STUDIES ON RESISTANCE GENES ASSOCIATED WITH *Staphylococcus aureus* IN WOUND INFECTIONS AMONG PATIENTS ATTENDING MURTALA MUHAMMAD SPECIALIST HOSPITAL (MMSH), KANO

BY

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DECLARATION

I hereby declare that this work is the product of my research efforts undertaken under the supervision of Dr Usman Aliyu Dutsinma and has not been presented elsewhere for the award of a degree or certificate. All sources have been duly acknowledged.

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CERTIFICATION

This is to certify that this research work for this dissertation and subsequent write up [Faiza Musa with registration number SPS/13/MMB/00037] were carried out under my supervision.

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DEDICATION

I dedicate this work to my father Alhaji Musa Kallam Yusuf and my Mother Hajiya Khadija Suleiman Sambo and to Khadijah Ahmad(Aman)

I also dedicate this work to late Asma'u Abba Abdullahi (MCB class 2012), Late Hajiya AishaAlhaji Ali Mai Dara and to Late Hajiya Hafsa (Me man gelo) may their souls rest in peace.

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ABSTRACT

This study was carried out to determine the antibiotic susceptibility pattern of *Staphylococcus aureus* isolated from wounds and to study some of the resistance genes. The study was conducted on patients with wound infection attending Murtala Muhammad Hospital, Kano. A sterile swab stick was used to collect the sample before wound dressing and it was cultured on mannitol salt agar (MSA). Biochemical test was carried out to identify the *S. aureus* followed by MRSA screening using cefoxitin (30mcg) disc. Antibiotic susceptibility pattern of the isolates was determine using agar diffusion method. PCR was carried out to detect the resistant genes. Out of the 180 wound swabs collected *S. aureus* was isolated from 51 (28%), and all were sensitive to ciprofloxacin. Methicillin resistance *Staphylococcus aureus* (MRSA) was found to be 19.61%. There was no significant difference between *S. aureus* isolated among age groups ($P>0.05$). There was significant difference in *S. aureus* isolated between different wound type ($P< 0.05$). Five (9.8%) of the isolates were screened for the presence of *MecA* and *FemB* gene. *MecA* gene was found in 20% of the screened samples and *FemB* was also found in 20% of the screened samples. Occurance of *FemB* and *MecA* genes among the isolates should be considered as a menace that could cause havoc to effective chemotherapy of wound infections in the population studied.

CHAPTER ONE

1.0 INTRODUCTION

1.1. Background

The human skin is established to constitute the first line of defense against pathogens and disease elements. Consequently, a breach in this order translates to infiltration of the immune status of susceptible individuals (Bowler, *et al., 2001*). This results to wound and subsequently infection.

A wound is the disruption in the continuity of soft parts of the body structure (Singleton and Sainsburg1978; Torpy, *et al.,*2005). However, a wound is said to be infected when virulent factors expressed by one or more micro-organisms in a wound out-compete the host natural immune system and subsequently invade and disseminate microorganisms in various tissues thus provoking a series of normal and systemic host responses (Bowler, *et al.,* 2001).

Post-operative wound infections are the major global problem in the field of surgery leading to many complications, in-creased morbidity and mortality (Anguzu and Olila, 2007,; Raza, *et al.,* 2013). Most post-surgical wound infections are hospital acquired and vary from one hospital to another (Isibo *et al., 2008*). Lack of standardized criteria for diagnosis presents a challenge to monitor the global epidemiology of surgical site infection. In addition to this, emerging of high anti-microbial resistance among bacterial pathogens has made the management and treatment of post-operative wound infections difficult (Andhoga *et al.,* 2002). The situation is serious in developing countries due to irrational prescriptions of antimicrobial agents (Fadeyi *et al.,* 2008). In Ethiopia, different studies reported that the prevalence of post surgical wound infection ranges from 14.8% -60% (Taye 2005; Endalafer *et al.,*2011; Godebu *et al.,* 2013; Tesfahunegn*et al.,* 2009). *Staphylococcus aureus, Klebsiella* species*, E.coli, Proteus* species, *Streptococcus*

species, Enterobacter species, *Pseudomonas* species and Coagulase negative *Staphylococci* were reported as the most common pathogens (Godebu *et al.,* 2013). *Pseudomona.aeruginosa* is an epitome of opportunistic nosocomial pathogen, which causes a wide spectrum of infections and leads to substantial morbidity in immune compromised patients. Due to it s high drug resistance to many antibiotics, the mortality rate is substantial (Goswami *et al.,* 2011). Data on the spectrum of bacteria isolated from hospitalized patients and their antimicrobial susceptibility patterns to guide post operative wound infection in the region is scarce.

*Staphylococcus aureus*is a major cause of serious hospital and community-acquired infections associated with morbidity and mortality rates with rapid development of resistance (Al-Jumaily *et al.,* 2012). Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the highest ranking pathogens worldwide and represents a real challenge to the clinical practice with significant public health concern (WHO, 2011). The global problem of increasing trend in antimicrobial resistance is particularly pressing in the developing countries, where the MRSA is often the severe casual agent in hospital acquired infections (Madani *et al.,* 2001). *S. aureus* have been associated with a variety of diseases, such as skin and soft tissue infections, endovascular infections, pneumonia, endocarditis and septic shock (Liu *et al.,* 2011). These strains show resistance to a wide range of antimicrobials including vancomycin, the drug of last resort for MRSA infections (Relm, 2008). The introduction of methicillin in 1960s had an important impact on the treatment of infections caused by penicillinase producing *S. aureus* (Johnson, 2011). Methicillin resistant *Staphylococcus aureus* (MRSA) was first isolated soon after introduction of methicillin into clinical use in 1960 (Barber, 1961; Jevons, 1961). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious threat to hospitalized patients globally and it now represents a challenge for public health; as community associated infections appear to be on the increase in both adults and children in various regions and countries (Layton

et al., 1995). The overall incidence of MRSA isolation has gradually increased to reach levels of around 30% or more in some countries (Ayliffe, 1997). It was estimated that MRSA strains accounted for 84% of hospital-acquired *S. aureus* isolates and 45% of nonhospital acquired *S. aureus* isolates in Taiwan in 1998 (Ho *et al.,* 1999).

Resistance in MRSA is related to a chromosomal mec*A* gene that specifies the production of an abnormal penicillin binding protein called PBP2a or PBP21.Penicillin–binding proteins are membrane-bound enzymes, which targets for all β-lactam antibiotics.PBP2a has a decreased affinity for binding β-lactam antibiotics resulting in resistance not only to methicillin but also to all β-lactams including penicillins and cephalosporins (Weems 2001). The mec*A* gene complex also contains insertion sites for plasmids and transposons that facilitate acquisition of resistance to other antibiotics. Thus, cross-resistance to non-β-lactam antibiotics such as erythromycin, clindamycin, gentamicin, co-trimoxazole and ciprofloxacin is common (Chambers, 1979; Chambers, 2001).

1.2 Justification

Several cases of wound infections are being encountered where different pathogenic bacteria are implicated. *Staphylococcus aureus* is a virulent pathogen that is currently the most common cause of infections in wounds(Goswami *et al.,* 2011). The increase in the resistance of this virulent pathogen to antibacterial agents, coupled with its increasing prevalence as a nosocomial pathogen is of major concern considering the threat it poses to health. Therefore there is the need to determine some of the genes associated with its resistance.

1.3 Aim and Objectives

Aim

This research is aimed at detecting resistance genes of *Staphylococcus aureus* isolated from wound infections in patients at Murtala Muhammad specialist hospital Kano, Nigeria. This would be achieved through the following objectives

Objectives

1. To isolate *Staphylococcus aureus* involved in wound infections at Murtala Muhammad specialist hospital Kano.
2. To screen for Methicillin Resistant *Staphylococcus aureus* (MRSA).
3. To assess the antibiotic resistance pattern of the isolates.
4. To detect some of the resistance genes associated with antibiotic resistance using molecular techniques.

CHAPTER TWO

2.0 LITERATURE REVIEW

From a microbiological perspective, the primary function of normal, intact skin is to control microbial populations that live on the skin surface and to prevent underlying tissue from becoming colonized and invaded by potential pathogens (Bowler *et al.,* 2001). Exposure of subcutaneous tissue following a loss of integrity (i.e. a wound) provides a moist, warm, and nutritious environment that is conducive to microbial colonization and proliferation. However, the abundance and diversity of microorganisms in any wound would be influenced by factors such as wound type, depth, location and quality, the level of tissue perfusion, and the antimicrobial efficacy of the host immune response. Whereas the micro-flora associated with clean, surgical wounds would be expected to be minimal, the presence of foreign material and devitalized tissue in a traumatic wound is likely to facilitate microbial proliferation unless early prophylactic antibiotic treatment and surgical debridement is implemented (Robson, 1997).

Although it is estimated that 20–30% of the general human population are carriers of *Staphylococcus aureus,* this bacterium is one of the most important etiological agents responsible for healthcare-associated infections (Konrad *et al.,* 2009). Staphylococci are among the most robust microbes that infect humans. This and its propensity to develop antibiotic resistance establish this microbe as a major human pathogen (Campbell, 2002).

The most commonly affected part of the body due to *S. aureus* infection is the skin*(* (Lowy, 1998). More serious infections associated with *S. aureus* include endocarditis, mastitis, meningitis, osteomyelitis and pneumonia (Lowy, 1998; Bhatia and Zahoor,2007). *Staphylococcus aureus* has also been implicated in a number of acute food poisoning outbreaks world wide due to the production of the heat stable enterotoxin B that is pre-produced in food by

the bacterim (Leloir *et al*., 2003). Various other diseases can be linked to *S. aureus* specific toxins including Staphylococal scalded skin syndrome (SSSS) and toxic shock syndrome (TSS) (Slayers and whitt,2002).

Other species such as *Staphylococcus epidermidis* cause infections associated within dwelling medical devices (Vadyvaloo and Otoo, 2008). *Staphylococcus saprophyticus* causes urinary tract infections commonly associated with young girls (Horowitz and Cohen,2007).

*Staphylococcus aureus*is a coloniser of the nasal passages, causing skin infections, which range from boils, furuncles and impetigo to more serious complications such as endocarditis, scalded skin syndrome, surgical wound infections and toxic shock syndrome (Prescott*et al.*,2002).

2.1 Types of wound

Robson *(*1997) outlined different types of wound as follows;

a. Incision;

An incision wound is a cut on the skin caused by a sharp object such as a knife, broken glass, scissors or surgeon scalpel. Incision wounds are neat and the edges of the skin are usually smooth. Incisions tend to bleed freely because the blood vessels are cut cleanly and without ragged edges. There is little damage to the surrounding tissues. Of all classes of wounds, incisions are the least likely to become infected, since the free flow of blood washes out many of the microorganisms that cause infection (Eyad, 2014).

b. Laceration;

A laceration is injury to skin that results in the skin being cut or torn open, which can be shallow only injuring the surface skin, or deep, causing injury to the muscles, tendons, ligaments, blood vessels or nerves. Lacerations are most commonly caused a sort of blunt trauma such as

being hit with a fist or baseball bat. The difference between an incision wound and a laceration wound is that a laceration is usually jagged, since the skin is torn instead of cut.

c. Abrasion;

An abrasion is a type of wound in which the skin is scraped pr rubbed off. When skin is dragged on carpet, the resulting wound, often called a carpet burn, is an abrasion. Abrasions are usually superficial wounds, meaning that only the outer layers of the skin are affected. A deep abrasion, one that penetrates to the inner layer of skin, can leave a scar. Parts of the body with thin layers of skin such as the knee and elbow are most prone to abrasions (Robson,1997).

d. Contusion;

A contusion is a kind of closed wound, meaning that the skin is not broken. Contusions are caused by blunt force trauma to the skin that results in tissue damage. When the blood vessels under the skin are broken, blood pools under the skin causing a bruise (Robson,1997).

e. Puncture;

A puncture wound is created when a sharp object enters the skin. These wounds are usually small and do not bleed a lot. Although these wounds tend to close over quickly, they still need treatment as infection is a possibility. Puncture wounds are prone to tetanus infection, so it is important to seek medical advice for any puncture wound. Common types of puncture wounds include stepping on a nail or bites from animals (Robson,1997).

f. Avulsions

An avulsion is the tearing away of tissue from a body part. Bleeding is usually heavy (Eyad, 2014).

g. Amputations

A traumatic amputation is the nonsurgical removal of the limb from the body. Bleeding is heavy (Eyad, 2014).

h. Chronic wounds

Chronic wounds are most commonly caused by endogenous mechanisms associated with a predisposing condition that compromises the integrity of dermal and epidermal tissue. Pressure or decubitis ulcers have a different etiology from other chronic wounds in that they are caused by sustained external skin pressure (Robert, 2013).

2.2 Wound Infection

Staphylococcus aureus is the major causative agent of wound infection (Basak *et al.,* 1992; Adebayo *et al.,* 2003). Wound infection is not a modern phenomenon. As early as 14-37AD there was documentary evidence that Cornelius Celsus (a Roman physician) described the four principal signs of inflammation and used antiseptic solutions. Another Roman physician, Claudius Gelen (130-200 AD) had such an influence on the management of wounds that he is still thought of by many today as the father of surgery. It should also be remembered that he and some of his followers instigated the laudable plus theory, which incorrectly considered the development of pus in a wound as a positive part of healing process (Bibbings, 1984).

Surgical site infection is a type of healthcare-associated infection in which a wound infection occurs after an invasive (surgical) procedure. Other types of healthcare-associated infections that mainly affect surgical patients are postoperative respiratory and urinary tract infections,bacteraemias (including methicillin-resistant *Staphylococcus aureus* infections and

intravascular cannula infections) and antibiotic-related diarrhoeas (particularly *Clostridium difficile* enteritis).

Surgical site infections have been shown to compose up to 20% of all of healthcare-associated infections. At least 5% of patients undergoing a surgical procedure develop a surgical site infection (National Institute For Health And Clinical Excellence,2009).

Post-operative wound infections are major global problem in the field of surgery leading to many complications, in-creased morbidity and mortality (Anguzu and Olila, 2007; Raza *et al.,* 2013). Most post-surgical wound infections are hospital acquired and vary from one hospital to the other (Isibo *et al.,* 2008). Lack of standardized criteria for diagnosis presents a challenge to monitor the global epidemiology of surgical site infection. In addition to this, emerging of high anti-microbial resistance among bacterial pathogens has made the management and treatment of post-operative wound infections difficult (Andhoga *et al.,* 2002). The situation is serious in developing countries due to irrational prescriptions of antimicrobial agents (Fadeyi *et al.,* 2008). In Ethiopia, different studies reported that the prevalence of post surgical wound infection ranges from 14.8% -60% (Taye, 2005; Endalafer*et al.*, 2011; Godebu *et al.,* 2013; Tesfahunegn*et al.,* 2009). *S. aureus, Klebsiella* species, *Eschericia coli*, *Proteus* species, *Streptococcus* species, *Enterobacter* species, *Pseudomonas* species and Coagulase negative *Staphylococci* were reported as the most common pathogens (Godebu *et al.,* 2013). *Pseudomonas aeruginosa* is an epitome of opportunistic nosocomial pathogen, which causes a wide spectrum of infections and leads to substantial morbidity in immune compromised patients. Due to its high drug resistance to many antibiotics, the mortality rate is substantial (Goswami*et al.,* 2011). It is known that specific therapeutic options to patients with post surgical wound infections mainly depend on data from antimicrobial susceptibility tests generated by clinical

laboratories or sound epidemiological data from ongoing nosocomial infection surveillance (Mulu *et al .,* 2006).

S. aureus was the most prevalent isolate from infected wounds according to different findings. According to Reiye*et al*. (2014), in a research conducted in Ethiopia, it was having a prevalence of 35.77%. This was in line with previous surveillance conducted in Ethiopia (Godebo *et al.,* 2013), Uganda (Anguzu and Olila, 2007), India (Amrita *et al.,* 2010) and Nigeria (Sani *et al.,* 2012). The normal flora nature of *S. aureus* in the skin and anterior nares, which can enter to deep site during surgery of the natural barrier of the skin, could be the possible justification for its high prevalence (Anguzu and Olila, 2007, Bowler *et al.,* 2001).

2.3.1 Antibiotic Resistance

The spread of antibiotic resistance pathogen is one of the most serious threats to public health in the twenty-first century (Willey *et al.,* 2011).

Antibiotic have been effective in controlling many of the diseases that have been a scourge to humankind (Jerome and James, 1997).

Tuberculosis, bacterial pneumonia, syphilis and many other infectious diseases that were once fatal can now generally be treated with antibiotics. Antibiotics have been called “wonder drugs” because they affect a dramatic cure for what had previously been incurable. But there is a problem of “wonder drugs” that became evident after widespread use (Jerome and James, 1997). The application of penicillin as a chemotherapeutic agent led to the evolution of pathogenic bacteria strains that were unaffected by the drug. These resistant organisms retained their potent pathogenicity but were no longer controlled by the administration of penicillin. For example, when penicillin G was first introduced, virtually all strains of *Staphylococcus aureus* were sensitive to the drug. Within a span of only ten years, essentially all staph

infections acquired in hospitals were caused by strains that were resistant to penicillin (Jerome and James, 1997).

2.3.2 Cause of antibiotic resistance

Increase in antibiotic resistance in bacteria is largely due to the widespread use of antibiotic in medicine, in animal care and in agriculture. Drugs frequently have been overused. It has been estimated that over 50% of the antibiotic prescriptions in hospitals are given without clear evidence of infection or antibacterial drugs to patients with colds, influenza, viral pneumonia, and other viral diseases (Willey *et al.,* 2011).

Frequently antibiotics are prescribed without culturing and identifying the pathogen or without determining bacteria sensitivity to the drug. Toxic broad spectrum antibiotic are sometime given in place of narrow-spectrum antibiotic as a substitute for culture and sensitivity testing, with the consequent risk of dangerous side effects, opportunistic infections, and the selection of drug-resistant mutants (Willey *et al.,* 2011).

The situation is made worse by patients not completing their course of medication. When antibiotic is ended too early, drug resistant mutant may survive (Willey *et al.,* 2011).

The use of antibiotics in animal feeds is undoubtedly another contributing factor to increasing drug resistance. The addition of low levels of antibiotics to livestock feeds raises the efficiency and rate of weight gain in cattle, pigs, and chickens. However, treatment with low doses of antibiotics over extended periods of time selects for resistant strains of bacteria. These resistant strains may then be spread to humans through contact with the animals or through consumption of undercooked meat (Willey *et al.,* 2011).

Another cause of antibiotic resistance is the use of antibiotic on agricultural product. Such as the spraying of an aerosol form of antibiotics on fruits orchards (Levy, 1998).

The dosage is sufficiently high to kill all the bacteria on the trees when they are sprayed. However low dosage of the aerosol sprayed reaches the plants other than the targets. The low dosage creates selective pressure for resistant bacteria, promoting the growth of resistant strains on these other plants. The resultant antibiotic resistant bacteria may contaminate the human food chain, ultimately ending up in the human digestive system where a problematic infection may occur (Levy, 1998).

Additionally, small amounts of antibiotic spray may form a lasting residual layer on the treated fruit, killing sensitive bacteria but allowing bacteria with a resistance advantage to slowly proliferate and end up in the human food chain (Levy, 1998).

2.3.3 Strategy of Resistance to Antibiotics by Bacteria

Bacteria has developed several strategies to resist antibiotic

- Natural resistance: the bacteria may lack the structure that the antibiotic inhibits, as occurs with mycoplasma, which lacks cell wall and is thus unaffected by penicillin because it cannot penetrate the bacterial outer membrane (Jerome and James, 1997).

A decrease in permeability can lead to sulfonamide resistance. Mycobacteria resist many drugs because of the high content of mycolic acids in a complex lipid layer outside their peptidoglycan. This layer is impermeable to water-soluble drugs (Willey *et al.,* 2011)

- Pumping of the drug out of the cell after it has entered; some bacteria have plasma membrane translocase. E.g. *Escherichia coli, Pseudomonas aeruginosa* and

Staphylococcus aureus. It is often called efflux pumps that expel drugs. Because they are relatively non specific and pump many different drugs, these transport protein often are multidrug resistance pumps. Many are a drug/proton antiporter, that is, proton enters the cell as the drug leaves (Willey *et al.,* 2011). the emergence of resistance could be through:

i. Acquired resistance: A resistant bacterium may produce a substance that inactivates the antibiotic, as occurs with *Staphylococcus aureus* in producing the enzyme penicillinase which disrupts the penicillin molecule (Jerome and James, 1997).

A gradual accumulation of mutations in chromosomal DNA may result in cellular structures that will not bind the antibiotic. For example the gene for transpeptidase synthesis in *Staphylococci* can mutate so that the enzyme does not bind penicillin (Jerome and James. 1997).

ii. Use of alternate pathway: resistant bacteria may use alternate pathway to bypass the sequence inhibited by the agent or increase the production of the target metabolite. For example, some bacteria are resistant to sulfonamides simple because they use performed folic acid from their surrounding rather than synthesize it themselves. Other strains increase their rate of folic acid production and thus counteract sulfonamide inhibition (Willey *et al.,* 2011).

2.3.4 Criterion for Testing Antimicrobial Susceptibility of Bacteria

Antimicrobial susceptibility test are used to study the inhibition of a test organism by one or more antibiotics or chemotherapeutic agents. Two general methods are in use: the tube dilution method and the agar disk diffusion method (filter paper method) (Edward, 2001).the methods are:

i. **Tube dilution method**: this determines the smallest amount of antibiotic necessary to destroy a population of a test organism. This amount is

known as the Minimum Inhibitory Concentration (MIC). To determine the MIC, the microbiologist prepares a set of tubes with different concentrations of a particular antibiotic. The tubes are then inoculated with an identical population of the test organism, incubated, and examined for the growth of bacteria. The extent of growth diminishes as the concentration of antibiotic increases, and eventually an antibiotic concentration is observed at which growth fails to occur. This is the MIC (Edward, 2001).

ii. **Agar disk diffusion method or the filter paper method:** this is probably the most widely used; it is also known as the *Kirby-Bauer test.* A Petri plate containing an agar medium is inoculated uniformly over its entire surface with a standardized amount of a test organism. Next filter paper disks impregnated with known concentration of chemotherapeutic agents are placed on the solidified agar surface. During incubation, the chemotherapeutic agents diffuse from the disks into the agar. The farther the agent diffuses from the disk, the lower its concentration. If the chemotherapeutic agent is effective, a zone of inhibition forms around the disk of the disk after a standardized incubation. The diameter of the zone can be measured; in general, the larger the zone the more sensitive the microbe is to the antibiotic(Gerard, 2004).

2.4 Antimicrobial Resistance of *S. aureus* Strains

Staphylococcus aureus causes the greatest apprehension as a pathogen because of the intrinsic virulence that it has and the ability to rapidly adjust to different environmental conditions (lowy,1998). The trend of multidrug resistance in *S. aureus* is particularly alarming

because of the severity and diversity of diseases caused by this pathogen (Waldvogel ,2000).Despite the availability of novel drugs as an approach to staphylococcal theraphy,the bacteria seem to be able tp rapidly develop resistance to these drugs (Diekema *et al* .,2004). Perhaps the most commonly known resistance of*S. aureus* , is methicillin resistance, which has caused alarming reports with regard to the spread of *S. aureus* in hospitals and the community (Kowlski *et al.,* 2003)

Chromosomes or plasmid can mediate antibiotic resistance in *S. aureus* through various mechanism, including transduction and conjugation (Chambers, 1997). Although the mechanism of methicillin resistance in *S. aureus* is partly understood , there have been reports of low – level methicillin resistance in *mec A* negative strains of *S. aureus* (Unal *et al* .,1994). These *mec*A negative MRSA strains possibly arose from the hyper-production of beta-lactamase (McDougal and Thornsberry, 1986).

2.5 Methicillin Resistant *Staphylococcus aureus* (MRSA)

Methicillin a semi synthetic penicillin was introduced in 1960s as an alternative to penicillin therapy for the treatment of *S. aureus* infection (Chambers, 2001). Methicillin resistant *Staphylococcus aureus* (MRSA) was first isolated soon after introduction of methicillin into clinical use in 1960 (Terry *et al.,* 2011).

Community associated outbreaks of MRSA infection have occurred among prisoners, intravenous-drug users, athletes, military trainees, and men who have sex with men (Kazakova *et al., 2005 and* Zinderman *et al.,* 2004).

Methicillin resistant *Staphylococcus aureus* identification is based on phenotypic and genotypic investigation (Fluit *et al.,* 2001). Phenotypic identification of *S. aureus*includes gram-staining, catalase , coagulase, culture on mannitol salt agar (Waldvogel,2002). Upon

identifying *S. aureus* by Gram-staining (Gram-positive cocci), catalase (positive) and tube coagulase (positive), the sample is grown on mannitol salt agar at 37^{o}C for 24h (Brown *et al* .,2005).the colonies appear yellow on mannitol salt agar (Brown *et al.,* 2005) .Staphylococcus aureus colonies are subjected to antimicrobial susceptibility testing by the Kirby Bauer disk diffusion method (Brown *et al.,* 2005).

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been commonly reported to be one of the commonest causes of nosocomial infections worldwide. Also, recent reports describe methicillin-resistant *S. aureus* (MRSA) carriage in persons in the community (Adebola *et al.,* 2005). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious threat to hospitalized patients globally and it now represents a challenge for public health; as community-associated infections appear to be on the increase in both adults and children in various regions and countries (Layton *et al.,* 1995). The overall incidence of MRSA isolation has gradually increased to reach levels of around 30% or more in some countries (Ayliffe, 1997). It was estimated that MRSA strains accounted for 84% of hospital-acquired *S. aureus* isolates and 45% of non-hospital acquired *S. aureus* isolates in Taiwan in 1998 (Ho *et al.,* 1999). At present, MRSA infections have a higher frequency than methicillin-susceptible *S. aureus* (MSSA) infections in some settings (Konrad *et al.,* 2009). MRSA was accountable for 59% of skin and soft tissue infections diagnosed in eleven emergency departments in the United States (Okuma *et al.,* 2002). Moreover, MRSA accounted for 59.5% of all S. aureus infections in in-tensive care units patients in 2004 (Rice, 2006). Also, the occurrence of MRSA infections outside health -care facilities, in the community, more than doubled between 2002 and 2004. The increasing rate of MRSA infections has shifted chemotherapy away from β-lactam antibiotics toward antibiotics more effective against MRSA, such as vancomycin and daptomycin (Berger-Bachi *et al.,* 1992). Moreover, very low-level resistant MRSA strains are dangerous since they

can evade standard phenotypic detection while they appear phenotypically susceptible. These strains still carry the *mecA* determinant and express resistance heterogeneously and upon β-lactam exposure they are able to segregate highly resistant subpopulations at a frequency well above spontaneous mutation rate (Ender *et al.,* 2008).

2.6*mecA* (gene)

The *mecA* gene is a gene found in bacterial cells. The *mecA* gene allows a bacterium to be resistant to antibiotics such as methicillin, penicillin and other penicillin-like antibiotics (Ubukata *et al.,* 1989). The *mecA* gene does not allow the ringlike structure of penicillin-like antibiotics to bind to the enzymes that help form the cell wall of the bacterium (transpeptidases), and hence the bacteria is able to replicate as normal. The gene encodes the protein PBP2A (penicillin binding protein 2A). PBP2A has a low affinity for beta-lactam antibiotics such as methicillin and penicillin. This enables transpeptidase activity in the presence of beta-lactams, preventing them from inhibiting cell wall synthesis (Deurenberg and Stobberingh, 2009).

2.7*Fem B* (gene)

Fem B catalyses the formation of the pentaglycine interpeptide bridge, which is the characteristic of the *S. aureus* peptidoglycine bridge (Uniport, 2002). Since cross linking between the peptide strands is critical for maintaining stability of the cell wall, *Fem B* is a potential target for the development of new antibacterial agents (Uniport, 2002).*Fem B* is a factor essential for expression of methicillin resistance (Hoffman and Valencia, 2004).*Fem B* gene encode proteins which influence the level of methicillin resistance of *S. aureus* (Kobayashi *et al.,*1994). Although *fem B* genes are detectable only in *S. aureus,* absence of *fem B* gene dosnt mean the isolate is not *S. aureus* as shown in the work of Kobayashi *et al,*(1994) where three percent of the samples were negative for *Fem B*. Harleen *et al.* (2012) also recorded the absence of *fem B* in 27% of the samples despite being recomfirmed as *S. aureus* phenotypically. The in activation of

fem B by insertion of Tn551 in the central part of the *fem B* was shown to increase susceptibility of methicillin resistance strains towards beta lactam antibiotics (Uniport, 2002).

2.8 Polymerase Chain Reaction

Any attempt to document the development of the polymerase chain reaction will encounter nearly as much myth as science. The strict fact, at least as reiterated in the literature, is that the polymerase chain reaction was conceptualized and operationalized by Kary Mullis and colleagues at Cetus Corporation in the early 1980's (Saiki, 1985). The method was first formally presented at the American Society of Human Genetics Conference in October of 1985 and the first clinical application for PCR, an analysis of sickle cell anemia, was published the same year (Saiki,1985). In its initial form, PCR was tedious and labor intensive. However, the advent of a method by which a specific DNA sequence could be isolated from its genomic context and amplified virtually without limit would not long remain a tool of graduate student and post-doc abuse. The breakthrough came with the isolation and purification of thermostable DNA polymerases (Lawyer *et al.,* 1989). This allowed for PCR to be automated and soon the first programmable PCR thermal cyclers appeared on the market. Since that time, PCR has spread to literally every corner of the world and to every conceivable aspect of biology and chemistry. So profound was the impact of PCR that Kary Mullis was awarded the 1993 Nobel Prize in Chemistry, not even ten years after its introduction (Integrated DNA Technologies, 2011) .

2.9 The PCR Reaction Components

Despite the numerous variations on the basic theme of PCR, the reaction itself is composed of only a few components as outlined by the Integrated DNA Technologies (2011). These are as follows:

1. Water : while it may seem trivial, water can be a source of concern and frustration. Water is present to provide the liquid environment for the reaction to take place. It is the matrix in which the other components interact.
2. PCR: Buffer This reagent is supplied with commercial polymerase and most often as a 10x concentrate. The primary purpose of this component is to provide an optimal pH and monovalent salt environment for the final reaction volume.
3. $MgCl_2$: Many commercially supplied PCR buffers already contain magnesium chloride(MgCl2). MgCl2 supplies the Mg++ divalent cations required as a cofactor for Type II enzymes, which include restriction endonucleases and the polymerases used in PCR. The standard final concentration of this reagent for polymerases used in PCR is 1.5mM.
4. Deoxynucleotide Triphosphates (dNTPs): The purpose of thedNTPs is to supply the "bricks." Since the idea behind PCR is to synthesize a virtually unlimited amount of a specific stretch of double-stranded DNA, the individual DNA bases must be supplied to the polymerase enzyme. This much is obvious. What might not be as obvious is the fact that the PCR reaction requires energy. The only source of that energy is the β and γ phosphates of the individual dNTPs.
5. Target DNA : The quality and quantity of the target DNA is important. The phrase "garbage in-garbage out" is apt. The DNA used as the PCR target should be as pure as possible but also it should be uncontaminated by any other DNA source. The PCR reaction does not discriminate between targets. That is, DNA is DNA is DNA as far as the reaction is concerned. Thus care must be taken to ensure that the target DNA only contains the target of interest (Integrated DNA Technologies, 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1.1 Hospital as Source of Patients for the Study

Murtala Muhammad specialist hospital Kano Nigeria was the source of the samples. The hospital is located within the centre of Kano metropolitan area. It handles most referral cases from the fourty four local governments of the state and neighbouring states.

3.1.2 Study population

The study population consisted of patients who have under-gone surgery and develop surgical site infections (SSI), those with burnt wounds, leg ulcer, chronic oesteomyelities and soft tissue wounds.

3.1.3 Sample Size

Sample size is 180 and was obtained using the formular for minimum sample size

$N=z\ pq/d$

Where;

N= minimum sample size

z= point of normal distribution curve equivalent to 95% confidence interval

q= complementary probability of q= 1-p

p= prevalence rate from previous work = 12%

d= degree of precision margin of error = 0.05

3.1.4 Ethical Clearance

An ethical approval was obtained from Kano state Hospital management board (Appendix 1).

3.2 Sample Collection

Samples were collected from 29th June, 2015 to 2nd November, 2015. A total of 180 samples were collectedFrom patints with wound infection attending Murtala Muhammad Specialist Hospital Kano.Twenty five (13.89%) samples were collected from patients between the age group of 0-9years. Twenty eight (15.56%) samples were collected from patients between the age group of 10-19years. Fifty samples (27.78%) were collected from patients between the age group of 20-29years. Thirty three (18.33%) samples were collected from patients between the age group of 30-39years. Fifteen samples (8.33%) were collected from patients between the age group of 40-49years. There were 18 (10%) samples were collected from patients between the age group of 50-59years. Eleven (6.11%) samples were collected from patients between the age group of 60years and above The samples were collected from patients before wound dressing. A sterile swab stick was rotated on the infected wound.

3.2.1 Media preparation

All media used were prepared according to manufacturer's instructions. They are:

Nutrient Agar (NA)

Mannitol Salt Agar (MSA)

Mueller Hilton agar (MHA)

3.2.2 Innoculation and Isolation of Pure Culture

The manitol salt agar (MSA) plates were dried in an oven to remove moisture. The streak plate method was used according to Cheesbrough (2006). The swab stick was rotated at a point on the MSA plate to make a pool and it was then streaked on the plate in a zigzag pattern.

3.3 Preservation of the Test Organisms

Nutrient agar slants were prepared as instructed by the manufacturer, autoclaved at 121^0C for 15 minutes. These were allowed to dry in slanted position and stored at 4^0C. The test organism was inoculated on the surface of the slant using a sterile wire loop it was incubated at 37^oc for 24 hours. This was stored at 4^0C.

3.4 Biochemical test

Biochemical test are used for the identification of bacteria specie based on differences in the biochemical of different bacteria. The following biochemical test were carried out.

3.4.1 Gram Staining

The test organism was placed on a drop of normal saline contained on a centre of clean microscopic slide using a sterile wire loop, to make a thin smear. The smear was allowed to air dry. The slide was passed three times over the flame with the smear side upper most to heat fix the preparation to the slide (Cheesbrough, 2006).

The smear was covered with crystal violet for 60 seconds and then washed off with clean water, all the water is tipped off and the smear is covered with Lugol's iodine for 60 seconds. It was poured off and washed with water. It was decolorized rapidly with ethyl-alcohol and washed immediately with clean water. It was counterstained with safranin. The smear was covered with clean water. The back of the slide was wiped and placed on a draining rack for the smear to air-

dry .The slide was examined microscopically using x 100 objectives and the cells appear as gram positive cocci (Cheesbrough, 2006). Three to 5 colonies having similar morphological appearance (such as, elevation, shape, texture e.t.c) of the bacteria to be tested was picked up by a sterile wire loop by just touchingthe center and emulsified in 3ml of sterile saline (El-Mishad, 2010).

The density of the bacterial suspension is adjusted by matching it to turbidity standard of 0.5 barium chloride (McFarland's).Whenever growth is scanty inocula was added. Too much turbidity is adjusted by the additions of sterile physiological saline.

3.4.2 Catalase Test

Catalase production by the test organism was detected by picking the colony with the edge of clean microscopic slide and immersing it on drop of 3% hydrogen peroxide contained at the center of a clean microscopic slide.

Staphylococcal specie shows active bubbling while non staphylococcal specie does not show bubbling.

3.4.3 Coagulase Test

A drop of distilled water was placed on each end of a slide. A colony of the test organism was emulsified in each of the two drops and thick suspensions were made. A loopful of plasma was added to one of the suspensions, gently mixed and observed for clumping within ten seconds. Presence of clump indicates positive (i.e. conversion of fibrinogen to fibrin) and absence indicates the reverse (Cheesbrough, 2006).

3.5 Preparation of Inocula

Subcultures were made by carefully picking one colony using sterile inoculation loop, this was streaked on the surface of nutrient agar for confluent bacterial growth appears on the medium.

Such pure cultures were used for identification and antibiotic sensitivity tests (EI-Mishad, 2010). This was done so as to obtain fresh growth of the organism that are at the logarithmic/experimental phase of growth in which state the physiological activities of the test organism arc best modified (El-mishad, 2010).

3.6 Bioassay Procedure

Following identification of the bacterial isolate, a standard disc diffusion technique for drug susceptibility test (DST) using commercially prepared antibiotic disc was done.

Agar plates were dried in the hot air oven until no visible excess moisture is observed on the surface. A sterile swab stick was dipped in the suspension of the prepared inoculums and then excess fluid was removed by pressing and rotating it against the side of the tube above the level of the suspension. The swab was streaked evenly over the surface of the medium in three directions, whilst rotating the plate to ensure even distribution (El-Mishad, 2010).

With the Petri dish lid in place, the surface of the agar was allowed to dry for 3-5 minutes. A sterile forceps was used to place the antibiotic discs on the surface of the inoculated plate .the antibiotics are : Augumentin (10mcg)Erythromycin (30mcg)Amplicillin (30mcg) Chloramphenicol (30mcg) Septrin (30mcg)Ampiclox (20mcg)Cefoxitin (30mcg)Pefloxacin (10mcg)Gentamycin (10mcg)Levofloxacin (20mcg)Ciproflox (10mcg).

Plates were incubated at 37°C for 24 hours and then examined for the presence of zones inhibition of bacterial growth around antibiotic disc. According to El - Mishad (2010), they were measured bya ruler on the underside of the plate and the zones were interpreted according to Clinical and Laboratory Standard Institute (2015)

3.7 MRSA Screening

Screening for MRSA as done using the method recommended by the Clinical and Laboratory Standard Institute (CLSI) (2015). A suspension of each isolate as made so that the turbidity was

equal to 0.5 McFarlands Standard and then plated onto Mueller Hilton agar plate. A 30mcg cefoxitin disc was applied to each plate. After incubation for 24 hours zones were measured using meter rule.

3.8 Molecular Probe to Detect Resistance Gene

3.8.1 DNA Extraction

Bacterial DNA was extracted from overnight cultures of*S. aureus* at 37^{o}C isolated from wound swabs.PBS buffer (1000µl) was added to slant containing the bacteria using a pippet, and a plastic wire loop was used to emulsify the growth which was then emptied into eppendorf tube. The suspension(500µl) was pipetted and kept as back up. The eppendorf tube was then centrifuged at 14,000 rpm for 5minutes at 4^{o}C in a cold centrifuge and the supernatant was then discarded.Lyses buffer (400µl) was then added to the tubes and 25µl of proteinase K was then added and the mixture was vortexted and incubated at 65^{o}C for 1hour vortexting at 15minutes interval.Phenol chloroform (400µl) was then added and it was vortexted and centrifuged at 13,000rpm for 10 minutes. The supernatant was pippeted into a new tube and chloroform (400µl) was added and it was centrifuged at 13,000rpm for 5minutes and the supernatant was transferred into a new tube.Absolute ethanol(1000µl) was added followed by 40µl of three molar sodium acetate The tubes were then stored at -20^{o}C over night.The tubes were then centrifuged for 10minutes at 13000rpm at 4^{o}C and the supernatant was discarded. Then, 400µl of 70% ethanol was then added to the tubes and centrifuged for 10minutes at 13000rpm at 4^{o}C and the supernatant was discarded.It was centrifuged again for two minutes and a pipette was used to remove the remaining ethanol and it was allowed to dry at room temperature. Pure water (40µl) was added to the dried tubes.

The primers were reconstituted by adding 400µl of distilled water to the primer this was done in a sterile pcr hood.The mixture was then vortexted and the primers were diluted by adding 5µl of the primer to 45µl of pure water.The primers used for the detection of *mecA* gene were: *mecA* 1-5′ GAA ATG ACT GAA CGT CCG ATA A 3′ (25 nucleotides) The *mecA* 2- 5′ CCAATT CCA CAT TGT TTC GGT CTAA 3′ (25 nucleotides) yielding 300 bp, and *femB* gene was detected using the primers *femB* 1- 5′ TTA CAG AGT TAA CTG TTA CC 3′ (20nucleotides) *femB* 2- 5′ ATA CAA ATC CAG CAC GCT CT 3′ (20nucleotides) yielding a 700 bp PCR product.

3.8.2 PCR Amplification

One micro litre of each primer was added to the hot start, two micro litre of the DNA extracted was also added, 14µl of pure water was also added to the hot start.It was then centrifuged for few seconds using micro centrifuge and it was centrifuged for 30 seconds.It was then placed in a pcr machine with the following conditions:-

1. The machine was set at 94ºC for one minute. This was done to denature the target DNA so as to make it single-stranded and open up the complementary sequences of the primers.
2. The primer annealing temperature was set at 47ºC for 1 minute. The melting temperature of the primers determines this temperature.
3. The polymerase extension temperature was set to 72^0Cand the duration was 1 minute for 40 cycles.

Electrophoresis was done to detect the resistance genes.Gel was prepared according to manufacturer's instruction by adding 1.5g to 100ml of 1×TEA buffer and it was heated in a microwave until it melt.It was then allowed to cool for some minutes and ethidium bromite was

then added to it.It was then poured in a cast and form was put into the well to create wells, it was then allowed to solidify.DNA ladder was put in one of the wells and the samples were also loaded.It was switched on and the timing was set.The gel was then placed in a gel doc which was connected to a monitor and the bands were checked.

3.9 Statistic Analyses

Statistic analyses was done using Epi info version 7.chi – square test at 0.5 probability to detect significance difference in *S. aureus* isolated between different wound types (Appendix II) and between different age groups (appendix III)

CHAPTER FOUR

4.0 RESULTS

Out of the 180 wound swabs collected *Staphylococcus aureus* was isolated from 51 (28.3%) 18 samples (35.3%) were isolated from soft tissue would which constituted the highest sample size, surgical wound made up 27.5% (14) of the *Staphylococcus aureus* isolated followed by leg ulcer which constituted 15.7% (8), burn wound constituted 13.7% (7) and oesteomyelities constituted 7.8% (4) of the *S. aureus* isolated. One hundred and four (104) 57.8% were collected from male patients and 76 (42.2%) were collected from females.*MecA* gene was seen in only one (20%) of the five samples screened and also *femB* is seen in only one (20%) of the five samples screened.

Occurrence of *S. aureus* Based on Wound Type

In Table 1,91 (50.55%) samples were collected from soft tissue wound and *S. aureus* was isolated from 18 (35.29%) of them. Twenty one (11.67%) samples were collected from burn wound and *S. aureus* was isolated from 7(13.73%) of them. Thirty nine (31.67%) samples were collected from surgical wound and *S. aureus* was isolated from 14 (27.45%) of them. Nineteen (10.55%) samples were collected from leg ulcer wound and *S. aureus* was isolated from 8 (15.69%) of them. Ten (5.55%) samples were collected from oesteomyelities wound and *S. aureus* was isolated from 4 (7.84%) of them. Figure 1 is a bar chart showing the samples collected from different wound types. Significant difference ($P<0.05$) was seen in *Staphylococcus aureus* in different wound types (Appendix II).

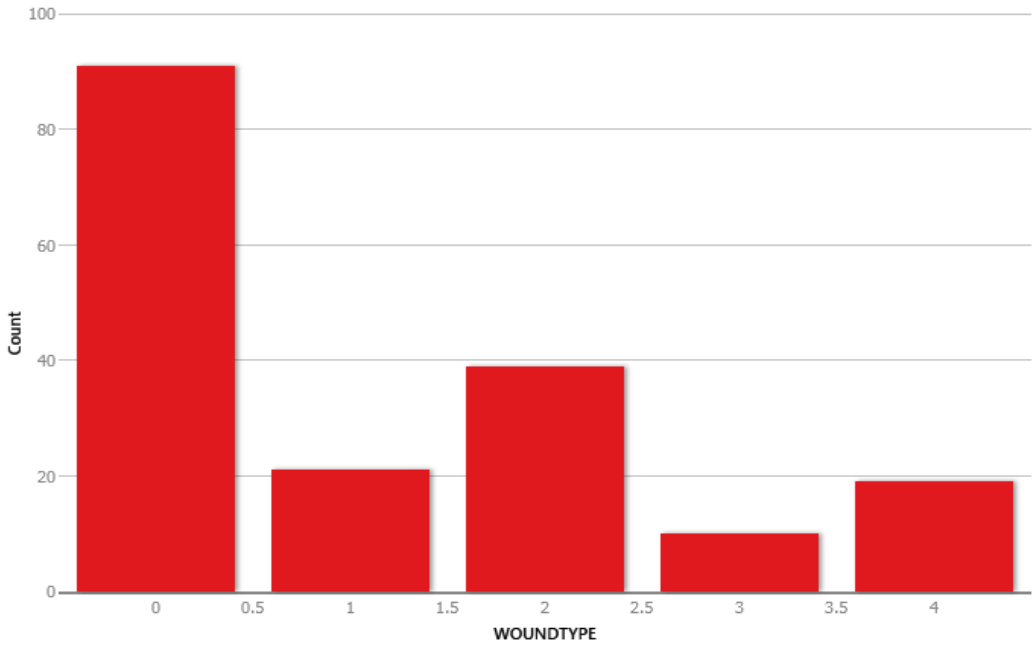

FIGURE 1: Bar chart showing samples collected from different wound types

Keys:

0= Soft Tissue Wound

1= Burn Wound

2= Surgical Wound

3= ostoesmylytis

4= leg Ulcer

Occurrence of *S. aureus* Among Age Group Based on Wound Type

From Table 2, a total of 180 samples were collected. There were 104 samples were collected from males and 76 from females (figure 2) Twenty five (13.89%) samples were collected from patients between the age group of 0-9years and *S. aureus* was isolated from 3 (16.67%) of the soft tissue wound, 1 (14.29%) from burn wound, 1 (7.14%) from surgical wound, 1 (12.5%) from leg ulcer and 2 (50%) from oesteomyelities. Twenty eight (15.56%) samples were collected from patients between the age group of 10-19years and *S. aureus* was isolated from 2 (11.11%) of the soft tissue wound, 1 (14.29%) from burn wound, 2 (14.28%)from surgical wound, 2 (25%)from leg ulcer and 1 (25%) from oesteomyelities. Fifty samples (27.78%) were collected from patients between the age group of 20-29years and *S. aureus* was isolated from 5 (27.78%) of the soft tissue wound, 1 (14.29%) from burn wound, 6 (4.85%) from surgical wound, 3 (37.5%) from leg ulcer and none from oesteomyelities. Thirty three (18.33%) samples were collected from patients between the age group of 30-39years and *S. aureus* was isolated from 4 (22.22%) of the soft tissue wound, 2 (28.57%) from burn wound, 2 (14.85%) from surgical wound, none from leg ulcer and 1 (25%)from oesteomyelities. Fifteen samples (8.33%) were collected from patients between the age group of 40-49years and *S. aureus* was isolated from 1 (5.55%) of the soft tissue wound, 1 (14.29%) from burn wound, 1 (7.14%) from surgical wound, none from leg ulcer and none from oesteomyelities. There were 18 (10%) samples were collected from patients between the age group of 50-59years and *S. aureus* was isolated from 2 (11.11%) of the soft tissue wound, none from burn wound, 2 (14.85%) from surgical wound, 1 (12.5%) from leg ulcer and none from oesteomyelities.

Table 1: Occurrence of *S. aureus* based on wound type

S. aureus	**Wound Type / Occurance of S. aureus (%)**					
Isolated	ST (%)	B (%)	S (%)	U (%)	Os (%)	Total
Yes	18 (35.29)	7 (13.73)	14 (27.45)	8 (15.69)	4 (7.84)	51(28)
No	73 (56.59)	14 (10.85)	25 (19.38)	11 (8.53)	6 (4.65)	129(72)
Total	91 (50.55)	21 (11.67)	39 (21.67)	19 (10.55)	10 (5.55)	180 (100)

Chi-square: 7.0810, df: 4 Probability: 0.1317

keys:

ST=soft tissue, B= burn, B = surgical, U=ulcer, Os=osteomylytis

Table 2: Occurrence of *S. aureus* among age group based on wound type

		Wound type/ Occurance of S. aureus %					
Age group (years)	No examined (%)	ST (%)	B (%)	S (%)	U (%)	OS(%)	Total
0 – 9	25 (13.89)	3 (16.67)	1 (14.29)	1 (7.14)	1 (12.5)	2 (50)	8
10 – 19	28 (15.56)	2 (11.11)	1 (14.29)	2 (14.28)	2 (25)	1 (25)	8
20 – 29	50 (27.78)	5 (27.78)	1 (14.29)	6 (42.85)	3 (37.5)	0 (0)	14
30 – 39	33 (18.33)	4 (22.22)	2 (28.57)	2 (14.85)	0 (0)	1 (25)	9
40 – 49	15 (8.33)	1 (5.55)	1 (14.29)	1 (7.14)	0 (0)	0 (0)	3
50 – 59	18 (10)	2 (11.11)	0 (0)	2 (14.85)	1 (12.5)	0 (0)	5
60 – above	11 (6.11)	1 (5.55)	1 (14.29)	0 (0)	1 (12.5)	0 (0)	4
Total	180	18	7	14	8	4	51

Chi-square: 45.6526, df: 24, probability: 0.0049

Keys:

ST=soft tissue, B= burn, B = surgical, U=ulcer, Os=osteomylytis

Eleven (6.11%) samples were collected from patients between the age group of 60years and above and *S. aureus* was isolated from 1 (5.55%) of the soft tissue wound, 1 (14.29%) from burn wound, none from surgical wound, 1 (12.5%) from leg ulcer and none from oesteomyelities. There was no significant difference ($p>0.05$) in the occurrence of *Staphylococcus aureus* isolated between age groups (Appendix III).

Antibiotic Susceptibility Pattern of *S. aureus* Isolated from Soft Tissue Wound

Table 3 shows the antibiotic susceptibility pattern of *S. aureus* isolated from soft tissue wound with various susceptibility to the antimicrobial agents and the zones were interpreted using CLSI guidline (2015). A total of 18(35.29%) isolates were tested and 7(38.89%) were resistant to Augumentin (10mcg), 8 (44.44%) to Erythromycin (30mcg), 7(38.89%) to Amplicillin (30mcg), 5 (27.78%) to Chloramphenicol (30mcg), 15 (83.33%) to Septrin (30mcg), 2 (11.11%) to Ampiclox (20mcg), 4 (22.22%) to Cefoxitin (30mcg), 2 (11.11%) to Streptomycin (30mcg), 2 (11.11%)to Pefloxacin (10mcg) 3 (16.67%) to Gentamycin (10mcg), 4 (22.22%) to Levofloxancin (20mcg), and none was resistant to Ciproflox (10mcg). The isolates were intermediately sensitive to the antibiotics as follows:9 (50%) to Erythromycin (30mcg), 10 (55.56%) to Chloramphenicol (30mcg), 1 (5.56%) to Septrin (30mcg), 13 (72.22%) to Ampiclox (20mcg), 10 (55.56%)to Streptomycin (30mcg), 10 (55.6%)to Pefloxacin (10mcg) 3 (16.67%) to Gentamycin (10mcg), 5 (27.78%) to Levofloxancin (20mcg), and 9 (50%) was resistant to Ciproflox (10mcg).

Antibiotic Susceptibility Pattern of *S. aureus* Isolated from Burnt Wound

Table 4 shows the antibiotic susceptibility pattern of *S. aureus* isolated from burnt wound with various susceptibility to the antimicrobial agents.

Table 3: Antibiotic susceptibility pattern of *S. aureus* isolated from soft tissue wound

	Isolates																	
Antibiotic (conc/mcg)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Augumentin (10)	14^{R}	27^{S}	20^{S}	18^{R}	12^{S}	21^{S}	25^{S}	15^{R}	23^{S}	20^{S}	18^{R}	14^{R}	19^{S}	32^{S}	18^{R}	28^{S}	29^{S}	16^{R}
Erythromycin 30	13^{R}	17^{I}	10^{R}	18^{R}	15^{I}	18^{I}	12^{I}	10^{R}	0^{R}	12^{R}	19^{I}	17^{I}	18^{I}	0^{R}	17^{I}	20^{S}	0^{R}	21^{I}
Amplicillin (30)	8^{R}	12^{R}	13^{R}	9^{R}	16^{R}	25^{S}	0^{R}	7^{R}	0^{R}	0^{R}	0^{R}	20^{R}	16^{R}	13^{R}	0^{R}	9^{R}	11^{R}	12^{R}
Chloramphenicol (30)	14^{I}	17^{I}	20^{I}	19^{I}	12^{R}	16^{I}	20^{S}	14^{I}	10^{R}	21^{I}	27^{S}	13^{I}	23^{S}	14^{I}	11^{R}	17^{I}	12^{R}	0^{R}
Septrin (30)	18^{S}	10^{S}	0^{R}	0^{R}	8^{R}	10^{R}	0^{R}	9^{R}	7^{R}	0^{R}	11^{R}	10^{R}	9^{R}	8^{R}	7^{R}	8^{R}	16^{I}	0^{R}
Ampiclox (20)	14^{I}	12^{R}	20^{S}	16^{I}	12^{R}	18^{I}	17^{I}	26^{S}	12^{I}	18^{I}	0^{R}	14^{I}	17^{I}	18^{I}	20^{I}	14^{I}	18^{I}	16^{I}
Cefoxitin (30)	23^{S}	11^{R}	23^{S}	22^{S}	10^{R}	23^{S}	24^{S}	22^{S}	24^{S}	23^{S}	22^{S}	12^{R}	23^{S}	0^{R}	24^{S}	23^{S}	22^{S}	25^{S}
Streptomycin (30)	17^{I}	19^{S}	18^{I}	20^{S}	14^{I}	26^{S}	14^{I}	24^{S}	16^{I}	20^{S}	15^{I}	10^{R}	14^{S}	20^{R}	13^{I}	15^{I}	14^{I}	18^{I}
Pefloxacin (10)	12^{I}	15^{I}	20^{S}	22^{S}	20^{S}	13^{I}	16^{I}	10^{R}	12^{R}	19^{S}	16^{I}	13^{I}	20^{S}	13^{I}	20^{I}	27^{S}	18^{I}	15^{I}
Gentamycin (10)	20^{S}	22^{S}	18^{S}	17^{S}	18^{S}	21^{S}	10^{R}	13^{S}	16^{I}	20^{S}	22^{S}	12^{R}	23^{S}	12^{R}	19^{S}	14^{I}	16^{I}	20^{S}
Levofloxancin (20)	17^{I}	12^{R}	21^{S}	23^{S}	7^{R}	17^{I}	20^{S}	23^{S}	18^{I}	19^{S}	15^{I}	20^{S}	12^{R}	22^{R}	19^{S}	16^{I}	14^{R}	22^{S}
Ciproflox (10)	18^{I}	22^{S}	25^{S}	17^{I}	18^{I}	17^{I}	20^{S}	22^{S}	23^{S}	25^{S}	15^{I}	18^{I}	23^{S}	15^{I}	17^{I}	24^{S}	25^{S}	19^{I}

Keys:

$S_{st1} - S_{st18}$= *S.aures* isolated from soft tissue wound

^{R}= resistant ^{I}=intermediate ^{S}=sensitive

Table 4: Antibiotic Susceptibility Pattern of *S. aureus* Isolated from Burnt Wound

	Isolates						
Antibiotic (conc/mcg)	1	2	3	4	5	6	7
Augumentin (10)	16^{R}	18^{R}	15^{R}	0^{R}	14^{R}	20^{I}	27^{I}
Erythromycin 30	12^{R}	18^{I}	24^{S}	16^{I}	20^{I}	7^{R}	14^{I}
Amplicillin (30)	0^{R}	0^{R}	20^{S}	0^{R}	14^{I}	0^{R}	0^{R}
Cholramphenicol (30)	12^{R}	15^{R}	19^{R}	28^{S}	20^{R}	18^{R}	14^{R}
Septrin (30)	0^{R}	14^{I}	18^{S}	9^{R}	14^{R}	20^{R}	10^{R}
Ampiclox (20)	14^{I}	17^{I}	14^{I}	0^{R}	7^{R}	16^{S}	14^{I}
cefoxitin (30)	12^{R}	24^{S}	22^{S}	23^{S}	13^{R}	22^{S}	23^{S}
Streptomycin (30)	13^{I}	14^{I}	15^{I}	12^{I}	20^{S}	14^{I}	20^{S}
Pefloxacin (10)	7^{R}	23^{S}	17^{I}	14^{I}	8^{R}	20^{I}	10^{R}
Gentamycin (10)	0^{R}	24^{S}	26^{S}	20^{S}	8^{R}	20^{S}	16^{I}
Levofloxancin (20)	15^{I}	18^{I}	20^{S}	14^{I}	18^{I}	20^{S}	18^{I}
Ciproflox (10)	17^{I}	20^{I}	23^{S}	18^{S}	17^{I}	26^{S}	20^{S}

Keys: ^{R} = resistant ^{I}=intermediate ^{S} =sensitive

Seven (13.73%) isolates were tested and 5 (71.43%) were resistant to Augumentin (10mcg), 2 (28.57%) to Erythromycin (30mcg), 5 (71.43%) to Amplicillin (30mcg), 6 (85.72%) to

Chloramphenicol (30mcg), 5 (71.43%) to Septrin (30mcg), 2 (28.57%) to Ampiclox (20mcg), 2 (28.57%)to Cefoxitin (30mcg), none was resistant to Streptomycin (30mcg). Similarly, 3 (42.86%) to Pefloxacin (10mcg), 2 (28.57%) to Gentamycin(10mcg), none was resistant to Levofloxancin (20mcg), and Ciproflox (10mcg). The isolates were intermediately sensitivity to the antibiotics as follows: 2 (28.57%) were intermediately sensitive to Augumentin (10mcg), 4 (57.14%) to Erythromycin (30mcg), 1 (14.29%) to Amplicillin (30mcg), 1 (14.29 %) to Septrin (30mcg), 4 (57.14%) to Ampiclox (20mcg), 5 (71.42%) to Streptomycin (30mcg), 3 (42.86%)to Pefloxacin (10mcg), 1 (14.29%) to Gentamycin (10mcg), 5 (71.42%) to Levofloxancin (20mcg), and 3 (42.86) to Ciproflox (10mcg).

Antibiotic Susceptibility Pattern of *S. aureus* isolated from Surgical Wound

Table 5 shows the antibiotic susceptibility pattern of *S. aureus* isolated from surgical wound wound with various susceptibility to the antimicrobial agents. A total number of 14(27.45%) isolates were tested and 4 (28.57 %) were resistant to Augumentin (10mcg), 3 (21.43%) to Erythromycin (30mcg), 12 (85.72 %) to Amplicillin (30mcg), 8 (57.14%) to Chloramphenicol (30mcg), 9 (64.29%) to Septrin (30mcg), 6 (42.86%) to Ampiclox (20mcg), 1(7.14%)to Cefoxitin (30mcg), none was resistant to Streptomycin (30mcg). Similarly, 3 (21.43%) to Pefloxacin (10mcg), 5 (35.72%) to Gentamycin (10mcg), none to Levofloxacin (20mcg), and Ciproflox (10mcg). The isolates were intermediately sensitive to the antibiotics as follows: 5(35.71%) were intermediately sensitive to Augumentin (10mcg), 6 (42.86%) to Erythromycin (30mcg), 2(14.29%) to Amplicillin (30mcg), 2 (14.29%) to Chloramphenicol (30mcg), 3 (21.43%) to Septrin (30mcg), 4 (28.57%) to Ampiclox (20mcg), 9 (64.29%) to Streptomycin (30mcg), 4 (28.57%)to Pefloxacin (10mcg),3 (21.43%) to Gentamycin (10mcg), 6 (42.86%) to Levofloxancin (20mcg), and 7 (50%) to Ciproflox (10mcg).

Table 5: antibiotic susceptibility pattern of *S. aureus* isolated from surgical wound

	Isolates													
Antibiotic (conc/mcg)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Augumentin (10)	19^{I}	8^{R}	20^{S}	24^{S}	18^{I}	10^{R}	17^{R}	21^{S}	25^{S}	19^{I}	19^{I}	18^{I}	22^{S}	7^{R}
Erythromycin (30)	8^{R}	20^{S}	18^{I}	7^{R}	20^{S}	14^{I}	20^{S}	18^{S}	19^{I}	8^{R}	14^{I}	25^{S}	16^{I}	14^{I}
Amplicillin (30)	9^{R}	18^{I}	7^{R}	8^{R}	10^{R}	0^{R}	11^{R}	10^{R}	0^{R}	12^{R}	13^{R}	0^{R}	0^{R}	20^{I}
Cholramphenicol (30)	30^{S}	0^{R}	17^{S}	25^{S}	21^{R}	17^{R}	10^{R}	12^{R}	11^{R}	19^{I}	8^{R}	18^{R}	21^{I}	28^{S}
Septrin (30)	0^{R}	16^{I}	12^{I}	7^{R}	20^{S}	9^{R}	0^{R}	18^{S}	0^{R}	7^{R}	12^{I}	0^{R}	13^{R}	0^{R}
Ampiclox (20)	20^{S}	24^{S}	14^{I}	16^{I}	25^{S}	12^{R}	16^{I}	0^{R}	20^{S}	17^{I}	19^{R}	18^{R}	7^{R}	12^{R}
Cefoxitin (30)	23^{S}	21^{S}	24^{S}	23^{S}	22^{S}	12^{R}	24^{S}	22^{S}	23^{S}	26^{S}	22^{S}	23^{S}	23^{S}	22^{S}
Streptomycin (30)	23^{S}	18^{I}	14^{I}	24^{I}	15^{I}	17^{I}	24^{S}	26^{S}	24^{S}	14^{I}	16^{I}	20^{S}	18^{I}	14^{I}
Pefloxacin (10)	13^{I}	14^{I}	22^{S}	23^{S}	18^{I}	10^{R}	14^{I}	24^{S}	23^{S}	26^{S}	20^{S}	12^{R}	0^{R}	23^{S}
-Gentamycin (10)	20^{S}	20^{S}	12^{R}	23^{I}	14^{R}	8^{R}	20^{S}	26^{S}	8^{R}	25^{S}	17^{I}	26^{S}	16^{I}	0^{R}
Levofloxancin (20)	22^{S}	20^{S}	16^{I}	17^{I}	16^{I}	10^{R}	18^{I}	14^{R}	19^{S}	16^{I}	13^{R}	18^{I}	23^{S}	25^{S}
Ciproflox (10)	24^{S}	20^{S}	18^{I}	26^{S}	22^{S}	24^{S}	16^{I}	21^{I}	18^{I}	15^{I}	20^{I}	17^{I}	22^{S}	23^{S}

Keys:^{R}= resistant ^{I} =intermediate^{S} =sensitive

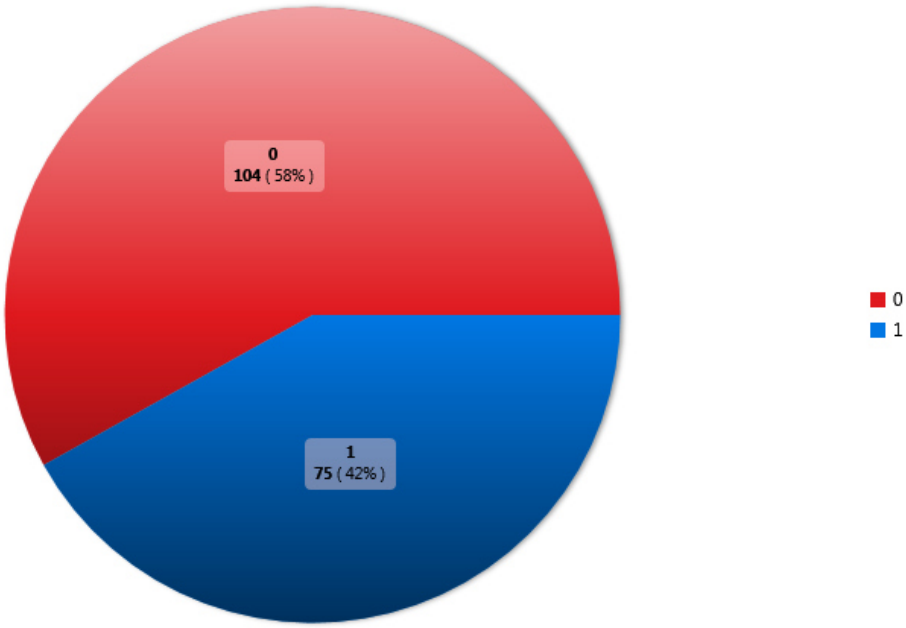

FIGURE 2: pie chart showing distribution of samples collected based on sex

Antibiotic Susceptibility Pattern of *S. aureus* Isolated from Leg Ulcer

From Table 6, the antibiotic susceptibility pattern of *S. aureus* isolated from leg ulcer wound shows various susceptibility of the isolates to the antimicrobial agents. A total number of 8(15.69%) isolates were tested and 2 (25%) were resistant to Augumentin (10mcg), none was resistant to Erythromycin (30mcg). Similarly, 7 (87.5%) were resistant to Amplicillin (30mcg), 2 (25%) to Chloramphenicol (30mcg), 6 (75%) to Septrin (30mcg), 1 (12.5%) to Ampiclox (20mcg), 2 (25%) to Cefoxitin (30mcg), 1 (12.25)to Streptomycin (30mcg), none to Pefloxacin (10mcg). Similarly,1 (12.5%) was resistant to Gentamycin (10mcg), noneto Levofloxancin (20mcg), and Ciproflox (10mcg). The isolates were intermediately sensitivity to the antibiotics as follows: 4(50%) were intermediately sensitive to Augumentin (10mcg), 6 (75%) to Erythromycin (30mcg), 5 (62.5%) to Chloramphenicol (30mcg), 2 (25%) to Septrin (30mcg), 4 (50%) to Ampiclox (20mcg), 4 (50%) to Streptomycin (30mcg), 3 (37.5%)to Pefloxacin (10mcg),4 (50%) to Gentamycin (10mcg), 4 (50%) to Levofloxancin (20mcg), and 3 (37.5%) to Ciproflox (10mcg).

Antibiotic Susceptibility Pattern of *S. aureus* Isolated from Osteomylytis

From Table 7 the antibiotic susceptibility pattern of *S. aureus* isolated from osteomylytiswound shows various susceptibility to the antimicrobial agents. A total number of 4 (7.84%) isolates were tested and 1 (25%) was resistant to Augumentin (10mcg), 1 (25%) to Erythromycin (30mcg), 4 (100%) to Amplicillin (30mcg), 1 (25%) to Chloramphenicol (30mcg), 4 (100%) to Septrin (30mcg), 2 (50%) to Ampiclox (20mcg), 1 (25%)to Cefoxitin (30mcg), noneto Streptomycin (30mcg) and Pefloxacin (10mcg). Similarly,1 (25%) was resistant to Gentamycin (10mcg), 2 (50%) to Levofloxancin (20mcg), and none to Ciproflox (10mcg).

Table 6: Antibiotic susceptibility pattern of *S. aureus* isolated from leg ulcer

	Isolates							
Antibiotic (conc/mcg)	1	2	3	4	5	6	7	8
Augmentin (10)	20^{R}	11^{R}	19^{I}	25^{I}	15^{I}	30^{S}	19^{I}	7^{R}
Erythromycin (30)	18^{I}	18^{I}	19^{S}	13^{I}	27^{S}	23^{I}	15^{I}	18^{I}
Amplicillin (30)	11^{R}	21^{R}	22^{R}	25^{R}	30^{S}	16^{R}	10^{R}	8^{R}
Cholramphenicol (30)	18^{I}	14^{I}	17^{I}	11^{R}	20^{S}	16^{I}	17^{I}	7^{R}
Septrin (30)	14^{I}	12^{R}	0^{R}	8^{R}	18^{I}	9^{R}	0^{R}	10^{R}
Ampiclox (20)	18^{I}	20^{S}	16^{I}	20^{S}	18^{I}	$I7^{R}$	20^{S}	18^{I}
cefoxitin (30)	23^{S}	23^{S}	11^{R}	24^{S}	21^{S}	17^{R}	21^{S}	29^{S}
Streptomycin (30)	14^{I}	10^{R}	15^{I}	20^{S}	13^{I}	22^{S}	18^{I}	19^{S}
Pefloxacin (10)	20^{S}	24^{S}	23^{S}	16^{I}	19^{S}	24^{S}	18^{I}	17^{I}
Gentamycin (10)	13^{R}	16^{I}	22^{S}	15^{I}	20^{S}	19^{S}	16^{I}	14^{I}
Levofloxacin (20)	15^{I}	19^{S}	25^{S}	18^{I}	20^{S}	16^{I}	22^{S}	15^{I}
Ciproflox (10)	13^{S}	15^{I}	15^{I}	26^{S}	20^{S}	22^{S}	23^{S}	14^{I}

Keys :^{R} = resistant ^{I} =intermediate ^{S} =sensitive

Table 7: Antibiotic susceptibility pattern of *S. aureus* isolated from osteomylytis

	Isolates			
Antibiotic (conc/mcg)	1	2	3	4
Augumentin (10)	20^{S}	18^{R}	24^{S}	26^{S}
Erythromycin (30)	20^{S}	10^{R}	24^{S}	16^{S}
Amplicillin (30)	0^{R}	16^{R}	12^{R}	17^{R}
Chloramphenicol (30)	27^{S}	15^{I}	20^{I}	10^{R}
Septrin (30)	0^{R}	8^{R}	10^{R}	0^{R}
Ampiclox (20)	8^{R}	0^{R}	14^{I}	16^{I}
Cefoxitin (30)	22^{S}	10^{R}	23^{S}	23^{S}
Streptomycin (30)	15^{I}	18^{I}	14^{I}	26^{S}
Pefloxacin (10)	13^{I}	16^{I}	19^{I}	20^{S}
Gentamycin (10)	17^{I}	12^{R}	16^{I}	24^{S}
Levofloxacin (20)	18^{I}	10^{R}	21^{S}	12^{R}
Ciproflox (10)	20^{S}	16^{I}	23^{S}	26^{S}

Keys: ^{R} = resistant ^{I} =intermediate ^{S} =sensitive

The isolates were intermediately sensitivity to the antibiotics as follows: 2(50%) were intermediately sensitiveto Chloramphenicol (30mcg), 2 (50%)to Ampiclox (20mcg), 3 (75%) to Streptomycin (30mcg), 3 (75%)to Pefloxacin (10mcg),2 (50%) to Gentamycin (10mcg), 1 (25%) to Levofloxancin (20mcg), 1 (25%) to Ciproflox (10mcg).

Summary of Antibiotic Susceptibility Pattern of *S. aureus* Isolated from Infected Wounds

Table 8 shows the summary of the susceptibility pattern. All the isolates were susceptible to ciproflox while only 6 (11.76%) were susceptible to ampicillin

Resistance Genes Detected

The *mec A*gene (300bp) as found in 1 (20%) of the isolates and the fem B gene (700bp) wasalso found in 1 (20%) of the isolates. There was high prevalence of the genes in the screened samples. Figure 3is the result for the PCR.

Table 8: summary of antibiotic susceptibility pattern of *S. aureus* isolated from infected wounds

Antibiotics (conc/mcg)	No sensitive/51	Percentage sensitive
Augumentin (10)	31	60.78
Erythromycin (30)	38	73.55
Amplicillin (30)	6	11.76
Chloramphenicol (30)	29	56.86
Septrin (30)	13	25.49
Ampiclox (20)	23	45.10
Cefoxitin (30)	41	80.39
Streptomycin (30)	49	17.65
Pefloxacin (10)	43	84.31
Gentamycin (10)	38	74.51
Levofloxacin (20)	41	80.39
Ciproflox (10)	51	100

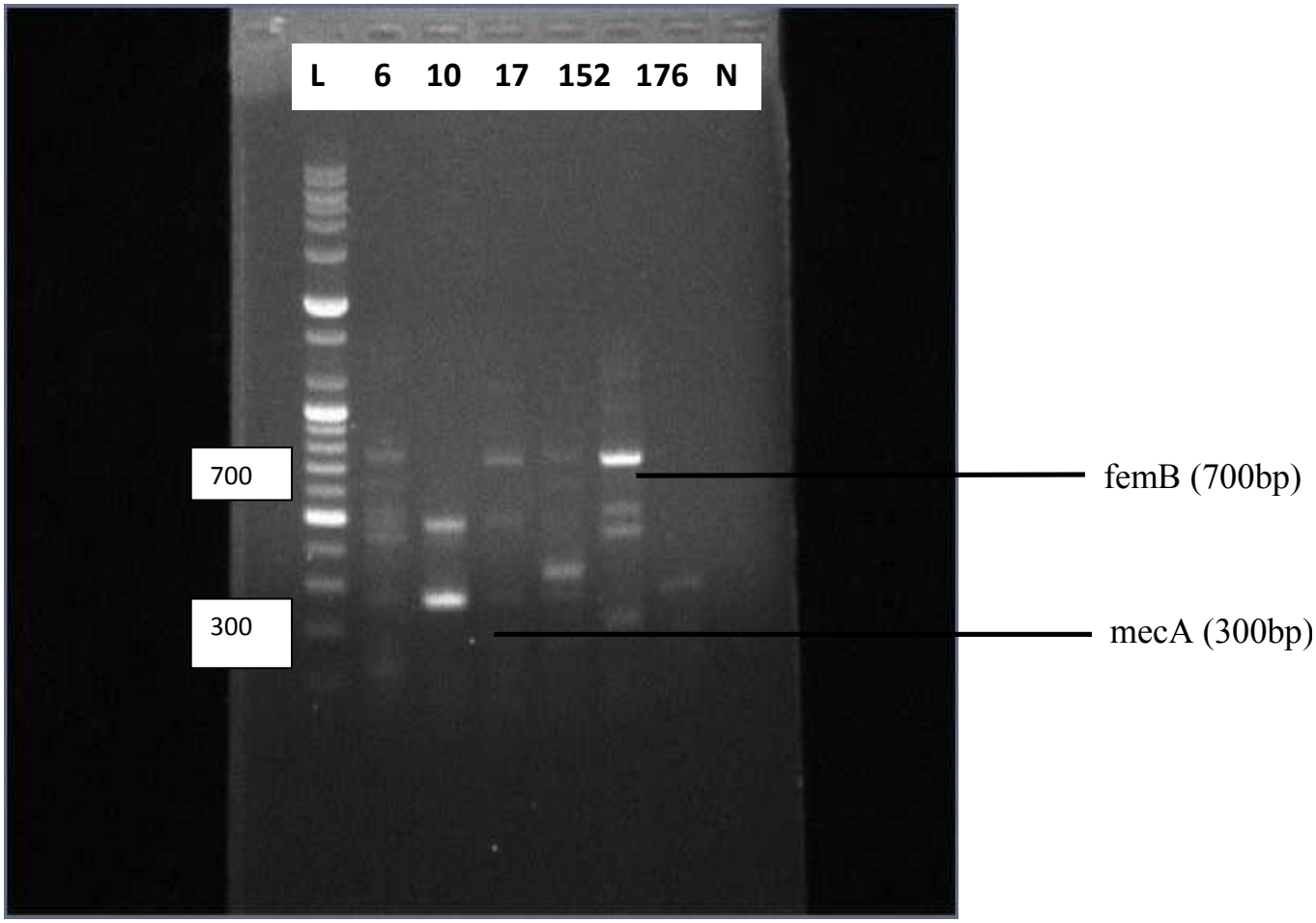

FIGURE 3 : Gel Electrophoresis for *mecA* (300 bp) and *femB* (700 bp) genes

L = ladder, 6-176 are the samples, N-Negative control

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Out of the 180 swabs collected from infected wounds *S. aureus* was isolated in 51 (28%) and it was similar to the findings of Ezekiel *et al*, (2014) in a study conducted in Obafemi Awolowo University teaching hospital complex Ille Ife, Nigeria where *S. aureus* was isolated from 23% of the samples studied. This Study also agrees with the Nosocornial Infection National Surveillance Service (NINSS) survey of (1997 – 2001) which reported *Staphylococcus* including *S. aureus* and *S.epidermidis* are the common organisms causing surgical site infections with a prevalence of 47% (NINSS, 2001). Similar reports have been documented in Indian hospital (Kownhar *et al.,* 2008). Prevalence of *S. aureus* in surgical wound infections have been attributed to the high rate of nasal carriage of the organism in patient and health care workers involved in the treatment of the patients and because of the close proximity to humans (Dixon, 2002).

Susceptibility pattern of *S. aureus* in the study showed 100% sensitivity to ciproflox (10mcg) which was similar to the findings of Shamweel (2013), where 94% sensitivity to ciproflox was reported but slightly differs from the finding of Neeta and Mohluddin (2013) where they reported 92.5% resistance of *S. aureus* to ciproflox. Susceptibility to erythromycin was found to be 76% which was similar to the finding of Misko *et al.,* (1995) where susceptibility to erythromycin was found to be 74%. Also 22% resisitance to erythromycin was reported by Misko *et al.,* (1995) a similar to this findings. The resistance rate of streptomycin was 22% which was similar to the findings of Ako-Nai *et al.,* (2013) where resistance of 25%

was reported. MRSA was found to be 19.61% which was similar to the findings of Shittu *et al.,* (2011) where MRSA was found to be 16.1 % in a study conducted in Nigeria, but differs from the findings of Onanuga *et al.,* (2005) in a study conducted in Zaria where he found MRSA to be 71%. It is also similar to the findings of Adebayo *et al.,* (2011) where 16% of the samples were found to be resistant to methicillin. Most of the isolates were highly susceptible to gentamicin, levofloxacin and pefloxacin, which is in agreement with previous reports (Umolu *et al.,* 2002; Ehinmidu, 2003; Olayinka *et al.,* 2004). High susceptibility to gentamicin though very cheap, may be due to the complexity of the aminoglycoside and the route of administration. The fluoroquinolones are newer drugs with mode of action central on inhibition of the DNA replication which stops the multiplication of the bacteria cells and are relatively expensive therefore they are more likely less available for abuse.

The prevalence of *MecA* gene was 20% which was higher than the findings of Alli *et al.,* (2015) in a study conducted in Nigeria and finding of Kumurya *et al*.,(2013) where *MecA* gene was seen in only 5% of the samples but lower than the finding of Mounir (2014) where *MecA* gene was found in 100% of the samples but similar to the study of Rehm (2008) where *MecA* gene was found in 17% of the samples. *FemB* gene was also found to be 20% which was lower to the finding of Mounir (2014) where he found *FemB* in 100% of his samples but similar to the findings of Abazar *et al.,* (2014) where *FemB* gene was reported to be found in only 7.8% of the samples. Although some isolates did not reveal the presence of *femB* genes even though these were positive for free coagulaseand mannitol fermentation tests, Kobayashi *et al., (*2008) have reported that though *femB* genes are detectable only in *S. aureus,* an absence of *femB* gene does not mean that the isolate is not *S. aureus*. The low rate of the genes screen is probably due to the storage period before the screening was done as was show in the work of Kumurya *et al*., (2013).

5.2 Conclusion

From the study conducted, it can be concluded that *S. aureus* is having a high prevalence within infected wound in patients attending Murtala Muhammad Specialist Hospital Kano. The Isolates showed various degree of susceptibility to the different antibiotics they were tested against. 100% susceptibility was recorded in ciproflox .MRSA was found to be 19.61% There was no significant difference in *S. aureus* isolated between different age groups (P>0.005). There was significant difference (P<0.005)in *S. aureus* isolated between different age groups. *MecA* gene was found in 20% of the samples and also *femB* was found in 20% of the samples screened.

5.3 Recommendations

1. Antimicrobial susceptibility testing should be done in the case of infected wounds before
2. Self-medication and misuse of antibiotics should be avoided.
3. Equipments use for gene detection should be provided to our hospitals so that diagnosis can be done up to molecular level.

Equipment use for gene detection should be provided to our schools to enable students carry out hitch free researches and avoid unnecessary waste of time.

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Fa'iza Musa,
Department of Microbiology,
Bayero University, Kano,
Kano.

PROVISIONAL ETHICAL CLEARANCE

Sequel to conduct a research titled "STUDIES ON RESISTANCE GENES ASSOCIATED WITH STAPHYLOCOCCUS AUREUS IN WOUND INFECTIONS AMONG PATIENT ATTENDING MURTALA MUHAMMAD SPECIALIST HOSPITAL, KANO". In the light of the above, I am mandated to convey provisional clearance to proceed on your study based on the following conditions.

i. That the consent of all participants must be obtained by filling in consent form.
ii. That you should liase with the Management of the facility for appropriate guidance.
iii. That any publication related to the study should be brought to the knowledge of the Ethical Committee for approval.
iv. That a copy of your finding should be submitted for documentation, record and final approval, please.

Best regards.

MAIMUNAH KHALID ABDUL'AZIZ
Asst. Secretary II
FOR: EXECUTIVE SECRETARY

CC:

Chief Medical Director,
MMSH,
Hospitals Management Board,
kano.

Above is for your information and noting, please.

MAIMUNAH KHALID ABDULAZIZ
ASSISTANT SECRETARY II
FOR: EXECUTIVE SECRETARY

APPENDIX II: Statistical analyses

Chi-square

WOUND TYPE	Yes	No	TOTAL
Soft tissue	18	73	**91**
Burn	7	14	**21**
Surgical	14	25	**39**
oesteomylities	4	6	**10**
Ulcer	8	11	**19**
TOTAL	**51**	**129**	**180**

Chi-square	df	Probability
7.081	4	0.1317

APPENDIX III

Statistical analyses

AGE_GROUP	Soft TISSUE	BURN	SURGICAL	OESTEOMYLITIES	ULCER	TOTAL
0 - <10	1	2	0	3	1	**7**
10 - <20	3	0	4	1	0	**8**
20 - <30	8	2	8	0	3	**21**
30 - <40	3	2	1	0	0	**6**
40 - <50	0	1	0	0	0	**1**
50 - <60	3	0	1	0	2	**6**
60 - < HIVALUE	0	0	0	0	12	**2**
TOTAL	**18**	**7**	**14**	**4**	**8**	**51**

Chi-square	df	Probability
45.6526	24	0.0049