IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF METHICILLIN AND ERYTHROMYCIN RESISTANT *Staphylococcus aureus* FROM CLINICAL AND ENVIRONMENTAL SAMPLES IN MINNA, NIGERIA

By

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(MTech/ SLS/2017/7343)

DEPARTMENT OF MICROBIOLOGY

FEDERAL UNIVERSITY OF TECHNOLOGY

MINNA

JULY, 2021

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ABSTRACT

Methicillin resistance in *Staphylococcus aureus* (MRSA) is progressively increasing globally and has become a global health concern. Antibiotic susceptibility profile of *Staphylococcus aureus* was determined using disc diffusion method. A total of 21.9 % (73/360) from clinical and environmental samples tested positive for *Staphylococcus aureus*. The prevalence of *Staphylococcus aureus* in environmental samples was 24 % while it was 20.5 % in clinical samples. The age group 18 to 49 years had the highest prevalence of *Staphylococcus aureus* (74 %) followed by 0 to 17 years (42 %) while 50 to 70 years had the least (4 %). The prevalence of *Staphylococcus aureus* in female was 22.4 % while in male, it was 20 %. The resistance of *Staphylococcus aureus* to Oxacillin, Cefoxitin, Ampicillin, Vancomycin, Erythromycin, Norfloxacin, Rifampicin, Amoxacillin and Gentamycin were 88.60 %, 45.60 %, 34.20 %, 21.50 %, 18.90 %, 11.40 %, 8.90 %, 6.30 %, 5.10 % respectively. Septrin and Levofloxacin were 100 % active on all the 79 *Staphylococcus aureus* isolates. Methicillin (mecA) and Erythromycin (ermA and ermC) genes were molecularly identified from the isolates. These findings showed relatively high prevalence of *Staphylococcus aureus* from the samples and revealed poor personal hygienic practices amongst clinicians and patients.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Staphylococcus aureus is a coagulase-positive, Gram-positive Coccus that forms clusters resembling grapes. *Staphylococcus aureus* is a bacterium that lives asymptomatically on healthy people, including their, mucous membranes, noses, guts, and skin glands (Sahreena and Kunyan, 2018).This ubiquitous bacterium is a major pathogen due to its combination of toxin-mediated virulence, invasiveness, and antibiotic resistance (Rachel *et al.,* 2016; Sahreena and Kunyan, 2018).

This organism has been a leading cause of nosocomial and community-acquired infections. *Staphylococcus aureus* does not produce spores, it can contaminate food during preparation and processing. *Staphylococcus aureus* can thrive in a wide range of temperatures (7 to 48.5 degrees Celsius, optimum 30 to 37 degrees Celsius), pH (4.2 to 9.3, optimum 7 to 7.5), and sodium chloride concentrations up to 15%. *Staphylococcus aureus* is a desiccation-tolerant organism that can survive in potentially dry and stressful environments like the nose, skin, and inanimate surfaces like clothing, tables, and workbench surfaces (Rachel *et al.,* 2016). Many food products benefit from these characteristics because they promote organisms growth and development (Rachel *et al.,* 2016). After initial contact with *S. aureus*, it can survive about 48 hours on hands and environmental surfaces (Tong *et al.,* 2015; Rachel *et al.,* 2016).

About 30% of the global population have been colonized by *S. aureus* (Rachel *et al.,* 2016). Bacteremia, infective endocarditis (IE), osteoarticular, skin, and soft tissue infections, pleuropulmonary and device-related infections are among the most common infections caused by *Staphylococcus aureus* (Tong *et al.,* 2015; Rachel *et al*., 2016). The most well-known

Staphylococcus aureus infection is bacteremia. Numerous research on the prevalence, prognosis, and outcome of *S. aureus* have been reported. Infections caused by *S aureus* bacteremia (SAB) is a condition that mostly affects people in developed countries.. In addition, there is still a scarcity of high-quality evidence to direct SAB management (Tong *et al.,* 2015). The pathogen's propensity for acquiring antibiotic resistance makes treatment of these infections difficult. Community-acquired methicillin-resistant *S. aureus* (CA-MRSA) skin infections, in particular, are becoming more common in healthy people who have no known healthcare-related risk factors (Tattevin *et al.,* 2012).

Resistance to commonly used antimicrobial drugs is frequently encountered in *S. aureus*. Some of the mechanisms of resistance include; inactivation of antibiotics by enzymes, decreased affinity for the antibiotics caused by alteration of the target, efflux pumps, and trapping of the antibiotic (Gitau *et al.,* 2018).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is defined as any strain of *S. aureus* that has acquired resistance to methicillin and other beta lactam antibiotics It is also responsible for a number of human infections that are difficult to treat (Brown *et al.,* 2012; Dangler *et al.,* 2013).

Staphylococcus aureus and MRSA are both shed by swimmers (Plano *et al.,* 2011) and have been reported in seawater and beach sand(Plano *et al.,* 2011; Goodwin *et al.,* 2012). *Staphylococcus aureus* has a high resistance. The production of penicillin-binding protein 2a (PBP2a), which is encoded by the mecA gene on the mobile gene element (MGE) of the staphylococcal chromosome cassette mec (SCCmec), that has a low affinity for beta-lactam antibiotics, causes *S. aureus* to be resistant to methicillin (Akanbi *et al.,* 2017).

The macrolide-lincosamide-streptogramin B resistance phenotype in *Staphylococcus aureus* includes erythromycin resistance. Chabbert was the first to identify this phenotype shortly after

the use of erythromycin in clinical practice (Chabbert, 1956). Erythromycin was found to induce spiramycin in four clinical strains of *S. aureus*. This phenotype in *S. aureus* has been shown to be due to an erythromycin resistance mythylase (erm gene product) which renders newly synthesized ribosomes resistant to macrolide-lincosamide-streptogramin B antibiotics by methylating a specific adenosine residue of the 23S rRNA (Akanbi *et al.,* 2017). In *S. aureus*, the genes encoding the methylase have been designated ermA, ermB, and ermC. ermA was first described by in 1969 in a clinical stain, designated 1206, with inducible resistance (Rahimi *et al.,* 2016). Antimicrobials work by inhibiting key bacterial functions like cell walls synthesis (beta-lactams and glycopeptides), protein synthesis (aminoglycosides, tetracyclines, macrolides, lincosamides, chloramphenicol, mupirocin, and fusidic acid), nucleic acid synthesis (quinolones), RNA synthesis (rifampin), and metabolic pathways like folic acid metabolism (Rahimi *et al.,* 2016). Antimicrobial resistance develops as a result of overuse, either by acquisition of foreign resistance genes or point mutations, resulting in a change in the antimicrobial target, degradation of the antimicrobial, or a decrease in the cell's internal antimicrobial concentration (Rahimi *et al.,* 2016;Bitrus *et al.,* 2018).

Methicillin and erythromycin resistant *S. aureus* are also found to be resistant to other antibiotics such as oxacillin, amoxicillin and penicillin. These bacteria may also develop resistance to antibiotics such as clindamycin, cotrimoxazole, and gentamicin (Bale *et al.,* 2018). The MecA gene, which codes for the penicillin-binding protein PBP 2A, is responsible for methicillin resistance (Cuny *et al.,* 2015; Bitrus *et al.,* 2017). Clinical isolates with the erm (A) and erm(C) genes, which code for rRNA methylases, are erythromycin resistant (Rahimi *et al.,* 2016)

Healthy humans and animals have been found to have a new methicillin resistance gene called mecC (Harrison *et al.,* 2013; Perrero *et al.,* 2014). The mecC gene has been reported to be

responsible for morbidity and mortality in different parts of the world (Persoons *et al.,* 2013), and is referred to as community acquired methicillin-resistant *S. aureus* (CA-MRSA) (Perrero *et al.,* 2014).

1.2 Statement of the Research Problem

Antibiotic-resistant bacteria have emerged as a major problem in antibiotic therapy. Methicillin resistant *Staphylococcus aureus* is a major cause of hospital and community acquired infections that are becoming increasingly difficult to combat because methicillin resistant *S. aureus* has the ability to develop resistance to any antibiotic to which it has been exposed (Bitrus *et al.,* 2017; Cheung*et al*., 2021). Community acquired MRSA is the major cause of bacteremia and infective endocarditis (IE) as well as osteoarticular, skin and soft tissue infections (SSTIs), pleuropulmonary, and device related infections that has become increasingly problematic due to its high virulence and the ease with which they spread in the community (Reyes *et al.,* 2011; Tacconelli and Magrini , 2017).

Staphylococcus aureus was found in the majority of clinical trials involving *Staphylococci.* Some species are considered non-pathogenic, while *S. aureus* is considered a pathogen. Approximately 30% of the human population are colonized with *S. aureus* (Tong *et al.,* 2015).

Here in Nigeria, some studies conducted across the country showed that methicillin resistant *S. aureus* has been isolated and is a common cause of hospital and community acquired infections with varying prevalence (Fayomi *et al.,* 2011; Sina *et al.,* 2011; Adeiza *et al.*, 2020). Also, nasal carriage of *S. aureus* amongst students has been reported (Rasheed and Hussein, 2020).

1.3 Justification for the Study

Staphylococcus aureus is responsible for many human diseases with high morbidity and mortality rates. The increasing morbidity and mortality rate has been reported by various studies to be strongly linked to corresponding upsurge in the occurrence of resistant *Staphylococcus aureus*. Diseases caused by methicillin resistant *Staphylococcus aureus* and other non-methicillin resistant *Staphylococcus aureus* are challenging.

The prevalence of MRSA and non-MRSA in various clinical and environmental sources and their antibiotic profile will serve very useful purpose to clinicians for management of disease cases involving *S. aureus*. To the best of my knowledge the prevalence of antibiotic susceptibility profile of methicillin and erythromycin resistant *S. aureus* in clinical and environmental samples is yet to be documented for Minna Niger state. It is hoped that information from this research work will increase knowledge on the prevalence of methicillin and erythromycin resistant *S. aureus* and the most suitable antibiotics for managing such strains.

1.4 Aim and Objectives of the Study

The aim of this study was to identify and determine antibiotic susceptibility profile of methicillin and erythromycin resistant *Staphylococcus aureus* from clinical and environmental samples of Minna Nigeria.

The objectives of the study were to:

i. isolate *Staphylococcus aureus* from clinical and environmental sources in General Hospital Minna Niger State;

ii. determine antibiotic susceptibility profile of methicillin and non-methicillin resistant *Staphylococcus aureus*;

iii. detect the occurrence of methicillin and erythromycin resistant genes in the *Staphylococcus aureus*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of *Staphylococcus aureus*

Staphylococcus aureus was isolated from a surgical wound infection for the first time by Alexander Ogston in 1880. The isolated organism caused abscesses in guinea pigs and mice after being injected into them (David *et al.,* 2011). The pus from human *Staphylococcal* infections was then injected into animals, causing abscesses. The genus *Staphylococcus* was named after Ogston in 1882, and Rosenbach separated it into species in 1884. *Staphylococcus aureus* and *S. albus* are two bacteria that can cause infections (David *et al.,* 2011). These names remained in use until 1939, when Cowan differentiated *S. aureus* from *S. epidermidis. Staphylococcus aureus* was one of the first pathogens discovered (Gnanamani *et al.,* 2017).

Staphylococcus aureus is a common cause of infections in hospitals and the community. with severe consequences. It may cause infections in the bloodstream, skin and soft tissues, and the lower respiratory tract, such as CLABSI (central line associated bloodstream infection) and some severe deep-seated infections like endocarditis and osteomyelitis (Sejvar 2013; Tong *et al.,* 2015).

Staphylococcus aureus is equipped with a repertoire of virulence factors and toxins, often making it responsible for many toxin mediated diseases, including toxic shock syndrome, *styphylococcal* food borne diseases (SFD),and scalded skin syndrome. These virulence factors and toxins allow *S. aureus* to address challenges presented by the human immune system, because it has such elaborate tools, one might think that humankind would be highly vulnerable to severe infections by *S. aureus.* Interestingly, however *S. aureus* maintains a fine control of its virulence factors and for the most part rarely causes severe life threating infections in

otherwise-healthy individuals. Clinically, a major issue associated with *S.aureus* is the remarkable level of acquisition of resistance to multiple antibiotic classes, complicating treatment (Tong *et al.,* 2015; Serafini *et al.,* 2016; Gnanamani *et al.,* 2017)

Historically, *S. aureus* resistance emerged within two years of penicillin's introduction. In 1942, the first Penicillin-resistant *S. aureus* was detected (Hussein, 2016). In the late 1950s, the semi-synthetic antibiotic methicillin was developed, and MRSA was first clinically documented in 1960 (Hussein, 2016). Outbreaks of *S. aureus* linked to antibiotic resistance do happen (Stefani *et al.,* 2012). "Archaic" MRSA strains were discovered after the discovery of penicillin-resistant epidemic *S. aureus* strains, which were first discovered in the United Kingdom. To begin with, the epidemic was mostly confined to Europe. Nonetheless, new lineages began to emerge in the 1980s, resulting in a global disaster that is still ongoing. Infections due to Methicillin-resistant strains of *S. aureus* are associated with high mortality rates than infections caused by methicillin-susceptible strains. In addition they result in increased length of hospital stay as well as associated health care costs (Antonanzas *et al.,* 2015).

Methicillin resistant *Staphylococcus aureus* strains have a changed penicillin-binding protein (PBP) that has a lower affinity for most Synthetic penicillins. MecA, an acquired gene, encodes the protein (Stefani *et al.,* 2012). This methicillin-resistant genetic component is carried on a mobile genetic element (MGE) designated *staphylococcal* cassette chromosome mec (SCCmec) (Mejia *et al.,* 2010; Stefani *et al.,* 2012).

These mobile genetic elements are acquired and inserted into the chromosomes of susceptible *Staphylococci* strains, resulting in methicillin resistant strains. When it comes to treating and managing *Staphylococcal* infections, antimicrobial resistance has placed medical professionals at risk. In most cases, MRSA is responsible for at least 25 to 50 percent of *S. aureus* infections

in hospitals (Trakulsomboom and Thamlikitkul, 2008; Stefani *et al.,* 2012). They are a major source of concern due to their high morbidity and mortality, as well as their susceptibility to both penicillins and most other B-lactam antibiotics (except ceftaroline and ceftobiprole). Methicillin resistant *Staphylococcus aureus* has previously been linked to health-care environments like hospitals and other medical facilities, as well as the people who worked there. It has, however, formed reservoirs in the ecosystem and has become a major source of community-acquired infections, as a result of this methicillin resistant *Staphylococcus aureus* is no longer considered a nosocomial pathogen (Kandala *et al.,,* 2017).

Community-acquired MRSA infections have been on the rise since their detection in the 1980s (Tenover and Goering, 2009; Sahreena and Kunyan, 2018). These strains were previously associated to skin and soft tissue infesctions (SSTIs), but they are now also linked to infections in healthcare facilities (Otter and French, 2011; Sahreena and Kunyan, 2018). Community acquired-MRSA is distinguished from hospital-MRSA by its resistance to less non-B-lactam antibiotics, a smaller variant of SCCmec, and a high level of Panton-Valentine leukocidin output (PVL) (Oliveira *et al.,* 2018).

Community acquired-MRSA strains have historically been restricted to populations outside of health-cares environments, as previously stated. They were mainly responsible for only minor diseases, such as uncomplicated skin and soft tissue infections, at the time of their emergence. As a result, distinguishing CA-MRSA from HA-MRSA used to be based on this. However, this classification has recently become vague, and CA-MRSA strains have become more common. As several studies of CA-MRSA has been established as the etiological agent of nosocomial outbreaks in health care environments, but the epidemiological and molecular distinction between these two strains has become less well defined, as numerous reports of CA-MRSA

invading health care settings have identified CA-MRSA as the etiological agent of nosocomial outbreaks (Teare*et al.,* 2010; Collins and O'Connell, 2012; Sahreena and Kunyan, 2018).

2.1.1 Classification of *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive, non-motile, non-spore ssforming facultative anaerobe. It is catalase and coagulase positive. It occurs in irregular grape-like clusters and sometimes singly or in pairs. Typical colonies are smooth, raised, yellow to golden yellow in color and hemolytic on blood agar containing 5% sheep or horse blood (Rasheed and Hussein, 2021). Table 1 shows the classification of *Staphylococcus aureus*

Table 2.1:Classification of *Staphylococcus aureus*

Taxonomic group	**Bacteria**
Kingdom	Animalia
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Staphylococcaceae
Genus	*Staphylococcus*
Species	*Aureus*

2.1.2 Laboratory identification and cultural characteristics

Staphylococci grow rapidly on most bacteriological media under aerobic or microaerophilic conditions. *Staphylococci* grow most rapidly at 37°C, but form pigment best at room temperature (30 to 37 °C).The most important *Staphylococcal* species is S. *aureus*, which is named for its yellow-pigmented colonies (*aureus* = golden) as the result of the carotenoid

pigments that form during their growth. Colonies on solid media are round, smooth, elevated, and glistening. *Staphylococcus aureus* usually forms grey to deep golden yellow colonies. *Staphylococcus epidermidis* colonies usually are grey to white on primary isolation; many colonies develop pigment only upon prolonged incubation. No pigment is produced anaerobically or in broth. Various degrees of haemolysis are produced by *S. aureus* and occasionally by other species. *Peptostreptococcus* species, which are anaerobic cocci, often resemble *staphylococci* in morphology (Oliveira *et al.,* 2018; Rasheed and Hussein2021).

Staphylococcus aureus are Gram positive cocci in grape-like clusters. Figure 2.1 shows a Gram reaction of *Staphylococcus aureus.*

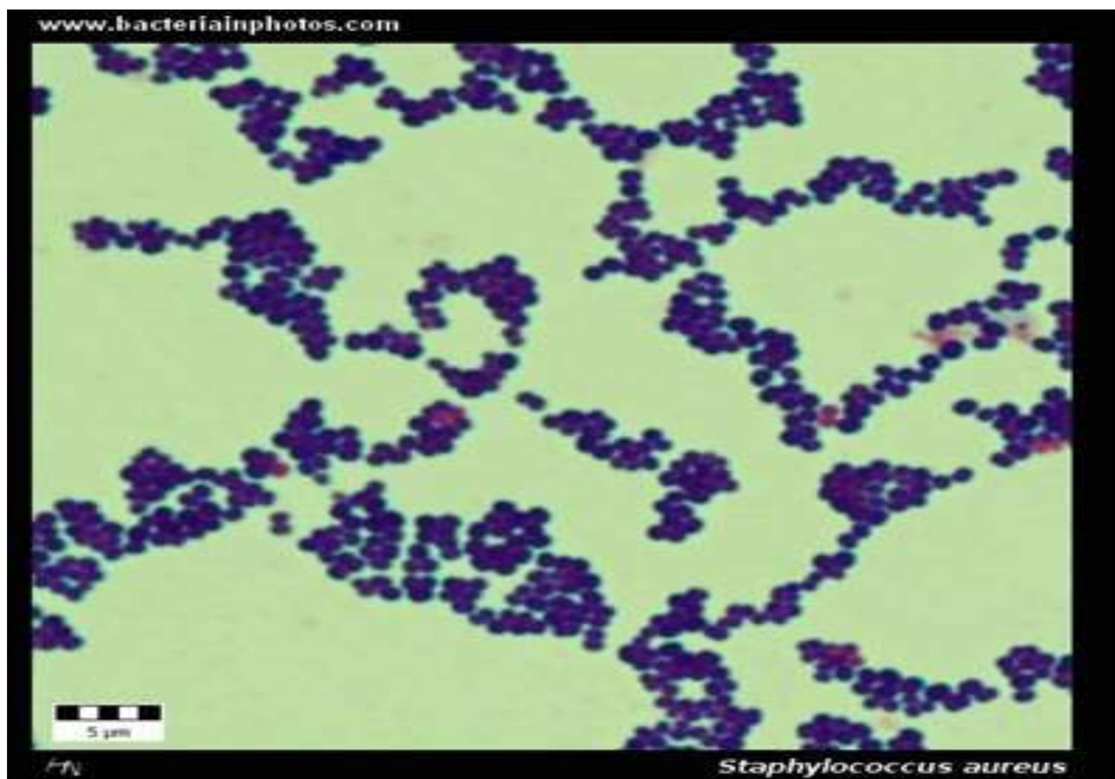

Figure 2:1: Gram stain of *Staphylococcus aureus* under oil immersion (X100) objective lens

(Cheesbrough, 2018).

2.2 Methicillin Resistance *Staphylococcus aureus*

2.2.1 History and epidemiology

Methicillin, introduced in 1961 was the first synthetic penicillinase-resistant antibiotic. Its introduction was followed by reports of methicillin resistant isolates (Turner *et al.,* 2019). Clinicians have been concerned about the spread of methicillin-resistant strains. *Staphylococcus aureus* is resistant to the antibiotic methicillin. Methicillin-resistant *S. aureus* (MRSA) infections have a greater clinical outcome than methicillin-sensitive *S. aureus* infections (Plano *et al.,* 2011; Gnanamani*et al.,* 2017). After being found in a British hospital, MRSA clones spread rapidly across international borders. When these rare MRSA clones are discovered in a new environment, they rapidly spread, becoming resident clones and accounting for an increasing proportion of nosocomial infections (Oliveira*et al.,* 2018; Bitrus *et al.,* 2018).

Methicillin resistant *Staphylococcus aureus* isolates, including penicillin-resistant strains, contain antimicrobial resistance genes (Malachowa and DeLeo, 2010). Methicillin resistant *Staphylococcus aureus* is spreading in a similar way to how penicillin resistance spread in the 1940s. Methicillin resistance was first discovered in hospitals in the 1960s, and it is now becoming more commonly recognized in the general public (Kandala *et al.,* 2017). While many of these diseases occurred in patients who had previously visited a hospital, the number of patients who had never visited a hospital has recently increased (Kandala *et al.,* 2017)). Both rural and urban patients have been identified with these community-based infections (Kandala *et al.,* 2017; Tong *et al.,* 2015).

The evolutionary origins of MRSA are poorly understood and there is no consensus on the nomenclature, the number of major MRSA clones, or the relatedness of clones described from different countries (Paterson *et al.,* 2014; Thomer *et al.,* 2016). Oliveira *et al.* (2018) reported that over 3,000 MRSA isolates from different regions of the world characterized, confirms the

existence of only a few epidemic clones worldwide, namely the Iberia, Brazilian, Hungarian, New York/Japan and Paediatric clones. This suggests that acquisition of the *Staphylococcal* cassette chromosome SCC mec, the mobile genetic elements harboring the mecA gene that confer methicillin resistance, has been a rare event (Brurec *et al.,* 2011).

The evolutionary changes of MRSA have resulted in its continuing threat to public health. The rising prevalence of MRSA infections in hospitals has resulted in a rise in the use of vancomycin, the last antibiotic that MRSA strains were reliably susceptible to (Howden *et al.,* 2011; Raymund *et al.,* 2013).

2.2.2 Evolution of methicillin resistant *Staphylococcus aureus*

The understanding of the evolution of MRSA has been aided immensely by the development of molecular methods that provide characterization of both the strain phylogeny (evolutionary history) and the methicillin resistance determinants (Wertheim *et al.,* 2004; Bitrus *et al.,* 2018).

Resolution of MRSA strain on the basis of its sequence at seven housekeeping genes (Brurec *et al.,* 2011) and whole genome typing techniques, such as amplified fragment length polymorphism (AFLP), which records nucleotide sequence variation, insertions, and deletions across the genome (Taylor *et al.,* 2020) have provided consistent molecular epidemiological evidence that supports the view that the evolution of MRSA and of *S. aureus* as a species is predominantly clonal (Wolk *et al.,* 2009; Taylor *et al.,* 2020).

However, horizontal transfer of DNA from other strains or species has been documented and plays an important part in the resistance acquisition in *S. aureus*(Brurec *et al.,* 2011). This horizontal transfer is brought about mainly different events (Brurec *et al.,* 2011). It is believed that, MRSA strains have emerged from the introduction of a large mobile genetic element,

Staphylococcal Cassette Chromosome mec (SCC mec) into a prevalent methicillin susceptible *S. aureus* strain that continue to flourish ((Bitrus *et al.,* 2018; Taylor *et al.,* 2020).

2.2.3 Mechanism of methicillin resistance

Methicillin resistance requires the presence of the chromosomally localized mecA gene (Oliveira *et al.*, 2018). Penicillin binding protein 2a is a protein that binds to penicillin (PBP2a, also known as PBP21) is a 78-kilodalton protein that is synthesized by *mecA*. Penicillin binding proteins are membrane-bound enzymes that catalyze the transpeptidation reaction needed for peptidoglycan chain cross-linking (Li *et al.,* 2011; Oliveira *et al.,* 2018). They have behavior that is close to that of serine proteases, from which they seem to have descended. Due to its low affinity for -lactam antibiotics, PBP2a serves as a stand-in for the other PBPs, allowing *Staphylococci* to tolerate large concentrations of them. Some studies determined the crystal structure of a soluble derivative of PBP2a, implying that resistance to methicillin confers resistance to all -lactam agents. Penicillin binding protein 2a's active site is distinct from that of other PBPs in that it prevents all -lactams from binding while allowing the transpeptidation reaction to continue (Turner *et al.,* 2019). Phenotypic expression of methicillin resistance is variable and each MRSA strain has a characteristic problem of the proportion of bacterial cells that grow at specific concentrations of methicillin(Proctor, 2012; Cheung *et al.,* 2021).

In certain MRSA strains, homologues of the blaz regulatory genes control resistance expression. These genes, mecI and mecRI, regulate the mecA response to β-lactam antibiotics in a fashion similar to that of the regulation of blaZ by the genes blaR1 and blaI upon exposure to penicillin (Cheung *et al.,* 2021)recently discovered that either mecI or blaI must be active in all MRSA strains, implying that this is a defense mechanism to avoid toxin protein overproduction. The fem genes are another group of genes involved in crosslinking

peptidoglycan strands and leading to methicillin resistance heterogeneity (factor important for methicillin resistance) (Cheung *et al.,* 2021).

Most clinical bisolates display a heterogeneous pattern of resistance under routine culture conditions. Heterogeneous strains can appear homogeneous under certain culture conditions, such as growth in hypertonic culture medium supplemented with NaCl or sucrose or incubation at 30℃ (i.e. at 50mg of methicillin per ml, 1% or more of the cell develops) (Brown *et al.,* 2012). The addition of EDTA (PH 5.2) or incubation at 37°C to 43°C favors a heterogeneous pattern and may completely suppress resistance. Methicillin resistance expression variations with various culture conditions are considered to be intermittent and phenotypic. Methicillin resistance expression is seen in the borderline (or low level) methicillin-resistant strains of *S. aureus*. Methicillin MICs equal to or just above the susceptibility break point (e.g. Oxacillin MICs of 4.8 mg/ml) define such borderline methicillin resistance (BORSA) strains (Brown *et al.,* 2012). Borderline *Staphylococcus aureus* strains containing mecA develop PBP2a and are highly heterogeneous methicillin resistant strains. These strains have a small number of cells that are resistant to the drug and can spread at high concentrations (Cheung *et al.,* 2021). Borderline *Staphylococcus aureus* that lack the mecA gene fall into the second group. This can be distinguished from extremely heterogeneous mecA-positive BORSA strains phenotypically by the absence of highly resistant clones in the population of cells. The hypothesis is that BORSA in mecA negative strains is either as a result of modification of normal PBP genes or overproduction of *staphylococcal* β-lactamase (Brown *et al.,* 2012).

Altered penicillin binding protein (PBP 2a) of MRSA differ generally from MSSA isolates by the presence, in the chromosome of large stretch of foreign DNA (40-60kb), referred to as the mec element, the presence of the mecA gene that codes for the 76 KDa penicillin binding protein, PBP 2a (also referred to as PBP21). The mecA gene has been proposed to originate

from *S. sciuri* (Chakraborty *et al.,*2011). Although the mechanism of gene acquisition in this species is unknown, two genes on the mec element of one isolate, ccrA and ccrB, have been found to code for recombinase proteins capable of excising and incorporating the mec element into the chromosome (Chakraborty *et al.,*2011; Magiorakos *et al*., 2012).Penicillin binding protein 2a shares the common structure motifs associated with penicillin binding with other PBPs, but its affinity for beta-lactam antibiotics is significantly reduced. As a result, PBP2a remains active, ensuring the crosslinking of the glycan in peptidoglycan even at therapeutic levels of methicillin, which would inhibit other PBPs' transpeptidational activities. Since cells grown in the presence of methicillin show a significant reduction in crosslinking, PBP2a cannot fully compensate for the other PBPs. The cell's survival is, however, ensured by the minimal degree of crosslinking.

2.2.3.1 Altered penicillin binding protein

Methicillin binding protein vary generally from methicillin-sensitive *S. aureus* isolates by the existence of the mecA gene, which encodes the 76 KDa penicillin binding protein, PBP2a is found on the chromosome of the mec element, which is a 40-60kb stretch of foreign DNA (also referred to as PBP21). *Staphylococcus sciuri* is thought to be the source of the mecA gene (Bitrus *et al.,* 2018). Although the mechanism of gene acquisition from this species is not known, two genes, ccrA and ccrB, present on the mec element from one isolate, have been shown to code for recombinase proteins that are capable of excising and integrating the mec element into the chromosome (Chakraborty *et al.,* 2011). Penicillin binding protein 2a, like other PBPs, has structure motifs linked to penicillin binding, but it has a low affinity for -lactam antibiotics. Penicillin binding protein 2a remains active even at therapeutic levels of methicillin, which would otherwise inhibit the transpeptidational activities of other PBPs and avoid peptidoglycan crosslinking. Penicillin binding protein 2a is unable to completely

compensate for the other PBPs because cells developed in the presence of methicillin display a substantial reduction in crosslinking. The small degree of crosslinking, on the other hand, is sufficient to ensure cell survival (Chakraborty *et al.,* 2011).

2.2.3.2 Expression of penicillin binding protein 2a regulation

Two genes, mecR1 and mecI, are located next to mecA on the *Staphylococcal* chromosome and are co-transcribed in a different way than mecA. Membrane bound signal transduction protein (MecR1) is encoded by the mecR1 gene, while mecI (transcriptional regulator) is encoded by the mecI gene (MecI). The promoters for these genes are located between mecA and mecR1, as well as an operator region that includes mecR1's -10 sequence (Bitrus *et al.,* 2017). The B1aR1 and B1aI, which are involved in the inducible expression of the plasmid-mediated *Staphylococcal* -lactamase gene, blaZ, MecR1 and MecI have a lot of protein sequence homology. The arrangement of the B1aR1 and BlaI genes is close to that of the mecA system, indicating that mecA may have inherited the blaZ system's regulatory genes at some stage in the past (Sowash *et al.,* 2014). The operator regions are similar enough to allow BlaI to regular PBP2a expression . As a result, the presence of a plasmid containing the blaZ regulatory genes can make PBP2a expression inducible under the influence of BlaR1 and BlaI, which is normal in clinical MRSA isolates (Shibabaw *et al.,* 2013).

The inducible -lactamese speech signaling mechanism has been deciphered (Shibabaw *et al.,* 2013). BlaI is a DNA-binding protein that forms a homodimer with the operator region and prevents blaZ and BlaR1-blal from trancribing RNA(Shibabaw *et al.,* 2013).

In the absence of a -lactam antibiotic, -lactamase is expressed at low levels. Bla1 detects the presence of the -lactam using an extracellular penicillin-binding domain and sends the signal to the cytoplasmic membrane through a second intercellular zinc metalloprotease signaling domain. As -lactam binds to BlaR1, the intracellular zinc metalloprotease domain converts

from an inactive proenzyme to an active protease (Bitrus *et al.,* 2017). The activated form of BlaR1 is thought to directly or indirectly cleave Blal resulting in fragments that are incapable of forming dimers and binding DNA (Bitrus *et al.,* 2018). Transcription of both blaZ and blalR1-blal can begin without Blal bound to the operator site, and -lactamase synthesis can confer -lactam resistance. Another gene product, BlaR2, regulates -lactamse synthesis as well, though its function is unknown. It's still unclear whether or not other proteins are involved in the signaling mechanism. In isolates carrying the normal regulatory genes (mecA and mecR1-mecI), PBP2a expression is not strongly inducible, and induction is much slower (15 minutes for -lactmase expression vs. up to 48 hours for PBP2a synthesis), unlike -lactmase synthesis. This is because MecI controls mecA transcription very tightly (Shibabaw *et al.,* 2013), and most pre-MRSA isolates, while carrying the mecA gene, are methicillin-resistant. Antibiotic use, on the other hand, has exerted selective pressure on methicillin-resistant *S. aureus* isolates with mutations or deletions in the mecI or mecA promoter/operator regions, resulting in an inactive repressor and constitutive PBP2a expression (Shibabaw *et al.,* 2013; Bitrus *et al.,* 2018).

2.2.4 Virulence factors of *Staphylococcus aureus*

Toxin production or direct invasion and degradation of tissue are two ways that *S. aureus* causes disease. *Staphylococcus aureus* causes impetigo, *Staphylococcal* scalded skin syndrome (SSSS), *Staphylococcal* food poisoning, and Toxic Shock Syndrome (TSS), among other infections. Other diseases are caused by the species spreading, resulting in abscesses and tissue destruction.

2.2.4.1 Beta toxin

Majority of *S. aureus* strains produce ß toxin, ß toxin also known as Sphingomyelinase C is a heat-labile 35 KDa enzyme found in *S. aureus*. This enzyme is toxic to erythrocytes, leukocytes, macrophages, and fibroblasts, among other cells, and has sphingomyelin and lysophosphatidylcholine specificity. In susceptible cells, it catalyzes membrane phospholipid hydrolysis, with the amount of sphingomyelin exposed on the cell surface determining the degree of lysis. The differences in toxin sensitivity between organisms are thought to be the result of this. The development of beta-toxin is influenced by the species. The erythrocytes of sheep, cows, and goats are most vulnerable. Human erythrocytes are the most responsive, followed by murine and canine erythrocytes. The amount of sphingomyelin in the membrane affects the erythrocytes' sensitivity. The function of ß toxin in human disease is unknown, but it is thought to be involved in tissue destruction and abscess formation in *staphylococcal* diseases when combined with a toxin (Cheung *et al.,* 2021).

2.2.4.2 Gamma-toxin

Gamma-toxin can lyze erythrocytes from human, sheep and rabbits, as well as human lymphoblastic cells. Gamma-toxin, leukocidin, and other bicomponent toxins are a family of proteins encoded by the Hlg and luk PV loci. All of the toxins in this family contain two synergistically acting proteins: one S component (LukS-PV, H1gA, or H1gC) and one F component (LukF-PV, H1gA, or H1gB) designated on the basis of their mobility (slow or fast) in ion-exchange chromatography. Panton-Valentine leukocidin (PVL) and gamma-toxin are the prototype bicomponent toxins. The LukS-PV and LukF-PV are the PVL S and F elements, and are designated as HIgA and H1gB, respectively. are S and F elements of gamma-toxin (Cheung *et al.,* 2021). Synergistic function involves sequential binding of the H1gB (F) followed by HIgA (S) and subsequent generation of a pore (Cheung *et al.,* 2021).

2.2.4.3 Delta toxin

Almost every strain of *S. aureus*, as well as the majority of other *Staphylococci,* produces δ toxin, a 3 KDa polypeptide. The toxin affects erythrocytes, other eukaryotic cells, Cell membrane structures, organelles, spheroplasts and protoplasts, and certain mammalian cells. The non-specific membrane toxicity of the toxin backs up the hypothesis that it acts like a surfactant, disrupting cellular membranes in a detergent-like manner. When used at high concentrations, in laboratory animals, it has also been stated to be dermonecrotic and lethal. Delta-toxin action is inhibited by phospholipids. The hlg gene produces a 26-residue long peptide that peaks at the end of the exponential growth period and can be purified in many ways (Charaborty *et al.*, 2011).

Neutrophils, monocytes, lymphocytes, and erythrocytes have different toxin affinities are all distinct (Cheung *et al.,* 2021). The toxin induces erythrocytes and other mammalian cells to lyse by allowing pores in the membrane to form. Human and canine strains of *S. aureus* express at least two different variants of δ toxin, they are just 62% identical and are immunologically distinct (Cheung *et al.,* 2021).

2.2.4.4 Leukocidin

Staphylococcus aureus has the ability to produce a toxin that targets polymorphonuclear leukocytes. Leukocidin can be a virulence factor since phagocytosis is a key defense against *Staphylococcal* infection. The fact that it can destroy leukocytes gives it its name (of which neutrophils are one type). The PVL is made up of two proteins: S and F. In that it binds to GM1 gangliosides, the S portion is close to the B element of an A-B toxin. Both, however, have enzymatic activity and are active in the metabolism of phospholipids and phosphatidylinositol.In eukaryotic cells, phosphatidylinositol, an essential signaling molecule, regulates a range of cellular processes. As a consequence, it appears that this two-component toxin disrupts normal cellular functions by changing phospholipid metabolism (Cheung *et al.*,

2021). According to most studies, the S portion binds first and then forms pores, the PVL's two components bind to human neutrophils in a particular order (Cheung *et al.,* 2021).

2.2.4.5 Exfoliative toxins A and B

Exfoliative toxin A (ETA) and exfoliative toxin B (ETB) are two serologically distinct exfoliative toxins that have been identified . These toxins cause *staphylococcal* scalded skin syndrome (SSSS), which is marked by intraepidermal separation of skin layers at the desmosomes and is most commonly seen in newborns. The illness starts with a generalized erythema near the mouth that quickly spreads across the body. The epidermal layer wrinkles irreversibly when the skin is gently rubbed, resulting in the classic positive Nikolsky symbolWide flaccid sterile bullae occur later, leading to the stratum granulosum layer being separated. It takes 7 to 10 days from the start of the illness to complete recovery. There are no long-term scars on the skin, and the toxins that cause the infection are not toxic to the host (Cheung *et al.,* 2021).

2.2.4.6 Toxic shock syndrome toxin-1

Fever, hypotension, and rash, as well as desquamation and multiple organ system involvement, are all symptoms of TSS. It is brought about by a toxin. Toxic shock syndrome toxin-1 (TSST-1) is an exotoxin developed by some *S. aureus* strains in a rabbit model, *S. aureus* can mimic many of the clinical symptoms of TSS. It was previously known as enterotoxin F and pyrogenic exotoxin C (there is no rash or desquamation). While not all *Staphylococcal* isolates from TSS patients tested positive for enterotoxin B, the vast majority did. This second toxin's role in TSS is unknown. Coagulase-negative *staphylococci* may also produce TSS (Chakroborty *et al.,* 2011).

2.2.4.7 Capsule

Staphylococci cultured in vitro seldom have a polysaccharide layer that fits loosely (slisme layer), but it is thought to be more normal in vivo. *Staphylococcus. aureus* clinical isolates account for more than 90% of all *S. aureus* isolates. Capsular polysaccharides are generated by *S. aureus*. In *S. aureus*, eleven capsular serotypes have been described. *Staphylococcus aureus* is the most common cause of infection. serotypes 5 and 8 of *Staphylococcus aureus* (Griffiths and O'Neill, 2012). Using colony morphology as a guide, these capsules may also be divided into two classes. The serotype 1 and 2 capsules are mucoid on solid medium, and the strains that produce them are heavily encapsulated. The remaining serotype 3 to 11 capsules are classified as microcapsules; strains with these capsules have a thin capsular layer and develop in non-mucoid colonies on solid medium. Antiphagocytic virulence factors have been discovered, such as mucoid-type capsules, that mask C3b accumulated on bacterial cell walls and prevent it from being recognized by phagocytic cell receptors (Chakroborty *et al.,* 2011; Cheung *et al.,* 2021).

The capsule defends the bacteria against polymorphonuclear leukocytes and prevents mononuclear cell proliferation after mitogen exposure. Bacterial adhesion to catheters and other synthetic materials is also made easier. Examples include Grafts, shunts, prosthetic valves, and joints are all examples of grafts. This property is especially essential for coagulase-negative *staphylococci* survival, which are relatively virulent. (Chakroborty *et al.,* 2011).

The ability of *S. aureus* Microcapsule-deficient strains to induce experimental infective endocarditis (IE) has been demonstrated, it indicates that the microcapsule can obscure important surface-expressed cell wall proteins involved in IE pathogenesis (Cheung *et al.,* 2021).

2.2.4.8 Protein A

Protein A is evenly coated on the surface of most S. aureus strains (except those that are coagulase-negative). This protein binds to the peptidoglycan layer covalently and Immunoglobulins (Ig) IgG1, IgG2, and IgG4 bind to the Fc receptor, effectively preventing antibody-mediated immune clearance. Protein A's function in *Staphylococcal* infections isn't entirely clear. Protein A can obstruct opsonized bacteria phagocytosis by binding IgG to receptors on the host cell. Antibodies can bind to extracellular protein A, forming specific antibodies that are then consumed by the complement system (Chakraborty *et al.,* 2011).

Endocarditis, pneumonia, empyema, and osteomyelitis are all examples of skin infections and septic arthritis are only a few of the diseases that can be caused by it, protein A has been shown to mediate bacterial adherence to von Willebrand factor. Significantly fewer *staphylococci* are needed to develop disease in the presence of a foreign body, such as a splinter, catheter, shunt, prosthetic valve, or joint. Patients with congenital conditions that impair chemotactic or phagocytic responses, such as Job-Buckley syndrome, Wiskott-Aldrich syndrome, and chronic granulomatous disease, are more likely to develop *staphylococcal* infections(Foster, 2016; Cheung *et al.,* 2021).

2.2.5 Staphylococcal enzymes

2.2.5.1 Coagulase

The generation of coagulase is the most common criterion for identifying *S. aureus* in the clinical microbiology laboratory. Despite the fact that a small number of *S. aureus* strains do not produce detectable amounts of coagulase, all strains tend to have a coagulase gene (coa) (Foster, 2016). Bound (also known as clumping factor) and free coagulase are both present in *S. aureus* strains. Coagulase attached to the cell wall of *Staphylococci* can convert fibrinogen to insoluble fibrin and cause the bacteria to clump. By interacting with a globulin plasma factor (coagulase-reacting factor) to form staphylothrombin, a thrombinlike factor, the

same result can be achieved with cell-free coagulase. The conversion of fibrinogen to insoluble fibrin is catalyzed by this element. Coagulase is a virulence marker for *S. aureus*. Coagulase may form a fibrin layer around a focal *staphylococcal* abscess, which helps to localize the infection and protect the bacteria from phagocytosis, but its role in disease pathogenesis is unknown. Coagulase-negative mutants, on the other hand, in a blood-borne *Staphylococcal* pneumonia mouse model, they were less virulent than the parental strain. This suggests that in some pathogens, coagulase is more essential than in others (Foster, 2016).

2.2.5.2 Catalase

Catalase is an enzyme that catalyzes the conversion of harmful hydrogen peroxide to harmless water and oxygen, is produced by all *staphylococci*. Hydrogen peroxide can build up during bacterial metabolism or phagocytosis. Various toxic forms of oxygen are released as accidental by-products during the reduction of O_2 to H_2O in respiration. From DNA strand damage to membrane lipid peroxidation, these reactive oxygen intermediates have a broad range of effects on living organisms. Bacteria have also developed enzymes that break down harmful oxygen products. Catalase, which attacks hydrogen peroxide (H_2O_2), is the most common enzyme in this group (Foster, 2016).

2.2.5.3 Hyaluronidase

Hyaluronidase is an enzyme that breaks down hyaluronic acids, which are acidic mucopolysaccharides that hold the body's cells together, in connective tissue, this is especially true. This digesting activity is thought to cause infected wound tissue to blacken and assist the microorganism in spreading away from its original infection site. Hyaluronidase may be combined with a medication to help it spread through a body tissue for medicinal purposes. About 90% of S. This enzyme is generated by strains of *Staphylococcus aureus* (Hiramatsu *et al.,* 2014; Egea *et al.,* 2014).

2.2.5.4 Fibrinolysin

All the *S. aureus* contain fibrinolysin, also known as staphylokinase. *S. aureus* strains has the ability to remove fibrin clots. The fibrinolytic enzymes released by *streptococci* are not the same as staphylokinase (Jiménez *et al.,* 2012; Iyamba *et al.,* 2014).

2.2.5.5 Lipases

All strains of *S. aureus* and more than 30% of coagulase-negative *Staphylococci* produce different lipases as their names implies, these enzymes hydrolyse lipids, an essential function to ensure the survival *Staphylococci* in the sebaceous areas of the body. It is believed that these enzymes must be preset for *Staphylococci* to invade cutaneous and subcutaneous tissues and for superficial skin infections such as furuncles (boils) and carbuncles. Lipases are enzymes that break down fats into fatty acid and glycerol components. After that, each part is metabolized separately (Iyamba *et al.,* 2014).

2.2.5.6 Nuclease

Almost all *S. aureus* strains contain a thermo stable nuclease (TNase), that is been used as a criterion for diagnosing *S. aureus* as a type of bacteria. The TNase hydrolyzes single-stranded and double-stranded DNA and RNA at the 5' position of phosphodiester bonds using a calcium-dependent mechanism. The function of this enzyme in infection pathogenesis is unknown (Iyamba *et al.,* 2014).

2.2.5.7 Penicillinase

In 1941, when penicillin was first used clinically, the antibiotic was effective against more than 90% of *Staphylococcal* isolates. Due to bacteria's ability to develop penicillinase, penicillin resistance developed quickly (beta- lactamase). The enzyme cleaves the penicillin molecule's ß-lactam ring. This enzyme was widely spread since it was discovered on transmissible plasmids (Iyamba *et al.,* 2014).

2.2.6 Regulation of virulence determinants

Staphyloccocus aureus was tested in vitro and found to be effective. During the early stages of *S. aureus* life cycle, Surface proteins including adhesins and protein A, a major surface antigen, are found on *S. aureus*, are formed and then down-regulated. Among the most widely secreted proteins are toxins, haemolysins, and tissue-degrading enzymes. The agr (Tong *et al.,* 2015; Gnanamani *et al.,* 2017) global regulatory locus, which has been shown to be necessary for virulence in several experimental infection models, is in charge of the development of all of these factors (the virulence response) (Yoong and Torres, 2013). The agr locus (Yoong and Torres, 2013) is made up of two distinct transcription units that are related by promoters P2 and P3 (Gnanamani *et al.,* 2017).

The genes, AgrA, B, C, and D are found in the P2 operon. A signal transduction pathway with two components is encoded by the genes agrA and C, with agrC coding for the signal receptor and agrA coding for the response regulator (Tong *et al.,* 2015). The auto inducer is a small peptide that binds to and activates AgrC when the other two genes, agrB and D, are combined (Rasheed and Hussein, 2021).The auto inducer pro peptide is encoded by AgrD, and AgrB is a protein that is necessary for processing and possibly secretion of the auto inducer (Gnanamani *et al.,* 2017). In vitro, AgrC is a transmembrane protein that responds to the auto inducer by phosphorylating a conserved histidine residue (Rasheed and Hussein, 2021).

For auto inducer binding and subsequent phosphorylation activation, AgrC's last extracellular loop is sufficient. The main role of the agrAC signaling pathway, in combination with another transcription factor named SarA, is to improve the expression of both the P2 and P3 agr promoters (Rasheed and Hussein, 2021) discovered that it can also up regulate the coagulase gene, but that none of the other agr-dependent genes are.

The RNA III effector molecule, which is a 512-nucleotide transcript that encodes the S-hemolysin, is a 26-amino-acid protein that isn't involved in control, is specified by the P3 operon (Rasheed and Hussein, 2021).At least one element, a protein, is regulated by RNA III. -hemolysin is a form of hemolysin, which folds into an untranslatable configuration in the absence of RN III, by pairing with the hla mRNA's leader zone (Tong *et al.,* 2015).

2.2.6.1 Bacteraemia and infective endocarditis

Staphylococcus aureus and coagulase-negative *Staphylococci* (CoNS) are ideal infective endocarditis (IE) pathogens, in that they possess a number of virulence factors, which enable them to establish IE. Acute endocarditis caused by *S. aureus* is a serious disease, with a mortality rate approaching 50% .Although patients with *S. aureus* with *S. aureus* endocarditis may initially have non-specific influenza-like symptoms, their condition can deteriorate rapidly and include disruption of cardiac output and peripheral evidence of septic embolization. Unless appropriate medical and surgical intervention is instituted immediately, the patient's prognosis is poor. *Staphylococcus aureus* can be acquired in two ways: in the community (CA) or in the hospital (HA), bacteremia continue to be caused by *S. aureus.* Diabetes mellitus and renal insufficiency are two of the most common predisposing medical conditions. Patients infected with *S. aureus* make up the vast majority of cases. An intravascular catheter is normally the source of infection in *S. aureus* bacteremia. *Staphylococcus aureus* patients have a higher overall mortality rate. The rate of *S. aureus* bacteremia remains high in the absence of a

removable primary target, older patients, increasingly lethal comorbid conditions, underlying respiratory or cardiac disease, related infective endocarditis (IE), and septic shock (Rasheed and Hussein, 2021)..

2.2.6.2 Wound infections

Staphylococcal skin infections can be mild or life-threatening, depending on the skin's resistance to infection and the bacterial strain's invasiveness. The most common species recovered from patients who develop a wound infection after surgery is *Staphylococcus aureus* (*S. aureus*). The most common aerobic bacteria associated with wound infections are *S. aureus* and the CoNS, which rank first and seventh, respectively. *Staphylococci* that cause wound infections are normally found in the patient's flora or on the surgical team (Rasheed and Hussein, 2021)..

2.2.6.3 Infections of the skin and soft tissues

Staphylococci, a saprophytic bacterium, live on the skin and mucous membranes of mammals. Several causes, such as tight clothing or scratching, immunosuppression, or the insertion of a prosthesis, may all contribute to this condition, may all contribute to the development of a prosthesis, may all contribute to this condition, and may trigger disease by altering the host-parasite relationship. *S. aureus* colonized the nares; for example, it causes hand carriage, and species are often distributed from the hands to other parts of the body. People are commonly infected with *Staphylococci* through their noses, palms, and wounds(Cheung *et al.,* 2021).

Staphylococcus aureus makes a lot of enzymes and toxins. *S. aureus* induces a wide range of symptoms, some of which are specifically caused by toxin damage and others are caused by a confluence of pathogenicity factors. Pyomyositis (purulent skeletal muscle infection), folliculitis and furuncles, persistent furunculosis, impetigo, botryomycosis, and secondary

cutaneous infection all occur frequently in eczema patients. *S. aureus* infections are a form of *Staphylococcal* infection caused by the bacteria *S. aureus* (Cheung *et al.,* 2021).

2.3 Methicillin Resistant *Staphylococcus aureus* in the Healthcare Sector

Methicillin Resistance *Staphylococcus aureus* (MRSA) isolates from hospital settings have been gradually growing . Methicillin resistant *Staphylococcus aureus* infections in hospitals have been decreasing recently, according to data from a 2011 screening program in the United States (Raymund *et al.,* 2013).

High rates of MRSA (>50 percent) have been reports in the US, Asia and Malta, intermediate rates (25-50%) reported in Africa, China, and Europe while in some part of Europe, the prevelence rate is relatively lower than 50%, depending on the study region and sample size (Meja *et al.,* 2010; Tacconelli and Magrini, 2017). The prevalence of HA-MRSA has decreased in a number of European countries, including France, Ireland, and the United Kingdom, according to Stafeni *et al.* (2012). Infections with HA-MRSA are still prevalent in Asia particularly South Korea (77.6%), Vietnam (74.1%), Taiwan (65%), and Hong Kong (65%).Hospital acquired-Methicillin resistant *Staphylococcus aureus* is still high and the major linage responsible for the spread between these continents the CC8 (ST239) (Harris *et al.,* 2010; Tacconelli and Magrini, 2017). Hospital acquired Methicillin resistant *S. aureus* is more likely to become infected after colonization (Tacconelli and Magrini, 2017). Hospital colonization may occur as a result of contact with MRSA-colonized patients or contaminated objects. Methicillin resistant *S. aureus* transmission by aerosols is made more likely by respiratory infection, which can lead to severe infections and complications (Tacconelli and Magrini, 2017). Hospital aquired-Methicillin *S. aureus* is most often found in immune compromised people and causes dermatitis, septicemias, heart and lung disease. Risk factors

include hospitalization, surgery, dialysis and previous history of MRSA infections (Umaru *et al.,* 2011).

Table 2.2: Methicillin resistant *Staphylococcus aureus* in certain parts of the world

Country	Samplesize	Prevalence %	Source	References
Columbia	538	92.4,65.1,43.6	Hospital	Jiménez *et al.,* 2012
Congo		60	patients	Iyamba *et al.,* 2014
Indonesia	1502	4.3	Hospital	Santosaningsih *et al.,* 2014
Kenya	950	7.0	Hospital	Aiken *et al.,* 2014
Cameroon	295	34.6	Hospital	Gonsu *et al.,* 2013
Nigeria	208	19.2	Hospital	Olowe *et al.,* 2013
North India	6743	46	Hospital	Arora *et al.,* 2010
Bolivia	585	0.5	community	Bartoloni *et al.,* 2013
Ethiopia	118	44.1	Hospital	Shibabaw *et al.,* 2013
Sudan	426	69.4	Hospital	Elimam *et al.,* 2014
Argentina	591	16	Hospital	Egea *et al.,* 2014

2.4 Community-Associated Methicillin Resistant *Staphylococcus aureus*

In the late 1990s, MRSA strains were discovered in the population for the first time in patients who had never been exposed to healthcare before (Stefani *et al.,* 2012; Hussein, 2016). In this case, USA300 was the most common lineage in the United States (CC8-ST8). Skin and soft tissue infections are the most common infections caused by these strains. In Europe, infection

caused by the CC80 lineage is the most common (ST80). The strain USA300, on the other hand, has been found in Europe (Umaru *et al.,* 2011; Stefani *et al.,* 2012). Methicillin resistant *S. aureus* strains have been found to cross international borders, including those between North and South America and Middle East, Asia (Stefani *et al.,* 2012).

Community associated-Methicillin resistant *Staphylococcus aureus* (CA-MRSA) has spread to health-care facilities in the United States and France (Sowash and Uhlemann, 2014; Tenenbaum, *et al.,* 2016), to prisons (Stefani *et al.,* 2012), day care centers (Hussein, 2016), military quarters (Tenenbaum, *et al.,* 2016), homeless people (Umaru *et al.,* 2011), and intravenous drug users (Tenenbaum, *et al.,* 2016), CA-MRSA outbreaks are most common in sports teams (Sowash and Uhlemann, 2014; Hussein, 2016). Overcrowding, itchy eyes, poor hygiene, and sharing towels, sports equipment, and unsterilized first-aid devices are all risk factors (Stefani *et al.,* 2012; Sowash and Uhlemann, 2014; Tenenbaum *et al.,* 2016).

2.5 Antibiotic Resistance in *Staphylococcus aureus*

Antibiotic resistance in *Staphylococcus aureus* is presumed to have occurred in four waves, the most recent of which was the appearance of community-acquired methicillin-resistant *S. aureus* (Hiramatsu *et al.,* 2014). Prior to the use of antibiotics to treat *S. aureus* infections, resistant strains outside of hospital settings were rarely encountered (Bitrus *et al.,* 2018).

Following World War II and the widespread use of antibiotics, penicillin-resistant *S. aureus* emerged. The incidence of resistant *S. aureus* has increased. Resistance to penicillin is associated with production of penicillinase, a predominantly extracellular enzyme that hydrolyses the β-lactam carrying antibiotics encoded by blaZ gene controlled by two adjacent regulatory genes, the anti-repressor bla RI and the repressor blaI (Bitrus *et al.,* 2018). Regardless of the environment, the vast majority of *Staphylococcal* isolates now develop penicillinase. The gene for -lactamase, as well as other antimicrobial resistance genes, is

located on a large plasmid (genes for gentamycin and erythromycin), and resistance to penicillin is spread primarily through the spread of resistance strains(Bitrus *et al.,* 2018).

Penincillin was first used in the early 1940s, and it dramatically improved the prognosis of patients with *Staphylococcal i*nfections. Penicillin-resistant *Staphylococci*, on the other hand, appeared shortly after the antibiotic was introduced, first in hospitals and then in the population. This pattern of resistance is well-established and repeats itself with each new wave of antimicrobial resistance. It started in hospitals and then spread to the general public (Bitrus *et al.,* 2017).

The introduction of methicillin in 1959 led to a drop in the prevalence of penicillin resistant *S. aureus* (Bitrus *et al.,* 2018). However, in less than a year of its introduction methicillin resistant *S. aureus* was identified (Bitrus *et al.,* 2017; Rasheed and Hussein, 2020). Antibiotic use in animals for therapeutics, food processing, and disease prevention, in addition to human use, has contributed to antibiotic resistance in humans (Ndi and Barton, 2012). Under doses of antibiotics to food producing animals can result in bacterial resistance in livestock, elevating the potential for resistant bacterial strains to cross species boundaries, especially livestock imported from countries where antibiotic use is indiscriminate (Bitrus *et al.,* 2017; Rasheed and Hussein, 2020).

What makes *S. aureus* a dangerous pathogen is the combination of antibiotic resistance and high virulence. The relative ease with which *S. aureus* exchanges genetic material encoding antibiotic resistance and virulence determinants amongst strains and other species such as *S. epidemidis* suggest an emerging hyper virulent, multidrug-resistant superbug (Davies and Davies, 2010; De Smalen *et al.,* 2017).

As a "superbug" *S. aureus* has higher morbidity and mortality due to multiple mutations encoding it with high levels of resistance to different antibiotic classes specifically

recommended for their treatment. The *Staphylococcal* genome's complexity and maturity have enabled it to modify and adapt to a variety of situations, including exposure to a new antibiotic, adherence to a clinical device, and transition from an animal to a human host (Humphreys, 2012).

Staphylococcus aureus is unlikely to show a change in sensitivity to a drug administered for a single short course, unless the mutation rate of resistance to that drug is very high, like in the case of streptomycin (aminoglycoside) and erythromycin (macrolide) (Rahimi, 2016).

Staphylococcus aureus is less sensitive to erythromycin than *Pneurmococci* or *Haemolytic, Streptococci* and rapid development of resistance has been observed, especially of *Staphylococci* in vitro (Rahimi, 2016). It was noticed in vivo that resistance is usually not a serious clinical problem with short course of treatment with erythromycin but resistance is more likely to develop with prolonged use.

2.5.1 Multidrug resistant *Staphylococcus aureus*

The problems posed by the increasing spread of multidrug resistant *S. aureus* (MDR-SA) in clinical setting is compounded by spectacular adaptive capacity of this pathogen resulting in the emergence and worldwide spread of lineage that acquired resistance to the majority of available antimicrobial agents, narrowing choice of therapy to a few antibacterial agents, among them the glycopeptide antibiotic vancomycin, which has become the mainstay of therapy worldwide (Tacconelli and Magrini, 2017).

The environment and especially freshwater, constitute ambulance for the evolution and rise of new resistance that provides „environmental-hot spots" for antibiotics and other pollutants from different sources, environmental species with intrinsic antibiotic resistance mechanism

and bacteria from different antibiotic sources to interact to acquire resistance determinants which may involve phages and integrons and introducing same into clinics (Lupo *et al.,* 2012).

The 1950s were marked by an increased prevalence of both virulent and multiple antibiotic resistant *S. aureus*. Since, the 1960s, however, there has been an improvement in this position through the interaction of several agents that were less subject to less of activity through the emergence of resistant variants (Tacconelli and Magrini, 2017). The most important of these agents are the semisynthetic penicillinase stable penicillins (Methicillin, cloxacillin oxacillin and nafcillin) and the cephalosporins (cephalothin, cephaloridine and cephalexin) (Piso *et al.,* 2017).

A series of new antibiotics; streptomycin, the tetracycline chloramphenicol, erythromycin and novobiocin, effective against *S. aureus* was introduced during the period of increasing prevalence of penicillin-resistant penicillinase producing *S. aureus* (Kandala *et al.,* 2017). The increased use of these agents in the treatment of infection causes by the penicillin-resistant *Staphylococci* was soon followed by the emergence of strains resistant to these antibiotics (Piso *et al.,* 2017) after periods of time that varied with the type of antibiotics and with the amount of time that was used.

Resistance to erythromycin developed rapidly, with resistant strains spreading fast in hospital where the antibiotic was used. Resistance was also readily produced to novobiocin but it was not often seen because the antibiotic was little used (Piso *et al.,* 2017). *Staphylococcus aureus* is predominant in most developed countries where they occur as multidrug resistant pathogen (Tacconelli and Magrini, 2017). Multidrug resistant bacteria such as MRSA are endemic in healthcare environment and serves as a potential source for outbreaks in these settings (Tacconelli and Magrini, 2017). Methicillin Resistant *Staphylococcus aureus* is said to be multidrug resistant because of its non-susceptibility to at least one antimicrobial agent in three

or more categories and whose resistance to oxacillin or cefoxitin predicts non-susceptibility to all categories of β-lactam antimicrobials with the exception of the anti MRSA cephalosporins (Magiroakos *et al.,* 2012).

The improved medi care due to antibiotic discovery in the 20th century has been eclipsed by the upsurge of antibiotic resistance in hospitals, community and the environment attributed to extraordinary genetic versatility of microbes that have capitalized anthropogenic activities to utilize every sources of resistance genes and horizontal gene transfer mechanism to develop multiple mechanisms of resistance for each and every antibiotic introduced into clinical and agricultural practice (Davies and Davies, 2010). *Staphylococcus aureus* is antimicrobial resistant. The appearance of strains resistant to vancomycin and daptomycin, both last-line antimicrobials, has made *S. aureus* a major public health concern (Howden *et al.,* 2011).

Antibiotic resistance has spread in microorganisms as a result of widespread prescribing and unregulated widespread use of antibiotics (Davies and Davies, 2010). Antibiotic use provides a favorable environment for bacteria with resistance mechanisms to thrive. These processes are triggered not only by mutations in microbial genes that code for antibiotic absorption or binding sites, but also by horizontal or longitudinal transfer of resistance determinants (Tacconelli and Magrini, 2017),and have contributed to antibiotic resistance and virulence in *Staphylococcus aureus* (Chan *et al.,* 2011; Rasheed and Hussein, 2020).

2.5.2 *Staphylococcal* cassette chromosome

Staphylococcal cassette chromosomes (SCCs) are relatively large fragments of DNA that always insert into the orfX gene on the *S. aureus* chromosome and can encode antibiotic resistance and/or virulence determinants (Malachowa and Deleo, 2010). It is a mobile genetic element that carries the central determinant for broad spectrum beta-lactam resistance encoded

by the MecA gene. The emergence of methicillin resistant *Staphylococcal* lineage is due to the acquisition and insertion of the SCC mec element into the chromosome of susceptible strains.

The SCC mec types differ from one another by the number of genes in their architecture (Rasheed and Hussein, 2021). Some SCCmec are carriers of resistance genes that are determinants of multiple antibacterial drugs which include β-lactam antibiotics, macrolides, lincosamides, streptogramins, aminoglycosides and tetracycline and bacterial cell acquiring such SCCmec, acquires a multiple resistance phenotype (Ito *et al.,* 2012; Saleem, 2017).

Staphylococcal cassette chromosome mec type I, IV, V and VI encode resistance to β-lactam antibiotics only, while SCCmec type II and III carry multi-resistant genes some on plasmids and transposons (Rasheed and Hussein, 2020). The initial reservoir of SCCmec is not very clear but is suspected to have arisen from coagulase-negative *Staphylococcal* species (Saleem, 2017). SCCmec may have originated from a primordial mobile element, the *Staphylococcal* Cassette Chromosome (SCC), into which the mec complex was inserted, and it is probable that SCCmec serves as the carrier of the mecA gene moving through *Staphylococcal* species, as mecA genes in other *Staphylococcal* species have never been identified without the presence of an SCC mec-like structure.

Using a combination of ccr allotype and mec class, the SCC mec element type has been identified. Several forms of SCCmec elements have been identified in MRSA strains, and these SCCmec elements have been distinguished further by variations in regions other than – ccr and mec, which are referred to as junkyard (j) regions. These constitute non-essential component of the cassette, but some cases these regions carry additional antibiotic resistance determinants (Ito *et al.,* 2012; Saleem, 2017). The region between ccr and the right junction chromosome, J2 the region between the mec complex and the left extremity orfX, and J3 (the region between the mec complex and the left extremity orfX) are the three parts that make up the J regions

(Rasheed and Hussein, 2021). The SCCmec components have four structural features: first, they bear the mec gene complex, which includes the methicillin resistance determinant mecA and its regulatory gene system (mec1 and mec R1 or Dmec1), as well as the insertion sequence, IS. Second, they contain the ccr gene complex, which contains the ccr genes (ccrA and ccrB, or ccrC in form VSCC mec) that encode SCCmec factor mobility recombinases (insertion and excision) (Rasheed and Hussein, 2021). At both ends, they have reversed complementary sequences and characteristic directly replicated nucleotide sequences (Saleem, 2017).

Finally, they attach to the orfX 3 1 end of an open reading frame (ORF) (Ito *et al.,* 2012). Eleven SCCmec elements are reported to date SSC mec I to XI (Li *et al.,* 2011;Ito *et al.,* 2012). Among these, SCCmec type I-V are the most commonly reported (Saleem, 2017). Three types of SCC mec (I, II and III) are carried mostly by healthcare- associated MRSA strains throughout the world (Kandala *et al.,* 2017; Sultan and Al Meani, 2019).While type IV and V are widely disseminated among community acquired MRSA infections (Sultan and Al Meani, 2019). The SCC mec IV and V allotypes are smaller than the other SCCmec element I, II and III, more genetically mobile being readily transmissible between *Staphylococci* and does not have any additional antimicrobial resistance genes at this time (Ito *et al.,* 2012; Kandala *et al.,* 2017).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Study area, Minna, is the capital of Niger State, Nigeria. It is situated on Latitude 9.61 N and Longitude 6.56 E at an elevation of 299 m above sea level. It is bordered to the North by Sokoto State, West by Kebbi State, and South by Kogi and South-West by Kwara State. Niger State has a common boundary with the Republic of Benin along New Bussa, Agwara and Wushishi Local Government Area. Samples were collected from General hospitals in Minna Nigeria (GH) shown in Figure 3.1.

Figure 3.1 Map of the study area

3.2 Sample Size Determination

Naing (2011) equation was used to determine the sample size.

The prevalence of 30% in a similar study obtained by Tong *et al.* (2015) was used to determine the sample size using the formular below: -

$$N = \frac{PQ}{(E/Z)^2}$$

P = prevalence of previous study 30%

Q = 100 – p = 100 – 30 = 70

E = Allowable error = 5%

Z = Standard normal distribution at 95%, CI = Confidence interval, 1.96.

N = Number of sample to be collected

$$N = \frac{30 \times 70}{(5/1.96)^2} = \frac{2100}{(2.55)^2} = \frac{2100}{6.5} = 323.1$$

However, for the purpose of obtaining precise results in this research, 360 samples were collected comprising of Clinical samples and environmental samples.

3.2.1 Ethical clearance

The approval for this study was obtained from the Ethical Committee of the Niger State Hospital Management Board, General hospital Minna, Niger State.

3.2.2 Preparation of media

Nutrient Agar, Mueller Hinton agar, and Mannitol Salt Agar were among the media used. All media were prepared according to manufacturer's instruction respectively and were sterilized by autoclaving at 121℃ for 15 minutes. Glassware used were thoroughly washed with detergent and clean water, and sterilized for 15 minutes at 160°C in an oven, then allowed to dry for an hour at 70°C in a hot air oven.

3.2.3 Collection of sample

Wound, ear, and nasal samples were collected from in-ward patients using sterile swab sticks. For the blood samples, a blood culture bottle was used to collect 10ml of blood sample. Urine was collected using sterile universal bottles (midstream urine). The specimens were labeled

with the site of specimen collection's first initial (E for ear swabs and W for wound). For the skin swab, a small portion of skin around the leg was swabbed in a zigzag fashion, as well as some selected portion of areas within the hospital laboratory floor and areas on the work bench were used as environmental samples (area size of approximately 2x2cm). Air samples were taken using the 1/1/1 method (1 meter above the floor, 1 meter away from walls or any major obstacles for 1 hour). For this study, all of the samples were taken to the Microbiology Laboratory at the Federal University of Technology Minna.

3.2.6 Isolation and identification

Each sample collected was inoculated onto Mannitol Salt Agar (MSA) and incubated at 37°C for 24 hours. To obtain pure culture and for further analysis, suspected distinct colonies of *Staphylococcus aureus* were subcultured onto Mannitol Salt Agar (MSA) and then onto nutrient agar slant. Each isolate was identified based on colonial appearance, which included size, form, consistency, color, and elevation, as well as its distinguishing characteristics, which included pigmentation, lactose fermentation on Mannitol Salt Agar, and Gram staining (Cheesbrough, 2018).

3.2.7 Gram staining and microscopy

Gram staining was carried out using the method described by Cheersbrough (2018). The dried smear of the isolate obtained was heat fixed and stained for 60 seconds with crystal violet solution (primary dye), rinsed with tap waterand drained. It was further flooded with Iodine solution (mordant) for 60 seconds and rinsed. Acetone was carefully added to the slide for 3 seconds until all free colours were removed and subsequently rinsed with tap water. The slides were further flooded with safranin for 30 seconds, rinsed and air dried. Finally, all slides were examined using Microscope with x100 oil immersion objective. *Staphylococcusaureus*

appeared as purple cocci, grape-like clusters. This indicated that it is Gram-positive bacteria (Cheesbrough, 2018).

3.2.8 Biochemical screening of *Staphylococcal isolates*

The following biochemical tests were carried out as recommended for the identification of *Staphylococcus aureus*; Catalase test, Coagulase test (slide test to detect bound coagulase) and Mannitol fermentation (Cheesbrough, 2018).

3.2.8.1 Catalase test procedure

A glass slide was taken that was clean and grease-free. A drop of freshly prepared 3 percent H_2O_2 solution was dropped onto the slide. A sterile glass rod was used to take a pure colony from the Nutrient Agar plate, which was then transferred to the 3 percent H_2O_2 solution. A catalase-positive test was shown by the rapid evolution of gas bubbles on the slide (Leber, 2016).

3.2.8.2 Coagulase test procedure (clumping factor/bound coagulase)

The test was used to differentiate *Staphylococcus aureus* from other *Staphylococci.* A drop of physiological saline was placed onto a clean, grease-free slide. Using a glass rod, a colony of the test organism was transferred onto it, resulting in a thick suspension. A drop of plasma was applied to the suspension and gently mixed in. A positive slide coagulase (clumping factor) test was indicated by the presence of clumping within 10 seconds, while a negative test was indicated by the absence of clumping (Leber, 2016).

3.2.9 Antibiotics susceptibility testing

Antimicrobial susceptibility test against *S. aureus* isolates was carried out using Kirby-Bauer disc diffusion techniques guideline established by Clinical and Laboratory Standard

Institude(2017). Ciprofloxacin (10g), chloramphenicol (30g), gentamacin (10g), amoxacillin (20g), septrin (30g), rifampicin (20g), erythromycin (30g), nofloxacin (10g), ampiclox (20g), and levoflaoxacin (20g) antibiotic discs were used. Before inoculation, pure isolates of labeled *S. aureus* were suspended in sterile water and diluted in steps of 1:10 to achieve turbidity equal to the 0.5 McFarland levels (a density of $1x10^8$ cells/ml). For about 5 minutes, the inoculated plates were allowed to dry.

3.2.10 Application of discs to inoculated agar plates

Using a sterile forcep, the antibiotic discs were gently pressed onto prepared Mueller-Hinton Agar plate to ensure full contact and Incubated at 37°C for 24 hours after the plates were inverted.

3.2.11 Examination of plates and interpretation of results

Each plate was examined after 24 hours of incubation. The diameters of the zones of complete inhibition were measured to the nearest whole millimeter, using a ruler. The results were interpreted as either susceptible, intermediate, or resistant according to Clinical and Laboratory Standard Institute guideline (CLSI, 2016).

3.2.12 Determination of multiple antibiotic resistance index

The MAR index was determined for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotic tested (Osundiya *et al.,* 2013).

$$\text{MAR Index} = \frac{\text{Antibiotics to which the isolate is resistant}}{\text{Antibiotics that have been tested in total}}$$

3.2.11 Detection of methicillin resistant *Staphylococcus aureus*

The MRSA screening was performed using Oxacillin 1 µg, Vancomycin 30µg and Cefoxitin 30µg antibiotics disc which are surogates of methillcin (Oxoid, UKS). *Staphylococcus aureus* pure isolate before inoculation, was suspended in sterile water and diluted in 1:10 to achieve turbidity equal to the 0.5 McFarland levels (a density of $1x10^8$ cells/ml). The Mueller-Hinton agar was prepared as directed by the manufacturer (3.2.9). Making use of sterile forceps, the antibiotic discs were uniformly placed onto the inoculated Agar plate's surface and gently pressed down to ensure full contact with the Agar surface. The plates were inverted and incubated for 24 hours at 37 °C. Using a ruler, the diameters of the full inhibition zones were determined to the nearest whole millimeter. According to the Clinical and Laboratory Standard Institute's guidelines, the findings were classified as susceptible, intermediate, or resistant (CLSI, 2016).

3.3 Isolate Recognition by Molecular Markers

3.3.1 Extraction of DNA

Nadeem *et al* (2018) technique was used to extract DNA. Single colonies were transferred from medium to 1.5 mL liquid medium (nutrient broth) and cultured for 48 hours at 28°C in a shaker. The cultures were then centrifuged for 5 minutes at 4600g. The pellets were then resuspended in a 520-liter solution of Tris-EDTA (TE) buffer (10 mMTris-HCl, 1mM EDTA, pH 8.0). 15 µl of 20% SDS and 3 liters of Proteinase K (20 mg/ml) were then added. After being incubated for 1 hour at 37 ºC, the mixture was votexed with 100 l of 5 M NaCl and 80 l of a 10% CTAB (cetyl trimethylammonium bromide) solution in 0.7 M NaCl. The suspension was held on ice for 15 minutes after a 10-minute incubation time at 65 degrees Fahrenheit. The mixture was put on ice for 5 minutes before centrifuging at 7200 g force for 20 minutes after adding an equivalent amount of chloroform to isoamyl alcohol (24:1). After that, the aqueous phase was

transferred to a new tube and treated with isopropanol (1: 0.6) before being frozen for 16 hours at –20°C.

3.3.2 Polymerase chain reaction

Polymerase chain reaction cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25 mM MgCl2, µl of 10 mM dNTPs mix, 1 µl of 10 pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5′- AAGGAGGTGATCCAGCC-3′ primers, and 0.3 units of Taq DNA polymerase (Promega, USA) A GeneAmp 9700 PCR System was used to perform the PCR (Nadeem *et al*., 2018). Thermalcycler (Applied Biosystems Inc., USA) with a profile that included a 5-minute initial denaturation at 94°C, followed by 30 cycles of 30 seconds at 94°C, 60 seconds at 50°C, and 1 minute 30 seconds at 72°C, and a 10-minute final termination at 72°C and left at 4°C holding (Ouyang *et al.,* 2021).

3.3.3 Integrity test

Before beginning to make the wells, the gel was permitted to set for 20 minutes. 1XTAE buffer was poured into the gel tank, and the gel was barely submerged. Following the loading of the 100bp DNA ladder into well 1, four microliters (4µl) of each PCR product were combined with two microliters (2 µl) of 10X blue gel loading dye and loaded into the wells. The gel was electrophoresed for 45 minutes at 120V, then photographed with ultraviolet trans-illumination.

The mobility of a 100bp molecular weight ladder, which was run alongside experimental samples in the gel, was used to estimate the sizes of the PCR products.

3.3.4 Amplified product purification

The amplified fragments were ethanol filtered to eliminate any PCR reagents after the gel integrity was established. Each 40-liter PCR amplified product was switched to a different sterile 1.5-liter Eppendorf tube containing 7.6 liters Sodium acetate (NaOAc) 3M and 240 liters 95 percent ethanol, vortexing thoroughly, and storing for at least 30 minutes at -20°C. Following a 10-minute centrifugation at 13000 g force for 10 minutes at 4°C, the pellets were washed in 150 L of 70% ethanol and blended before centrifugation at 7500 g force and 4°C for 15 minutes. The tube was inverted into paper tissue and allowed to dry for 15 minutes in the fume hood at room temperature before being resuspended in 20 liters of sterile distilled water and deposited at -20°C. A 1.5 percent agarose gel was run at 110V for about 1 hour to confirm the presence of the purified fragment, and the results were quantified using a thermo science nanodrop model 2000.

3.3.5 Sequencing

The amplified fragments were sequenced with an Applied Biosystems Genetic Analyzer 3130xl sequencer and a BigDye terminator v3.1 cycle sequencing package, according to the manufacturer's instructions. All genetic research was done with Bio-Edit software and MEGA 6.

3.3.6 Molecular identification of *mecA, ermA,* and*ermC* coding genes

Easy PCR on the extracted DNA MecA, ermA, and ermC coding regions unique primers are used to investigate the MecA, ermA, and ermC coding genes in the 10 molecularly described *Staphylococcus aureus*. The 8000U taq DNA polymerase, MgCl2 (0.75), 10pM DNTP (0.25), 10pM, 5X PCR SYBR green buffer (2.5), MgCl2 (0.75), 10pM DNTP (0.25), 10pM forward and back primer (0.25), 5X PCR SYBR green buffer (2.5), MgCl2 (0.75), 10pM

forward and back primer (0.25), 5X PCR SYBR green buffer (0.75), 10pM forward and back primer (0.25), 5X PCR SYBR green buffer (0.06), and sterile distilled water yielded a final concentration of 10.5 to which 2 l template was added. The appropriate profile for each primer pair was used in a GeneAmp 9700 PCR Device Thermalcyzer (Applied Biosystems Inc., USA), as shown in the Table below:

Table 3.1: Primers Used for the Study

Gene	Primer	Sequence	Profile	Reference
MecA	mecA-F	GTT GTA GTT GTC GGG TTT GG	After a 5-minute denaturing phase at 94°C,	Wielders *et al.*(2002)
	mecA-R	CTT CCA CAT ACC ATC TTC TTT AAC	then 35 94°C cycles	
			56°C for 30 minutes	
			and 72°C for 30s and terminate at 72° C for 10min	
ErmA	ermA-F	AAG CGG TAA AAC CCC TCT GAG	After a 5-minute denaturation phase at 94°C,	Jensen *et al* (1999)
	ermA-R	TCA AAG CCT GTC GGA ATT GG-3'	after that, 35 94°C cycles	
			30 minutes at 60°C	
ErmC	ermC-1	CAA ACC CGT ATT CCA CGA TT	After a 5-minute denaturing phase at 94 degrees Celsius,	Jensen *et al* (1999)
	ermC- 2	ATC TTT GAA ATC GGC TCA GG	after that, 35 times	
			30s at 94°c, 50°c, and 72°c for 30°C	
			terminate at 72° C for 10min	

3.4 Data Analysis

Microsoft Excel 2021 was used to enter the data, which was then transferred to SPSS for review. Comparism between proportions were made using one-way ANOVA for three means. Differences showing a critical value less than F value confidence level 0.05 or 0.01 were considered significant.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 The prevalence and distribution of *Staphylococcus aureus* in various samples

Table 4.1 shows the prevalence of *Staphylococcus aureus* in various samples. The highest prevalence was obtained from laboratory floor (48%) while the least prevalence was obtained on workbench (8%).

Table 4.1: The Prevalence and Distribution of *Staphylococcus aureus* in Various Samples

SAMPLE TYPE	NUMBER OF SAMPLE	NUMBER OF ISOLATES	Prevalence (%)
BLOOD	70	12	17.1
URINE	70	12	17.1
WOUND	70	17	24.3
SKIN	25	9	36
NOSE	25	6	24
EAR	25	4	16
WORK BENCH	25	2	8
AIR	25	5	20
LAB FLOOR	25	12	48
Total	360	79	21.9

4.1.2. Prevalence of *Staphylococcus aureus* from clinical and environmental samples

The 360 samples collected were classified into clinical and environmental sources with clinical sources having a total of 210 and 150 for environmental samples. Environmental samples was higher with the prevalence rate of 24.0% while clinical samples was lower with prevalence rate of 20.5%. The prevalence of *S. aureus* was 21.9% as shown in table 4.2.

Table 4.2: Prevalence of *Staphylococcus aureus* from Clinical and Environmental Samples

Sample sources	Number (%)	Number of *S. aureus* isolate	Prevalence of *S. aureus* (%)
Clinical	210 (58)	43	20.5
Environmental	150 (42)	36	24.0
Total	360 (100)	79	21.9

4.1.2. Prevalence of *Staphylococcus aureus*on different age groups and gender

The prevalence rate of *S. aureus* with respect to age group and gender was determined (Table 4.3). The age range of 0-17 years had the highest prevalence rate of 25.9% followed by age range 18-49 years with prevalence rate of 20.6% while age group 50-70 years had the least prevalence rate of 8.3%. Prevalence of *S. aureus* was higher in females 22.4% than in males 20.0%.

Table 4.3: Prevalence of *Staphylococcus aureus* on Different Age Groups and Gender

Parameter	Number of Sample	Number of isolated *S. aureus*	Prevalence of *S. aureus*
Age Groups	81	21	25.9
0-17	180	37	20.6
18-49			
50-70	24	2	8.3
Gender			
Male	160	32	20.0
Female	125	28	22.4

4.1.3 Antibiotic susceptibility profile

The number of isolates resistant to each of the antibiotics is shown in Table 4.4. Out of the 79 *S. aureus* isolates obtained from clinical and environmental samples, high percentage of resistance against oxacillin 88.6 % followed by cefoxitin 45.6%, ampicillin 34.2% and vancomycin 21.5% were observed. Only 18.9% were resistant to erythromycin, the least resistance was recorded against ciprofloxacin and chloramphenicol (3.8%) each. Gentamycin and Ciprofloxacin showed high activity against *S. aureus* with 92.4% and 89.9% respectively. Rifamspicin 88.6% chloramphenicol 86.1% %, Amoxicillin 84.8%and Norfloxacin 82.3 %. Two antibiotics Septrin and Levofloxacin were 100% active against all the isolates.

Comparing the three methicillin (oxacillin, cefoxitin and vancomycin) and erythromycin antibiotics, higher resistance was seen in *S. aureus* against Oxacillin 88.6%, followed by Cefoxitin 45.6%, Vancomycin 21.5%. while for erythromycin it was 18.9%. Erythromycin showed high activity against S.aureus 53.1% followed by cefoxitin 31.60%, Vancomycin 26.60 while least was against oxacillin 11.40% as shown in Table 4.4. Hence, $P < 0.05$ shows that

erythromycin and methicillin has a significant effect on the *S. aureus* susceptibility profile. The interpretative chart for the susceptibility test is as shown in appendix A.

Table 4.4: Antibiotics Susceptibility Profile of *Staphylococcus aureus*

No. of *S.aureus*	**Susceptibility profile (n=79)**	**S**	**I**	**R**	**$\chi 2$**	**P-value**
N=79	Ciorofloxacin	71(89.9)	5(6.3)	3(3.8)	636.1	0.001
	Nalidixic acid	65(82.3)	5(6.3)	9(11.4)		
	Gentamycin	73(92.4)	2(2.5)	4(5.1)		
	Amoxacillin	67(84.8)	7(8.9)	5(6.3)		
	Septin	79(100)	0(0.0)	0(0.0)		
	Rifampicin	70(88.6)	2(2.5)	7(8.9)		
	Erythromycin	56(70.9)	20(25.3)	3(3.8)		
	Chlorophenicol	68(86.1)	8(10.1)	3(3.8)		
	Apiclox	41(51.9)	11(13.9)	27(34.2)		
	Levofloxacin	79(100)	0(0.0)	0(0.0)		
	Vancomycin	21(26.6)	41(51.9)	17(21.5)		
	Oxacillin	9(11.4)	0(0.0)	70(88.6)		
	Cifoxitin	25(31.6)	18(22.8)	36(45.6)		

$P < 0.05$ shows that the antibiotics have a significant effect on the bacteria susceptibility profile

Table 4.5: Erythromycin and Methicillin Susceptibility Profile of *Staphylococcus aureus*

Number of *Staphylococcus aureus* (n)	Susceptibility profile	E	VA	OX	FOX	X^2	P-Value
N=79	S	56 (70.9)	21 (26.6)	9 (11.4)	25(31.6)	166.27	0.001
	I	20 (25.3)	41 (51.9)	0 (0.0)	18(22.8)		
	R	3 (3.8)	17 (21.5)	70(88.6)	36(45.6)		

E: Erthromycin, V:Vancomycin, OX: Oxacillin, FOX:Cefoxitin

4.1.4. Susceptibility of *Staphylococcus aureus* to methicillin

Table 4.6. Shows that there is very high resistance of 88.6% to oxacillin followed by 45.6% resistanceto cefoxitin while low resistance was against vancomycin 24.1%. Susceptibility to cefoxitin was 32.9% followed by vancomycin 26.6% while the least susceptibility (high resistance) was against Oxacillin 12.7%.

Table 4.6: Susceptibility of *Staphylococcus aureus* to Methicillins

Antibiotics	Susceptibility	Intermediate	Resistance
Vancomycin VA	26.6%	51.9%	21.5%
Oxacillin OX	11.4%	0	88.6%
Cefoxitin FOX	31.6%	22.8%	45.6%

4.1.5. Sex distribution of methicillin resistant isolates

The resistant pattern of *S. aureus*to oxacillin in males was 93.8% while in females was 85.7%. Males also had higher resistance of 28.1% and female had lower 25.0% to vancomycin while for cefoxitin, females had higher resistance of 53.6% and males had the lower resistance of 37.5% as shown in Table 4.7.

Table 4.7: Sex Distribution of Methicillin Resistant *Staphylococcus aureus* Isolates

SEX	MRSA		
N=60	OX	VA	FOX
MALE	93.8%	28.1%	37.5%
FEMALE	85.7%	25.0%	53.6%

E: Erthromycin,V:Vancomycin, OX: Oxacillin, FOX: Cefoxitin

4.1.6. Multiple Antibiotic resistant indices of *Staphylococcus aureus*isolates

The results revealed that 20 isolates (26.3%) were resistant to three or more antibiotics.multiple antibiotic resistant indices ≥ 0.3 indicated that the isolates originated from an environment where antibiotics were frequently used. Therefore, Multi drug resistant (MDR) is determined by the isolates that show resistance to three and above antibiotics Tables 4.8 and 4.9 respectively.

Table 4.8: Multiple Antibiotic Resistant Indices of *Staphylococcus aureus* Isolates

No. of antibiotic to which resistant (n=13)	**Number of isolates with same no. of antibiotic resistance**	**MAR index**	**Percentage of *S. aureus* with corresponding MARI**
1	24	0.1	31.6
2,3	40	0.2	52.6
4	4	0.3	5.3
5	2	0.4	2.6
6,7	6	0.5	7.9
TOTAL	76		100

Table 4.10: Antibiotics Resistant Profile of Multidrug Resistant *Staphylococcus aureus*

ISOLATE CODES	ANTIBIOTICS RESISTANT PATTERN	MARI	NO. ANTIBIOTICS RESISTANT TO	RESISTANT CATEGORY
W15	VA, OX, FOX	0.2	3	MDR
W45	VA, OX, FOX	0.2	3	MDR
W35	OX, FOX NB CN, E, CH	0.5	6	MDR
W26	OX, FOX, CN, RD, APX	0.3	5	MDR
W30	VA, OX, CPX, NB,AMX, APX	0.5	6	MDR
W27	OX, FOX, CPX, NB, AMX, RD, APX	0.6	7	MDR
U09	VA, OX, FOX	0.2	3	MDR
U30	OX, FOX, CN, CH	0.3	4	MDR
U69	OX, FOX, NB, RD, E, CH, APX	0.6	7	MDR
U46	VA, OX, FOX	0.2	3	MDR
S01	OX, NB, AMX, E, APX	0.3	5	MDR
S15	VA, OX, FOX	0.2	3	MDR
N07	VA, OX, FOX, APX	0.3	4	MDR
N10	OX, FOX, CPX, APX	0.3	4	MDR
N08	OX, FOX, NB, APX	0.3	4	MDR
N09	OX, FOX, AMX, RD, APX	0.3	5	MDR
E19	OX, VA, FOX	0.2	3	MDR
E05	VA, OX, FOX	0.2	3	MDR
E09	VA, OX, FOX, NB, CN, RD, APX	0.6	7	MDR
LF05	OX, FOX, APX	0.2	3	MDR

CPX: Ciprofloxacin, LEV: Levofloxacin, CN: Gentamicin, RD: rimfampicin, APX: amplicillin, S: streptomycin, AMX: amoxicillin, OX: Oxacillin, VA: vancomycin, NB: Norfloxacin, FOX: Cefoxitin, CH: Chloramphemicol, E: Erythromycin, MDR: multidrug resistant, MARI: multi antibiotics resistant indices.

4.1.7 Molecular characteristics of the isolates

4.1.7.1 Agarose gel electrophoresis indicating a positive amplification of the 16S region in the bacterial isolates

Plate 1. Shows Agarose gel electrophoresis indicating a positive amplification of the 16S region in the bacterial isolates. Lane 1 shows the markers, lane 2 -11 are the bacterial isolates that were amplified while lane 12 is the buffer.

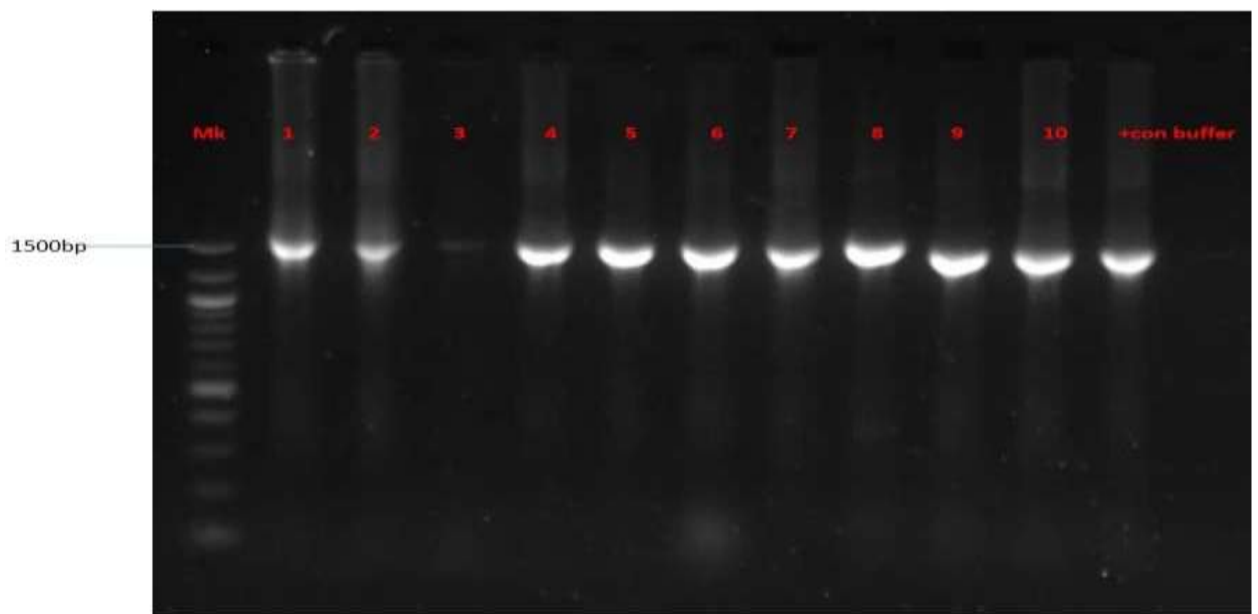

Plate I: Agarose gel electrophoresis indicating a positive amplification of the 16S region in the bacterial isolates

4.1.7.2.The polymerase chain reaction of the mecA gene amplified from bacterial isolates on an agarose gel electrophoresis

Plate II demonstrates agarose gel electrophoresis of mecA gene PCR from bacterial isolates known as *Staphylococcus aureus* using mecA specific primers. Positive amplification is confirmed by a band size of roughly 330pb. *Staphylococcus aureus* received ten (10) points out of a possible ten. Five (5) of the methicillin-

resistant*Staphylococcus aureus* isolates had the MecA gene.

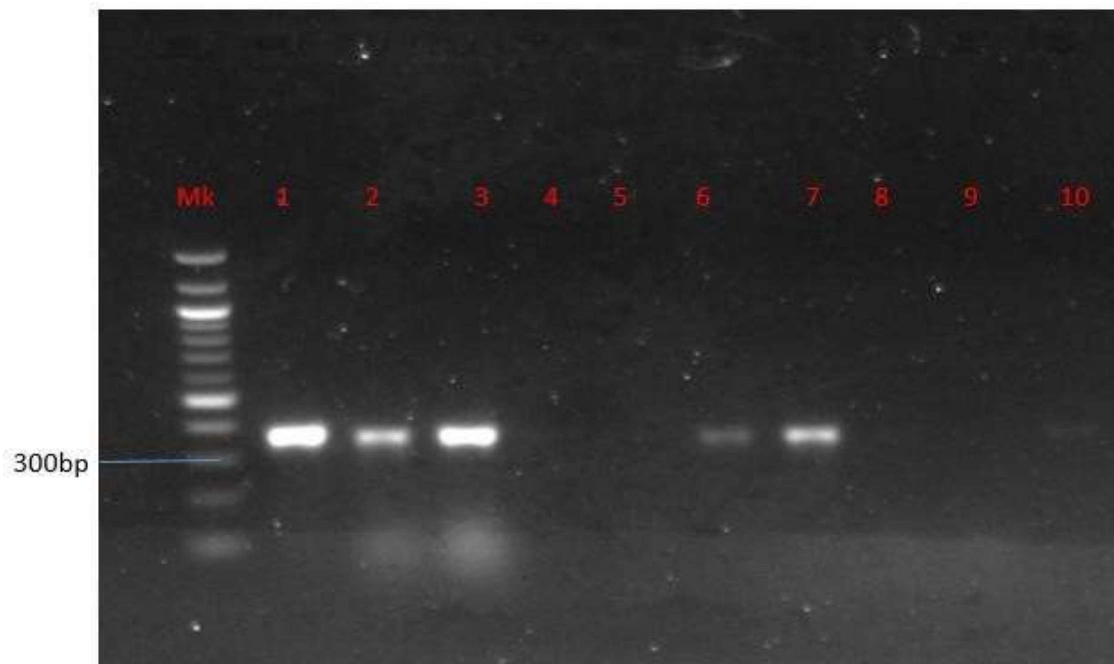

Plate II: Agarose gel electrophoresis of the PCR of mecA gene amplified from bacterial isolates identified as*Staphylococcus aureus*

Lane 1= mbp= 100bp

Lane 2- 11 samples

4.1.7.3. The polymerase chain reaction of the ermA gene amplified from bacterial isolates on an agarose gel electrophoresis

Plate III shows agarose gel electrophoresis of the PCR of *ermA* gene amplified from bacterial isolates identifiedas*Staphylococcus aureus*, Using *ermA* specific primers. Band size approximately 450pb confirmed positive amplification. Result indicates 3 out of 10 were positives for *ermA*.

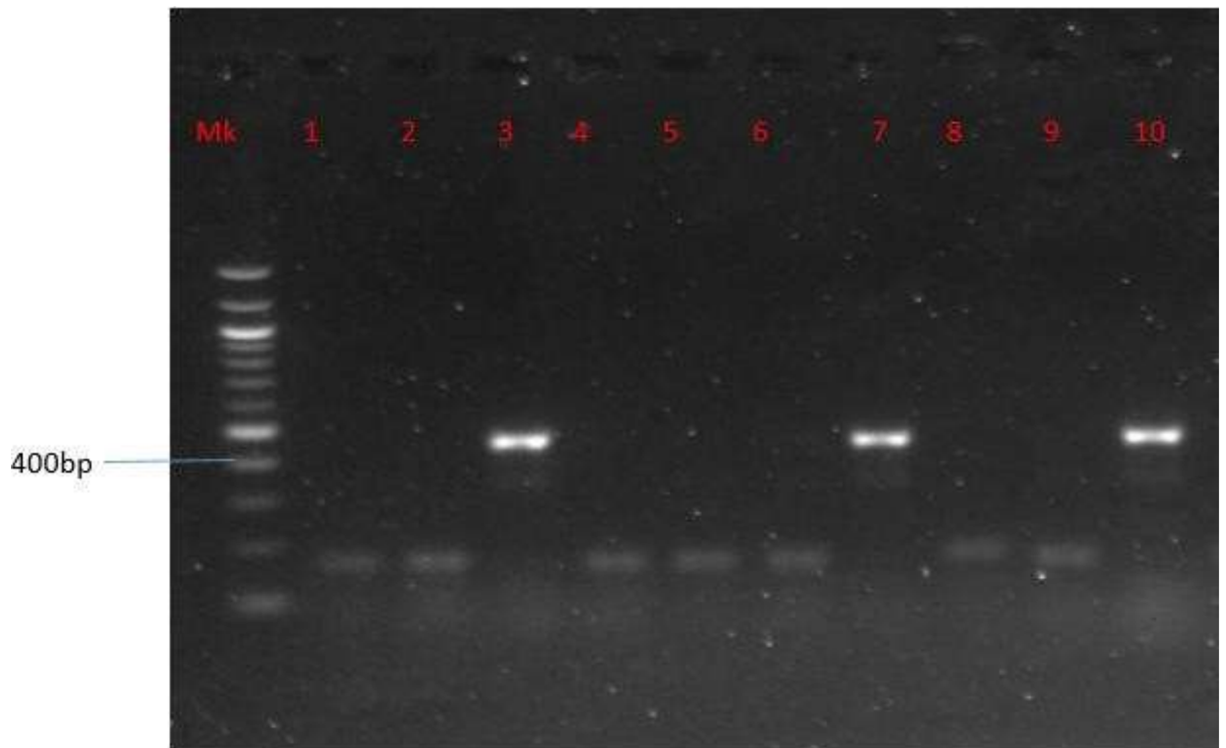

Plate III. Polymerize Chain Reaction of the ermA gene amplified from bacterial isolates as *Staphylococcus aureus* on an agarose gel electrophoresis.

Lane 1= mbp= 100bp

Lane 2- 11 samples

4.1.7.4. The polymerase chain reaction of the ermC gene amplified from bacterial electrophoresis on an agarose gel

PCR of the ermC gene amplified from bacteria isolates known as Staphylococcus aureus on an agarose gel electrophoresis with ermC specific primers. Band size approximately 380pb confirmed positive amplification. Result indicates 6 out of 10 were positives for *ermC.*

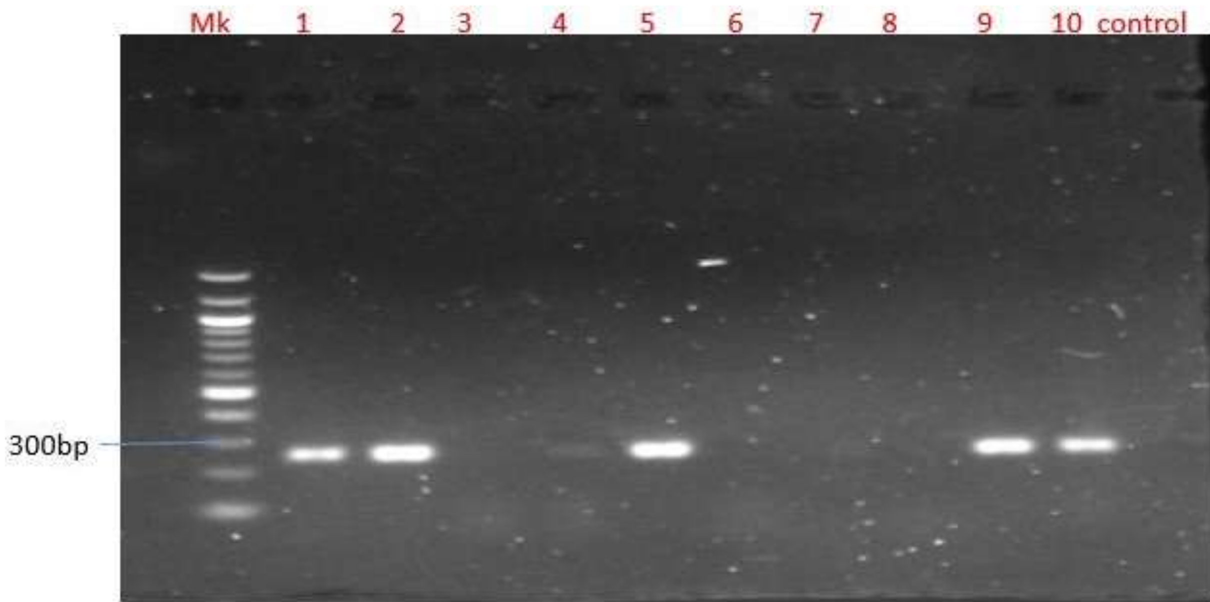

Plate IV. Agarose gel electrophoresis of the PCR of *ermC* gene amplified from bacteria isolates identified as *Staphylococcus aureus.*

Lane 1= mbp= 100bp

Lane 2- 11 samples

4.1.7.5 DNA Sequencing

Ten isolates were selected for DNA sequencing of the PCR product. The phylogenetic tree comparing the nucleotide sequence of the selected isolates with the other *Enterobacteriaece* from the gene bank data base was shown in plate 4.

As observed from the phylogenetic tree in this study, there are two main clusters based on their similarities and members within a cluster are more related than between clusters. It is also observed that isolates are mostly clustered together which further confirmed their similarities except for two isolates (U69 and N10) MN814062 and MN814061 which may be either from where the sample originated or mutation of the gene. There is a relationship between isolate MN814064 (B08) and MN 814065 (B26) which was from Blood and the members of the cluster which were from the lab floor and the difference in

the time of divergence is not wide. This showed that the organisms originated from the same source, indicating the possible transmission either from humans to environment or environment to humans.

4.2 DISCUSSION

Staphylococcus aureus is a major cause of community-acquired and nosocomial infection, and it has spread all over the world, being widespread in many countries (Khadri and Alzohairy, 2010).In this study, 360 samples were collected and screened for the presences of *S. aureus* from patients and environmental samples in General Hospital Minna. Out of the 360 samples, 210 were from clinical sources while 150 were from environmental sources.

Theprevalence of *S. aureus* isolated was 44.5% (clinical sample 20.5% and environmental samples 24.0%). The high prevalence observed in environmental samples could be due to poor infection control (sanitation) in the hospital environment, which could serve as a reservoir of the organism. It was also reported by Thongchai *et al.*(2018) that isolation of *S. aureus* from clinical and environmental sources such as blood, workbench, air and laboratory floor shows the ubiquitous prevalence of *S. aureus*. *Staphylococcus aureus* has been used in clinical and environmental samples in a variety of tests (Olowe *et al.,* 2013; Ugwu *et al.,* 2016; Thongchai *et al.,* 2018). Cleaning procedures are carried out on a regular basis in these areas, but they are not always successful (Boyce, 2016; Bale, *et al.,* 2018). The highest prevalence of *S. aureus* was found in the age group 0-17 years (25.9%) while age group 50-70 years had the lowest prevalence (8.3%). The high prevalence among age group of 0-17 could be due to the fact that children are most vulnerable in an area where there is lack of water and poor environmental hygienes.

The prevalence of *S. aureus* among the females was higher (22.4%), than in males (20.0%). The difference in prevalence among the gender could be attributed to the fact that anatomy of females exposes them to easy contamination of *S. aureus* by colonizing the vagina. This findings is in agreement with the findings of Tong *et al.* (2015) who reported that females anatomy are exposed to easy contamination of pathogens which is endogenous colonizing the vagina vault of healthy women.

In this study 45.6% of the isolates were found to be methicillin resistant *Staphylococcus aureus* (MRSA) while 88.6% were resistant to oxacillin. Muralidharan (2009) reported similar prevalence of 40.6% to 59.3% in India and Kshetry *et al.* (2016) reported prevalence of 43% in Jos, while Onemu and Ophori (2013) reported a higher prevalence of 79% in Benin.

The prevalence rate in this study was higher than the prevalence rates of 34.7 percent in Ilorin, 30.4 percent in Ibadan, 20.1 percent in Enugu, and 12.5 percent in Maiduguri (Okon *et al.,* 2013; Udeani *et al.,* 2016). This study found that Switzerland had the highest prevalence rate of MRSA (0.09 percent), the United Kingdom had the lowest (0.005 percent) (Pallab *et al.,* 2011), and Ullah *et al.* (2016) found that Pakistan had the highest prevalence rate of MRSA (36.1 percent)

The high resistance to oxacillin in this study may be due to overproduction of -lactamase, which could lead to phenotypic oxacillin resistance, resulting in oxacillin-resistant clinical and environmental isolates that lack the normal genetic mechanism for such resistance. Under antibiotic attack, such strains are likely to evolve into completely resistant strains. According to Kshetry *et al.* (2016), differences in MRSA prevalence rates between studies may be due to differences in study locations and time periods, as well as differences in hygienic conditions maintained in different hospitals. Mir and

Srikanth (2013) have mentioned the hospital's healthcare facilities, as well as the introduction of an infection management program and the rational use of antibiotics, all of which can differ from one hospital to the next. The use of methicillin disc for MRSA detection might have been responsible for the higher prevalence over-the-counter and has been misused over the years (Adeiza*et al.,* 2020). Motayo *et al.* (2012) and Fayomi *et al*. (2011) recorded similarly high prevalence rates.

Several studies reported by CLSI, (2017), Faiqa *et al.* (2016), Adeiza*et al.,* (2020) have recommend Cefoxitin disc diffusion to be more to other commonly recommended phenotypic methods, such as oxacillin disc diffusion. Cefoxitin has also been used as a surrogate marker for the mec A gene; it provided simpler end points, was easier to interpret, and was more consistent than the Oxacillin test (Faiqa *et al.,* 2016). MRSA strains with inducible resistance to methicillin developed much faster in the presence of cefoxitin than in the presence of oxacillin, owing to cefoxitin's enhanced induction of PBP 2a (Faiqa *et al.,* 2016).

The characteristic MDR feature of MRSA was well observed in this study with respect to amoxicillin, ampicillin, norfloxacin, rafampicin, and erythromycin. Previous studies elsewhere have made similar observation (Motayo *et al.,* 2012; Adeiza*et al.,* 2020). It has been stated that the presence of insertion sites for plasmids and transposons in mecA complex of MRSA which often carry antibiotics resistance genes account for the resistance to several classes of antibiotics (Motayo *et al.,* 2012; Adeiza*et al.,* 2020).

As reported by Davies and Davies (2010), the huge prescription and extensive use of antibiotics without control and caution accounts for widespread of antibiotic resistant organisms. A large percentage of the isolates (26.6%) were immune to three or more antibiotics. Multiple Antibiotic Resistance Indices (MARI) $\geq$ 0.3 indicated that the

isolates originated from the environment where antibiotics were frequently used (Christopher, 2013).

The highest (26.6%) prevalent multidrug resistance pattern was observed against ampicillin, oxacillin, cefoxitin, and chloramphenicol and erythromycin respectively. According to Lupo *et al.* (2012) who stated MRSA adaptability and propensity for acquiring multidrug resistance genes among species and from the environment by horizontal gene transfer accounts for its multidrug resistance.

Methicillin Resistant *Staphylococcus aureus* isolates had a very high susceptibility rate to the antibiotics tested in this study. Clinical and environmental isolates were susceptible to gentamycin with 92.4%, ciprofloxacin 89.9%, rifampicin 88.6%, chloramphenicol 86.1% amoxicillin 84.8%, norfloxacin 82.3% and erythromycin 53.1% while two antibiotics septrin and levofloxacin were 100% active on all the isolates. This result is in agreement with the findings of Kadora's (2010) who stated that gentamycin had 100 % activity on *S. aureus*. gentamycin is an injectable aminoglycoside, so its use limited compared to other antibiotics that come in tablet form. This study also discovered that isolates had a high resistance to ampicillin (76.9%) as was also reported by Okwu *et al.* (2012); Ugwu *et al.* (2016) and Nsofor *et al.* (2016). Resistance to beta-lactam antibiotics is usually mediated when the enzyme beta-lactamase disrupts the beta-lactam ring, deactivating the molecule's antibacterial property. This study found that isolates had a high level of resistance to ampicillin (Nsofor *et al.,* 2016).

Other causes of antibiotics resistance may be attributed to non-compliance to prescription (drug abuse). It is also evident that such habits equally impact the exponential growth of this organism because most individuals resort to patronizing patent medicine stores without correct prescription, dosage and shelf life in the community. Very low resistance

(3.8%) to ciprofloxacin, a fluoroquinolone recorded in this study has been reported by another study with 16.6% (Al-Mohana *et al.,* 2012). Antibiotics such as ampicillin, rifampicin, erythromycin, and norfloxacin were inactive on isolates. This supports previous findings that most *S. aureus* isolates are resistant to antibiotics. Many widely prescribed antibiotics wereinactive against *S. aureus* (Okwu *et al.,* 2012; Bektas *et al.,* 2016). Antibiotic resistance has been linked to self-medication and indiscriminate antibiotic use, and systemic antibiotics have been shown to alter MRSA in *S. aureus* (Okwu *et al.,* 2012; Ghidey *et al.,* 2014).

The majority of isolates were susceptible to widely used antimicrobial agents. Multiple drug resistance was widespread in the case of MRSA, and only a few antibiotics were effective against these isolates. High resistance was recorded against oxacillin (88.6%), cifoxitin (45.6%) and Vancomycin (21.5%) while commonly used antibiotics; ciprofloxacin, chloramphenicol, gentamycin, amoxicillin, rifampicin and norfloxacin had low resistance rate.

The MRSA strains from clinical samples should be considered resistant to all B-lactam antibiotics, with the exception of those with anti-MRSA activity, according to CLSI (2017). Methicillin Resistant *Staphylococcus aureus* is more susceptible to ciprofloxacin, according to Zabielinski *et al.* (2013), while MSSA showed improved tolerance to the antibiotic. This could be due to the fact that some antibiotics are commonly prescribed, available as over the counter antibiotics, and the organisms may have developed resistance due to selective pressure from inappropriate use (Zabielinski 2013).

Self-medication could be blamed for the rise in resistance, which may lead to a proliferation of antibiotics being used at sub-therapeutic levels, allowing resistant strains

to develop. Antimicrobial resistance trends showed seventy phenotype patterns, with oxacillin responsible for 70 (88.6%) of the isolates (Jain *et al*., 2011).

Antibiotic resistance was found in 19 (24.1%) of the isolates (multi-drug resistant). 10 (12.7%) of the multidrug resistant isolates came from clinical samples, all of which were MRSA, and 9 (11.4%) came from environmental samples. The hospital's uncontrolled handling of antibiotics and chemicals, which puts selective pressure on the organisms, could have resulted in the multi-drug resistant strain. According to Piso *et al*. (2017), unregulated antibiotic usage in hospitals and the general public developed reservoirs of bacteria that could become resistant. As a consequence, this condition is thought to be a factor in the multi-drug resistance MRSA discovered in this study. As a result, it is possible that this condition is responsible for the multidrug resistance MRSA found in this report.

Erythromycin resistance in *Staphylococcus aureus* is predominantly mediated by erythromycin resistance methylase encoded by erm genes (Weisblum, 1995). In human infections caused by *Staphylococci*, ermA and ermC are the most common methylase genes (Nicola *et al.,* 1998). For erythromycin-resistant *S. aureus* isolated from clinical and environmental samples, the incidence of ermA was 3.8 percent (3/79) and ermC was 6.3 percent (5/79) in this report. In erythromycin-resistant *S. aureus*, Westh *et al*. (1995) and Nicola *et al.* (1998) found a high prevalence of ermA (82-94 percent).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Staphylococcus aureus was isolated from clinical and environmental samples in General hospital Minna, Niger State.

The resistance of *Staphylococcus aureus* to oxacillin,cefoxitin, ampicillin, vancomycin, erythromycin. Norfloxacin, Rifampicin, Amoxacillin, Gentamycin were 88.6 %, 45.6 %, 34.2 %, 21.5 %, 18.9 %, 11.4 %, 8.9 %, 6.3% and 5.1 % respectively.

Methicillin (mecA) and erythromycin (ermA and ermC) genes were isolated from *Staphylococcus aureus* strains obtained from clinical and environmental samples in this study.

5.2 Recommendations

From the findings of this study the following recommendations are made:

1. The MRSA isolates were found to be resistant to the majority of antibiotics. This result necessitates immediate attention, with a strict policy being implemented to reduce irrational antibiotic use.
2. Antimicrobial profile of MRSA isolates should be monitored on a regular basis to assist clinicians in selecting suitable antimicrobial therapy.
3. High hygienic standards in hospital and Hospital environmental are recommended to avoid contamination and spread of MRSA. Hand washing remains the single most important infection control that prevents the spread of infectious diseases.

4. To provide a better understanding of the prevalence and epidemiology of MRSA, rapid and accurate detection of methicillin resistance in *S. aureus* is important for the use of appropriate antimicrobial therapy and for the control of Hospital Acquired-MRSA and Community Acquired-MRSA.

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Appendix A: MCFARLAND STANDARD

One percent (1%) v/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of distilled water. Exactly 1% v/ v solution of barium chloride was prepared by dispensing 0.5g of dehydrated Barium chloride in 50ml of distilled water. Then, 0.05ml of barium chloride and 9.95ml of sulphuric acid were mixed to give precipitate equivalent to Mcfarland Standard which is equivalent to 1.5x 10^8 cfu/ml (Cheesbrough, 2002).

Appendix B: STANDARD FOR ZONES OF INHIBITION IN MILLIMETER

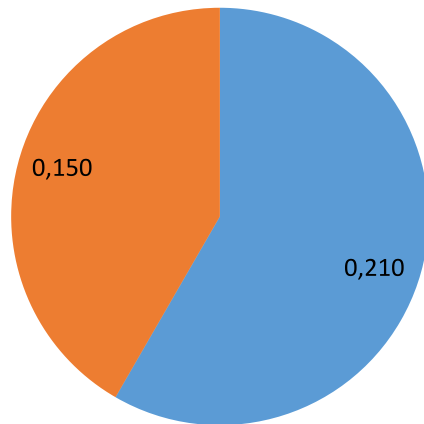
Appendix B: STANDARD FOR ZONES OF INHIBITION IN MILLIMETER

AGENT	CONTENT	S	I	R
CIPROFLOXACIN	5ug	≥21	16-20	≤15
NORFLOXACIN	10ug	>17	13-16	<12
GENTAMYCIN	10ug	>15	13-16	<12
AMOXACILLIN	20ug	>18	14-17	<13
SEPTRIN	10ug	>15	12_14	<11
RIFAMPICIN	5ug	>20	17-19	<16
ERYTHROMYCIN	15ug	>23	14-22	<13
CHLOROPHENICOL	30ug	>18	13-17	<12
AMPICLOX	10ug	>22	19-21	<18
LEVOFLOXACIN	5ug	>17	14-16	<18
VANCOMYCIN	30ug	>17	15-16	<14
OXACILLIN	1ug	>18		<17
CEFOXITIN	30ug	>28	24-27	<23

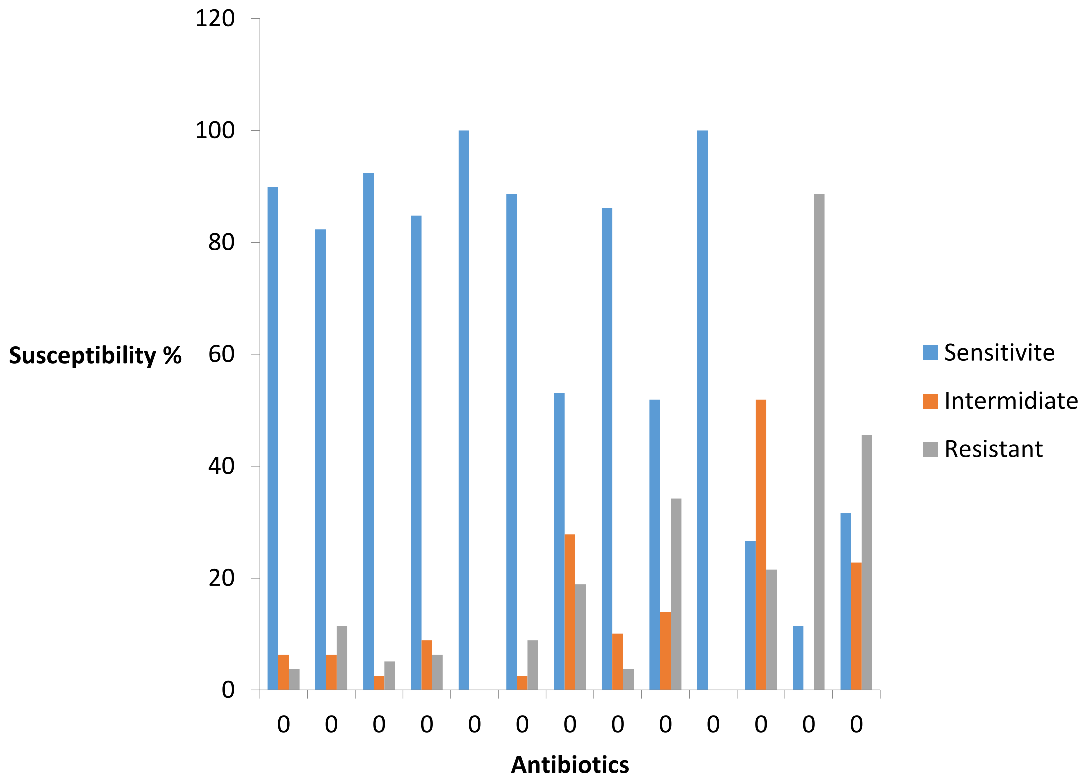
S: susceptibility, I: intermediate, R: resistant

Note: Interpretive Criteria (nearest whole mm)

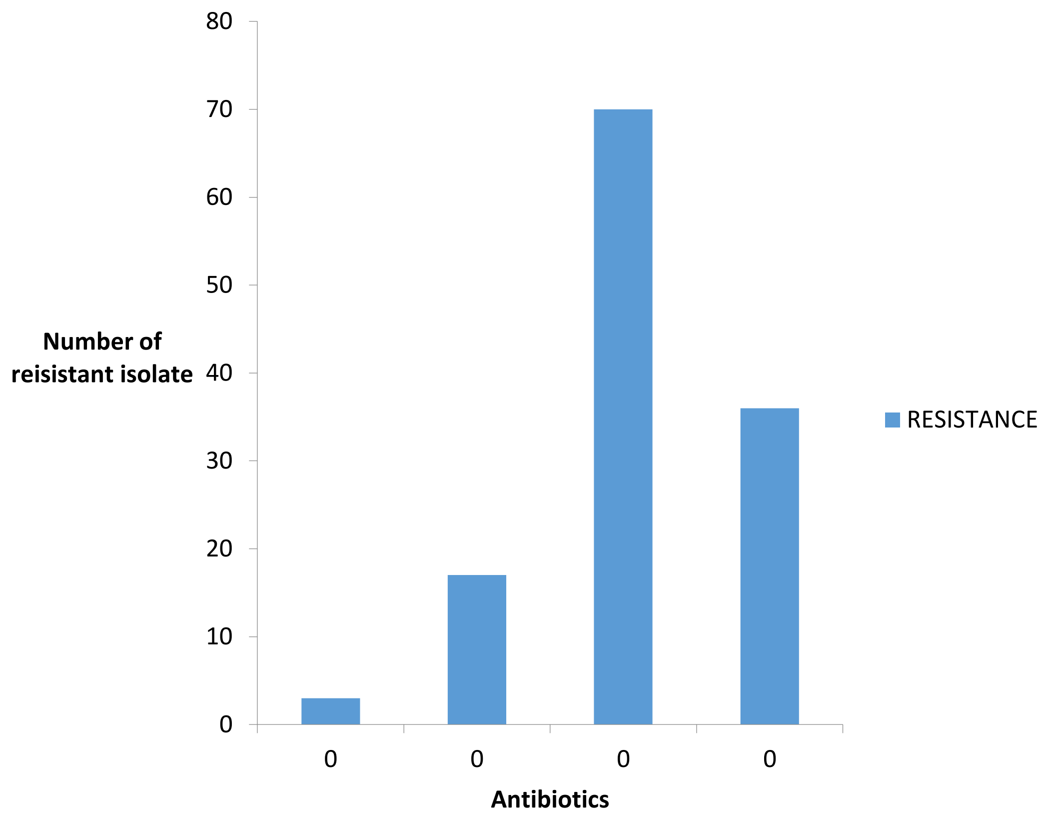
APPENDIX C: SAMPLE SOURCE AND DISTRIBUTION



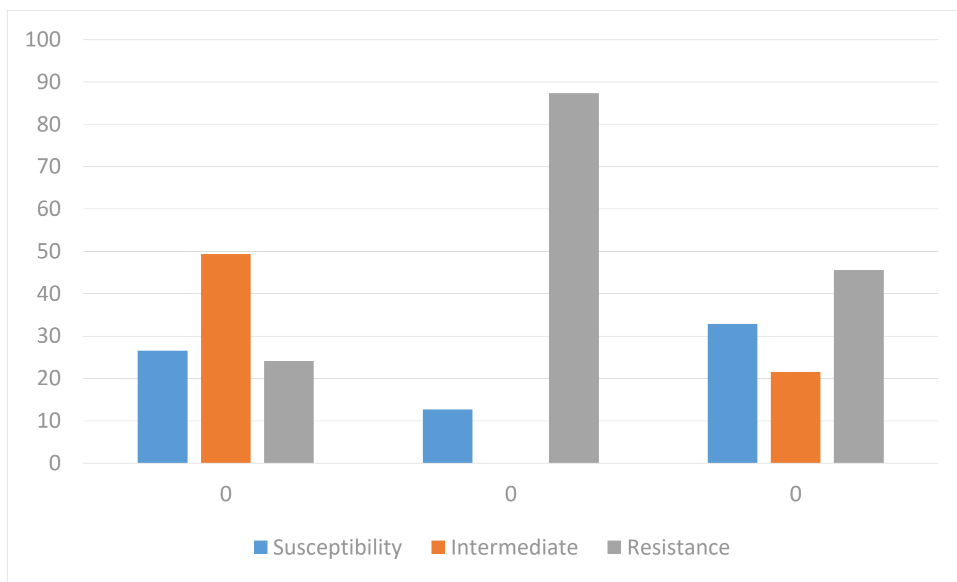
APPENDIX D: ANTIBIOTICS SUSCEPTIBILITY PROFILE



APPENDIX E: ERYTHROMYCIN AND METHICILLIN RESISTANCE PROFILE



APPENDIX F: METHICILLIN PROFILE



Appendix G: AMPLIFIED 16S REGION IN BACTERIA ISOLATES

>MN814061 Staphylococcus aureus strain GodMam-N10

GGCCGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACGGACGAGAA
GCTTGCTTCTCTGATGTTAGCGCGGAC
GGGTGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGA
AACCGGAGCTAATACGGATAATATTTT
GAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGG
ATCCGCGCTGCATTGCTAGTTGGTAAG
GTAACGGCTTACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGG
CCACACTGGAACTAGACACGGTCCAG
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTG
ACGGAGCAACGCCCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTT
ATTAGGGAAGAACATATGTGTAAGTAACTGTGCACATCTTGACGGTACTAA
TC
AGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGC
AAGCGTTATCCGGATTATTGGGCGTA
AAGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAAC
CGTGGAGGGTCATTGAAACTGGAAAA
CTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGC
AGAGATATGGAGGACACCAGTGGCG
AAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGAT
CAAACAGGATTAGAACCCTGGTAGTCC
ACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCT
GCAGCTAACGCATTAGCACTCCGCCT
GGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGC
ACAAGCGGTGGAGCTGTGGTTTAATTC
GAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACAACTCTAG
AGATAGAGCCTTCCCTTCGGGGGACAA
AGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGGTCGTGAGATGTTGGGTTA
AGTCCCGCAACGACGCAACCCTTAAG
CTTAGTTGCCATCATTAATTGGCACTCTAAGTTGACTGCCGGTGACAAACCG
GAGGAAGGTGGGGTGACGTCAAATCA
TCATGCCCTTATGATTTGGGCTACACACGTGCTACAATGGACAATACAAAG
GGCAGCGAAACCGCAGGTCAAGCAAAT
CCCATAAAGTTGTTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAA
GCTGGAATCGCTAGAATCGTAGATCAG
CATGCTACGGTGAATACGTTCCCGGGTCTTGTACACCCCGCCCGTCACACCA
CGAGAGTTTGTAACCCCGAAGCCGGT
GGAGTAACCTTTTAGGAGCTAGCCGTCGAAGGTGGGACAAATGGGGG

>MN814062 Staphylococcus aureus strain GodMam-U69

CCATGTCGAGCGAACGGACGAGAAGCTTGCTTCTCTGATGTTAGCGGCGGA
CGGGTGAGTAACACGTGGATAACCTAC
CTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAATATT
TTGAACCGCATGGTTAAAAGTGAAAGA
CGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCATTAGCTAGTTGGTAA
GGTAACGGCTTACCAGGCAACGATGC
ATAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCA
GACTCCTACGGGAGCAGCAGTAGGG
AATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATG
AAGGTCTTCGGATCTAAAACTCTGTTA
TTAGGGAAGAACATATGTGTAAGTAACTGTGCCTTGACGGTACCTAATCAG
AAAGCCACGGCTAATACGTGCCAGCAG
CCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAG
CGCGCGTAGGCGGTTTTACATTAAGTC
TGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAA
CTTGAGTGCAGAAAGGAAAGTGGAA
TTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGAACACCAGTGGCGA
AGGCGACTTTCTGGCTGTAACTGACG
CTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATAGCCCTGGTAGTC
CACGCCGTAAACGAGAGTGCTAAGTG
TTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACCCGCCT
GGGGAGTACGACCCAAGGTTGAAACT
CAAAGGAATTGACGGGGACCCGCACAAGCGGCGGAGTGTGGTTTAATTCG
AAGCAACGCGAAGACCTTACCAAATC
TTGACATCCTTTGAACAACTCTAGAGTAGAGCCTTCCCCCTTCGGAGGAAAA
AGTGACAGGTGTGCTGAAGTCGTCAG
CTCGTGTCAGTGAAGATGTGGTTAGTCCCGCAACGAGCGCACCGTAGGCTA
GTGCATCATTAGTGCACTCTAGTGACT
GCCGTGAACAACGGAAGAAGGTGGGATGACGTCATCATCATGCCCTTATGA
AATGGGGGCTTAACACG

>MN814063 Staphylococcus aureus strain GodMam-U30

CATTGAAGCTTGCTTCTCTGATGTTAGCGGCGGACGGGTGAGTAACACGTG
GATAACCTACCTATAAGACTGGGATAAC
TTCGGGAAACCGGAGCTAATACCGGATAATATTTTGAACCGCATGGTTCAA
AAGTGAAAGACGGTTTGCTGTCACTTAT
AGATGGATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGG
CAACGATGCATAGCGACCTGAGAGGG
TGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAG
CAGTAGGGAATCTTCGCAATGGGCGA
AAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAA
ACTCTGTTATTAGGAAGAACATATGT

GTAAGTAACTGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAA
CTACGTGCCAGCAGCGCGGTAATACGT
AGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTT
TTTTAAGTCTGATGGAAAGCCCACGGC
TCAACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAAGAGGA
AAGTGGAATTCCATTGTAGCGGTGAAA
TGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGT
AACTGACGCTGATGGCGAAAGCGTGG
GGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTG
CTAAGTGTTAGGGGTTTCCGCCCCTTA
GTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGGTAGACCGCAAGGTT
GAAACTCAAAGGAATGACGGGGACCC

GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTA
CCAAATCTTGACATCCTTTGACAACTCTA
GAGATAGAGCTTTCCCCTTCGGGGGACAAAGTGACAGGTGGTGCATGGTTG
TCGTCAGCTCGTGTGTGAGATGTTGG
GTTAAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAGTT
GGGCACTCTAAGTTACTGCCGGTGACA
AACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGATTTG
GGCTACACACGTGCACAAATGGACAAT
ACAAAGGGCAGCGAAACCGCGAGGTCAAGCAAATCCCATAAAGTTGTTCTC
AGTTCGGATTAGTCGCAACTCGACTAC
ATGAAGCTGGAATCGCTAGTAATCGTAGATCAGCATGCTACGGTGAATACG
TTCCCGGGTCTTGTAACACCGCCCGTC
ACACCACGAGAGTTTGTAACACCCGAAGCCGGTGGAGTAACCTTTTAGGAG
CAACAT

>MN814064 Staphylococcus aureus strain GodMam-B08

AACATGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCG
CGTGAGTGATGAAGGTCTTCGGATCGT
AAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGCACATCTTG
ACGGTACCTAATCAAAAGCCACGGCT
AACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGA
ATTATTGGGCGTAAGCGCGCGTAGGC
GGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTG
GAAACTGGAAAACTGAGTGCAGAAG
AGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGG
AACACCAGTGGCGAGGCGACTTTCTG
GTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGA
TACCCTGGTAGTCCCGCCGTAAACGAT
GAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATT
AAGCACTCCGCCTGGGAGTACGACCG
CAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGC

ATGTGGTTTAATTCGAGCAACGCGAAG
AACCTTACCAAATCTTGACATCCTTTGACAACTCTAGAGATAGAGTTCCCCT
TCGGGGGACAAAGTACAGGTGGTGCAT
GGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTAAGTCCCGCAACGAGCG
CAACCCTTAAGCTTATTGCCATCATTAA
GTTGGGCACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGAT
GACGTCAAATCATCTGCCCCTTATGAT
TTGGGCTACACACGTGCTACAATGGACAATACAAAGGGCAGCGAAACCGCG
AGGTCAAGCAAATCCATAAAGTTGTTC
TCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGT
AATCGTAGATCAGCTGCTACGGTGAAT
ACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACA
CCCGAAGCCGGTGGGTAACCTTTTAG GAGCTAGCCGTCGA

>MN814065 Staphylococcus aureus strain GodMam-B26

AAATTGCAAGTCGAGCGAACGGACGAGAAGCTTGCTTCTCTGATGTTAGCG
GCGGACGGGTGAGTAACACGTGGATAA
CCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATA
ATATTTTGAACCGCAGGTTCAAAAGTG
AAAGACGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCATTAGCTAGTT
GGTAAGGTAACGGCTACCAAGGCAAC
GATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACG
GTCCAGACTCCTACGGAGGCAGCAGT
AGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGACAACGCCGCGTGAGTG
ATGAAGGTCTTCGATCGTAAAACTCT
GTTATTAGGGAAGAACATATGTGTAAGTAACTGTGCACATCTTGACGGTAC
CTAATCAGAAAGCCAGGCTAACTACGC
CAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGC
GTAAAGCGCGCGTGGCGGTTTTTTAA
GTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGA
AAACTTGAGTGCAGAGAGGAAAGTG
GAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTG
GCGAAGGCGACTTCTGGTCTGTAACT
GACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTA
GTCCACGCCGTAAAGATGAGTGCTAA
GTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCC
GCCTGGGGAGTACACCGCAAGGTTGA
AACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTA
ATTCGAAGCAACGGAAGAACCTTACC
AAATCTTGACATCCTTTGACAACTCTAGAGATAGAGCTTCCCCTTCGGGGGA
CAAAGTGACAGGTGTGCATGGTTGTC
GTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCGCAACGAGCGCAACCCTT
AAGCTTAGTTGCCACATTAAGTTGGG

CACTCTAAGTTGACTGCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCA
AATCATCATGCCCCTATGATTTGGGCT
ACACACGTGCTACAATGGACAATACAAAGGGCAGCGAAACCGCGAGGTCA
AGCAAATCCCATAAGTTGTTCTCAGTTC
GGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGTAATCGT
AGATCAGCATGCTACGTGAATACGTTC

CCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGA
AGCCGGTGGAGTAACCTTTTAGGAGCTA GCCGTCGAAGGCCATT

>MN814066 Staphylococcus aureus strain GodMam-LF07

CCATACGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCT
AATACCAGATAATATTTTGAACCGCATG
GTTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGC
ATTAGCTAGTTGGTAGGTAACGGCTT
ACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGA
ACTGAGACACGGTCAGACTCCTACGG
GAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAA
CGCCGCGTGAGTGAGAAGGTCTTCGG
ATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGCAC
ATCTTGACGGTACCTATCAGAAAGCCAC
GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATC
CGGAATTATTGGGCTAAAGCGCGCGT
AGGCGGCCCTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGT
CATTGGAAACTGGAAACTTGAGTGCA
GAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATG
GAGGAACACCAGTGCGAAGGCGACTT
TCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGAT
TAGATACCCTGGTATCCACGCCGTAAA
CATGAGTGCTAAGTGTTAGGGGGCCCCCGCCCCTTAGTGCTGCAGCTAACG
CATTAAGCACTCCGCTGGCAGGAGTAC
GACCGCAAGGTTGAAACTCAAAGGTTGACGGGGACCCGCACAAGCGGTGG
AGCATGTGGTTTAATCGAAGCAACGCG
AAGAACCTTACCAAATCTTGACATCCTTTGACAACTCTAGAGATCCCGCCTT
CCCCTTCGGGGGACAAGTGACAGGTGG
TGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC
GAGCGCAACCCTTAGCTCCCTTGCCAT
CATTAAGTTGGGCACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAGGT
GGGGATGACGTCAATCATCATGCCCCT
TATGATTTGGGCTACACACGTGCTACAATGACAATACAAAGGGCAGCGAAA
CCGCGAGGTCCCGCAATCCCATAAAGT
TGTTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCG
CTAGTAATCGTAGACAGCATGCTACGG
TGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTT

GCATTCC

>MN814067 Staphylococcus aureus strain GodMam-A04

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AAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCATT
AGCTAGTTGGTAAGGAACGGCTTACCA
AGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTG
AGACACGGTCCAGCTCCTACGGGAGG
CAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCG
CGTGAGTGATGAAGTCTTCGGATCGT
AAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGCACATCTTG
ACGGTACCTAATCAAAAGCCACGGCT
AACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGA
ATTATTGGGCGTAAGCGCGCGTAGGC
GGCCCTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATT
GGAAACTGGAAAACTGAGTGCAGAAG
AGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGG
AACACCAGTGGCGAGGCGACCCTCTG
GTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGA
TACCCTGGTAGTCCCGCCGTAAACGAT
GAGTGCTAAGTGTTACCGGGTTTCCGCCCCTTATGCTGCAGCTAACGCATTA
AGCACTCCGCCTGGGAGTACGACCGC
AAGGTTGAAACTCAAAGGAATTACGGGGACCCGCACAAGCGGTGGAGCAT
GTGGTTTAATTCGAACAACGCGAAGAA
CCTTACCCCATCTTGACATCCTTTGACAACTCTAGAGATAGAGCCTTCCCCT
TCGGGGGACAAAGTGCAGGTGGTGCAT
GGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG
CAACCCTTAAGCTTGTTGCCATCATTA
AGTTGGGCACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGA
TGACGTCAAATCATATGCCCCTTATG
ATTTGGGCTACACACGTGCTACAATGGACAATACAAAGGGCAGCGAAACCG
CGAGGTCAAGCAAACCCATAAAGTTGT
TCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTA
GTAATCGTAGATCACATGCTACGGTGA
ATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTTGAT
CA

>MN814068 Staphylococcus aureus strain GodMam-LF005

AAATGCCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACC
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AAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCATTAGC
TAGTTGGTAAGGTACGGCTTACCAACGCAACGATGCATAGCCGACCTGAGA

GGGTGATCGKCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGG
CA
GCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCG
TGAGTGATGAAGGCTTCGGATCGTCC
CACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGCACATCTTGAC
GGTACCTAATCAGAAGCCACGGCTAAC
TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATT
ATTGGGCGTAAAGCCGCGTAGGCGGT
TTTTYAAGTCTGATGTGAAAGCCCACGGCTCCCCCGTGGAGGGTCATTGGA
AACTGGAAAACTTGATGCAGAAGAGG
AAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACA
CCAGTGGCGAAGGGACTTTCTGGTCT
GTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACC
CTGGTAGTCCACGCGTAAACGATCCCT
GCTARGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGC
ACTCCGCCTGGGGATACGACCGCAAG
GTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTG
GTTTAATTCGAAGCACGCGAAGAACC
TTACCAAATCTTGACATCCTTTGACAACTCTAGAGATAGAGCCTTCCCCTTC
GGGGGACAAAGTGAAGGTGGTGCATG
GTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC
AACCCTTAAGCTTATTGCCATCATTCC
CTTGGGCACTCTAAGTTGACTGCCGGTGACAACGGAGGAAGGTGGGGATGA
CGTCAAATCATCATCCCCTTATGATTT
GGGCTACACACGTGCTACAATGACAATACAAAGGGCAGCGAAACCGCGAG
GTCAAGCAAATCCCAAAAGTTGTTCTCA
GTTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGTAAT
CGTAGATCAGCATTTACGGTGAATACG
TTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTA

>MN814069 Staphylococcus aureus strain GodMam-W28

CCACCTTTAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAA
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AAGACGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCATTAGCTAGTTG
GTAAGGTAACGGCTACCAAGGCAACG
ATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGG
TCCAGACTCCTACGGAGGCAGCAGTA
GGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTG
ATGAAGGTCTTCGATCGTAAAACTCT
GTTATTAGGGAAGAACATATGTGTAAGTAACTGTGCACATCTTGACGGTAC
CTAATCAGAAAGCCAGGCTAACTACGT
GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGG
GCGTAAAGCGCGCGAGGCGGTCCCTT

AAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTG
GAAAACTTGAGTGCGAAGAGGAAAGT
GGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGT
GGCGAAGGCGACCCCTGGTCTGTAAC
TGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGT
AGTCCACGCCGTAACTGAGTGCTCCCT
GTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGC
CTGGGAGTACGACCCAAGGTTGAAAC
TCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATT
CGAAGCAACGCGAGAACCCCCCCAAA
TCTTGACATCCTTTGACAACTCTAGAGATAGAGCCTTCCCCTTCGGGGGACA
AAGTGACAGGTGGTCATGGTTGTCGTC
AGCTCGTGTCGTGAGATGTTGGGTTAAGTCCACGCAACGAGCGCAACCCTT
AAGCTTAGTTGCCATATCCCGTTGGGC
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AATCATCATGCCCCTATGATTTGGGCT
ACACACGTGCTACCCCGGACAATACAAAGGGCAGCGAAACCGCGAGGTCA
AGCAAATCCCATAAATTGTTCTCAGTTC
GGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGTAATCGT
AGATCAGCATGCTACGTGAATACGTTC
CCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTTAGA

>MN814070 Staphylococcus aureus strain GodMam-W06

GATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACC
GGATAATATTTTGAACCGCATGGTTCAA
AAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCATTAGC
TAGTTGGTAAGGTACGGCTTACCAAG
GCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAG
ACACGGTCCAGACTCTACGGGAGGCA
GCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCG
TGAGTGATGAAGGCTTCGGATCGTAA
AACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGCACATCTTGAC
GGTACCTAATCAGAAGCCACGGCTAAC
TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATT
ATTGGGCGTAAAGCCGCGTAGGCGGC
CCTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGA
AACTGGAAAACTTGGTGCAGAAGAGG

AAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACA
CCAGTGGCGAAGGCGACCCCTGGTCT
GTACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCC
TGGTAGTCCACGCCTAAACGATGAGT
GCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGC
ACTCCGCCTGGGGGTACGACCGCAAG

GTTGAAACTCAAAGGAATTGACGGCCCCCGCACAAGCGGTGGAGCATGTG
GTTTAATTCGAAGCACGCGAAGAACC
TTACCAAATCTTGACATCCTTTGACAACTCTAGAGATAGAGCCTTCCCCTTC
GGGGGACAAAGTGAAGGTGGTGCATG
GTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC
AACCCTTAAGCTTAGTGCCATCATTAA
GTTGGGCACTCTAAGTTGACTGCCGGTACAAACCGGAGGAAGGTGGGGATG
ACGTCAAATCATCAGCCCCTTATGATT
TGGGCCCCACACGTGCTACAATGGACAATACAAAGGGCAGCGAAACCGCG
AGGTCAAGCAAATCCATAAAGTTGTTCT
CAGTTCGGATTGTAGTCTGCAACTCGACTACATGCCCCTGGAATCGCTAGTA
ATCGTAGATCAGCAGCTACGGTGAATA
CGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAATCA
A