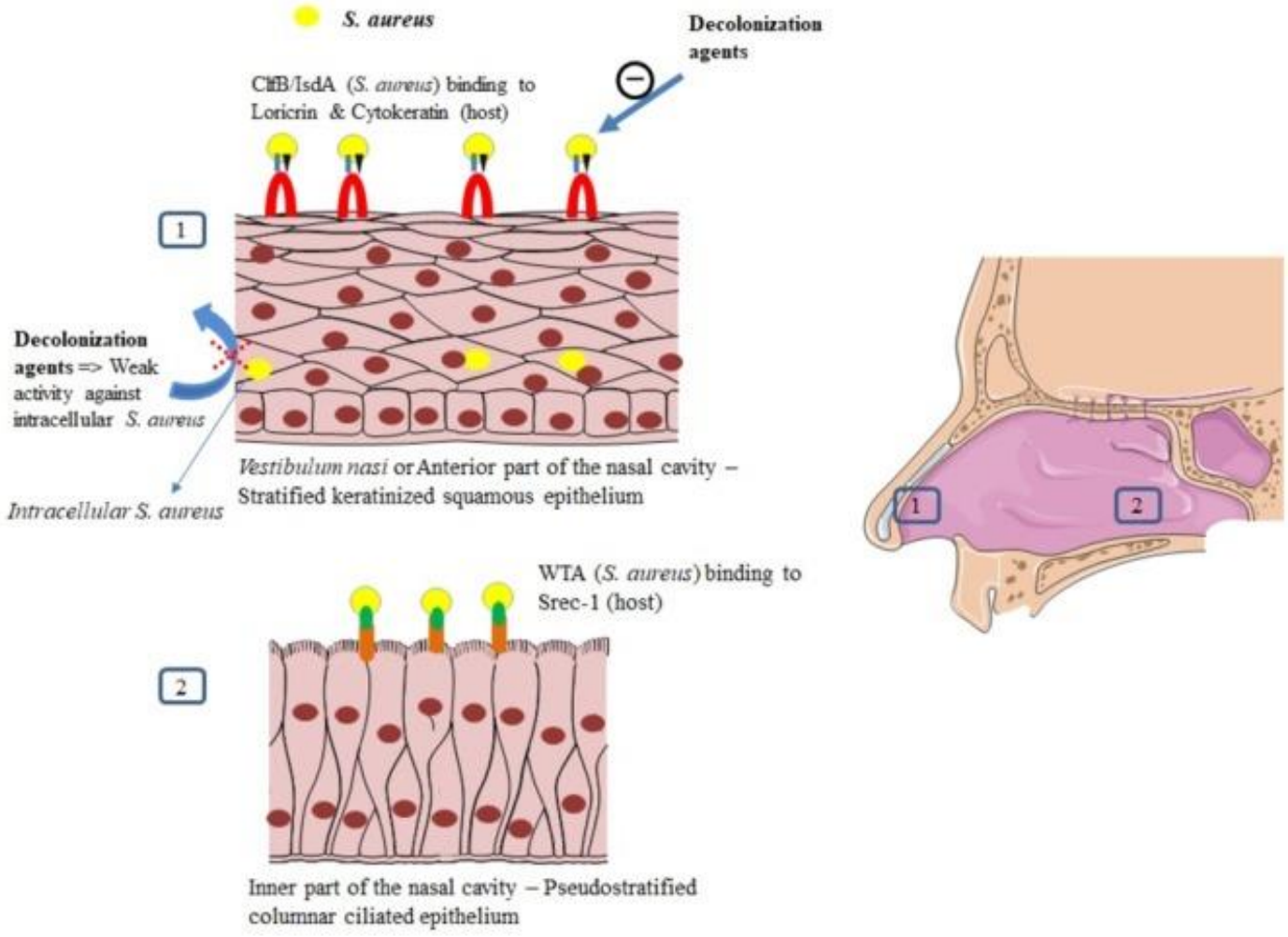

Figure 2.2: Mechanisms of *Staphylococcus aureus* nasal colonization (Sakr *et al*., 2018).

Staphylococcus aureus is a major human pathogen and has been shown to colonize mucosal surfaces and the skin in approximately 30-50% of individuals. Mulcahy *et al*., (2012) reported that *Staphylococcus aureus* asymptomatically colonizes the anterior nares and is permanently present in noses of approximately 20% of the population, and thus represents a significant risk factor for *Staphylococcus aureus* associated infections. The bacterial and host factors that facilitate colonization of the nasal cavity with *Staphylococcus aureus* remain to be fully elucidated (Mulcahy *et al*., 2012). According to research, *Staphylococcus aureus* adheres to the squamous epithelial cells present in the nose. Proteins expressed on the surface of *Staphylococcus aureus* such as the clumping factor B (ClfB) facilitate this interaction (Brown *et al.,* 2014). Mulcahy *et al*., (2012) showed that loricrin, which is a major component of the squamous epithelial cell envelope represents the principal ligand for ClfB. Additionally, it was reported in the study that the interaction between loricrin and ClfB is a requirement for efficient colonization of the nasal cavity with *Staphylococcus aureus* (Wertheim *et al*., 2008).

2.11 Risk factors of Drug-Resistant *Staphylococcus aureus*

Nasal colonization depends on host factors, such as the underlying condition or diseases. Some studies have found that nasal carriage was more frequent in human immunodeficiency virus (HIV)-infected (Kotpal *et al*., 2016) or obese patients (Olsen *et al*., 2013), compared to healthy individuals. This higher prevalence was also found among diabetic patients undergoing dialysis compared to non-diabetic patients in the same population. Other diseases such as granulomatosiss with polyangiitis (formerly known as Wegener's granulomatosis), rheumatoid arthritis (Laudien *et al*., 2010), skin and soft tissue infections (Immergluck *et al*., 2017) atopic

dermatitis (Breuer *et al*., 2002), and recurrent furunculosis (Demos *et al*., 2012) have been related with an increased carriage rate.

In healthy subjects, Liu C.M. *et al*. (2015) found similar carriage rates in men and women, while men had higher bacterial density. Reports of a higher risk of nasal carriage of *Staphylococcus aureus* among hospital workers than the rest of the population have not been confirmed (Elie-Turenne *et al*., 2010; Saadatian-Elahi *et al*., 2013; Chen *et al*., 2015; Price *et al*., 2017). The association between smoking and nasal carriage seems also controversial. In a study by Olsen *et al*. (2012), active smoking in healthy adults was found to be a protective factor for carriage of *Staphylococcus aureus*, with a hypothesized bactericidal activity of cigarette smokers. Conversely, a recent study showed that smokers were more frequently colonized than non-smokers, and cessation from smoking improved clearance of nasal *Staphylococcus aureus* in an experimental inoculation study (Cole *et al*., 2018). Many other host conditions have been punctually studied and reported as additional predisposing factor such as hormonal contraception (Zanger *et al*., 2012) and presence of hemoglobin in nasal secretions (Pynnonen *et al*., 2011).

At the genetic level, no correlation was found between genetic factors and *Staphylococcus aureus* carriage. No significant heritability for *Staphylococcus aureus* nasal colonization was detected in twins and family studies (Roghmann *et al*., 2011; Andersen *et al*., 2012). Interestingly, some polymorphisms in host inflammatory response genes have been associated with *Staphylococcus aureus* nasal carriage. The presence of the histocompatibility antigen phenotype HLA-DR3 could be a predisposition (Cole *et al*., 2018).

As previously said, at the immune system level, polymorphisms in genes encoding some proteins and differential expression profiles of AMPs could be the determinants of the various carriage states.

In a study involving 93 type 1 diabetes patients, vitamin D receptor polymorphisms were determined in Deoxyribonucleic acid (DNA) extracted from peripheral blood leukocytes. Analysis showed that presence of specific alleles coding for vitamin D receptors were associated with an increased rate of *Staphylococcus aureus* colonization (Panierakis *et al*., 2009).

Staphylococcus aureus nasal colonization has been identified as a major risk factor for the development of patent staphylococcal infections, weather community acquired, or nosocomial (Von Eiff *et al*., 2001; Wertheim *et al*., 2004) which increases the risk by 2 to 10 times. The risk of infection in nasal carriers has been mainly studied in surgical patients (general, orthopedic, cardiac, and neurosurgeries) (Perl *et al*., 2002; Bode *et al*., 2010; Walsh *et al*., 2017), patients on hemodialysis (Katneni and Hedayati, 2007), patients on chronic ambulatory peritoneal dialysis (CAPD) (Luzar *et al*., 2019), HIV-infected patients (Sissolak *et al*., 2012), and intensive care unit patients (Nardi *et al*., 2011). It has also been shown to be the primary risk factor for recurrent furunculosis, nasal colonization being present in almost 60% of individuals with furuncles and impetigo (Durupt *et al*., 2007)

2.12 Transmission, Prevention and Control of Drug-Resistant *Staphylococcus aureus*

2.12.1 Transmission

Staphylococcus aureus can be found in different body sites like the skin, rectum, vagina, gastrointestinal tract and axilla, the anterior nares appearing as the main reservoir. From a cutaneous commensal site, *Staphylococcus aureus* can enter in contact with the nasal mucosa, then interact with epithelial cell ligands such as loricrin and cytokeratin 10 (K10). Once the host's defenses are overcome, *Staphylococcus aureus* can propagate into the anterior nares so that the host becomes an *Staphylococcus aureus* nasal carrier (Wertheim *et al*., 2005). In human,

nasal colonization may begin within the first days of life (Maayan-Metzger *et al*., 2017). This has been demonstrated in a cohort study evaluating nasal carriage of *Staphylococcus aureus* in 100 pairs of infant–mother for a period of 6 months following delivery (Peacock *et al*., 2003). The carriage rate in the first 8 weeks of life was around 40–50%, thereafter it dropped to 21% at 6 months. In addition, this study found a nasal carriage concordance in 68% of infant–mother pairs attesting the role of environmental factors in *Staphylococcus aureus* carriage (Peacock *et al*., 2003). Another study found identical strains in 80% of infant–mother pairs. In 90% of these newborns, the source of *Staphylococcus aureus* was the maternal nasal strain as represented in figure 2.2 (Leshem *et al*., 2012)

After birth, hands are the main vector for *Staphylococcus aureus* transmission from surfaces to the nose (Wertheim *et al.*, 2005). The hypothesis of a link between hand and nose *Staphylococcus aureus* carriage is supported by the double blind randomized placebo controlled trial from Reagan *et al*. (2019), who demonstrated that nasal decolonization with mupirocin applied to health-care workers resulted in a decrease of nose and hand carriage. In a cohort study including outpatients and healthy hospital employees, nasal carriage was evaluated by a single or several swabs. Participants completed a questionnaire about their nose picking behavior, a positive correlation between this habit and nasal carriage of *Staphylococcus aureus* was found. However, it is unknown whether nose-picking patients were more frequently colonized at extra nasal sites (Wertheim *et al*., 2006).

Studies realized in individuals living in the same households have revealed that these people tend to carry genetically similar strains in their nares (Nouwen and Optima Grafische Communicatie, 2004; Muthukrishnan *et al*., 2013) suggesting horizontal transmission. Multisite MRSA carriage increases the risk for nasal MRSA colonization (Harbarth *et al*., 2000).

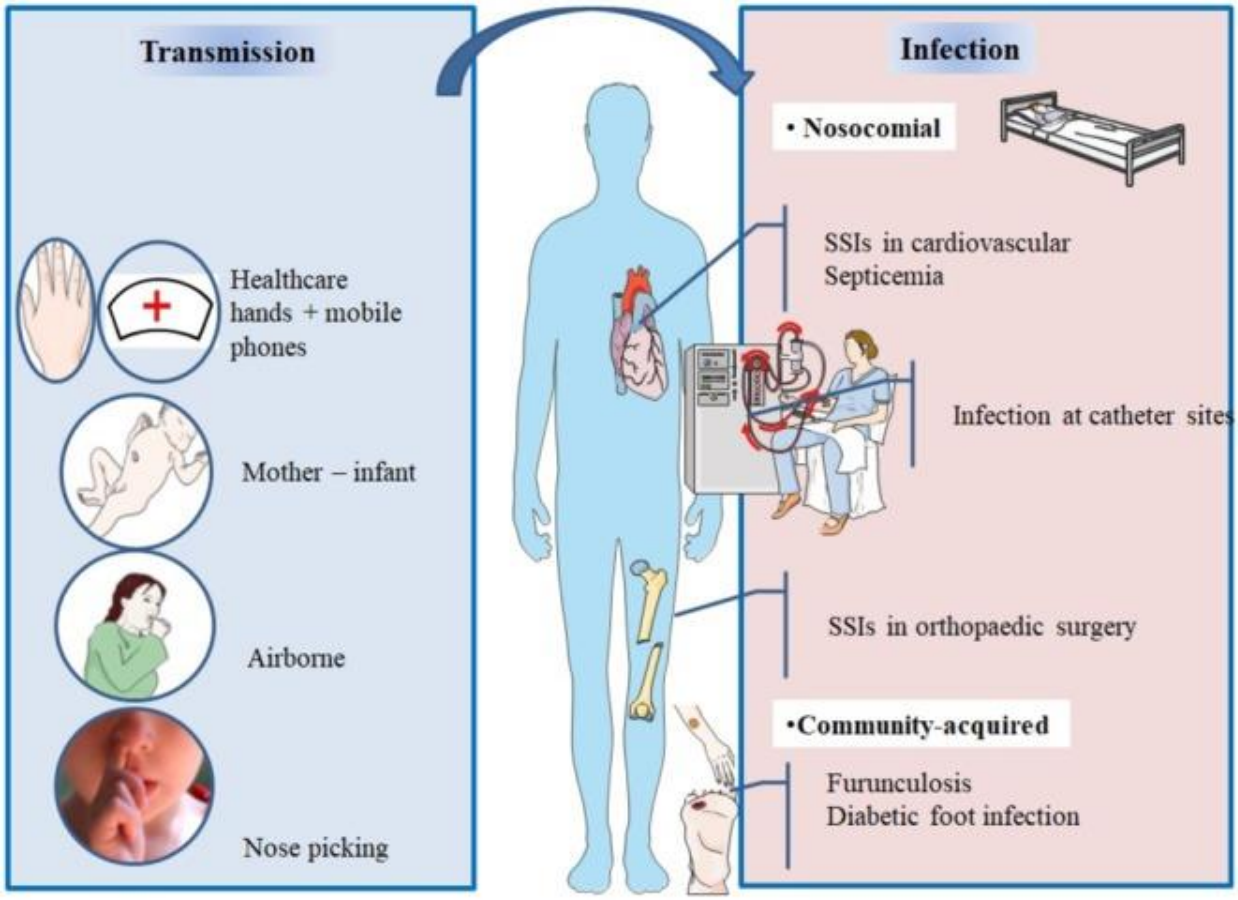

Figure 2 Main spread and transmission mechanisms of *Staphylococcus aureus* and impact of nasal carriage on subsequent infections (Sakr *et al*., 2018).

In spite infrequent, airborne transmission is another possible route of *Staphylococcus aureus* dissemination (Wertheim *et al*., 2005a) and may play a role in hospital outbreaks (Sherertz *et al.*, 2016).

During viral upper respiratory infections, the risk of disseminating endogenous *Staphylococcus aureus* in the air increases and infection outbreaks may occur. In 1996, an MRSA outbreak involving 8 of 43 patients occurred in a surgical ICU of a university hospital in the United States. Investigations of the cause concluded that a single physician was the source of this outbreak; he was a nasal carrier of MRSA and suffered an upper respiratory infection. To assess airborne dispersal of *Staphylococcus aureus*, the authors completed their findings by an experimental clinical test on this physician and showed that transmission of the bacterium increased by 40-fold when he was infected by a rhinovirus infection than when he was not. The use of a mask significantly reduced dispersal ($P = 0.015$) (Sherertz *et al*., 2016).

Healthcare workers who are asymptomatic nasal carriers can sometimes be the source of MRSA outbreaks (Wang *et al.*, 2001; Vonberg *et al*., 2006; Haill *et al*., 2013; Lamanna *et al*., 2017). On the other hand, in non-outbreak situations and in presence of control measures, healthcare workers are infrequently sources of transmission of *Staphylococcus aureus* (Price *et al*., 2017).

Mobile phones of healthcare workers may be a reservoir of *Staphylococcus aureus* (Chang *et al*., 2017). A recent study evaluated incidence of bacterial contamination of mobile phones belonging to medical staff working in the operating room. Seventy two healthcare professionals took bacterial cultures from their phones, anterior nares, and hands. The results revealed that 31 staff had *Staphylococcus aureus* isolated from their nares, 8 from their mobile phones, and 4 from their hands. Genotyping confirmed that 7/8 of the mobile phones strains were identical to the ones isolated from the nares (Chang *et al*., 2017).

2.12.2 Prevention and Control of Drug-Resistant *Staphylococcus aureus*

Treatment of *Staphylococcus aureus* infections depends largely on the type of infection as well as the presence or absence of drug resistant strains (Guo *et al*., 2020). When antimicrobial therapy is needed, the duration and mode of therapy are largely dependent on the infection type as well as other factors (Foster, 2017). In general, penicillin remains the drug of choice if isolates are sensitive (MSSA, or methicillin sensitive *Staphylococcus aureus* strains) and vancomycin for MRSA strains. In some cases, alternative therapy is necessary for addition to antimicrobial therapy (Turner *et al*., 2019). For example, fluid-replacement management is often required for toxin-mediated illness and removal of foreign devices for prosthetic value endocarditis or catheter-associated infections. Because many MRSA strains are resistant to multiple antibiotics, MRSA infections are emerging as serious pathogens in both the hospital and the community settings (Mutua *et al*., 2017).

CHAPTER THREE

3.0 MATERIAL AND METHOD

3.1 Study Area

Caleb University is a private University, located in Ikorodu-Itokin Road, Imota, Lagos and it has over 4,500 Students Population. Caleb University has a peaceful environment that is ideal for learning and character development.

3.2 SAMPLE COLLECTION AND STUDY POPULATION

One hundred Nasal swabs of 100 students, whom were randomly selected amongst the students, 25 male students and 25 female students was collected. The nasal samples were collected by sterile swab stick immersed into sterile saline water, then collected from the left and right nostrils. The swab sticks were immersed into peptone water prior analysis.

3.3 Laboratory Procedures

In the laboratory the sample in the tube was thoroughly mixed to suspend the microorganisms into the buffered peptone water solution. The suspension was inoculated into Mannitol salt agar media plates. The samples were then incubated at 35˚C for 22 hours. Mannitol Salt Agar is used for the selective isolation and enumeration of *Staphylococcus aureus* from clinical and nonclinical materials. Only *Staphylococcus aureus* 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 was indicated by a change in the phenol red indicator, which will 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.

3.4 ISOLATION OF ORGANISM

Nasal swabs were immediately inoculated on mannitol salt agar, which is a selective medium for *Staphylococcus aureus* isolation. For 48 hours, the infected plate will be incubated at 37°C. After incubation, the culture plate was checked for appearance, size, color, and morphology of the colonies.

To assess for beta hemolysis features, mannitol fermenting colonies from the mannitol salt agar plate was sub cultured on blood on blood agar and incubated at 37°C overnight.

3.5 GRAM STAINING

The isolates were stained to determine their gram reaction. *Staphylococcus aureus* are clusters of gram-positive cocci. Gram-positive bacteria retain the color of crystal violet, which is the color of the principal dye used in gram staining. The thick peptidoglycan found in all gram-positive bacteria is responsible for the color retention (Boyanova, 2018).

3.5 BIOCHEMICAL TEST

3.5.1 CATALASE TEST

Staphylococcus aureus is catalase-positive because they produce a catalase enzyme that converts hydrogen peroxide to water and oxygen (Chen *et al*., 2019).

3.5.2 UREASE TEST

This test is used to detect alkaline urea fermentation and the formation of ammonia as a result. The urea fermentation process takes place in the presence of the urease enzyme as well as carbon dioxide. Because *Staphylococcus aureus* generates the urease enzyme, it is urease positive (Javid *et al*., 2018).

3.5.3 SUGAR FEMENTATION TEST

This test was carried out to identify microorganisms that are able to ferment sugars, such as: glucose, fructose, galactose, lactose, mannitol and sucrose. It was used to differentiate organisms based on carbohydrate fermentation patterns (Chesebrough, 2000). Using an Inoculating loop, a colony was picked from a pure culture; the colony was inoculated into the sugar by shaking the loop in it. The cap was closed and incubated for 18-24 hours at 35ºC-37ºC in atmospheric air, change of color from red to yellow indicates positive while no change was interpreted negative.

3.5.4 MOTILITY TEST

This test was carried out to determine the ability of an organism to move by itself, this test was always carried out using a semi solid agar (a half strength nutrient agar). A sterile inoculating needle was used to pick a colony of bacteria and was stabbed at the centre of the tube containing the agar to over half the depth, it was then incubated at 35ºC-37ºC for 48 hours. The motile organisms grew out of the line of inoculation and the non-motile organisms grew only along the line of inoculation (Chesebrough, 2000).

3.5.5 HEMOLYSIS TEST

This test was carried out to determine the ability of an organism to produce hemolysins, an enzyme that damages red blood cells. An inoculating loop was used to pick a colony of organisms and streaked on a blood agar plate, the plate was incubated at 35ºC-37ºC for 24 hours. The plate was then inspected for signs of beta, alpha and gamma hemolysis. Beta hemolysis indicated by a complete hemolysis, a clear zone surrounding the colonies, alpha hemolysis indicated by a green, opaque zone and gamma hemolysis indicated by no zone around the colonies (Chesebrough, 2000).

3.6 ANTIBIOTIC SUSCEPTIBILITY TESTING

The disk diffusion method was used to test commercially manufactured antibiotics such as Cefuroxime, Ofloxacin, Erythromycin, Ceftriaxone, Gentamicin, Amoxicillin, and Ceftazidime. A colony of the test organism will be chosen and injected into peptone water using a sterile wire loop. The turbidity was equivalent to 0.5 macfarlard standard. The organism's suspension was swabbed throughout the Mueller Hinton agar plate's whole surface, and the antibiotic disks was be placed on the media with sterile forceps. Overnight, the plate was be incubated. After incubation, the zone inhibitor was assessed using the Clinical and Laboratory Standard Institute (C.L.S.I 2020) (Garoy *et al*., 2019).

3.6.1 Detection Of Methicillin Resistant *Staphylococcus aureus*

Resistance of the isolates to the third generation cephalosporin, cefoxitin, was used as a substitute marker for the detection of methicillin-resistant *Staphylococcus aureus* (Clarence *et al.*, 2005).

3.6.2 DOUBLE DISC SYNERGY TEST

This test was carried out to detect the production of ESBL by members of *Staphylococcus aureus.* The test was carried out by using a disc of amoxicillin-clavulanate (30μg) along with the third generation cephalosporin; ceftazidime (30μg) and cefotaxime (30μg). An amoxicillin-clavulanate disc was placed in the center of the plate and the other two discs were placed 15mm apart, in the centre to that of amoxicillin-clavulanate disc.

CHAPTER FOUR

RESULTS

4.1 GENERAL CHARACTERISTICS OF THE STUDY POPULATION

A total of 50 students (25males and 25 females) nasal swabs were collected for drug resistant *Staphylococcus aureus* bacteria. The characteristics of the study population shows that majority of the male students(56%) and females students(56%) were between 20-24 years as shown in table 4.1 and the female students(36%) and the male students(24%) were between 15-19 years while female students (8%) and the male (20%) were between 25-29 years. Activities of the students that exposes them into harboring bacteria in their nose, in the study it was found that 76% of the female students often use hand sanitizer, 52% of male students deep their hand in their nose, 64% of males and 84% of female always use nose mask [Table 4.2].

TABLE 4.1 AGE DIFFERENCE BETWEEN THE STUDY POPULATION

	Male n(%)	Female n(%)
AGE 15 -19	6(24%)	9 (36%)
20-24	14(56%)	14(56%)
25-29	5(20%)	2(8%)
FAMILY SIZE 1-3	3(12%)	2(8%)
4-6	20(80%)	18(72%)
7-9	2(8%)	5(20%)
9-above		

TABLE 4.2 DATA OF ACTIVITIES DERIVED FROM QUESTIONAIRES ANSWERED BY STUDENTS

QUESTIONS	MALE (%)	FEMALE (%)
Deeping of hands in the nose	52%	56%
Cleaning of the nose	80%	60%
Use of alcohol based hand sanitizer	88%	76%
Currently on antibiotics drugs	4%	12%
Deeping of hand into the nose regularly	52%	56%
Use of antibiotics without doctor's prescription	68%	84%
Use of nose mask	64%	84%
Practice of regular hand washing	80%	56%
Running nose	8%	4%
Deeping of hand in nose after use of toilet	16%	4%

4.3 IDENTIFICATION OF *STAPHYLOCOCCUS AUREUS*:

Staphylococcus aureus was isolated from the nose of a few male and female students respectively. All isolates were identified using gram staining, biochemical tests and cultural characteristics as shown in tables 4.3-4.5. The percentage of isolated *Staphylococcus aureus* with the same number 9 each in both gender (18%).and other bacteria species were found.

Table 4.3: CELLULAR MORPHOLOGY OF ISOLATED *Staphylococcus aureus*

Sample	Shape
Mno1 R	Cocci
Mno1 L	Cocci
Mno4 R	Cocci
Mno4 L	Cocci
Mno9 R	Cocci
Mno11 R	Cocci
Mno11 L	Cocci
Mno13 L	Cocci
Mno14 R	Cocci
Fno1r	Cocci
Fno1L	Cocci
Fno9 R	Cocci
Fno9L	Cocci
Fno10R	Cocci
Fno12R	Cocci
Fno13R	Cocci
Fno13L	Cocci
Fno14R	Cocci

TABLE 4.4: COLONIAL MORPHOLOGY OF *STAPHYLOCOCCUS AUREUS* ISOLATES OF MALE AND FEMALE STUDENTS

Samples	Mannitol salt agar	Nutrient Agar	Macconkey agar	Eosin Methylene Blue Agar	Suspected Organisms
Mno1 r	White colonies	White colony	NG	NG	*Staphylococcus aureus*
Mno1 l	Whitish pink colonies	White colony	NG	NG	*Staphylococcus aureus*
Mno2 r	Pink colony	Pink colony	NG	NG	*Staphylococcus aureus*
Mno2 l	Pink colony	Pink colony	NG	NG	*Staphylococcus aureus*
Mno4 r	Yellow pigment	Yellow pigment	NG	NG	*Staphylococcus aureus*
Mno4 l	Yellow pigment	Yellow pigment	NG	NG	*Staphylococcus aureus*
Mno9 r	Yellow pigment	White colony	Round pink colony	Purple colony	*Staphylococcus aureus*
Mno11 r	Yellow pigment	NG	NG	NG	*Staphylococcus aureus*
Mno11 l	Yellow	Cream	NG	NG	*Staphylococcus*

	pigment	mucoid colony			*aureus*
Mno13 l	Pink colony	Pink,colorless, colony	Pink colony	Pink colony	*Staphylococcus aureus*
Mno14r	Yellow pigment	White colony	Pink colony	Purple colony	*Staphylococcus aureus*
Fno1 r	Yellow pigment	Pink colony	NG	NG	*Staphylococcus aureus*
Fno1 l	Yellow pigment	Pink colony	NG	NG	*Staphylococcus aureus*
Fno9 r	Yellow pigment	White mucoid colony	Round pink colony	Purple colony	*Staphylococcus aureus*
Fno9 l	Yellow pigment pink colony	White mucoid colony	Round pink colony	Purple colony	*Staphylococcus aureus*
Fno10 r	Yellow pigment	Cream mucoid colony	Pink colony	Purple colony	*Staphylococcus aureus*
Fno12 r	Pink colony	White mucoid colony	Purple colony	Purple mucoid colony	*Staphylococcus aureus*
Fno13 r	Yellow	White colony	Purple	Purple	*Staphylococcus*

	pigment		colony	mucoid colony	*aureus*
Fno13 r	Yellow pigment	White colony	Purple colony	Purple mucoid colony	*Staphylococcus aureus*
Fno14 r	Yellow pigment	White mucoid colony	Purple colony	Purple mucoid colony	*Staphylococcus aureus*

KEY: NG: NO GROWTH, NU: NOT USED.

TABLE 4.5: BIOCHEMICAL TEST FOR MALE AND FEMALE SAMPLES

Sample	Catalase	Hemolysis	Motility	Urease	Citrate	Indole	Lactose	Sucrose	Fructose	Glucose	Galactose	Mannitol
Mno1R	+	Beta	-	+	+	-	-	+	+	+	+	+
Mno1L	+	Beta	+	+	+	-	-	-	+	+	+	-
Mno4R	+	Beta	-	+	+	-	+	+	+	+	+	+
Mno4L	+	Beta	-	+	+	-	+	+	+	+	+	+
Mno9R	+	Beta	-	+	+	-	+	+	+	+	+	+
Mno11	+	Beta	-	+	+	-	+	+	+	+	+	+

R												
Mno11 L	+	Beta	-	+	+	-	+	+	+	+	+	+
Mno13 L	+	Beta	-	+	+	-	+	+	+	+	+	+
Mno14 R	+	Beta	-	+	+	-	+	+	+	+	+	+
Fno1r	+	Beta	-	+	+	-	+	+	+	+	+	+
Fno1L	+	Beta	-	+	+	-	+	+	+	+	+	+
Fno9 R	+	Beta	-	+	+	-	+	+	+	+	+	+
Fno9L	+	Beta	-	+	+	-	+	+	+	+	+	+
Fno10R	+	Beta	-	+	+	-	+	+	+	+	+	+

Fno12R	+	Beta	-	+	+	-	+	+	+	+	+	+
Fno13R	+	Beta	-	+	+	-	+	+	+	+	+	+
Fno13L	+	Beta	-	+	+	-	+	+	+	+	+	+
Fno14R	+	Beta	-	+	+	-	+	+	+	+	+	+

Figure 4.1a: Positive DDST for *S. auerus* isolated from nasal swab of female students

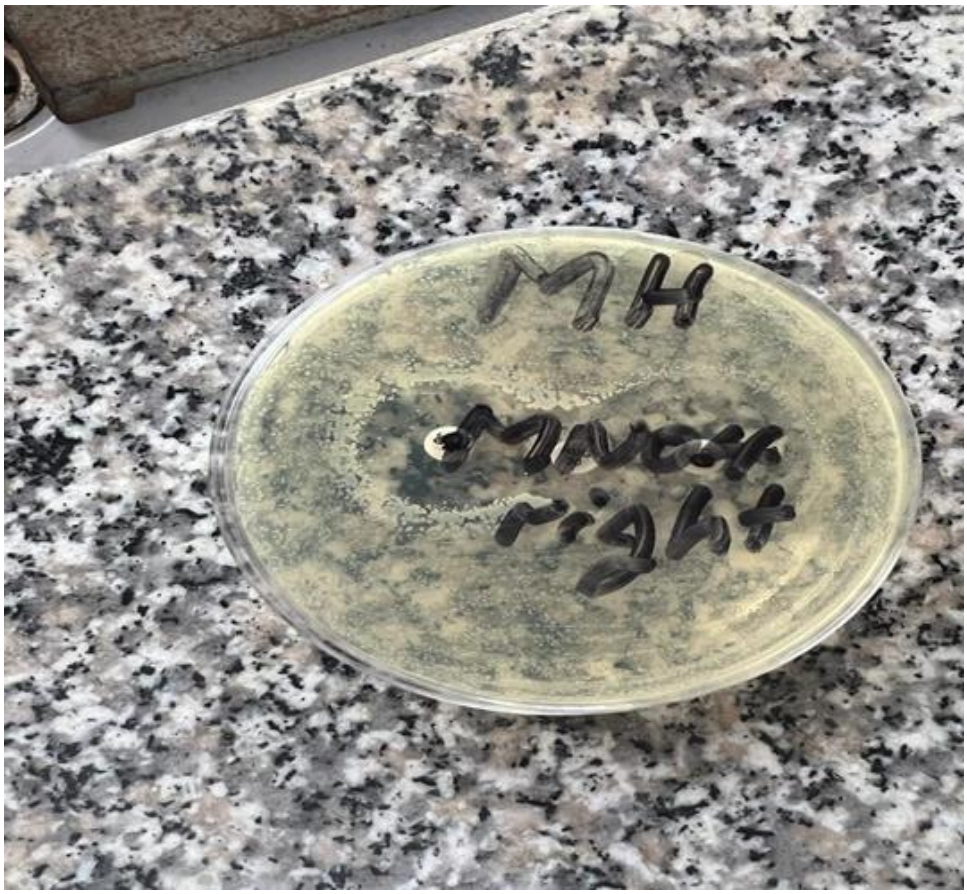

Figure 4.1b: Positive DDST for *S. auerus* isolated from nasal swab of male students

4.6 ANTIMICROBIAL SUSCEPTIBILITY TESTING: Multi-drug resistant *Staphylococcus aureus* was found (100%), the antibiotics with demonstrated multi-drug resistance were Amoxicillin-Clavalanate (50%), Ceftazidime(39%), Cefotaxime(28%), Ceftriaxone(44%), Cefoxitin(67%), Clotrimazole(100%), Imipenum(50%), Tetracycline(28%), Erythromycin(50%), Ciprofloxacin(44%), Gentamicin(11%) [Table 4.6-4.7].

TABLE 4.6 ANTIBIOTICS ZONE OF INHIBITION (MM)

Sample	Suspected Organisms	AUG (30ng)	CAZ (30ng)	CTX (30ng)	CRO (30ng)	FOX (30ng)	CLO (50ng)	IMI (10ng)	TE (30ng)	E (15ng)	CIP (5ng)	CN (10ng)
Mno1 R	*Staphylococcus aureus*	18	21	35	16	9	0	25	27	23	33	20
Mno1 L	*Staphylococcus aureus*	23	21	30	20	11	0	22	25	25	30	21
Mno4 R	*Staphylococcus aureus*	18	24	29	11	0	0	10	0	0	14	0
Mno4 L	*Staphylococcus aureus*	30	24	33	0	10	0	12	15	0	20	27
Mno9 R	*Staphylococcus aureus*	30	43	0	20	19	0	19	20	29	29	15
Mno11 R	*Staphylococcus aureus*	20	14	19	25	0	0	0	11	0	19	20
Mno11	*Staphylococcus*	0	13	15	20	10	0	20	15	16	30	35

L	*aureus*											
Mno13 L	*Staphylococcus aureus*	19	10	16	16	29	0	31	25	19	22	10
Mno14 R	*Staphylococcus aureus*	0	19	25	40	32	0	18	15	13	0	19
Fno1 R	*Staphylococcus aureus*	13	0	24	20	16	0	30	25	20	31	23
Fno1 L	*Staphylococcus aureus*	30	21	29	18	10	0	26	29	23	32	23
Fno9 R	*Staphylococcus aureus*	27	30	0	27	28	0	22	19	26	14	10
Fno9 L	*Staphylococcus aureus*	10	31	22	40	27	0	17	28	0	11	9
Fno10 r	*Staphylococcus aureus*	0	19	14	23	29	0	0	21	10	15	0
Fno12	*Staphylococcus*	20	28	0	15	10	0	18	0	20	10	19

r	*aureus*											
Fno13r	*Staphylococcus aureus*	19	35	22	0	29	0	11	17	12	20	19
Fno13L	*Staphylococcus aureus*	30	23	24	0	0	0	13	13	16	15	16
Fno14r	*Staphylococcus aureus*	10	14	20	35	30	0	10	20	24	15	20

KEY: AUG: Amoxicillin-Clavulanic acid, CAZ: Ceftazidime, CTX: Ceftaxime, IMI: Imipenem, CRO: Ceftriaxone, CLO: Clotrimazole, TE: Tetracycline, E: Erythromycin, CN: entamicin, FOX: Cefoxin, R: Resistance, S: Sensitive, I: Intermediate

TABLE 4.7: Antimicrobial Susceptibility testing of *Staphylococcus aureus* isolate for both male and female

S/N	Sample	AUG	CAZ	CTX	CRO	FOX	CLO	IMI	TE	E	CIP	CN
1	MNO1 r	S	R	S	S	R	R	R	R	R	S	S
2	MNO1 l	R	R	S	S	R	R	S	S	S	S	S
3	MNO4 r	R	S	S	R	R	R	R	R	R	R	R
4	MNO4l	S	S	S	R	R	R	R	S	R	S	S
5	MNO9 r	S	R	R	I	R	R	R	S	S	S	S
6	MNO11 r	S	R	I	S	R	R	R	R	R	I	S
7	MNO11 l	R	I	I	R	R	R	S	I	I	S	S
8	MNO13 l	S	S	R	I	R	R	S	S	S	S	S
9	MNO14 r	R	S	S	S	S	R	S	I	R	R	S
10	FNO1 r	R	R	S	R	R	R	S	S	R	S	S
11	FNO1 l	S	S	S	R	R	R	S	S	S	S	S
12	FNO9 r	S	S	R	S	S	R	S	S	S	R	S
13	FNO9 l	R	S	I	S	S	R	S	S	R	R	S

14	FNO10 r	R	S	R	S	S	R	R	S	R	R	R
15	FNO12 r	S	R	R	R	R	R	S	R	I	R	S
16	FNO13 r	R	S	I	R	S	R	R	I	R	I	S
17	FNO13 l	S	S	S	R	R	R	R	R	I	R	S
18	FNO14 r	R	R	I	S	S	R	R	S	S	R	S

Keys – S- Susceptible, I- Intermediate, R- Resistance

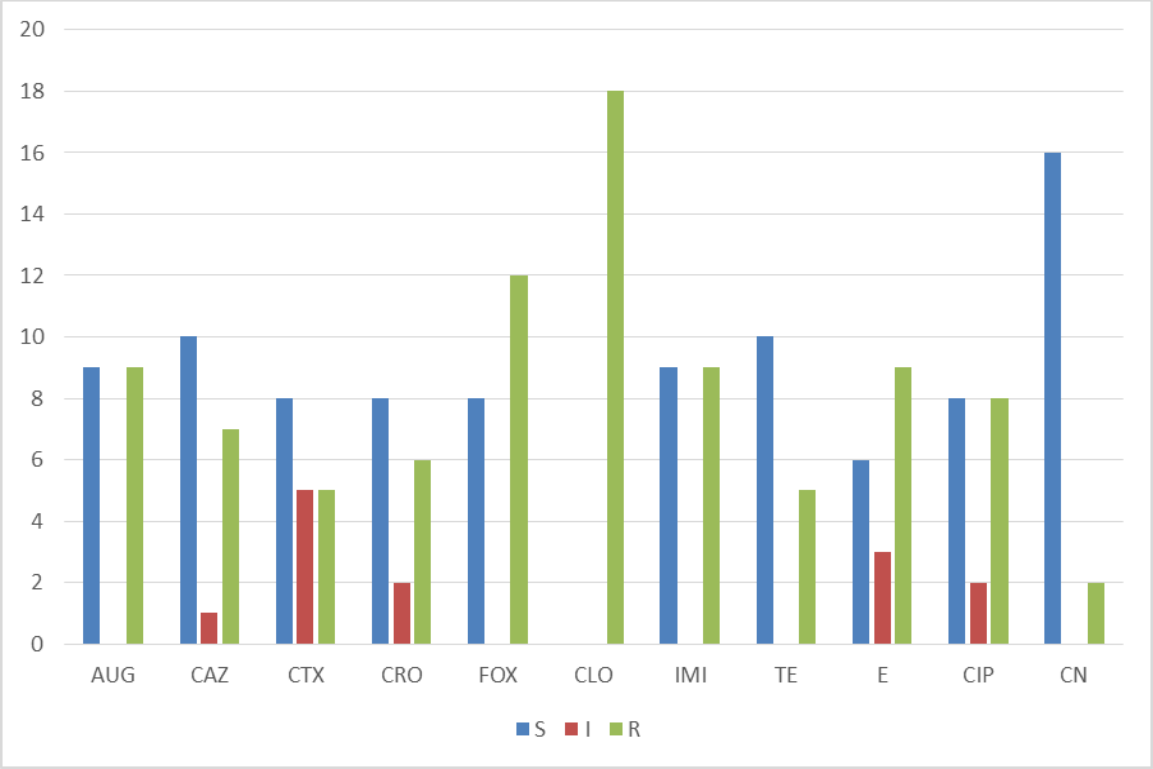

Figure 4.2 Frequency of the antimicrobial susceptibility testing of *Staphylococcus aureus* isolate for both male and female

Keys: S- Susceptible, I- Intermediate, R- Resistance, AUG: Amoxicillin-Clavulanic acid, CAZ: Ceftazidime, CTX: Ceftaxime, IMI: Imipenem, CRO: Ceftriaxone, CLO: Clotrimazole, TE: Tetracycline, E: Erythromycin, CN: entamicin, FOX: Cefoxin

CHAPTER FIVE

5.0 DISCUSSION

Staphylococcus aureus is a well-known pathogen with an alarmingly increasing level of developing resistance to most available antimicrobial agents. Nasal *Staphylococcus aureus* have been implicated in community associated infections like soft tissue infections and hospital infections like bacteremia.

This study showed an overall prevalence of 100% of *Staphylococcus aureus* in the nostrils of the students used for this study. In contrary, in Abia state of Nigeria, Chigbu and Ezeronye (2003) reported 50% nasal colonization in both hospital and non-hospital subjects. Whilst, Adesida *et al*. (2007) reported a much lower (14.0%) nasal colonization in medical students in Lagos, Nigeria. These variations may be attributed to the characteristics of the population under study. Other factors that can cause variations may be sampling and culture techniques (Adesida *et al*., 2007).

Multi-drug resistant *Staphylococcus aureus* was found to be (100%). According to (Magiorakos *et al.,* 2015) multi-drug resistance is defined as non-susceptibility to at least one agent in three or more antimicrobial categories. The antibiotics that demonstrated to be multi-drug resistance were Amoxicillin-Clavalanate (50%), Ceftazidime (39%), Cefotaxime (28%), Ceftriaxone (44%), Cefoxitin (67%), Clotrimazole(100%), Imipenum (50%), Tetracycline (28%), Erythromycin (50%), Ciprofloxacin (44%), Gentamicin (11%). Methicillin-resistant *Staphylococcus aureus* was found to be 67% in this study. The result is in accordance with studies done that Methicillin-resistant *Staphylococcus aureus* constituted nearly 40% of *Staphylococcus aureus* infection in the last year of this study (Johnson, 2011; Dantes *et al.*, 2013; Song *et al*., 2013). Extended

spectrum beta lactamase was detected in 60% of *Staphylococcus aureus*, resistance to third generation *cephalosporins* (Ceftazidime and Ceftaxime). This is in accordance by (Sirichoat *et al.,* 2016)

The susceptibility test results showed Clotrimazole to be the least effective agent with 100% bacterial resistance, this of course have been widely reported for *Staphylococcus aureus* from various sites of healthy subjects (Soysal *et al.,* 2006; Onanuga, and Temedie, 2011) and nosocomial infections (Umolu *et al.,* 2002, Hoerlle and Brandelli, 2009). This is basically due to the effect of beta-lactamases produced by *Staphylococcus aureus*. The uncontrolled availability of the agent in every drug vendors, which leads to its frequent use and misuse exert greater selection pressure for the resistant strains (Okeke *et al.,* 2019) thereby makes this agent almost useless in the treatment of staphylococcal infections. The observed moderately high resistance to Amoxicillin-Clavalanate, Imipenum, erythromycin, Cefoxitin and Ciprofloxacin may also be as a result of their uncontrolled usage in the environment which favours the increasing number of resistant strains due to selection pressure (Okeke *et al.,* 2019).

5.2 CONCLUSION

In this current research, the nose of randomly selected male and female students of Caleb University, Imota, Lagos were detected to be contaminated with *Stapylococcus aureus* and other bacteria species. It was concluded that the nose can serve as a means of transmitting diseases/ microorganisms by sneezing, because it is a depository for microorganisms. It is advisable to use tissue paper, neat handkerchief to clean the nose, prevent microorganisms from entering the nose with the use of nose mask, deeping of hand in the nose after eating or use of toilet is not advisable and use of alcohol based hand sanitizer regularly before deeping of hand in the nose, in

accordance with studies done by (Johnson et al, 2012). Further, nasal studies are also needed to establish and characterize resistant strains of *Staphylococcus aureus.*

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