ISOLATION AND CHARACTERIZATION OF VANCOMYCIN RESISTANT STAPHYLOCOCCUS AUREUS FROM WOUND INFECTIONS IN PATIENTS ATTENDING SELECTED HOSPITALS IN MINNA, NIGER STATE, NIGERIA

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MTech/SLS/2017/7399

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FEDERAL UNIVERSITY OF TECHNOLOGY MINNA

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ABSTRACT

This study determined the antibiotic sensitivity of *Staphylococcus aureus* isolated from wounds samples in patients attending General Hospital (GH), Minna and IBB Specialist Hospital, Minna, Niger State. Wounds samples from two hundred (200) patients were collected and analyzed for *Staphylococcus aureus*. The resistance pattern of the *S. aureus* isolates for vancomycin using disc diffusion techniques was also determined. Out of the 200 samples collected from both hospitals, 97 (48.50 %) were positive for *S. aureus*. GH Minna had higher occurrence of *S. aureus* 76 (50.70 %) than IBB specialist Minna with 21 (42.00 %). Wounds samples from male had higher frequency of occurrence 65 (67.00 %) compared to female 32 (33.00 %). In these findings, it was observed that *S. aureus* had a high resistance rate of vancomycin and also resistance to other antibiotics such as; Amoxicillin, Ampicillin, Oxacillin and Erythromycin, although resistance to Erythromycin was very low in this study. The molecular characterization identified the VRSA isolates to be *S. aureus* strain GSI17, *S. aureus* strain GSI36, *S. aureus* strain GSI93, *S. aureus* strain GSI112 and *S. aureus* strain GSI114. vanA, van B and vanXY from the isolates were resistant genes detected in the isolates with band size approximately 713 bp, 430 bp and 550 bp respectively. This study established the active circulation of vanA, vanB and vanXY resistant genes among *S. aureus* in the study area.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Staphylococcus aureus is a Gram-positive, non-spore-forming spherical bacterium that belongs to the *Staphylococcus* genus. This class is subdivided into 32 species and subspecies. *S. aureus* is widely found in the atmosphere (soil and water), and is also found in the nose and on the skin of humans (Montville and Matthews, 2008; Food and Drug Administration (FDA), 2012). *S. aureus* is the most pathogenic specie of the genus *Staphylococcus*, being involved in both community-acquired and nosocomial contaminations (Costa *et al.,* 2013). Despite constant improvement in patient care, *S. aureus* infections remain associated with considerable morbidity and mortality, both in hospitals and in the community (Rasigade *et al.,* 2014).

It has been estimated that about 20-30 % of the population are permanently colonized by this bacterium, while other 30 % are transient carriers (Wertheim *et al.,* 2005). This colonization represents an increased risk of infection by providing a reservoir from which bacteria are introduced when the host defense is compromised (Costa *et al.,* 2013). Due to the importance of *S. aureus* infections and the increasing prevalence of antibiotic-resistant strains, this bacterium has become the most studied staphylococcal species (Costa *et al.,* 2013).

Skin diseases are the highly popular type of *S. aureus* infection (Tong *et al.,* 2015). This may manifest itself in a variety of ways, including folliculitis, impetigo, cellulitis, and other serious, obtrusive soft-tissue diseases. People with atopic dermatitis, also known as skin inflammation, have a great prevalence of *S. aureus* (Tong *et al.,* 2015).

S. aureus may also manifest itself in one of three ways: osteomyelitis, septic joint pain, or contamination after a replacement joint operation (Rasmussen *et al.*, 2011; Tong *et al.*, 2015).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an infection caused by *Staphylococcus* bacteria. This type of bacteria is resistant to many different antibiotics (Lights, 2019). MRSA is responsible for several difficult-to-treat infections in humans. MRSA is any strain of *S. aureus* that has developed (through natural selection) or acquired (through horizontal gene transfer) a multiple drug resistance to beta-lactam antibiotics (Gurusamy *et al.*, 2013).

Methicillin-resistant *S. aureus* (MRSA) is one of a number of greatly feared strains of *S. aureus* which have become resistant to most β-lactam antibiotics. For this reason, vancomycin, a glycopeptide antibiotic, is commonly used to combat MRSA. Vancomycin inhibits the synthesis of peptidoglycan, but unlike β-lactam antibiotics, glycopeptide antibiotics target and bind to amino acids in the cell wall, preventing peptidoglycan cross-linkages from forming. MRSA strains are most often found associated with institutions such as hospitals, but are becoming increasingly prevalent in community-acquired infections (Bonomo *et al.*, 2007).

Vancomycin-resistant *S. aureus* (VRSA) is a strain of *S. aureus* that has established resistance to glycopeptides. In 1996, Japan defined the primary cause of vancomycin-intermediate *S. aureus* (VISA). However, it existed only in 2002 that the initial instance of *S. aureus* that was actually resistant to glycopeptide antibiotics stayed identified (Chang *et al.*, 2003). As of 2005, three circumstances of VRSA contamination had remained reported within the United States (Menichetti, 2005). Antimicrobial tolerance around *S. aureus* can be deduced from its capability to adjust in the tiniest of ways.

Different two-component flag transduction pathways aid *S. aureus* in acquiring unique qualities needed to survive antimicrobial stress (Sengupta *et al.*, 2012).

1.2 Statement of the Research Problems

The management of wound infection has continued to be a challenging issue in the clinical setting due to the globally growing problem of antibiotic resistance, particularly in *Staphylococcus aureus*.

The infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA) is a global threat to public health. Vancomycin remains one of the first-line drugs for the treatment of MRSA infections. However, *S. aureus* isolates with complete resistance to vancomycin have emerged in recent years (Cong *et al.*, 2020).

This study was carried out to know whether there exist strains of Vancomycin-resistant *S. aureus* in Minna, Niger State, Nigeria.

1.3 Justification for the Study

The trajectory nature of antibiotic resistance requires an immediate concerted effort to avail the next pandemic of untreatable bacterial infection. *S. aureus* that is vancomycin-resistant is also multi-drug resistant to a wide range of currently available antimicrobial agents, limiting treatment options and raising the risk of insufficient antimicrobial therapy, as well as an increase in morbidity and mortality (Tarai *et al.*, 2013). In recent years, the advent of VRSA has added to the therapeutic challenge created by the existence of multi-drug resistant species (Loomba *et al.*, 2010).

1.4 Aim and Objectives of the Study

The aim of this research was to isolate and characterize vancomycin resistant *Staphylococcus aureus* from wound infection of patients attending selected hospitals in Minna, Niger State, Nigeria

The objectives of the study were to;

i. isolate and characterize *Staphylococcus aureus* from wound infection in the study population
ii. determine the antibiotic susceptibility profile of *S. aureus* isolates
iii. detect vancomycin resistant genes in the *S. aureus* isolated

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biology of *Staphylococcus aureus*

Staphlococcus aureus is a main human pathogen that triggers infections in hospitals and the environment (Tong *et al.,* 2015). It could be a profoundly harmful living being that shows noteworthy anti-microbial resistance (Penetrate *et al.,* 2018). Methicillin-resistant *S. aureus* (MRSA) emerged around 1960s, just some limited years after the presentation of penicillin as a first-line treatment for diseases produced by penicillin-resistant *S. aureus*. Since the 1980s, vancomycin has been the treatment of choice for genuine MRSA diseases in many healthcare institutions (Appelbaum, 2006). Despite the fact that vancomycin has long been the most effective antibiotic against MRSA infections, there has been an alarming increase in *S. aureus* strains that are less susceptible to vancomycin and other glycopeptides (Apalbaum, 2006; Gardete and Thomas, 2014).

The clinical burden of diseases caused by antibiotic-resistant pathogenic microscopic organisms is an expanding challenge around the world. Particularly the nosocomial diseases with multi-resistant microbes are risky since of the rising troubles in focused on treatment that result in expanded dismalness and mortality especially in immunocompromised patients (Whisplinghoff *et al.,* 2004; Hidron *et al.,* 2007). Over the past few decades, there has been a disturbing increment within the predominance of antibiotic-resistant pathogens and strains in genuine diseases (Dutta *et al.,* 2013). In hospitals, ensuring an isolate's antimicrobial resistance is critical for optimal care (Matthew *et al.,* 2010).

Hospitalization raises a patient's risk of illness, and hospital-acquired diseases are related with higher death rates, prolonged hospital stays, and advanced healthcare charges as compared to community-acquired diseases (Lipsky *et al.,* 2007; Zervos *et al.,* 2012).

Hospitalized patients' wounds may be caused by surgery, weight ulcers, diabetic ulcers, or wounds obtained in the clinic or in the community, and wound infections may lead to repeated hospitalization. The relative frequency of living beings that cause wound contamination varies greatly between factors (Almeida *et al.,* 2014). *Staphylococcus aureus* and *Pseudomonas aeruginosa* are two of the most common bacteria isolated from serious wounds, and colonization by these organisms necessitates caution due to their ability to produce antimicrobial resistance and their connection to nosocomial infections (Albaugh *et al.,* 2013; Abdallah *et al.,* 2013). Bacterial colonization of wounds can exacerbate wound severity and impede healing (Albaugh *et al.,* 2013).

2.2 Diseases Caused by *Staphylococcus aureus*

2.2.1 Bacteremia caused by *Staphylococcus aureus*

Bacteremia is possibly the most well-known symptom of *S. aureus* disease. Several reports have now recorded the occurrence, prediction, and result of *S. aureus* bacteremia (SAB) in developed areas worldwide. In any case, numerous fundamental enquiries about the study of SAB disease transmission, especially in non-industrialized areas of the world, stay unanswered. Moreover, there remains to be a lack of excellent suggestion to monitor SAB administration (Tong *et al.,* 2015).

2.2.1.1 Clinical manifestations

Despite the fact that SAB has a variety of important clinical foci or appearances, there are uniform practice throughout cohorts. In a few later reports, continuous patients with also

SAB (MSSA and MRSA) (Tong *et al.,* 2012; Hewagama *et al.,* 2012) or MRSA bacteremia (Pastagia *et al.,* 2012), Vascular catheter related diseases, SSTIs, pleuropulmonary contaminations, and osteo-articular contaminations are all usual critical medical concentrations or sources of contamination (Tong *et al.,* 2015). These usual critical medical concentrations cover a subdivision of the most common *S. aureus* contamination symptoms. In either case, a disease centre isn't detected in 25 % of cases (Tong *et al.,* 2015).

SAB is divided into two categories: "complicated" and "uncomplicated." These tasks provide important recommendations for the degree and form of demonstrative estimate, the period of antimicrobial care, then a general guess. 724 SABs were studied in some single-centre study scenes, complex contamination was described as any that bring about inferred death, CNS inclusion, an embolic wonder, metastatic disease locations, or repeated contamination within 12 weeks (Tong *et al.,* 2015). Community procurement, certain follow-through blood societies at 48-96hours, decided temperature at 72 hours, and skin discoveries indicating an intense systemic contamination were all indicators of difficult SAB (Fowler *et al.,* 2003).

Furthermore, the main source of disease expects 30 days' death, with advanced rates for bacteremia without a core (22-48 %), and aspiratory diseases (39-67 %), associated to lower rates for catheter related bacteremia (7-21 %), SSTIs (15-17 %), and urinary tract infections (UTIs) (10 %) (van Hal *et al.,* 2012).

2.2.2 Skin and Soft tissue infections

S. aureus affects a wide range of SSTIs, varying from mild to dangerous (e.g., impetigo and simple cellulitis). We analyse the spread, pathophysiology, medical highlights, and

cure of *S. aureus* SSTIs in this article, with a focus on the later scourge of community associated MRSA (CA-MRSA) (Tong *et al.,* 2015).

2.2.2.1 Medical reports and results

Indeed, the increase of CA-MRSA, *S. aureus*, and other pathogens has occurred recently. SSTIs had a lot of support from aureus. The most widespread bacterial skin infection in teenagers is impetigo (Bangert *et al.,* 2012). Impetigo most often manifests as bullous or papular injuries improvement to coated injuries on open areas of the body without accompanying systemic symptoms (ordinarily the confront or limits). Later reports of impetiginous injuries discovered curing rates of 29 %-90 % for *Streptococcus pyogenes* and 57-81 % for *S. aureus*, correspondingly (McDonald *et al.,* 2006; Bowen *et al.,* 2014).

Whereas the trademark disease of *S. aureus* SSTI is by and large respected as the cutaneous boil (Fridkin *et al.,* 2005; Kaplan *et al.,* 2005; Liu *et al.,* 2008; Tong *et al.,* 2009), other signs of skin contamination are too experienced clinically. *S. aureus* can cause non-purulent cellulitis in a lesser case, despite the absence of a demonstrative gold standard and the flexibility posed by various biological strategies clouding the real microbiology of this situation (Chambers, 2013). Although *S. aureus* cellulitis most frequently affects the lesser edges, it may also affect the higher edges, stomach divider, and face. Such as a source of preseptal and orbital cellulitis, it competes with streptococci for supremacy (Chaudhry *et al.,* 2008; Seltz *et al.,* 2011).

2.2.3 Meningitis

Meningitis may occur as a result of haematogenous increase from a non-CNS source of contamination or as a side effect of neurosurgical intervention (Teh and Slavin 2012; Pintado *et al.,* 2012). Haematogenous *S. aureus* meningitis is generally developed in the population (Aguilar *et al.,* 2010; Teh and Slavin, 2012) and, compared with postsurgical

S. aureus meningitis, ordinarily influences more seasoned people with serious restorative comorbidities such as diabetes or incessant kidney illness (Pintado *et al.,* 2002; Aguilar *et al.,* 2010) and postsurgical (Lu and Chang 2000; Pintado *et al.,* 2012).

2.2.3.1 Clinical signs and symptoms

Since meningitis is an unusual problem for SAB patients, happening in just 1.7 % of 724 prospectively differentiated patients with SAB, physicians must remain wary of its chance. Meningitis is usually accompanied by at least one of the following symptoms/signs: constant fever, cerebral discomfort, firm neck, as well as spewing (Pintado *et al.,* 2002; Logigan *et al.,* 2009; Pintado *et al.,* 2012). Two most common clinical side effects are fever and altered consciousness (Lu and Chang 2000; Huang *et al.,* 2010). Haematogenous meningitis patients usually have a higher level of CSF leucocytosis than postsurgical patients (Pintado *et al.,* 2002).

Patients with *S. aureus* have a number of risk factors for death. A haematogenous origin versus a postsurgical origin, advancing years and the number of comorbidities (Pedersen *et al.,* 2006), the presence of septic stun (Pintado *et al.,* 2002), and concurrent IE are all factors in meningitis (Huang *et al.,* 2010).

2.2.4 Toxic shock syndrome

Todd *et al.* (1978) first described *S. aureus* poisonous stun condition (TSS) in 1978, when they described the sickness in a group of seven teenagers (Tong *et al.,* 2015). *S. aureus* toxic shock syndrome come to be associated with super absorbent tampons in discharging ladies in the 1980s without further ado (Herzer, 2001; Issa and Thompson, 2001), resulting in a disease prevalence of 13.7 per 100,000 bleeding women per year (Tong *et al.,* 2015). Following the elimination of extremely permeable tampons from the market, yearly rates of *S. aureus* toxic shock syndrome dropped to 1/100,000 menstruating

women and 0.3 for every non-menstruating citizen (DeVries *et al.,* 2011). Since then, the occurrence of *S. aureus* TSS has stayed stable, with recent yearly frequencies of 0.69 per 100,000 bleeding ladies and 0.32 per 100,000 total populations (DeVries *et al.,* 2011). Presently, the records of menstrual and non-menstrual staphylococcal TSS cases are alike, with SSTIs being the most usual source of contamination in non-menstrual circumstances (Descloux *et al.,* 2008).

2.3 Wound Contamination

In spite of the advance made for contamination control and wound administration, wound contamination still remains a genuine and noteworthy clinical challenge especially in creating nations where wound location contaminations are a major source of postoperative sickness, a cause of passing among burn patients (De Macedo and Santos, 2005) and accounts for roughly a quarter of all nosocomial diseases (Nicholas, 2001). These contaminations have come about in expanded injury within the understanding, delayed hospitalization, expanded healing centre fetched as common wound administration hones ended up more resource-demanding (Kihla *et al.,* 2014).

The progression of a wound to an infected state involves a multitude of factors including old age, repeated trauma, blood perfusion, immune suppression and coexisting morbidity all of which impair wound healing, increasing the risk of infection. Other chance variables detailed incorporate sex, surgical procedure, way of life, hospitalization which increments the hazard of disease by drug-resistant living beings, and length of operation (Sorensen *et al.,* 2005). The sort, estimate, and profundity of the wound and the combined level of destructiveness communicated by the sorts of microorganisms included have too been detailed to encourage wound disease (Bowler *et al.,* 2001). Hence, information of hazard variables related with diseases may offer assistance fortify endeavours to diminish

their event, in this way decreasing horribleness and mortality for these diseases (Kihla *et al.,* 2014).

2.3.1 Wound classification

Wounds may be categorized based on their etiology, location, type of harm causing side effects, wound depth and tissue damage, or clinical appearance of the wound (Shankar *et al.,* 2014). Pressure Ulcers, Burns, Diabetic Foot Ulcers, and General Wounds all have isolated reviewing apparatuses (Shankar *et al.,* 2014). Wounds that are not life threatening are known as general wounds. Surface-level (loss of epidermis only), complete thickness (includes epidermis and dermis) and partial thickness (includes epidermis and dermis) (involves the dermis, subcutaneous fat and sometimes bone). The most popular method for determining a wound's classification is to look for evidence of transcendent tissue types at the wound bed, dark necrotic, and the individual sum of each communicated as a rate. This classification technique is extremely visual, supports excellent appraisal and planning, and aids in continuous reassessment (Shankar *et al.,* 2014).

Wounds can be divided into two categories: open and closed. Within the base of an open wound, there is exposed body tissue. Damage occurs in closed wounds without exposing the basic body tissue. Wounds may be caused by external factors such as entering objects or sustaining a limit injury, as well as internal factors for instance healthy, metabolic, and neurologic aetiologies.

2.4 Wounds and its Infections Symptoms

Wounds are not antiseptic, and all exposed wounds contain a definite number of microscopic organisms; however, this does not mean that the wound is contaminated. Ordinary recovery is still possible. When bacterial growth accelerates significantly, a

disease develops. If you see any signs of infection, contact your doctor or nurse (Gloviezki and Peter, 2020).

2.4.1 Infection symptoms include:

i. Excessive pain in the wound bed
ii. Warmth or redness
iii. Some flu-like signs such as fever/chills
iv. Pus draining from the wound bed
v. Increasing odour from the wound
vi. Increased firmness of skin or swelling around the wound bed.

2.4.2 Secondary symptoms of disease

Not all illnesses begin with the classic signs and symptoms of illness. There are a few more signs and symptoms to look out for a lesser review disease within the wound bed, which slows curing and can improve if left untreated (Garner *et al.*, 2001).

i. Increasing the amount of fluid draining from the wound bed
ii. Delay in wound healing
iii. Discoloration of the wound bed, which darkens in hue.
iv. Increased fragility of the wound bed
v. Wound enlargement

2.5 Wound Characteristics

When persistent cutaneous wounds are viewed as chronic diseases, many features and marvels associated with unremitting wounds are easily clarified. By carefully observing unobtrusive changes within the wound and linking these changes to symptomatic instruments and treatment responses, a black-and-white image of a few of the forms

forming on the wound bed begins to emerge. Understanding a few of these activities will help us better plan our wound care options (Wolcott *et al.*, 2010).

Most data seem to be abdicated at the edges of chronic wounds. Wound edges that gently slant down to the wound bed and "feather" a few millimetres into the wound suggest that the patient move about while recovering. Edges that are lifted off the wound bed (punched-out) or have an edge of undermining at the edge, on the other hand, strongly suggest bacterial association. Later studies on keratinocyte movement suggest that solvent substances released by microbes can obstruct keratinocytes, causing them to abandon these types of edges. Additionally, additional clinical data related to dampness can be discovered at the wound's edge. If the wound biofilm becomes more dynamic and up directs have a fiery reaction, this will result in increased exudate production and significant maceration. A complex biofilm is proposed by maceration of the wound edge at the side torment, swelling, and wound weakening (Wolcott *et al.*, 2010).

The forcefulness of the wound biofilm can also be determined by looking at the edge of the wound. A generally gleaming red/pink border that isolates the wound's keratinocyte edge may be a sign that wound biofilm is under control. Quagmire that laps over the edge of the wound onto the keratinocyte edge, on the other hand, suggests destitute have defences and destitute mending. Singing, maceration, and non-physiologic colour shifts of the wound edge all indicate that the patient's protected structure and wound care procedures are being overwhelmed by the persistent contamination (Wolcott *et al.*, 2010).

The wound bed near the middle is more fibrotic, less exudative, less fragile, and has less swamp. When wound biofilm is easily smothered, epithelial islands form in the mid-portion of the wound, which can be seen clinically. Since there are no keratinocytes

present and a less vigorous capillary bed, surface microscopic organisms inside the mid-portion of the wound should be less threatened by have defences (Wolcott *et al.*, 2010).

Tunnelling and undermining are words used often in wound treatment. These miracles can occur in a variety of wound types. Biofilm, we believe, is the common denominator. Tunnelling may occur in sensitive tissue structures like the subcutaneous layer, fat tissue, and muscle. Tissue becomes compromised over time as the form and shape of the tract changes. Tunnelling is often widely used to represent degeneration around strong structures like ligaments or bones, spreading the wound much deeper inside the have tissues. Undermining usually refers to the disintegration of the subcutaneous layer underneath the wound edge, which includes a significant portion of the wound edge's diameter (Wolcott *et al.*, 2010).

2.6 Wound Healing and Infection

Wound healing could be a complex handle that takes after a dynamic three-step arrangement: provocative, proliferative, and re-modelling. These stages can cover (Thomas and Bishop, 2007), and each stage's term will be impacted by an assortment of components. When a wound falls flat to advance to mending or react to treatment over the anticipated recuperating time outline (depending on the patient and wound sort), it as a rule slows down within the fiery stage. This non-healing stage, called 'chronicity', has various causative components (Penhallow, 2005). Wound contamination frequently causes chronicity and, thus, it is imperative to get it why wound contamination happens, and how to recognize and oversee it (Swanson *et al.*, 2014).

2.7 Phases of Wound Healing

The wound environment changes in reaction to the person's changing health status, making wound healing a difficult and energetic process. Physiology of the normal wound healing process as it progresses across the stages of haemostasis, inflammation, granulation, and growth offers a basis for accepting the fundamentals of wound healing (Shankar *et al.*, 2014).

Wounds recover in four stages, according to research on acute wounds in an animal model. Chronic wounds are thought to go through the same essential stages as acute wounds. The first two phases are often combined by some writers. The phases of wound healing are:

i. Haemostasis
ii. Inflammation
iii. Proliferation
iv. Maturation

Kane's analogy of wound repair to the repair of a damaged house offers a fantastic basis for learning about the basic physiology of wound healing (Shankar *et al.*, 2014).

2.7.1 Haemostasis phase

During wound healing, damaged blood vessels must be restored. The platelet is the cell that functions as a function specialist in wound healing, repairing harmed blood vessels. The blood vessels themselves suffocate in reaction to the wound, but this fits gradually dissipate. Platelets release vaso-constrictive constituents to aid in this method, but their primary function is to form a steady clot that seals the harmed vessel. Platelets aggregate and adhere to exposed collagen below the effect of ADP (adenosine diphosphate) spilling

from harmed tissues. They also produce variables that are linked to and energize the natural clotting cascade by producing thrombin, which begins the development of fibrin from fibrinogen. The platelet total is reinforced by the fibrin work, resulting in a steady haemostatic plug. Lastly, platelets secrete cytokines like platelet-derived growth factor (PDGF), which is one of the primary components released in the early stages of the process. Unless there are fundamental clotting disarrays, haemostasis arises within minutes of the first injury (Shankar *et al.*, 2014).

2.7.2 Inflammation phase

Inflammation, one of the first stages of wound healing, manifests as erythema, swelling, and warmth, both of which are commonly associated with pain. This arrangement usually lasts for up to four days after the injury. Once the utilities have been turned off, the primary job to be performed is to clean up the flotsam and jetsam, which is close to wound healing. Typically, this is a work for unskilled labourers. The neutrophils, also known as PMNs, are non-skilled workers in a wound (polymorpho-nucleocytes). The incendiary reaction causes blood vessels to develop clogged, allowing plasma and PMNs to leak into the immediate tissue. The neutrophils are the first line of protection against disease, phagocytizing flotsam and jetsam as well as microorganisms. They're backed up by pole cells in the area. As part of this clean-up, fibrin is broken down, and the debasement products pull in another cell. Modifying a house is a complicated task that necessitates the use of a coordinator or a temporary worker. The macrophage is a cell that serves as a temporary worker in wound healing (Shankar *et al.*, 2014).

2.7.3 Proliferative phase

During the proliferative process of wound healing, the wound is regenerated with fresh collagen and extracellular matrix tissue. During the proliferative process, the wound

shrinks as new tissues are formed. In order for the granulation tissue to be firm and receive enough oxygen and nutrients, a new system of blood vessels must be created. By gripping the wound margins and bringing them together, myofibroblasts use a technique similar to that of smooth muscle cells to reduce the wound. In the early stages of wound healing, granulation tissue is pink or red in colour and has an irregular appearance. Granulation tissue that is in good health is also resistant to bleeding. Dark granulation tissue can be caused by infection, ischemia, or inadequate perfusion. At the end of the proliferative stage of wound healing, epithelial cells emerge in the wound. It's worth noting that keeping wounds wet and moisturized promotes epithelialization. When occlusive or semi-occlusive bandages are applied within 48 hours following damage, tissue humidity is maintained, allowing epithelialization to occur (https://www.woundsource.com/ blog/ four-stages-wound-healing).

2.7.4 Maturation phase

When collagen is transformed from type III to type I and the wound varnishes entirely, the maturation method, also known as the re-modelling phase of wound healing, occurs. Apoptosis, or programmed cell death, eliminates cells that were formerly needed to heal the wound but are now no longer needed. As collagen is laid down throughout the proliferative process, the wound becomes impermeable and disordered. Collagen is found along tension lines, and as the collagen matures, water is reabsorbed, allowing collagen fibres to lie closer together and cross-link. Scar viscosity is reduced by collagen crosslinking, which also strengthens the skin around the incision. Remodelling usually begins about 21 days after an accident and lasts for a year or more. Healed wound areas are still weaker than uninjured tissue, with tensile strength averaging just 80% of that of unwounded skin, even after cross-linking (https://www.woundsource.com/blog/four-stages-wound-healing).

Wound healing is a delicate procedure. Failure to improve through the stages of wound healing might lead to chronic wounds. Chronic wounds can be caused by venous illness, infection, diabetes, or metabolic insufficiencies in the elderly. Wound care accelerates the phases of wound healing by keeping wounds moist, clean, and safe from re-injury and infection (https://www.woundsource.com/blog/four-stages-wound-healing).

2.8 Technique for Isolation of Microorganism (Bacteria)

2.8.1 Microscopy techniques

The microscope is an essential identification tool for microorganisms present in a natural sample. Microscopy images enable analysis of shape, tracking of motion, and classification of biological objects (Franco-Duarte *et al.,* 2019). The microscope-based observation is still frequently applied to define the morphological differences of interesting bacteria, such as streptococci, staphylococci, bacilli (e.g., *Listeria monocytogenes*, *E. coli* or *Salmonella spp*.), and *Vibrio*, in both clinical and research sceneries (Bayraktar, 2006; Dige, 2007; Rajwa *et al.,* 2010). Notwithstanding its significance, automated segmentation remains challenging for several widely-used non-fluorescence, interference-based microscopy imaging modalities (example; contrast microscopy) (Franco-Duarte *et al.,* 2019).

Nonetheless, microscopy alone is not sufficient for microorganism identifications for several reasons: small cells that are usually present are difficult to identify; prokaryotes vary widely in size and some cells are close to the resolution limits of the optical microscope; when observing natural samples, such cells can easily be missed, especially if the sample contains a large amount of particulate matter or a large number of larger cells; and it is often difficult to differentiate living cells from dead cells or cells from inanimate materials present in natural samples (Madigan *et al.,* 2012). Although, when

microscopic analysis is associated with other tools, it becomes more promising. In addition, there are techniques of electron microscopy that are powerful instruments in the identification of microorganisms: transmission electron microscopy (TEM); scanning electron microscopy (SEM), confocal microscopy (CLSM), and atomic force microscopy (ATM) (Schuler *et al.,* 2015; Cardinale *et al.,* 2017). These techniques have great value mainly in the identification of microorganisms in biofilms (Beier *et al.,* 2012).

2.8.2 Molecular methods used to detect bacteria

The advent of the "molecular biology age" has provided a plethora of tools and techniques for the detection, identification, characterization, and typing of bacteria for a range of clinical and research purposes (Spratt, 2004). Previously, the identification and characterization of bacterial species was largely done by phenotypic and biochemical methods, which relied on preliminary isolation and culture. While these methods continue to hold place in certain settings, molecular-based techniques have provided unprecedented insights into bacterial identification and typing (Franco-Duarte *et al.,* 2019). To name a few examples, genotypic methods have enabled the identification of a large diversity of previously unknown taxa, the characterization of uncultivable bacteria, and facilitated metagenomics studies on large and diverse bacterial communities (Franco-Duarte *et al.,* 2019). Both clinical and research setting have provided in depth insights into bacterial virulence, pathogenesis, antibiotic resistance, and epidemiological typing, as well as identification of novel, emerging, and re-emerging species (Galluzzi *et al.,* 2007).

Most molecular methods for bacterial identification are based on some variation of DNA analysis, either amplification or sequencing based. These methods range from relatively simple DNA amplification-based approaches (PCR, real-time PCR, RAPD-PCR) towards

more complex methods based on restriction fragment analysis, targeted gene and whole-genome sequencing, and mass spectrometry (Franco-Duarte *et al.,* 2019). While the advantages and limitations of these approaches vary, the choice of the technology employed depends on several factors including sample type (clinical or research, single-species or mixed-species), depth and accuracy of results generated, resources and cost factors, as well as the turn-around-times expected. Given that the present "molecular biology revolution" is resulting in a larger number of laboratories, including small-scale and resource-limited setups, having access to genomic approaches, it is imperative to understand the fundamental principles of these techniques, their applications, and their limitations (Franco-Duarte *et al.,* 2019).

2.8.3 16S rRNA PCR-sequencing

The rapid amplification of nucleic acid targets from relatively lower starting material, makes PCR one of the most sensitive techniques available for detection of bacterial targets. PCR-based identification of bacterial DNA through amplification and sequencing of the 16S rRNA gene has become a standard molecular method, both in the laboratory as well as in clinical settings. The 16S rRNA gene is highly specific to each bacterial species and this makes it an ideal target for identification (Franco-Duarte *et al.,* 2019). The standard method involves PCR amplification of the 16S rRNA gene, followed by sequencing and comparison to known databases for identification. PCR-based methods are not only faster than conventional culture-based methods but are also helpful in identification of bacteria that are difficult to grow in laboratory conditions. In one study, universal primers for the 16S rRNA gene were designed to identify bacteria in the root canals of patients with necrotic pulp tissue (Fouad *et al.,* 2002). The primers included ten putative bacterial pathogens commonly found in root canals with necrotic pulp. After DNA extraction from the necrotic pulp, a PCR was run using universal primers, as well

as species specific primers, and the products were analyzed using gel electrophoresis (Franco-Duarte *et al.,* 2019).

n research laboratories, PCR-based identification is a straightforward procedure with reliable results. However, when applied to clinical settings, various factors come into play that can influence PCR results. Clinical samples often have very few bacteria to begin with, they also require various pre-processing steps before the PCR is carried out, to remove PCR inhibitors and enable extraction of maximum bacteria from the sample without contamination (Yamamoto, 2002; Rådström *et al.,* 2004). Despite these concerns, PCR-based identification has been successfully and widely employed to detect and identify bacteria in clinical samples (Kai *et al.,* 2019).

2.8.4 Real-time PCR

Real-time PCR (RT-PCR) provides many advantages over conventional PCR, such as higher sensitivity and accuracy and the ability to monitor DNA amplification in real-time through fluorescence intensity, thereby negating the need for any post-PCR detection techniques. RT-PCR can also be quantitative or semi-quantitative, using the Cq value (cycle number at which fluorescence intensity rises above the detectable level) to quantify the amount of DNA. As with conventional PCR, RT-PCR also has wide applications within research laboratories as well as in the clinic. It is also applied to numerous kinds of samples, right from identification of bacteria in milk, which are otherwise non-culturable (Taponen *et al.,* 2009), to the identification of bacteria within soil ecosystems (Höppener-Ogawa *et al.,* 2007).

Using universal primers that target conserved regions of 16S rRNA gene, an assay has been developed that can detect *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Clifford *et al.,* 2012). The primers were

designed based on the alignment sequence of 962,279 bacterial 16S rRNA gene sequences, which revealed two regions that were highly conserved in more than 90% of all rRNA gene sequences. The primers were able to successfully detect less than 100 genomic DNA copies. This real-time based 16S rRNA PCR has also been used to identify or quantify bacterial loads in clinical infections such as chronic wound tissue (Melendez *et al.,* 2010) and gastrointestinal mucosal biopsies (Huijsdens *et al.,* 2002) and has also been applied in forensic investigations of saliva specimens (Jung *et al.,* 2018). In addition, high resolution melting (HRM) is a rapid, reliable, accurate, and cost-effective emerging tool for genotyping bacteria, such as from the *Lactobacillus casei* group and both Gram-positive and Gram-negative bacterial pathogens (Iacumin *et al.,* 2015; Tamburro and Ripabelli, 2017).

2.8.5 Whole-genome sequencing (WGS)

Whole-genome sequencing (WGS) has recently become a highly accessible and affordable tool for bacterial genotyping. Analysis of the entire bacterial genome not only provides unprecedented insights into bacterial typing and evolutionary lineages but has also revolutionized our approach to understanding antimicrobial resistance and outbreak investigations (Franco-Duarte *et al.,* 2019). Advances in WGS technologies and analysis pipelines have rapidly increased output and analysis speed, while reducing overall costs (Quainoo *et al.,* 2017). In spite of reservations from clinicians related to experimental protocols and cost factors, WGS-based approaches are being evaluated for the pathogen identification and antimicrobial resistance typing. In one study, WGS was used to investigate a fatal outbreak of vancomycin resistant *Enterococcus faecium* (VRE) involving three patients in an ICU (McGann *et al.,* 2016). Using an Illumina Miseq benchtop sequencer, WGS established that isolates from patient two and three differed from that of patient one only by a single, non-synonymous polymorphism, each pointing

to ICU transmission. In addition, the distinct SNPs in isolates from patient two and three also indicated two separate direct transmission events from patient one, rather than linear transmission from patient one to patient two to patient three. As expected, the isolates were shown to carry genes (vanA) conferring resistance to vancomycin. Therefore, the in-depth analysis offered by WGS was not only able to establish antibiotic resistance but could also infer transmission dynamics and evolutionary lineage of the outbreak strains (Franco-Duarte *et al.*, 2019).

2.8.6 MALDI-TOF-MS in bacteria

In addition to advancements in genomics, proteomics-based approaches for bacterial identification and characterization have emerged. These methods are primarily based on mass spectrometry, which enables rapid and high-throughput analysis of biomolecular signatures produced by a bacterial strain (Emerson *et al.*, 2008). In MALDI-TOF MS, the spectra patterns produced from bacterial cells contain characteristic information to identify and characterize bacterial species. For this, the bacterial sample to be analyzed is mixed with organic matrices and ionized by a laser beam. As the resulting ions move towards the mass analyzer, the mass: charge ratio is obtained which creates a spectra pattern. This pattern is then compared with a known library of fingerprints. In a sophisticated work, Edwards-Jones *et al.* (2000) developed a MALDI-TOF-MS based method to discriminate between methicillin-sensitive and methicillin-resistant S. aureus. Based on the distinct spectral patterns obtained, MSSA and MRSA could be rapidly differentiated, and this was determined to be highly reproducible. This not only underscores the relevance of mass spectrometry-based approaches for bacterial identification and typing, but also indicates that it could assist with clinical decisions such as the initiation of appropriate antibiotics for the treatment of *S. aureus* infections (Franco-Duarte *et al.*, 2019).

2.9 Antibiotic Resistance

2.9.1 History of antibiotic development

Since 1928, when Sir Alexander Fleming inadvertently found penicillin developing on a petri-dish of microscopic organisms, antimicrobials have spared the lives of millions of individuals and creatures. Their revelation is seen as one of the foremost imperative logical accomplishments of the 20th century. But abuse and abuse of antimicrobials have contributed to the rise of resistance. Sir Alexander Fleming himself, on collecting a Nobel Prize for his disclosure, anticipated the day break of this battle, saying, "It isn't difficult to create organisms safe to penicillin within the research facility by uncovering them to concentrations not adequate to murder them"

Table 2.1 Antibiotic Discovery and Resistance Development

Antibiotic	Discovered	Introduced into clinical use	Resistance Identified
Penicillin	1940	1943	1940 (methicillin 1965)
Streptomycin	1944	1947	1947, 1956
Erythromycin	1952	1955	1956
Vancomycin	1956	1972	1987
Gentamicin	1963	1967	1970

Source: Stuart (2001)

Resistance is a mechanism by which a drug-susceptible microorganism finds a way to avoid being affected by the drug (Scott, 2014). Microbial resistance to antimicrobial specialists isn't a different occurrence; it's been going on in soil microorganisms since the

dawn of time, as a competitive & survival tool used by microorganisms to compete with other microorganisms (Scott, 2014).

Understanding the tools of resistance is critical not only for identifying improved ways to have present agents useful for a little longer, but also for assisting in the development of superior antimicrobial specialists who are not affected by already identified, expected, or obscure tools of resistance (Scott, 2014).

Despite the fact that antimicrobial resistance is a normal occurrence, it is regularly increased as a significance of infectious agents' variation to antimicrobials used in people or agriculture, as well as the general use of disinfectants at the cultivate and family levels. Antimicrobial use is nowadays widely recognized as the only most important aspect contributing to improved antimicrobial resistance (Scott, 2014).

2.10 Classification of Antibiotics

Antimicrobials are categorized in a variability of ways, nonetheless, the most widely used classification schemes are based on their atomic structures, kind of action, and range of action (Calderon and Sabundayo, 2007). Others outline the establishment's strategy (injectable, verbal, and topical). Antimicrobials in the same supplementary sequence would generally have the same design of adequacy, poisonous stability, and unfavourably susceptible likely side effects. Beta-lactams, Macrolides, Tetracyclines, Quinolones, Aminoglycosides, Sulphonamides, Glycopeptides, and Oxazolidinones are some examples of antimicrobials with chemical or atomic structures (van Hoek et al., 2011; Frank and Tacconelli, 2012; Adzitey, 2015).

2.10.1 Beta-lactams

Members of this class of antibiotics contain a 3-carbon and 1-nitrogen ring that is highly reactive. They interfere with proteins essential for synthesis of bacterial cell wall, and in the process either kills or inhibits their growth. More succinctly, certain bacterial enzymes termed Penicillin-Binding Protein (PBP) are responsible for cross linking peptide units during synthesis of peptidoglycan. Members of beta-lactam antibiotics are able to bind themselves to these PBP enzymes, and in the process, they interfere with the synthesis of peptidoglycan resulting to lysis and cell death (Heesemann, 1993). The most prominent representatives of the beta-lactam class include Penicillins, Cephalosporins, Monobactams and Carbapenems (Etebu and Arikekpar, 2016).

2.10.2 Penicillins

Penicillin, the initial antibiotic discovered and published by Alexander Fleming in 1929, was afterward discovered to be one of numerous antibiotic compounds known as penicillins (McGeer et al., 2001). Penicillins are found in a wide range of compounds, the common of which conclude in the suffix-cillin. They're beta-lactams with a 6-aminopenicillanic acid (lactam plus thiazolidine) ring nucleus and additional ring side chains (Etebu and Arikekpar, 2016).

Penicillin G, Penicillin V, Oxacillin (dicloxacillin), Methicillin, Nafcillin, Ampicillin, Amoxicillin, Carbenicillin, Piperacillin, Mezlocillin, and Ticarcillin are all included in the Penicillin lesson (Boundless, 2016). In this group of antimicrobials, and indeed the whole antimicrobials, Penicillin G was the first to be developed. Furthermore, although Penicillin G was originally discovered and isolated from the fungus *P. notatum* by Alexander Flemming, a close relative Penicilliun chrysogenum is the preferred choice of

source. Also producing the antibiotics through biochemical microbial fermentation more cost effective as compared to synthesizing it from raw materials (Talaro and Chess, 2008).

2.10.3 Monobactams

The antibiotic came from the *Chromobacterium violaceum* bacteria. Monobactams are beta-lactams, their beta-lactam ring, unlike most other beta-lactams, stands alone and is not connected to another ring (Etebu and Arikekpar, 2016). Aztreonam is the only commercially available monobactam antibiotic with a narrow spectrum of activity. Aztreonam is only active against Gram-negative bacteria that are aerobic in nature, it is used to treat microorganisms that cause pneumonia, septicemia, and urinary tract infections, such as Neisseria and Pseudomonas. Gram-positive bacteria and anaerobes are not affected by monobactams. They're used as inhalers and injectable (Etebu and Arikekpar, 2016).

2.10.4 Carbapenems

In 1976, this class of antibiotic was discovered as a result of a need. Prior to this time in the late 1960s, the efficiency of penicillin was jeopardized due to the discovery of beta-lactamase in bacteria. Bacterial beta-lactamases presented penicillin resistance to bacteria (Papp-Wallace *et al.*, 2011).

Carbapenems occupy a very important place in our fight against bacterial infections. This is because they are able to resist the hydrolytic action of beta-lactamase enzyme. Among the several hundreds of known betalactams, carbapenems possess the broadest spectrum of activity and greatest potency against Gram-positive and Gram-negative bacteria. As a result, they are often called “antibiotics of last resort” and are administered when patients with infections become gravely ill or are suspected of harboring resistant bacteria (Torres *et al.*, 2007).

2.10.5 Quinolones

This class of antibiotics was first discovered as nalidixic acid by Scientists involved in search of antimalarial drugs. Throughout the improvement of quinine in the early 1960s, nalidixic corrosive was discovered as a debasement. In microbes, they can obstruct DNA replication and translation. Quinolones and naphthyridines, such as cinoxacin, norfloxacin, ofloxacin, ciprofloxacin, temafloxacin, sparfloxacin, nalidixic corrosive, and enoxacin, are antibiotics, have been developed from the fundamental atom. A few changes to the parent system have been made since it was first revealed in the early 1960s and this has driven to the improvement and union of numerous subsidiaries with tried anti-microbial power (Etebu and Arikekpar, 2016).

2.10.6 Aminoglycosides

Streptomycin was the first antimicrobial drug discovered by people in this class in 1943, it was discovered. (Mahajan and Balachandran, 2012). Streptomycin is still commonly used to treat tuberculosis, which is caused by the bacteria *Mycobacterium tuberculosis*. Aminoglycosides are glycosidic bond-linked complexes made up mostly of 3-amino sugars (Etebu and Arikekpar, 2016).

Streptomycin is the oldest known aminoglycoside, as previously mentioned, and has been used to treat bubonic torment, tularaemia, and tuberculosis (Talaro and Chess, 2008). Despite its usefulness against a wide range of diseases, streptomycin has been discovered to be extremely poisonous. This dreadful aspect of the sedate demanded the need to look for different aminoglycosides that were still active against microbes but were less poisonous to humans. Antimicrobials including Gentamicin, Neomycin, Tobramycin, and Amikacin were discovered as a result of the investigation. Gentamicin is a less toxic antibiotic that is widely used to treat gram-negative bar infections (*Escherichia*,

Pseudomonas, Shigella, and Salmonella). *Pseudomonas* disorders of cystic fibrosis patients are treated with tobramycin in particular (Gilbert, 2000).

2.10.7 Glycopeptides

Glycopeptides antimicrobials for the most part shortened as GPAs were initially gotten as normal items, but the last 20 years witnessed the emergence of semi-synthetic derivatives with improved activity and pharmacokinetic properties (Van Bambeke, 2004; Van Bambeke *et al.*, 2004; Kahne *et al.*, 2005). Actually, glycopeptides are made up of a 7-amino-acid cyclic peptide with two sugars attached to it, hence the name glycopeptides (Kang, 2015). Yim *et al*. (2014) clearly show the structures of various glycopeptide forms. The antimicrobial's authority over its goal is established by the formation of five hydrogen bonds with the medicate's peptidic spine. During amalgamation, extra chlorine and sugar are often added to the spine of the medication (as in oritavancin). Drugs with such extra connections are well-known to attach to the target more effectively (Allen and Nikas, 2003).

2.11 Vancomycin

Vancomycin is an antimicrobial utilized to treat a few bacterial contaminations (The American Society of Health-System, 2015). It's provided intravenously to treat complicated skin infections, circulatory system diseases, endocarditis, bone and joint diseases, and methicillin-resistant *Staphylococcus aureus* (Liu *et al.*, 2011). Blood levels may be measured to decide the proper measurements (Hamilton and Richard, 2015). Vancomycin is also used orally as a treatment for severe *Clostridium difficile* colitis, (The American Society of Health-System, 2015).

2.11.1 Vancomycin-Resistant *S. aureus* (VRSA)

Despite being approved for use in humans in 1958, vancomycin became an antibiotic of choice for treatment of MRSA infections in hospital settings in the late 1980s (Levine, 2006; D'Agata et al., 2009). Resistance to vancomycin was discovered in enterococci within the 1980s, and this finding inspired noteworthy concern almost end of the utilize of vancomycin as a compelling treatment for MRSA (Murray, 2000). In no time from that point, *S. aureus* separates with decreased vulnerability to teicoplanin; a basic relative of vancomycin emerged in Europe (McGuinness *et al.,* 2017). The primary VRSA segregates within the joined together States were detailed in 2002 (CDC, 2002; Chang *et al.,* 2003). Since that time, there have been an add up to of 14 separates detailed within the joined together States (Walters *et al.,* 2015).

2.11.2 Vancomycin resistance mechanisms

Glycopeptides (such as vancomycin and teicoplanin) work against bacteria by preventing the fusion of the *S. aureus* cell divider (Hiramatsu, 2001). Vancomycin resistance is currently thought to be caused by cell divider thickening and, probably, the exchange of genetic fibre. Vancomycin works by fixing conclusively to the fatal DalanylDalanine of bacterial cell divider signs, preventing cell divider generation by attacking the cell divider blend destinations (Sieradzki *et al.,* 1999).

Changes in the peptidoglycan blend are thought to cause resistance in VISA strains. VISA strains produce more peptidoglycan with higher levels of DalanylDalanine buildups. These clumps fix vancomycin atoms and successfully attach them, stopping them from getting their bacterial target (Lowy, 2003). Furthermore, newly modified cell dividers comprising certain vancomycin aid in the prevention of drug molecules from progressing.

The function of the bacterial cell divider in vancomycin resistance in *S. aureus* has been investigated in several studies, (Cui *et al.,* 2000). Greater peptidoglycan union, stiffening cell dividers, better glutamine non-amidated muropeptides, and lower peptidoglycan crosslinking have all been associated to increased vancomycin resistance in Mu50 *S. aureus* (Cui *et al.,* 2000). Thickening of cell dividers was linked to the capture of vancomycin within the exterior coatings and was thought to be a resistance tool (Cui *et al.,* 2000). The Mu3 separates moreover shown resistance to vancomycin, thickening of the cell divider, and peptidoglycan amalgamation exercises, in spite of the fact that not one or the other of these happened to the same degree as watched in Mu50 confines (Cui *et al.,* 2000). Other theories propose that basic and metabolic modifications in cell divider teichoic acids may also play a role in the resistance instrument by slowing cell divider debasement (rather than expanding the rate of cell divider blend), in this way keeping up a relationship between divider thickness and diminishing defencelessness to vancomycin (Sieradzki and Tomasz, 2003).

As of late, there has been prove to back the trade of hereditary fabric among VRSA microbes (Srinivasan *et al.,* 2002; Lowy, 2003; Weigel *et al.,* 2003). The Michigan VRSA confinement was generated by the in-vivo transference of vancomycin resistance from *E. faecalis* to an MRSA strain, according to familial investigations (Sievert *et al.,* 2002; Weigel *et al.,* 2003). The Tn1546 (the vanA transposon, which is housed within a multi-resistant conjugative plasmid) was transferred between co-isolated vancomycin-resistant *E. faecalis* strains to secure the vanA quality in Michigan (Weigel *et al.,* 2003). This disconnect achieved vancomycin resistance by converting the lethal peptide of DalanylDalanine to DalanylDlactate, which occurs as a result of exposure to higher vancomycin concentrations, and the unused dipeptide tends to have a decreased liking for vancomycin.

2.12 Antibiotics Mode of Action

Most antibiotics are effective against bacteria because they target a specific feature of their structure or metabolic processes. Antibiotics work by the following mechanism:

i. Cell wall synthesis inhibition
ii. Analysis of cell membrane structure or function
iii. Nucleic acid synthesis inhibition
iv. Protein synthesis inhibition
v. Blockage of key metabolic pathways (Madigan and Martinko, 2006; Talaro and Chess, 2008; Wright, 2010).

2.12.1 Cell wall synthesis inhibition

Most bacterial cells are coated in an unyielding coating of peptidoglycan (PG), also known as murein in more experienced causes), which protects the cells while also allowing them to win osmotic weight in repeatedly severe surroundings and circumstances in which they live. -(1-4)-N-acetyl Hexosamine is a type of cross-linking peptide bond found in peptidoglycan. Microscopic organisms must synthesize peptidoglycan to stay alive; this is accomplished by the action of PBPs, which are trans glycosylases and trans peptidases. These dual chemicals are extremely important since they add disaccharide pent peptides to existing peptidoglycan particles, which amplify the glycan strands and cross-link strands of younger peptidoglycan units (Stop and Uehara, 2008). By hindering the peptide bond structure catalysed by PBPs, drugs like penicillin, carbapenem, and cephalosporin will square the cross-linking of peptidoglycan units (Josephine *et al.*, 2004).

Most antimicrobials having a place to the glycopeptide course of antimicrobials (for case, vancomycin) can hinder bacterial development by obstructing the PG blend. They limit

the amount of PG in the mix by connecting to PG units and delaying trans glycosylase and trans peptidase movement. (Kahne *et al.*, 2005).

2.12.2 Nucleic acid synthesis inhibition

The metabolic pathways that develop from the union of nucleic acids are extremely important; disturbance of nucleic acid corrosive amalgamation is harmful to bacterial cell existence and offspring. Antimicrobials interfered with the nucleic acid corrosive mix by stopping replication or translation. The traditional twofold helix structure is loosening up during DNA replication, which is aided by the helicase chemicals (Etebu and Arikekpar, 2016). The quinolones gather of antimicrobials, for illustration, do meddled with the usefulness of the helicase chemical in this manner disturbs the protein from playing its work of loosening up DNA. This anti-microbial activity of the quinolones eventually truncates the method of DNA replication and repair among helpless microscopic organisms (Chen *et al.*, 1996). Antimicrobials with a nucleic acid corrosive blend as their mode of action often target topoisomerase II and IV in microscopic species. Disturbing the activities of these chemicals in microbes has a negative impact on RNA polymerase, which predicts RNA amalgamation. Quinolones that prevent bacterial nucleic acid synthesis in this method do not relate with mammalian RNA polymerase, creating them especially toxic to Gram-positive and Gram-negative bacteria (Etebu and Arikekpar, 2016).

2.12.3 Protein synthesis inhibition

Microbes and other living things are described by the amount and type of proteins they produce on a regular basis. Proteins consider auxiliary structure, metabolic and functional types, and reaction to antagonistic situations, among supplementary things. Regardless, the form and quantity of proteins delivered by a bacterium at any certain period are related

on facts found in another extremely important biomolecule-deoxyribonucleic acid corrosive (DNA). DNA decides the type of protein generated by a bacterial cell based on material stored within it. The material may be in the form of codons, which are hereditary codes passed down to an indistinguishable biomolecule called ribonucleic acid (RNA), especially courier RNA (mRNA). A similar biomolecule, transfer RNA (tRNA), is also formed by DNA's command. The biomolecule mRNA travels to the ribosomes, which are the plant for protein fusion in a living cell. At that point, the tRNA decodes the codons in the mRNA and encourages the translation of the codon arrangement into an arrangement of amino acids, which are the building blocks of proteins (Etebu, 2013).

The ribosome and some cytoplasmic adornment variables are involved in the translation of mRNA into proteins, which arises in three stages (start, stretching, and end) (Gualerzi *et al.*, 2000). Ribosomes, which are made up of RNA and proteins, are often referred to as ribonucleoproteins. Ribosomal RNA (rRNA) is the RNA portion, and it is made up of two subunits, one small subunit (SSU) and the other large subunit (LSU). These two subunits are generally represented in terms of their sedimentation amounts (i.e., their rate of sedimentation is ultracentrifuge) and are calculated in Svedberg units (images) called the 30S and 50S, respectively (Nissen *et al.*, 2000).

On their rRNA, bacteria have 5S, 16S, and 23S attributes (Moore, 2001). The 16S rRNA consistency is found in their SSU (16S), while the extra two rRNA qualities (23S and 5S) are found on the bacterial ribosome's LSU (Lafontaine and Tollervey, 2001). The change among prokaryotic and eukaryotic rRNA is enormous, and this discovery has importantly aided researchers in developing antimicrobials that target the rRNA of a wide variety of pathogenic microscopic organisms (Hong *et al.*, 2014).

2.13 Antibiotic Susceptibility Test Methods

2.13.1 Diffusion

The disk diffusion method is the gold standard for confirming the susceptibility of bacteria. Standardized disk diffusion was introduced by Bauer and Kirby's experiments in 1956, after finalizing all aspects of optimization by changing physical conditions (Khan *et al.,* 2019). In this method, the isolated bacterial colony is selected, suspended into growth media, and standardized through a turbidity test. The standardized suspension is then inoculated onto the solidified agar plate, and the antibiotic-treated paper is tapped on the inoculated plate. The disc containing the antibiotic is allowed to diffuse through the solidified agar, resulting in the formation of an inhibition zone after the overnight incubation at 35 °C. Thereafter, the size of the inhibition zone formed around the paper disc is measured; the size of the inhibition zone corresponds to the concentration of antibiotic (Jorgensen and Ferraro, 2009). Assessing and determining the susceptibility of bacteria generally takes 16-24 hours. Several diffusion-based experiments have been performed prior to the standardized disk diffusion method (Khan *et al.,* 2019).

In later years, the introduction of effective drugs and convenient means of susceptibility testing have prevailed with the increase of the deadly infections. Therefore, variations of the method have been adopted to expand its versatility and utility. In 1947, Hoyt, Levine, and Bondi introduced penicillin tablets and the standard 6.5-mm disk method separately to emphasize multiple targets (Khan *et al.,* 2019). All the proposed methods were inaccurate, unsuitable, and unreliable for routine testing because of discrepancies in results obtained from different labs (Mayrhofer *et al.,* 2004). However, along with these advantages, it also has some significant drawbacks: only semi-automation is available (Sirscan), insufficient data availability for many bacteria (strains of *Pseudomonas*,

Bacillus, and *Corynebacterium*), and it has a poor performance when analyzing slow-growing and fastidious bacteria (Khan *et al.*, 2019).

Recently, the emergence of various instruments for analyzing the zone of inhibition has added to the reliability of the disk diffusion results by reducing variability due to operator handling and interpretation. The camera or scanner takes the picture, and the inbuilt image analysis software displays the zone of inhibition and compares the obtained results with the various guidelines present in the database. Accuzone (AccuMed International, Hillsboro, Oregon, USA), Biomic (Giles Scientific, Santa Barbara, California, USA), Mastascan Elite (Mast, Bootle, UK), and Sirscan (Becton Dickinson, Oxford, UK) are a few of the instruments capable of analyzing the zone of inhibition, but all differ in data input, analysis, ease of use, and presentation of results (Felmingham and Brown, 2001).

2.13.2 Dilution

Dilution was one of the earliest tools in microbiological practice, starting in the early 1870s, and it allows the growth and identification of bacterial populations in suspension. Pasteur, Lister, Koch, and Ehrlich were listed as the pioneers in the field of bacteriology, and they worked on the concept of macrodilution. William Roberts and John Tyndall further contributed to the macrodilution method and observed bacterial growth in a diluted medium. The two basic types of dilution are microdilution and macrodilution, wherein broth and agar are the most commonly used mediums. In broth dilution, consecutive two-fold dilutions (1, 2, 4, 8, and 12 µL) of antibiotics are made and dispensed into micro-centrifuge tubes containing bacterial growth medium, followed by making up the final volume by adding the medium and incubating overnight at 35 °C. Finally, the growth examination is carried out for setting the breakpoint through the turbidity of culture media. In agar dilution, antibiotics are diluted into the agar medium,

followed by plate formation and application of bacterial cells to the surface of the agar plate (Khan *et al.*, 2019).

In the early 20th century, various scientists made efforts to introduce serial dilution. They set the dilution factor in terms of geometric progression, and derived the generalized mathematical equation for interpreting the dilution results (Khan *et al.*, 2019). In 1929, Alexander Fleming performed the serial dilution technique to understand the activity of antibiotics. In this technique, two-fold dilution of antibiotics is mixed with a pre-inoculated liquid medium to determine antibiotic actions by checking the turbidity. In 1942, Fleming modified the previous protocol by using pH instead of turbidity to identify antibacterial activity. In the same year, Rammelkamp and Maxon introduced broth macro dilution, or the "tube dilution method", which is regarded as the standardized dilution method for both minimum inhibitory concentration (MIC) and AST. CLSI recommends guidelines to set the breakpoints. The first attempt regarding AST was made by Schmith and Reymann using agar medium during the 1940s (Wheat, 2001).

Microdilution is a miniaturized prototype of the macrodilution method where susceptibility testing is performed on disposable 96-well microtiter plates, where each well has a sample capacity of ~0.1 mL (Tang and Stratton, 2012). To dispense the samples into microwells, mechanized dispensers are used to avoid the handling error. After overnight incubation, growth and MIC are assessed through specialized optical instruments. This method has been well standardized for most fastidious bacteria (Jorgensen and Ferraro, 2009).

The central drawback of dilution methods is the requirement of a large volume of reagents. Apart from that, other potential limitations include: experimental space, tedious dilution steps (macrodilution), the possibility of false positive results due to long

incubation times (Waites *et al.,* 2012), chances of cross-contamination, bacterial incompatibility for growth, and the inability of discriminating viable and nonviable bacteria. Maintaining the recommended optimum testing parameters like pH, temperature, media, and length of incubation are additional hurdles, and a control viability plate is mandatory in tests to achieve practical clinical relevance (Khan *et al.,* 2019).

2.13.3 Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS)

MALDI-TOF MS, introduced in 2000, is another sensitive method for bacterial identification. High sensitivity and accuracy are the key characteristics that make it a useful method for clinical relevance. Assorted studies reveal its significance in discriminating MRSA, MSSA, and other bacterial strains where susceptible and resistant bacteria have been evaluated through spectral peak analysis. Even the subtle difference in expression profiles have been noticed in isogenic strains of *S. aureus* (Bernardo *et al.,* 2002; Hrabák *et al.,* 2013). The efficiency of MALDI-TOF MS has been further investigated on vancomycin-resistant Enterococci, where sensitivity higher than 90% has been recorded. Furthermore, analysis of multiple targets with different resistant strains of *Pseudomonas spp.* against ciprofloxacin, tobramycin, and meropenem have been identified efficiently (Jung *et al.,* 2014). The newly developed MALDI biotyper antibiotic susceptibility test rapid assay (MBT-ASTRA) is a more-straightforward and cost-effective modulation of MALDI-TOF MS used for both AST and MIC determination (Zimmermann and Burckhardt, 2017). Despite all the advantage of MALDI-TOF MS, the expensive nature of the instrument and its maintenance are prime disadvantages for mass application (Khan *et al.,* 2019).

2.14 Mechanisms of Antibiotic Resistance

1. The changes that occur in the receptor that connected to the drug and the region of the connection 'Connection of the antibiotics' target areas are different. They can be various enzymes and ribosomes. Resistance associated with alterations in the ribosomal target are the most frequently observed in macrolide antibiotics. Mutations in penicillin-binding proteins (beta-lactamase enzymes) and *Staphylococcus aureus, Streptococcus pneumoniae, Neisseria meningitidis and Enterococcus faecium* strains can develop resistance to penicillin. Changes in the structure of the target, beta-laktam, quinolones, glycopeptides, macrolides, tetracycline and rifampicin resistance is an important mechanism in the development (Bassetti *et al.*, 2013).
2. Enzymatic inactivation of antibiotics: Most of Gram-positive and Gram-negative bacterias synthesize enzymes that degrade antibiotics. This enzymatic inactivation mechanism is one of the most important mechanisms of resistance. In this group, beta-lactamases, aminoglycosides, modifying enzymes (acetylase, fosforiaz adenilaz and enzymes) degrade beta-lactam antibiotics and continually increasing their number of which inactivates enzymes include chloramphenicol and erythromycin (Yüce 2001; Bassetti *et al.,* 2013).
3. Reduction of the inner and outer membrane permeability: This resistance due to changes in the internal and external membrane permeability, decrease in drug uptake into the cell or quickly ejected from the active resistance of the pump systems. As a result of a change in membrane permeability decreased porin mutations in resistant strains can occur in proteins. For example; in Pseudomonas aeruginosa strains a specific porin called OprD can cause to mutation carbapenem resistance. Reduction in permeability of the outer membrane may play an

important role in resistance to quinolones and aminoglycosides (Yüce 2001; Bassetti *et al.,* 2013).

4. Flush out of the drug (Active Pump System): Resistance developing through the active pump systems mostly common in tetracycline group of antibiotics. Tetracyclines is thrown out with energy-dependent active pumping system and cannot accumulate in the cell. Such resistance is in control of the plasmid and chromosomal. Active pumping systems are effective in resisting quinolones, 14-membered macrolides, streptogramins, chloramphenicol and beta-lactams (Cesur and Demiröz, 2013).
5. Using an alternative metabolic pathway: Unlike some of the changes in the target in bacteria, a new pathway for drug-susceptible eliminate the need to develop objective. In this way resistance seen among the sulfonamide and trimethoprim. Bacterias can gain property of getting ready folate from the environment instead of synthesizing folate (Cesur and Demiröz, 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The research was conducted in selected hospitals in Minna, Niger State, Nigeria. Based on the patient population, and referral rate. The selected hospitals are General Hospital Minna and Ibrahim Badamasi (IBB) Specialist Hospital Minna. Minna, a city in North central region of Nigeria is the capital of Niger State with an approximate population of 3954772 in 2017 (National Population Commission (NPC) 2006). Minna is located at 9.62 latitude and 6.55 longitude and the city is raised 243 metres above sea level. It is the biggest city in Niger State. The State has an area of 76,363 km^2.

3.2 Study Population

The examined population consisted of male and female patients of age range 0 and 90 years old with suspected wound infections in General Hospital and IBB specialist Hospital Minna, Niger State. This research was conducted for over a period of six (6) months from July 2019 to January 2020.

3.2.1 Wound infection studied

Surgical wound infections: The risk of infection is generally based on the susceptibility of a surgical wound to microbial contamination (Bowler *et al.*, 2001). Clean surgery carries a 1 to 5 % risk of postoperative wound infection, and in dirty procedures that are significantly more susceptible to endogenous contamination, a 27 % risk of infection has been estimated. Examples of the latter include surgery associated with the large intestine and the head and neck, where extensive endogenous wound contamination, and hence a higher probability of wound infection, is likely (Bowler *et al.*, 2001). The surgical wound

infections were characterized by liquid by liquid coming out from the wounds. Besides, the wounds had white patches due to contamination.

Burn wound infections: Infection is a major complication in burn wounds, and it is estimated that up to 75 % of deaths following burn injury are related to infection. Although exposed burned tissue is susceptible to contamination by microorganisms from the gastrointestinal and upper respiratory tracts, many studies have reported the prevalence of aerobes such as *P. aeruginosa*, *S. aureus*, *E. coli*, *Klebsiella* spp., *Enterococcus* spp., and *Candida* spp (Bowler *et al.*, 2001). The burn wound infections were characterized by swollen spots with liquid draining from the wound.

Diabetic foot ulcer infections: Plantar ulcers associated with diabetes mellitus are susceptible to infection due to the high incidence of mixed wound microflora and the inability of the PMNs to deal with invading microorganisms effectively (Bowler *et al.*, 2001). However, with optimal treatment involving debridement of devitalized tissue, the use of appropriate dressings, and pressure relief, wound infection can be minimized (Bowler *et al.*, 2001). The diabetic food ulcer infections were characterized by deep cut pus oozing out.

3.3 Sample Size

The sample size was estimated using the sampling approach, and 200 wound samples were employed in the study.

The sample size was determined by the formula below:

$$N = \frac{(Z-a)^2 \times P (1-P)}{D^2}$$

Where

N = required minimum sample size

Z_1-a = Confidence level at 95% was found to be 1.96

P = the estimated prevalence of *S. aureus* infection in Minna obtained from previous studies by Garba *et al*. (2017) was 17% (0.17)

D= Precision error at 5% was 0.05

$$N = \frac{(1.96)^2 * 0.17(1-0.17)}{0.05^2} = \frac{3.8416 * 0.1411}{0.05^2} = \frac{0.542}{0.0025} = 216.8 \text{ samples}$$

The minimum required number of samples for this study was 216.8. However 200 samples was used in this study.

3.4 Sample Collection

A total of 200 wound samples were collected aseptically with a sterile swab from in and outpatients attending General Hospital Minna and IBB Specialist Hospital Minna. Wounds are collected using sterile swab, the wounds are cleaned with normal saline to avoid contamination from wound normal flora. The swab sticks were transported in an ice pack under sterile condition after collection to Step B Laboratory, Federal University of Technology Minna, Niger State, Nigeria.

3.5 Isolation and identification of *Staphylococcus aureus*

For the isolation of *Staphylococcus aureus*, the samples were immediately inoculated on Mannitol salt agar (MSA) plates and incubated at 37 ^{o}C for 24 hours. After incubation, colonies of Staphylococci characteristics were sub-cultured and incubated at 37 ^{o}C for 24 hours (Abdel Rahman *et al.,* 2010; Kaiser *et al.,* 2011).

The Pure colony was subjected to Gram staining, and biochemical tests including production of catalase, coagulase, oxidase, indole, urease, haemolytic, citrate, motility tests and utilization of glucose, fructose, mannitol, lactose test (Aryal, 2019).

3.5.1 Catalase test

The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production. The culture should not be more than 24 hours old.

Bacteria thereby protect themselves from the lethal effect of Hydrogen peroxide which is accumulated as an end product of aerobic carbohydrate metabolism (Aryal, 2019).

Procedure of catalase test

A loop of a small amount of colony growth is transfer in the surface of a clean, dry glass slide. A drop of 3% H_2O_2 is placed in the glass slide, the evolution of oxygen bubbles was observed (Aryal, 2019).

3.5.2 Coagulase test

Coagulase is an enzymatic protein that is a thermostable thrombin-like substance, which converts fibrinogen into fibrin resulting in clotting or clumping (Sapkota, 2020).

About 10 µl of deionized water or physiological saline is added to a slide. Several colonies from a fresh culture are collected with an inoculating loop and are emulsified into the water to obtain a smooth milk-colored suspension. A drop of a rabbit or human plasma is added to the slide, and the clumping is observed immediately, not to exceed 10 seconds (Sapkota, 2020).

3.5.3 Oxidase tests

Oxidase is a terminal enzyme in aerobic respiration. The aerobic respiration mechanism is composed of a number of enzymes which alternatively oxidize and reduce each other by donating or accepting electrons derived from H_2. Hydrogen atoms are removed from various substrates during oxidation reaction and ultimately H_2 atoms are combined with O_2 to from H_2O. Thus, cytochrome C oxidase is the terminal electron acceptor in this electron transport chain (ETC) reaction and its function is to transfer electron from H_2 to O_2 to from H2O molecule. This is the classic aerobic respiration scheme that most organisms utilize to derive energy from food they take in as molecule of ATP (Karki, 2018).

Procedure of oxidase test:

A piece of filter paper was placed in a clean petridish and 2-3 drops of freshly prepared oxidase reagent (1 % tetra methyl-p-Phenylenediamine dihydrochloride) were add. A small portion of culture was placed on the filter paper with the help of a sterile glass rod and make a smear on it. Immediate colour change to blue purple was examined within 10 seconds (Karki, 2018).

3.5.4 Indole test

Procedure of indole test: Take a sterilized test tubes containing 4 ml of tryptophan broth, Inoculate the tube aseptically by taking the growth from 18 to 24 hrs culture Incubate the tube at 37 °C for 24-28 hours. Add 0.5 mL of Kovac's reagent to the broth culture. Observe for the presence or absence of ring.

3.5.5 Urease test

Colonies of the organism were inoculated on urea based agar medium in glass test tubes, streaked and incubated at 37 ^{o}C for 24 hours. A change in colour from yellow/orange-pink indicated urease production by the isolates. Hence urease positive result was confirmed. Urease positive organisms were able to hydrolyse urea with the help of the urease enzyme. Whereas no pink colour change was concluded as negative urease test (Cheesbrough, 2010).

3.5.6 Citrate test

Citrate agar is used to test an organism's ability to utilize citrate as a source of energy. The medium contains citrate as the sole carbon source and inorganic ammonium salts ($NH_4H_2PO_4$) as the sole source of nitrogen.

Procedure of Citrate Utilization Test

The slant was streak back and forth with a light inoculum picked from the center of a well-isolated colony. It was incubated aerobically at 35 ^{o}C to 37 ^{o}C for up to 4-7 days. A color change from green to blue along the slant was observed (Aryal, 2109).

3.5.7 Motility test

Motility by bacterium is mostly demonstrated in a semi solid agar medium. In semi-solid agar media, motile bacteria 'swarm' and give a diffuse spreading growth that is easily recognized by the naked eye. The medium mainly used for this purpose is SIM medium (Sulphide Indole Motility medium) which is a combination differential medium that tests three different parameters, Sulfur Reduction, Indole Production and Motility. This media has a very soft consistency that allows motile bacteria to migrate readily through them causing cloudiness. The inoculum is stabbed into the centre of a semisolid agar deep.

Bacterial motility is evident by a diffuse zone of growth extending out from the line of inoculation. Some organisms grow throughout the entire medium, whereas others show small areas or nodules that grow out from the line of inoculation. The non-motile bacteria will only grow in the soft agar tube and only the area where they are inoculated (Aryal, 2019).

Method

A colony of a young (18-24 hours) culture was touched with a straight needle on agar medium. It was stabbed once to a depth of only $^{1}/_{3}$ to ½ inch in the middle of the tube. It was then incubated at 35 °C-37 °C and examined daily for up to 7 days. A diffuse zone of growth flaring out from the line of inoculation was observed (Aryal, 2019).

3.5.8 Sugar fermentation test

The bacterial isolates were tested for sugar fermentation using four (4) sugars individually; Glucose, lactose, fructose, and mannitol. Nutrient broth containing 0.5 % of each of the sugars, phenol red indicator and inverted Durham tubes were prepared and sterilized by boiling at 121 °C for 15 minutes. The test isolates were inoculated in each mixture of the sugar solution and incubated at 37 °C for 24 hours. Colour change from red to yellow as a result of acid production indicated sugar fermentation while gas production was demonstrated by the presence of bubbles trapped in the inverted Durham tubes. However, if there was no colour change of the medium it was regarded as a negative result (Cheesbrough, 2010).

3.6 Antimicrobial Susceptibility Test

Antibiotic susceptibility profiles of 97 isolates of *Staphylococcus aureus* from wounds was verified using five (5) antibiotics used for the treatment of bacterial infection. The

antibiotics tested were Vancomycin, Amoxicillin, Ampicillin, Erythromycin and Oxacillin. *S. aureus* was evaluated by Kirby-Bauer modified disc agar diffusion technique as recommended by Clinical and Laboratory Standard Institute guidelines (CLSI, 2018). Isolated colonies of *S. aureus* strains from 24 hours culture of nutrient agar plates were suspended in 3ml of sterile physiological saline and the turbidity was adjusted to 0.5 mcfarland standard (10^{-8} cfu/mL). Sterile swab sticks were used to inoculate the bacterial suspensions evenly on the surface of the Mueller Hinton agar (MHA) plates in three directions, rotating the plates at 60 °C. The inoculated plates were allowed to dry for 15 minutes. Sterile forceps were used to place the antibiotic disc on the surface of the agar. The plates were inverted after 30 minutes of disc application and were incubated aerobically for 18-24 hours at 37 °C.

In accordance with the diameter of the zones of growth inhibition around the antibiotics discs, the *S. aureus* strains were interpreted as sensitive, intermediate and resistant to particular antibiotics (CLSI, 2018). All the antibiotic discs were ordered from Oxoid Ltd, UK.

3.7 Molecular Identification of Isolates

3.7.1 DNA extraction

DNA was extracted using the protocol stated by (Trindade *et al.*, 2007). Briefly, Single colonies grown on medium were transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 hours at 28 ºC. After this period, cultures were centrifuged at 4600 g for 5 min. The resulting pellets were re-suspended in 520 µL of TE buffer (10 mMTris-HCl, 1mM EDTA, pH 8.0). Fifteen microliters of 20 % SDS and 3 µL of Proteinase K (20 mg/mL) were then added. The mixture was incubated for 1 hour at 37 ºC, then 100 µL of 5M NaCl and 80 µL of a 10 % CTAB solution in 0.7 M NaCl were

added and votexed. The suspension was incubated for 10 mins at 65 ºC and kept on ice for 15 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min and centrifugation at 7200 g for 20 min. The aqueous phase was then transferred to a new tube and isopropanol (1: 0.6) was added and DNA precipitated at -20 ºC for 16 h. DNA was collected by centrifugation at 13000 g for 10 mins, washed with 500 µL of 70 % ethanol, air-dried at 25 ºC for approximately three hours and finally dissolved in 50 µL of TE buffer.

3.7.2 Polymerase chain reaction (PCR).

PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µL of 25 mM $MgCl_2$, 1 µL of 10 mM of 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 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µL with sterile distilled water 8 µL DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94 °C for 5 min; followed by a 30 cycles consisting of 94 °C for 30 s, 50 °C for 60 s and 72 °C for 1 minute 30 seconds; and a final termination at 72 °C for 10 mins. And chill at 4 ºC.GEL (Wawrik *et al.*, 2005, Frank *et al.,* 2008).

3.7.3 Integrity

The integrity of the amplified about 1.5 Mb gene fragment was checked on a 1 % Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5 % agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60 °C and stained with 3 µL of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray

and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 mL) of 10X blue gel loading dye (which gives color and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µl of each PCR product and loaded into the wells after the 100 bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120 V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100 bp molecular weight ladder that was ran alongside experimental samples in the gel.

3.7.4 Purification of amplified product

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µL of Na acetate 3 M and 240 µL of 95 % ethanol were added to each about 40 µL PCR amplified product in a new sterile 1.5 µL tube eppendorf, mix thoroughly by vortexing and keep at -20 °C for at least 30 min. Centrifugation for 10 min at 13000 g and 4 °C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µL of 70 % ethanol and mix then centrifuge for 15 min at 7500 g and 4 °C. Again remove all supernatant (invert tube on trash) and invert tube on paper tissue and let it dry in the fume hood at room temperature for 10-15 min. then re-suspend with 20 µL of sterile distilled water and kept in -20 ^{o}C prior to sequencing. The purified fragment was checked on a 1.5 % Agarose gel ran on a voltage of 110 V for about 1 hour as previous, to confirm the presence of the purified product and quantified using a nano drop of model 2000 from thermo scientific (Wawrik *et al.,* 2005).

3.7.5 Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130 xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis (Frank e*t al.,* 2008).

3.8 Molecular Characterization of VRSA Gene

Five (5) *S. aureus* isolates were characterized at the molecular level. The presence of VRSA in isolates with a VRSA index of less than 0.5 was determined using Polymerase Chain Reaction (PCR) and specific primers VRSA, the procedure described by Trindade, (2000). The DNA was isolated to confirm amplification; a 1 % Agarose gel was used to evaluate the integrity of the amplified 1.5 Mb gene ladder.

Ten liters of 5 x GoTaq colorless reaction, 3 L of 25 mM $MgCl_2$, 1 L of 10 mM dNTPs mix, and 1 L of 10 pmol per 27 F polymerase chain reaction sequencing preparation cocktail 5'-AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers and 0.3 units Taq DNA (Wawrik *et al.,* 2005; Frank *et al.,* 2008). The PCR amplicon was mounted onto a 1.5 % agarose gel.

Molecular studies of vanA, vanB and vanXY coding gene in the five selected bacterial isolates (VRSA) was done using a simple PCR on the extracted DNA vanA, vanB and vanXY single coding regions primers that are specific. Primer sequences are listed in Table 3 as previously published. 5X PCR SYBR green buffer (2.5), $MgCl_2$ (0.75), 10pM DNTP (0.25), 10pM of each forward and backwards primer (0.25), 8000U of taq DNA polymerase (0.06), brought up to 10.5 with sterile distilled water to which 2 L template was added Table 3.1 shows the PCR profile reaction cocktail utilized in each assay. In a GeneAmp 9700 PCR System Thermalcycler, 0 PCR was performed (Applied Biosystem

Inc., USA) utilizing the proper profile as suggested for each primer pair (Frank *et al.,* 2008).

Table 3.1: Primer Sequences used for Molecular Characterization of VRSA Genes in the Study

Gene	Primer	Target gene Sequence	Band size	PCR Condition	Reference
vanA	vanA-F	GCAAGTCAGGTGA AGATG	700bp	After a 5-minute denaturing step at 94°C,	Khaled *et al*., (2019)
	vanA-R	ATCAAGCGGTCAAT CAGTTC		then 35 cycles of 94oC for 30 seconds, 55oC for 45 seconds, and	
				72°C for 60 seconds, then 72°C for 10 minutes	
vanB	vanB-F	GTG ACA AAC CGG AGG CGA GGA	400bp	After a 5-minute denaturing step at 94°C,	Khaled *et al*., (2019)
	vanB-R	CCGCCATCCTCCTG CAAAAAA		then 35 cycles of 94oC for 30 seconds, 55oC for 45 seconds, and	
				72°C for 60 seconds, then 72°C for 10 minutes	
vanXY	vanXY-F	AATAGCTATTTTGA TTTCCCCGTTA	500bp	After a 5-minute denaturing step at 94°C,	Garba *et al*., (2018)
	vanXY-R	TCCTGAGAAAACAG TGCTTCATTAA		Then 35 cycles of 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 60 seconds	
				and then finish at 72°C for 10 minutes	

Source: Frank *et al.* (2008)

3.9 Data analysis

The data collected was analyzed using Microsoft excel 2013 and was transferred to Statistical Package for Social Sciences (SPSS) version 21 for further analysis and results were presented in percentage. P-value of less than or equal to 0.05 were considered as statistically significant.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Results

4.1.1 Occurrence of *S. aureus* among the collected isolates from the hospitals

A total of 200 samples were collected from patients from General Hospital Minna and IBB Specialist Hospital Minna *S. aureus* was isolated from 48.5 % of the patients. General Hospital Minna had the highest occurrence of *S. aureus* 76 (50.7 %) while IBB Specialist Hospital Minna *S. aureus* was found in the least amount of cases 21 (42 %), as presented in Table 4.1

Table 4.1: Occurrence of *S. aureus* among the Collected Isolates from the Hospitals

Hospital	Sample size	Number of *S.aureus* isolated	Prevalence (%)
General Hospital	150	76	50.7
IBB Specialist Hospital	50	21	42.0
Total	200	97	48.5

4.1.2 Distribution of *S. aureus* according to demographic characteristics of patients recruited from this study

The age group of 21-30 years recorded the frequency of occurrence of *S. aureus* 24 (24.7 %) while 71-90 had the least frequency occurrence of 3 (3.1 %). Wound sample from male had the high frequency of occurrence for *S. aureus* compared to female (Table 4.2).

Similarly, wound of married patients had the highest frequency of occurrence of *S. aureus* (56.7 %), and the least (8.2 %) was recorded against wound infection from divorced patients.

Furthermore, businessmen/women patients had the highest frequency of occurrence of *S. aureus* (36.1 %) while students wound infection was found to be the least with (15.5 %) *S. aureus* (Table 4.2)

4.1.3 Antibiotic susceptibility profile of *Staphylococcus aureus*

Out of the 97 *S. aureus* isolates obtained from clinical samples, high resistance of *S. aureus* was observed against oxacillin (60.97 %) followed by vamcomycin (60.80 %), amoxicillin (56.09 %), ampicillin (41.46 %) and the lowest resistance was erythromycin (14.63 %). The number of *S. aureus* isolates resistant to each of the antibiotics is shown in Table 4.3.

4.1.4 VRSA phenotypic prevalence in *S. aureus* isolates

Out of 97 (48.5 %) *S. aureus* isolates recorded from the wound samples analyzed, 23 (23.7 %) were susceptible to vancomycin, 15 (15.5 %) were intermediate and 59 (60.8 %) were resistant to vancomycin (Table 4.4).

Table 4.2: Demographic Characteristics of Patients in the Study Area

Parameter		Number	Number of isolated *S. aureus*	Percentage (%)	P-value
Age (Years)	**0-10**	**24**	**14**	**14.4**	0.017
	11-20	50	17	17.5	
	21-30	39	24	24.7	
	31-40	23	12	12.4	
	41-50	19	5	5.2	
	51-60	15	12	12.4	
	61-70	17	7	7.2	
	71-80	8	3	3.1	
	81-90	5	3	3.1	
Gender	**Male**	**130**	**65**	**67.0**	0.563
	Female	70	32	33.0	
Marital status	**Single**	**80**	**20**	**20.6**	0.001
	Married	92	55	56.7	
	Divorced	8	8	8.2	
	Widowed	20	14	14.4	
Occupation	**Civil Servants**	**50**	**27**	**27.8**	0.001
	Businessmen/women	57	35	36.1	
	Farmers	68	20	20.6	
	Students	25	15	15.5	

Table 4.3: Antibiotic Susceptibility Profile of *Staphylococcus aureus*

Antibiotics	Resistance (%)	Intermediate (%)	Susceptibility (%)
Vancomycin 30 µg	60.80	15.50	23.70
Oxacillin 1 µg	60.97	4.87	34.14
Amoxicillin 20/10 µg	56.09	0	43.90
Ampicillin 10 µg	41.46	2.43	56.09
Erythromycin 15 µg	14.63	9.75	75.60

CLSI (2018).

Table 4.4: Susceptibility Pattern of VRSA

Susceptibility Pattern	**Number of *S. aureus***	**P-value**
Resistance (%)	59 (60.8)	0.406
Intermediate (%)	15 (15.5)	
Sensitive (%)	23 (23.7)	
Total	97 (100)	

P > 0.05

4.1.5 Molecular characterization of five (5) *Staphylococcus aureus* isolates

The agarose gel electrophoresis of the PCR products of 16s rRNA gene fragment in selected five of *Staphylococcus aureus* isolates using 16s rRNA universal Primers is shown in (Plate I). The primer has a band size of approximately 1500 bp which indicates pure isolates. The DNA extracted from this analysis showed that all isolates on gel electrophoresis were *Staphylococcus aureus*. The gel image shows sample arrangements on white horizontal lines indicating that lane 1-5, represents molecular weight marker, GSI 17, GSI 114, GSI 93, GSI 36 and GSI 112 and buffer control respectively.

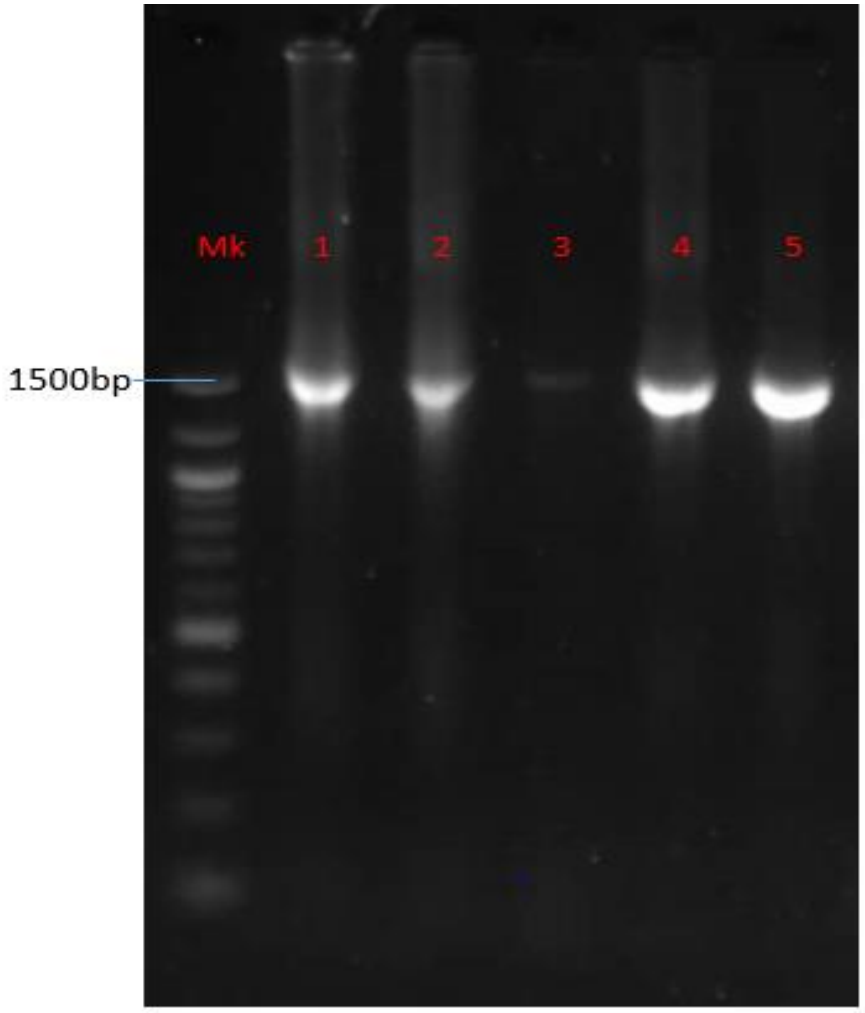

Plate I. Agarose gel electrophoresis of the PCR amplified products from the extracted DNA fragments of five (5) selected *S. aureus* strains.

4.1.6 Agarose Gel electrophoresis of *S. aureus* amplified vanA gene

The agarose gel electrophoresis of amplified PCR product vanA gene in selected *S. aureus* isolates shown in Plate II. The vanA gene primer has a band size approximately 713bp indicating the presence of vanA gene. The gel image shows white horizontal lines on lane 1-5, which represents molecular weight marker, GSI 17, GSI 114, GSI 93, GSI 36 and GSI 112 and buffer control respectively. Gel picture indicates the presence of vanA gene in all the samples.

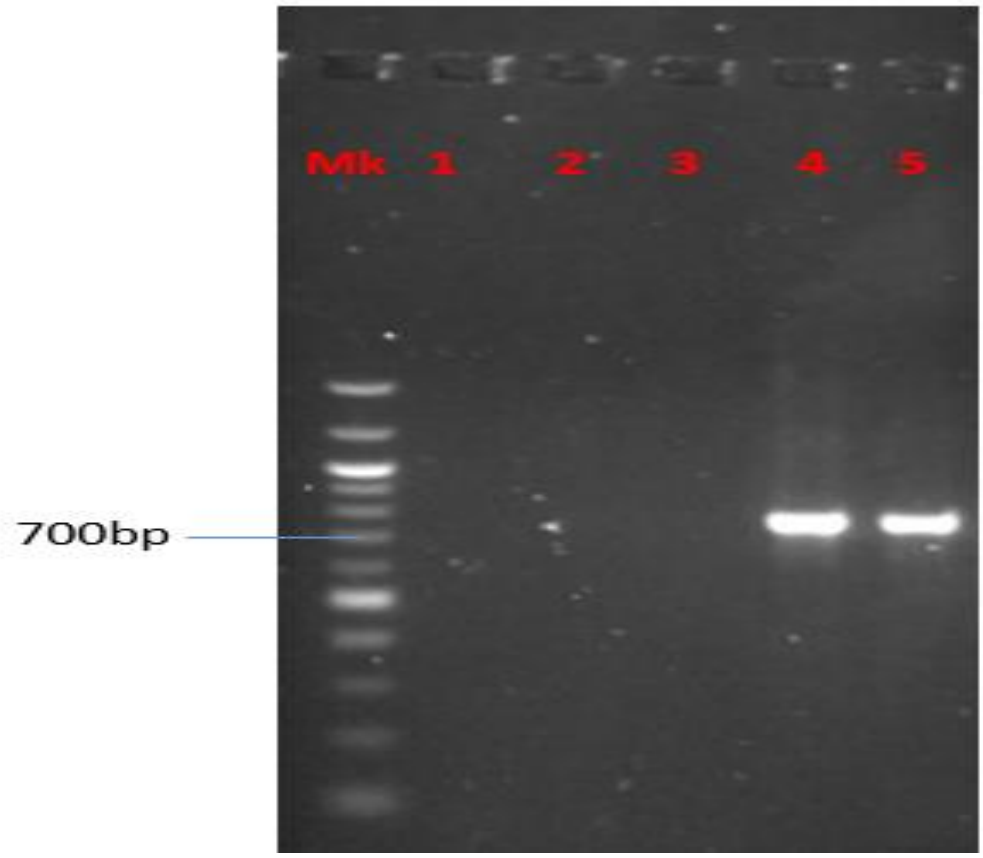

Plate II: Agarose gel electrophoresis of *S. aureus* amplified vanA gene

4.1.7 Agarose Gel electrophoresis of *S. aureus* amplified vanB gene

The agarose gel electrophoresis of amplified PCR product vanB gene in selected *S. aureus* isolates shown in Plate III. The vanB gene primer has a band size approximately 430bp indicating the presence of vanB gene. The gel image shows white horizontal lines on lane 1-5, which represents molecular weight marker, GSI 17, GSI 114, GSI 93, GSI 36 and GSI 112 and buffer control respectively. Gel picture indicates the presence of vanB gene in all the samples.

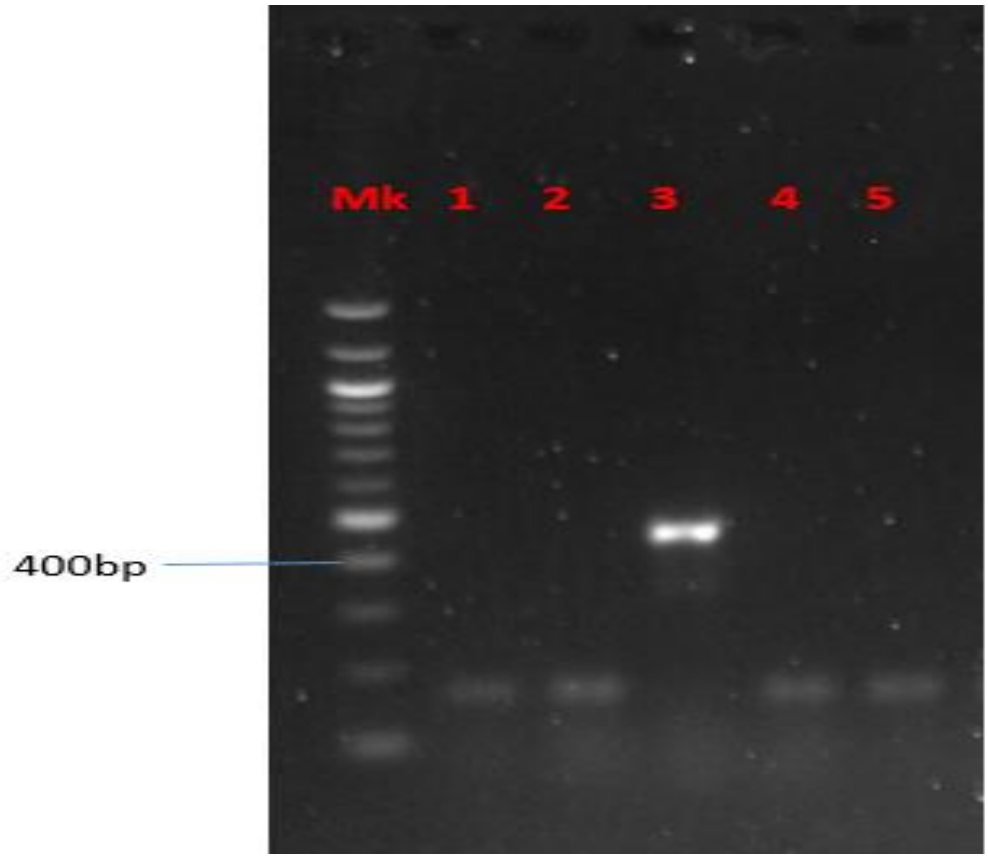

Plate III: Agarose gel electrophoresis of *S. aureus* amplified vanB gene

4.1.8 Agarose Gel electrophoresis of *S. aureus* amplified vanXY gene

The agarose gel electrophoresis of amplified PCR product vanXY gene in selected *S. aureus* isolates shown in Plate II. The vanXY gene primer has a band size approximately 550bp indicating the presence of vanXY gene. The gel image shows white horizontal lines on lane 1-5, which represents molecular weight marker, GSI 17, GSI 114, GSI 93, GSI 36 and GSI 112 and buffer control respectively. Gel picture indicates the presence of vanXY gene in all the samples.

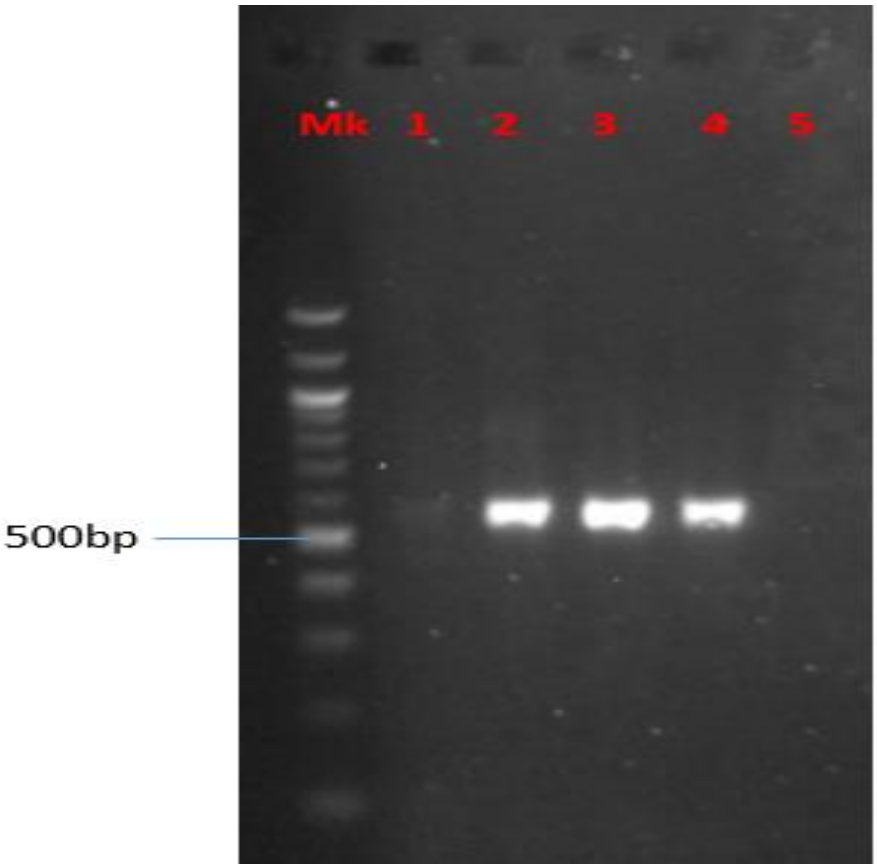

Plate IV: Agarose gel electrophoresis of *S. aureus* amplified vanXY gene

4.1.9: Presence and distribution of vancomycin genes among selected *S. aureus* isolates

The extracted DNA from the five selected *S. aureus* isolates were used to screen for the presence of vancomycin genes via polymerase chain reaction assay. vanA gene was present in 2 (40 %) of the isolates, vanB gene was present in 1 (20 %) of the isolates, and vanXY gene was present in only 2 (40 %) of the *S. aureus* strains (Table 4.11).

Table 4.5: Presence and Distribution of Vancomycin Genes among Selected *S. aureus* Isolates

Isolates	Vancomycin Genes vanA (%)	vanB (%)	vanXY (%)
GSI17	-	-	-
GSI114	-	-	+
GSI93	-	-	+
GSI36	+	+	+
GSI112	+	-	-
Total	(40)	(20)	(60)

Key:

vanA = Vancomycin A

vanB = Vancomycin B

vanXY = Vancomycin XY

4.2 Discussion

Staphylococcus aureus is a regular source of infection in hospitals and is most likely to infect infants, surgical patients, old and malnourished persons and patients with diabetes and other serious diseases (Nsofor *et al.,* 2016). It is one of the most infectious causes with high prevalence in several communities and healthcare organizations. In this study, the overall isolation rate was (48.70 %) out of 200 wound samples collected. *S. aureus* was isolated from all forms of wound samples and is endemic in this study area with prevalence rate 48.70 % which was comparable with the findings by Vijaylakshmi, (2015) who reported the prevalence of *S. aureus* isolated from surgical site wounds as 43.20 % in India. It is also in line with Kahsay *et al.* (2014) who reported a prevalence of 39.70 % from surgical wound in Ethiopia. Karmakar *et al.* (2016) was also reported a prevalence of 33.33 % isolated from burn and wound section in India, 20.90 % by Belbase *et al.* (2017) from pus/wound samples in Nepal, and 17.00 % reported by Garba *et al.* (2018) from wound in Nigeria. Although the result in this study is lower than the prevalent reported by Upreti *et al.* (2018), Neopane *et al.* (2018) both in Nepal, where the prevalence rate of wounds infection was reported as 56.9 % and 69.8 % respectively. It was observed that, the higher prevalence of *S. aureus* wound infection was from General Hospital Minna and this could be because the higher number of patients patronizing General Hospital compared to IBB Specialist Minna.

This study shows the highest frequency of *S. aureus* wound infection (Table 4.2) among age group of 21-30 (24.7 %), while 71-90 had the least frequency of (3.1 %), the rates of this group in this study is lower than Christopher *et al.* (2011) who reported a prevalence of 75.9 % and 65.8 % for age group of 21-25 and 26-30 respectively.

It was shown in Table 4.2 that male patients had highest percentage of *S. aureus* infection with (67.0 %) while female patients had (33.0 %). The high percentage of *S. aureus* of male patients in this study is higher than Offner *et al.* (1999) who reported a prevalence of 24.8 % from surgical wound. this is because male gender is associated with a dramatically increased risk of major infections following trauma.

Antibiotic susceptibility profiles of *S. aureus* isolates from wounds infections against five antibiotic tested (Table 4.5) revealed that *S. aureus* isolates were highly resistance to Oxacillin (60.97 %), Vancomycin (60.8 %), Amoxicillin (56.1 %), Ampicillin (41.4 %) and Erythromycin (14.6 %). The high antibiotic resistance profile by *S. aureus* isolates is comparable with Upreti *et al.* (2018) for Ampicillin with resistance rate of 92.4 %. Kahsay *et al.* (2014) reported the resistance rate of Ampicillin (82.2 %), Amoxicillin (82.2 %), and Erythromycin (95.9 %) which are higher in this study, while Vancomycin (4.1 %) and Oxacillin (49.3 %) are lower.

In this study, it was observed that 60.8 % of the VRSA strains were resistant; this is probably because these antibiotics are not used extensively in the therapy of *Staphylococcus aureus* bacterial diseases, among others in clinics in Nigeria and are most effective in the handling of gram-positive and gram-negative bacteria that are susceptible. *S. aureus* isolates in this study were all VRSA. This is important to note that if humans are infected with such strains, it could result in treatment failure, prolonged illnesses, higher health-care costs, and, in the worst-case scenario, death (Tanwar *et al.*, 2014). The evolution of antibiotic resistance among these isolates is possibly linked to the R-factor, a plasmid-mediated genetic determinant that transmits resistance (Akindolire, 2013).

In this research, it was also observed that *S. aureus* isolates with VRSA characteristics affected the cure of staphylococcal infections, particularly in children and people in their twenties.

The incidence of 40 % of vanA gene in this study is higher than that of Garba *et al.* (2018) who reported prevalence of 25 %, the incidence rate of vanB and vanXY genes are 20 % and 60 % respectively. The incidence rate of vanB gene (20 %) is lower compared to Garba *et al.* (2018) who reported 25 %; also incidence rate of vanXY (60 %) is also lower than Garba *et al.* (2018) who reported the incidence rate 83 %. Therefore, in this study isolate with vanB showed a high level of resistance, vanA showed moderates resistance, and vanXY showed a low level of resistance.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

S. aureus had a high resistance to vancomycin and to other antibiotics such as Amoxicillin, Ampicillin, Oxacillin and Erythromycin. Although resistance to Erythromycin was very low in this study. Vancomycin-resistant *S. aureus* (VRSA) were recorded to be 23.7 % sensitive to vancomycin while 15.5 % and 60.8 % isolates were intermediate and resistant respectively.

The molecular characterization reveals the *S. aureus* isolates to be GSI17, GSI36, GSI93, GSI112 and GSI114 were identified as *S. aureus* strain GHA10, SUSST48, pm37, SUSST48 and pm37 respectively. vanA, van B, and vanXY from the isolates are resistant genes detected in the isolates and other antibiotics in the study area were detected in all (100%) isolates.

This study had shown the presence of vancomycin resistant *S. aureus* (VRSA) encoding genes in General Hospital Minna and IBB Specialist Hospital Minna, and has also added to the limited reports on the prevalence of van resistant genes in Minna.

5.2 Recommendations

It is recommended that;

i. Intensive surveillance of vancomycin-resistance, proper use of antibiotics, and adherence to infection control guidelines in health-care settings are essential for preventing emergence and dissemination of VRSA strains.

ii. The application of anti-microbial combination treatment against VRSA, and support of legitimate cleanliness by hospitalized patients and staff should be exposed to successfully diminish the rate and dispersal of such cases.

iii. The prevention of emergence of multidrug-resistant organisms will require a comprehensive, systematic approach that integrates the health care and public health systems.

iv. The identification and study of new resistant determinants are important for surveillance of vancomycin-resistance.

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Appendix A: Colonial Morphology of *Staphylococcus aureus* Isolates

Isolates	MSA	G/RXN	SHAPE	Motility
GSI15	Yellow	+	S	NM
GSI16	Yellow	+	S	NM
GSI17	Yellow	+	S	NM
GSI19	Yellow	+	S	NM
GSI22	Yellow	+	S	NM
GSI23	Yellow	+	S	NM
GSI24	Yellow	+	S	NM
GSI25	Yellow	+	S	NM
GSI26	Yellow	+	S	NM
GSI27	Yellow	+	S	NM
GSI28	Yellow	+	S	NM
GSI29	Yellow	+	S	NM
GSI32	Yellow	+	S	NM
GSI33	Yellow	+	S	NM
GSI36	Yellow	+	S	NM

Key: MSA= Mannitol Salt agar, G/RXN= Gram reaction, + = Positive, S= Spherical, & NM= Non motile.

Appendix A: Continues

Isolates	MSA	G/RXN	SHAPE	Motility
GSI37	Yellow	+	S	NM
GSI38	Yellow	+	S	NM
GSI40	Yellow	+	S	NM
GSI41	Yellow	+	S	NM
GSI45	Yellow	+	S	NM
GSI47	Yellow	+	S	NM
GSI50	Yellow	+	S	NM
GSI52	Yellow	+	S	NM
GSI53	Yellow	+	S	NM
GSI54	Yellow	+	S	NM
GSI55	Yellow	+	S	NM
GSI57	Yellow	+	S	NM
GSI58	Yellow	+	S	NM
GSI59	Yellow	+	S	NM
GSI61	Yellow	+	S	NM

Appendix A: Continues

Isolates	MSA	G/RXN	SHAPE	Motility
GSI63	Yellow	+	S	NM
GSI64	Yellow	+	S	NM
GSI65	Yellow	+	S	NM
GSI69	Yellow	+	S	NM
GSI70	Yellow	+	S	NM
GSI71	Yellow	+	S	NM
GSI73	Yellow	+	S	NM
GSI75	Yellow	+	S	NM
GSI76	Yellow	+	S	NM
GSI78	Yellow	+	S	NM
GSI79	Yellow	+	S	NM
GSI79	Yellow	+	S	NM
GSI80	Yellow	+	S	NM
GSI81	Yellow	+	S	NM
GSI82	Yellow	+	S	NM

Appendix A: Continues

Isolates	MSA	G/RXN	SHAPE	Motility
GSI83	Yellow	+	S	NM
GSI84	Yellow	+	S	NM
GSI85	Yellow	+	S	NM
GSI86	Yellow	+	S	NM
GSI87	Yellow	+	S	NM
GSI90	Yellow	+	S	NM
GSI92	Yellow	+	S	NM
GSI93	Yellow	+	S	NM
GSI94	Yellow	+	S	NM
GSI95	Yellow	+	S	NM
GSI96	Yellow	+	S	NM
GSI100	Yellow	+	S	NM
GSI102	Yellow	+	S	NM
GSI104	Yellow	+	S	NM
GSI105	Yellow	+	S	NM

Appendix A: Continues

Isolates	MSA	G/RXN	SHAPE	Motility
GSI106	Yellow	+	S	NM
GSI107	Yellow	+	S	NM
GSI110	Yellow	+	S	NM
GSI111	Yellow	+	S	NM
GSI116	Yellow	+	S	NM
GSI120	Yellow	+	S	NM
GSI125	Yellow	+	S	NM
GSI127	Yellow	+	S	NM
GSI130	Yellow	+	S	NM
GSI135	Yellow	+	S	NM
GSI138	Yellow	+	S	NM
GSI140	Yellow	+	S	NM
GSI147	Yellow	+	S	NM
GSI148	Yellow	+	S	NM
GSI150	Yellow	+	S	NM

Appendix A: Continues

Isolates	MSA	G/RXN	SHAPE	Motility
ISI2	Yellow	+	S	NM
ISI4	Yellow	+	S	NM
ISI7	Yellow	+	S	NM
ISI8	Yellow	+	S	NM
ISI9	Yellow	+	S	NM
ISI13	Yellow	+	S	NM
ISI14	Yellow	+	S	NM
ISI15	Yellow	+	S	NM
ISI16	Yellow	+	S	NM
ISI17	Yellow	+	S	NM
ISI20	Yellow	+	S	NM
ISI25	Yellow	+	S	NM
ISI28	Yellow	+	S	NM
ISI29	Yellow	+	S	NM
ISI34	Yellow	+	S	NM

Appendix A: Continues

Isolates	MSA	G/RXN	SHAPE	Motility
ISI37	Yellow	+	S	NM
ISI45	Yellow	+	S	NM

Key: MSA= Mannitol Salt agar, G/RXN= Gram reaction, + = Positive, S= Spherical, & NM= Non motile.

Appendix B: Biochemical Characteristics of *Staphylococcus aureus* Isolates

Isolates	Catalase	Coagulase	Voges-Proskauer	Citrate	Indole	Hemolysis	Methyl Red	Oxidase
GSI15	+	+	+	+	-	+	-	-
GSI16	+	+	+	+	-	+	-	-
GSI17	+	+	+	+	-	+	-	-
GSI19	+	+	+	+	-	+	-	-
GSI22	+	+	+	+	-	+	-	-
GSI23	+	+	+	+	-	+	-	-
GSI24	+	+	+	+	-	+	-	-
GSI25	+	+	+	+	-	+	-	-
GSI26	+	+	+	+	-	+	-	-
GSI27	+	+	+	+	-	+	-	-
GSI28	+	+	+	+	-	+	-	-
GSI29	+	+	+	+	-	+	-	-
GSI32	+	+	+	+	-	+	-	-
GSI33	+	+	+	+	-	+	-	-
GSI36	+	+	+	+	-	+	-	-
GSI37	+	+	+	+	-	+	-	-
GSI38	+	+	+	+	-	+	-	-

Appendix B: Continues

Isolates	Catalase	Coagulase	Voges-Proskauer	Citrate	Indole	Hemolysis	Methyl Red	Oxidase
GSI40	+	+	+	+	-	+	-	-
GSI41	+	+	+	+	-	+	-	-
GSI45	+	+	+	+	-	+	-	-
GSI47	+	+	+	+	-	+	-	-
GSI50	+	+	+	+	-	+	-	-
GSI52	+	+	+	+	-	+	-	-
GSI53	+	+	+	+	-	+	-	-
GSI54	+	+	+	+	-	+	-	-
GSI55	+	+	+	+	-	+	-	-
GSI57	+	+	+	+	-	+	-	-
GSI58	+	+	+	+	-	+	-	-
GSI59	+	+	+	+	-	+	-	-
GSI61	+	+	+	+	-	+	-	-
GSI63	+	+	+	+	-	+	-	-
GSI64	+	+	+	+	-	+	-	-
GSI65	+	+	+	+	-	+	-	-
GSI69	+	+	+	+	-	+	-	-

Appendix B: Continues

Isolates	Catalase	Coagulase	Voges-Proskauer	Citrate	Indole	Hemolysis	Methyl Red	Oxidase
GSI70	+	+	+	+	-	+	-	-
GSI71	+	+	+	+	-	+	-	-
GSI73	+	+	+	+	-	+	-	-
GSI75	+	+	+	+	-	+	-	-
GSI76	+	+	+	+	-	+	-	-
GSI78	+	+	+	+	-	+	-	-
GSI79	+	+	+	+	-	+	-	-
GSI80	+	+	+	+	-	+	-	-
GSI81	+	+	+	+	-	+	-	-
GSI82	+	+	+	+	-	+	-	-
GSI83	+	+	+	+	-	+	-	-
GSI84	+	+	+	+	-	+	-	-
GSI85	+	+	+	+	-	+	-	-
GSI86	+	+	+	+	-	+	-	-
GSI87	+	+	+	+	-	+	-	-
GSI90	+	+	+	+	-	+	-	-
GSI92	+	+	+	+	-	+	-	-

Appendix B: Continues

Isolates	Catalase	Coagulase	Voges-Proskauer	Citrate	Indole	Hemolysis	Methyl Red	Oxidase
GSI93	+	+	+	+	-	+	-	-
GSI94	+	+	+	+	-	+	-	-
GSI95	+	+	+	+	-	+	-	-
GSI96	+	+	+	+	-	+	-	-
GSI100	+	+	+	+	-	+	-	-
GSI102	+	+	+	+	-	+	-	-
GSI104	+	+	+	+	-	+	-	-
GSI105	+	+	+	+	-	+	-	-
GSI106	+	+	+	+	-	+	-	-
GSI107	+	+	+	+	-	+	-	-
GSI110	+	+	+	+	-	+	-	-
GSI111	+	+	+	+	-	+	-	-
GSI116	+	+	+	+	-	+	-	-
GSI120	+	+	+	+	-	+	-	-
GSI125	+	+	+	+	-	+	-	-
GSI127	+	+	+	+	-	+	-	-
GSI130	+	+	+	+	-	+	-	-

Appendix B: Continues

Isolates	Catalase	Coagulase	Voges-Proskauer	Citrate	Indole	Hemolysis	Methyl Red	Oxidase
GSI135	+	+	+	+	-	+	-	-
GSI138	+	+	+	+	-	+	-	-
GSI140	+	+	+	+	-	+	-	-
GSI147	+	+	+	+	-	+	-	-
GSI148	+	+	+	+	-	+	-	-
GSI150	+	+	+	+	-	+	-	-
ISI2	+	+	+	+	-	+	-	-
ISI4	+	+	+	+	-	+	-	-
ISI7	+	+	+	+	-	+	-	-
ISI8	+	+	+	+	-	+	-	-
ISI9	+	+	+	+	-	+	-	-
ISI13	+	+	+	+	-	+	-	-
ISI14	+	+	+	+	-	+	-	-
ISI15	+	+	+	+	-	+	-	-
ISI16	+	+	+	+	-	+	-	-
ISI17	+	+	+	+	-	+	-	-
ISI20	+	+	+	+	-	+	-	-

Appendix B: Continues

Isolates	Catalase	Coagulase	Voges-Proskauer	Citrate	Indole	Hemolysis	Methyl Red	Oxidase
ISI25	+	+	+	+	-	+	-	-
ISI28	+	+	+	+	-	+	-	-
ISI29	+	+	+	+	-	+	-	-
ISI34	+	+	+	+	-	+	-	-
ISI37	+	+	+	+	-	+	-	-
ISI45	+	+	+	+	-	+	-	-

KEY: + (Positive) and – (Negative)

Appendix C: Antibiotic susceptibility test results in General Hospital Minna

Isolates	VA	AMC	AMP	AZM	CIT	E	OX
GSI24	I	R	S	S	R	S	R
GSI26	R	R	S	S	R	S	R
GSI27	R	R	R	S	R	S	R
GSI28	R	R	R	S	R	S	R
GSI36	R	R	R	S	R	S	R
GSI40	I	S	S	R	R	S	I
GSI41	R	S	S	R	R	S	R
GSI47	R	S	S	R	R	S	R
GSI59	R	S	S	R	R	S	R
GSI65	R	I	S	R	R	S	S
GSI69	S	R	R	S	S	S	R
GSI70	R	R	R	S	S	I	S
GSI71	R	R	R	S	S	I	R
GSI75	R	S	R	S	R	I	S

Appendix C: Continues

Isolates	VA	AMC	AMP	AZM	CIT	E	OX
GSI76	R	S	R	S	R	R	R
GSI79	S	R	R	R	S	R	S
GSI80	R	S	R	R	R	R	S
GSI83	R	I	S	S	S	S	R
GSI84	R	R	I	R	S	S	S
GSI85	R	I	S	R	S	S	R
GSI86	S	I	S	R	S	S	R
GSI93	S	R	R	S	I	I	R
GSI102	R	R	R	S	S	S	R
GSI105	I	R	R	S	S	S	R
GSI106	R	R	R	S	S	S	R
GSI112	R	R	S	S	R	S	S
GSI114	R	R	S	S	R	S	S
GSI115	S	S	R	S	S	S	S

Appendix C: Continues

Isolates	VA	AMC	AMP	AZM	CIT	E	OX
GSI116	R	R	I	S	R	S	S
GSI118	S	I	R	S	I	I	R
GSI120	R	R	S	S	R	R	R
GSI129	R	S	S	S	R	R	R
GSI138	R	S	S	I	R	S	R
GSI148	R	R	S	I	S	S	R
GSI150	S	S	S	S	S	S	R

KEY: VA (Vancomycin), AMC (Amoxicillin), AMP (Ampicillin), AZM (Azithromycin), CIT (Cefotetan), E (Erythromycin), OX (Oxacillin), S (Sensitive), I (Intermediate), R (Resistant).

Appendix D: Antibiotic susceptibility test results in IBB Specialis Hospital Minna

Isolates	VA	AMC	AMP	AZM	CIT	E	OX
ISI7	R	S	S	R	R	R	R
ISI8	R	S	S	S	R	R	R
ISI13	R	I	S	S	R	S	S
ISI16	S	S	S	S	R	S	S
ISI17	R	R	S	S	R	S	S
ISI18	S	R	R	S	R	R	S
ISI20	R	S	R	I	I	S	S

Appendix D: Continues

Isolates	VA	AMC	AMP	AZM	CIT	E	OX
ISI23	R	R	R	R	S	S	S
ISI28	I	I	R	R	S	S	S
ISI35	R	R	S	R	I	S	I

KEY: VA (Vancomycin), AMC (Amoxicillin), AMP (Ampicillin), AZM (Azithromycin), CIT (Cefotetan), E (Erythromycin), OX (Oxacillin), S (Sensitive), I (Intermediate), R (Resistant).

Appendix E: Nucleotide sequence of *Staphylococcus aureus* strain GHA10

GATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACGGACGAG
AAGCTTGCTTCTCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGATAA
CCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATA
ATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTGTCACTTAT
AGATGGATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGG
CAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGA
CACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGG
CGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGT
AAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGCACATCTTG
ACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTA
ATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTA
GGCGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTC
ATTGGAAACTGGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTG
TAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGAC
TTTCTGGTCTGTAACTGGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATT
AGATACCCTGGTAGTCCACGCCGTAAACGATGATGCTAAGTGTTAGGGGGT
TTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTC
GACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGA
CATCCTTGACAACTCTAGAGATAGAGCCTTCCCCTTCGGGGGACAAAGTGA
CAGGTGGTGCATGGTTGTCGTCAGCTCGGTCGTGAGATGTTGGGTTAAGTCC
CGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAGTTGGGCACTCT
AAGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCA
TCATGCCCCTTATGATTTGGGCTACACACGTGCTACAATGGACAATACAAAG
GGCAGCGAAACCGCGAGGTCAAGCAAATCCCATAAAGTTGTTCTCAGTTCG
GATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGTAATCGTAG
ATCAGCATGCTACGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCA
CACCACGAGAGTTTGTAACACCCGAAGCCGGTGGAGTAACCTTTTAGGAGC
TAGCCGTCGAAGGTGGGACAAATGATTGGGTG

Sample GSI17 is 99% identical to *Staphylococcus aureus* strain GHA10

Appendix E: Nucleotide sequence of *Staphylococcus aureus* strain SUSST48

CGGGCCGTGCTATACATGCAGTCGAGCGAACGGACGAGAAGCTTGCTTCTC
TGATGTTAGCGGCGGACGGGTGAGTAACACGTGGATAACCTACCTATAAGA
CTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATATATTTTGAACCGC
ATGGTTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGATCCGCGC
TGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCATAGC
CGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTC
CTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGG
AGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTA
GGGAAGAACATATGTGTAAGTAACTGTGCCTTGACGGTACCTAATCAGAAA
GCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCG
TTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTACATTAAGT
CTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAA
ACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATCGC
AGAGATATGGAGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGA
CGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATAGCCCTGGTAG
TCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGT
GCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTT
GAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTT
TAATTTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGAACA
ACTCTAGAGATAGAGCCTTCCCCCTTCGGAGGAAAAAGTGACAGGTGTGCA
TGAAGTCGTCAGCTCGTGTCAGTGAAGATGTGGTTAGTCCCGCAACGAGCG
CACCGTAGGCTAGTGCATCATTAGTGGCACTCTAGTGACTGCCGTGAACAAC
GGAAGAAGGTGGGATGACGTCATCATCATGCCCTTATGAAATGGGGGCTTA
ACACG

Sample GSI93 is 99% identical to *Staphylococcus aureus* strain SUSST48

Appendix E: Nucleotide sequence of *Staphylococcus aureus* strain pm37

GGAGCTATACATGCAGTCGAGCGAACAGATGAGAAGCTTGCTTCTCTGATG
TTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTACCTATAAGACTGGG
ATAACTCCGGGAAACCGGGGCTAATACCGGATAATATTTTGAACCGCATGG
TTCAATAGTGAAAGACGGTTTCGGCTGTCACTTATAGATGGACCCGCGCCGT
ATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGACGATACGTAGCCGA
CCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTA
CGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAG
CAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTGTTAGG
GAAGAACAAATTTGTTAGTAACTGAACAAGTCTTGACGGTACCTAACCAGA
AAGCCACGGCTAACTACGTGCCAGCAGCCGGTAATACGTAGGTGGCAAGCG
TTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTCTTAAGTCTGA
TGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGAACTGGGAAACTTGA
GTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAG
ATAGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAACTGACGCT
GATGTGCGAAAGCGTGGGGATAAACAGGATTAGATACCTGGTAGTCCACGC
CGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAG
CTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTC
AAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCG
AAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACCGCTCTAGA
GATAGAGTCTCCCCTTCGGGGGACAAAGTGACAGGTGGTGCATGGTTGTCG
TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTT
AAGCTTAGTTGCCATCATTAAGTTGGGCACTCTAGGTTGACTGCCGGTGACA
AACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGATTTG
GGCTACACACGTGCTACAATGGATAATACAAAGGGCAGCGAATCCGCGAGG
CCAAGCAAATCCCATAAAATTATTCCAGTTCGGATTGTAGTCTGCAACTCGA
CTACATGAAGCTGGAATCGCTAGTAATCGTAGATCAGCATGCTACGGTGAA
TACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAAC
ACCCGAAGCCGGTGGAGTAACCTTTTAGGAGCTAGCCGTCGAAGTGACAT

Sample GSI36 is 99% identical to *Staphylococcus aureus* strain pm37

Appendix E: Nucleotide sequence of *Staphylococcus aureus* strain SUSST48

GGAGCTATACATGCAGTCGAGCGAACAGATGAGAAGCTTGCTTCTCTGATG
TTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTACCTATAAGACTGGG
ATAACTCCGGGAAACCGGGGCTAATACCGGATAATATTTTGAACCGCATGG
TTCAATAGTGAAAGACGGTTTCGGCTGTCACTTATAGATGGACCCGCGCCGT
ATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGACGATACGTAGCCGA
CCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTA
CGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAG
CAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTGTTAGG
GAAGAACAAATTTGTTAGTAACTGAACAAGTCTTGACGGTACCTAACCAGA
AAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAG
CGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTCTTAAGTCT
GATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAAAC
TTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGCA
GAGATATGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAACTGA
CGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCTGGTAGTC
CACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGC
TGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGA
AACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTA
ATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACCGCTC
TAGAGATAGAGTCTCCCCTTCGGGGGACAAAGTGACAGGTGGTGCATGGTT
GTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA
CCCTTAAGCTTAGTTGCCATCATTAAGTTGGGCACTCTAGGTTGACTGCGGT
GACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGA
TTTGGGCTACACACTGCTACAATGGATAATACAAAGGGCAGCGAATCCGCG
AGGCCAAGCAAATCCCATAAAATTATTCTCAGTTCGGATTGTAGTCTGCAAC
TCGACTACATGAAGCTGGAATCGCTAGTAATCGTAGATCAGCATGCTACGG
TGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTG
TAACACCCGAAGCCGGTGGAGTAACCTTTTAGGAGCTAGCCGTCGAAGTGA
CAT

Sample GSI112 is 99% identical to *Staphylococcus aureus* strain SUSST48

Appendix E: Nucleotide sequence of *Staphylococcus aureus* strain pm37

CCATCCAGTCGAGCGAACGGACGAGAAGCTTGCTTCTCTGAAGTTAGCGGC
GGACGGGTGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACTTC
GGGAAACCGGAGCTAATACCGGATATATTTTGAACCGCATGGTTCAAAAGT
GAAAGACGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCATTAGCTAGT
TGGTAAGGTAACGGCTTACCAAGGCAACGATGCATAGCCGACCTGAGAGGG
TGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAG
CAGTAGGGAATCTTCCCCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGT
GAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATA
TGGGTAAGTAACTGTGCCTTGACGGTACCTAATCAGAAAGCCACGGCTAAC
TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATT
ATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTACATTAAGTCTGATGTGAAA
GCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCA
GAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATCGCAGAGATATGGA
GAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGC
GAAAGCGTGGGGATCAAACAGGATTAGATAGCCCTGGTAGTCCACGCCGTA
AACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAA
CGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAG
GAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTTCGAAG
CAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGAACAACTCTAGAGAT
AGAGCCTTCCCCCTTCGGAGGAAAAAGTGACAGGTGTGCATGAAGTCGTCA
GCTCGTGTCAGTGAAGATGTGGTTAGTCCCGCAACGAGCGCACCGTAGGCT
AGTGCATCATTAGTGGCACTCTAGTGACTGCCGTGAACAACGGAAGAAGGT
GGGATGACGTCATCATCATGCCCTTATGAAAAGGGCTAAC

Sample GSI114 is 99% identical to *Staphylococcus aureus* strain pm37