# MOLECULAR IDENTIFICATION OF SOME ANTIBIOTIC RESISTANCE GENES OF STAPHYLOCOCCI ISOLATED FROM CHRONIC SKIN ULCER PATIENTS IN KADUNA STATE, NIGERIA.

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**AUGUST, 2015**

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**DEPARTMENT OF MICROBIOLOGY**
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**AHMADU BELLO UNIVERSITY, ZARIA**
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## DECLARATION

I declare that the work in this dissertation entitled "**MOLECULAR IDENTIFICATION OF SOME ANTIBIOTIC RESISTANCE GENES OF STAPHYLOCOCCI ISOLATED FROM CHRONIC SKIN ULCER PATIENTS IN KADUNA STATE, NIGERIA"**, has been carried out by me in the Department of Microbiology. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.

| | | |
|---|---|---|
| Name of Student | Signature | Date |

## CERTIFICATION

This dissertation entitled "**MOLECULAR IDENTIFICATION OF SOMEANTIBIOTIC RESISTANCE GENES OF STAPHYLOCOCCI ISOLATED FROM CHRONIC SKIN ULCER PATIENTS IN KADUNA STATE, NIGERIA" by JOHN BABA**meets the regulations governing the award of the Degree of Doctor of Philosophy (Ph.D) of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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Prof. A. Z. Hassan Date
Dean, School of Postgraduate Studies

## DEDICATION

This dissertation is dedicated to the Glory of God, for sparing my life and bringing me to this height in the pursuit of my academic career, and to the memory of my late Father, Pa Reuben Babalola Ojimi. May his gentle soul rest in perfect peace, Amen.

## ACKNOWLEDGEMENT

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The place of a mother in the life of a child cannot be undermined. I wish to specially thank my mother, Mrs. Emilia Abeke Ojimi for her unflinching support and special prayers to God on my behalf, which have sustained me thus far in life. It is also very important to note here that this entire programme would have been difficult for me to start and complete, but for the moral and financial support, prayers and understanding I enjoyed from my jewel of an inestimable value, Mrs. Alice Olaoluwa John. I am grateful to God for having her as a wife.

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John Baba.

## ABSTRACT

Molecular identification of someantibiotic resistance genes of staphylococci isolated from chronic skin ulcer patients in Kaduna state was investigated. Pure staphylococci isolates were characterized phenotypically before subjecting them to screening for antibiotic resistance genes using using the Polymerase chain reaction (PCR) technique. The total number of samples collected from the patients across four hospitals in Kaduna state from January 2012 to January 2013 was two hundred and ninety-two (292). *Staphylococcus aureus* ranked highest among the species of staphylococci isolated from the chronic skin ulcer patients (n=18, 34%), while *Staphylococcus epidermidis*had the highest number of isolates (n= 10, 18.9%) among the coagulase-negative staphylococci.The highest number of the Staphylococci isolates was found among leg ulcers (n=28, 52%). The commonest resistance pattern demonstrated by the *Staphylococcus aureus* to some antibiotics is in the order, Cefoxitin (methicillin) , Amoxiclllin-clavulanic acid , which was exhibited by 72% of the isolates.The resistance pattern of coagulase-negative staphylococci (CoNS) followed the same trend as in *S.aureus*.*Staphylococcus aureus* exhibited 100% resistance to Amoxicillin - clavulanic acid. Percentage resistance of coagulase-negative staphylocci revealed that all the Coagulase-negative Staphylococci, except *Staphylococcus intermedius* showed 79% resistance to Cefoxitin on the average basis. The Minimum Inhibitory Concentrations (MICs) of the antibiotics on *S.aureus* isolates showed that 12(67%) of the isolates were susceptible to Amoxicillin - Clavulanic acid at the concentration of 32.0µg/ml. The same pattern was observed for the Coagulase-negative Staphylococci, where 13(52%) of the isolates were also susceptible to Amoxicillin - Clavulanic acid at the same concentration of 32.0µg/ml. For the Minimum Bactericidal Concentrations (MBCs), over 50% of the isolates of *S.aureus* and coagulase-negative staphylococci were killed at the highest concentration of 32.0µg/ml for almost all the antibacterial agents used, except for Ciprofloxacin where an

isolate each of *S.aureus* and coagulase-negative staphylococci were killed at the concentration of 8.0µg/ml. Polymerase chain reaction (PCR) analysis indicated the presence of resistance genes such as *blaZ* (173bp), *tetM (*158bp) and *mecA* (314bp) in the staphylococci species as depicted by the bands of the gel electrophoresis. The statistical analysis of the socio-demographic factors using the chi-square ($\chi^2$) test of association showed that variables such as age, sex, educational status, occupation, cause of ulcer, site of ulcer and type of urbanization were statistically significant for the occurrence of chronic skin ulcer in the patients at P- value $\leq 0.05$. The study showed that there was high prevalence of MRSA and MRCoNS with possible transmission from the hospital to the community and vice versa.The isolates of MRSA and MRCoNS were also resistant to other antibiotics apart from cefoxitin. The resistance to the antibiotics by the isolates is as a result of some antibiotic resistance genes in them.The socio-demographic factors could influence the occurrence of chronic skin ulcer in the patients as indicated by the result of the statistical analysis.

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**List of Abbreviations**

| **Accronyms** | **Meanings** |
|---|---|
| AGEs | Advanced glyciation endproducts |
| AMEs | Aminoglycoside modifying enzymes |
| ANT | Aminoglycoside nucleotidyltransferase |
| APH | Aminoglycoside phosphotransferase |
| ATP | Adenosine triphosphate |
| CDCP | Centre for Disease Control and Prevention |
| CoNS | Coagulase-negative staphylococci |
| DFUs | Diabetic foot ulcers |
| DHEA | Dehydroepiandrosterone |
| EPS | Extracellular polysaccharide |
| HBOT | Hyperbaric oxygen therapy |
| HIV | Human immuno deficiency virus |
| MMPs | Matrix metalloproteases |
| MRSA | Methicillin Resistant *Staphylococcus aureus* |
| NIAID | National Institute of Allergy and Infectious Diseases |
| PBP | Penicillin binding protein |

| | |
|---|---|
| PCR | Polymerase chain reaction |
| PFGE | Pulse field gel electrophoresis |
| PMNs | Polymorphonuclear Neutrophils |
| UTIs | Urinary tract infections |
| WHO | World Health Organization |
| +VE | Positive |
| -VE | Negative |

# CHAPTER ONE

## 1.0 INTRODUCTION

A wound can be defined as any injury that damages the skin and therefore compromise its protective function. However, a wound is said to be chronic when healing is not achieved within three months (Siddiqui and Bernstein, 2010). Ulcer appears to be the commonest type of chronic wound. Open skin wounds are colonized by bacteria, this does not mean that all wounds are infected. Many factors determine the progression of a wound from contamination to infection, which may include bacterial load, the types of bacteria present in such wounds, their synergistic effect including their virulent nature (Edwards and Harding, 2004; Siddiqui and Bernstein, 2010).The skin becomes broken when there is a cut or abrasion on the skin, and there is every likelihood that their protective defense mechanism becomes obstructed. When this happens, the environment becomes conducive for bacteria to contaminate the skin, increase in number and possibly cause an infection. The bacteria contaminating wounds are from the environment, through dust particles, bacteria on hands, clothing and equipment.

Different kinds of wounds exist, ranging from superficial burns, bite wounds and surgical wounds (Bowler *et al*., 2001). The activities of microorganisms in the wound could cause delayed healing in such wounds, thereby making the wound to be chronic. The most common type of chronic wound is an ulcer, which occurs usually in the lower leg of individual having underlying diabetes. The healings of such ulcer would delay, even when less pathogenic microorganisms are present (Williams *et al*., 2004). The initial colonizers of the skin are those bacteria that live symbiotically on the skin. The non-healing condition of a wound over time exposes it to different pathogenic bacteria(Bowler *et al*., 2001).Ulcerations of the lower leg of venous and diabetic origin are the most frequent of all chronic wounds. The delays of these wounds could be the disturbance in the supply of nutrients and removal

of metabolic waste products, caused by the pathology of blood vessels (Bowler, 1998). Generally, infection may be caused by pathogenic bacteria originating from the external environment, as well as bacteria forming physiological microflora of the skin (Schmidt *et al*., 2000).

*Staphylococcus aureus* has been reported to be a major cause of several infections that include, bacteremia, skin and soft tissue infections and osteomyelitis (Diekema *et al.*, 2001; Alam *et al.*, 2002). The root of most pyogenic local and systemic infections in both hospitals and community has been linked to *Staphylococcus aureus* (Arunava *et al*., 2013). Methicillin resistant *S. aureus* (MRSA) is often acquired when there is an individual exposure to hospitals, and other health care facilities, the consequence of this is a different serious healthcare- associated infections (Guidelines, 2008). MRSA isolates that were first recognized in the 1960's (Barber, 1963; Barret *et al*., 1968), and were found to be largely limited to those patients with certain health care exposures, have however been also recognized among previously healthy members of the community that lack health care exposures (Herold *et al.*, 1998). Therefore, community acquired MRSA (CA-MRSA) emerged throughout the world in the late 1990's (Otto, 2007).

Research has shown that *S. aureus* has for the past five decades acquired resistance to previously effective antibiotics that include penicillinase-resistant ones such as methicillin (Diekema *et al*., 2001). The case has however become worsen, that, in the present day, Methicillin resistant *staphylococcus aureus*(MRSA) now poses a serious therapeutic problem in the entire world (Engemann*etal*., 2003).Methicillin resistant *staphylococcus aureus* are strains of *S aureus* that show altered penicillin binding protein (PBP2a) thereby conferring resistance to beta-lactam antibiotics (Arunava *et al*., 2013). As a result of series of documented outbreaks of hospitals cross infection caused by MRSA in the entire universe

since 1970's, they have attracted special attention in hospital acquired infections (Shanson *et al*., 1976). Methicillin resistant *staphylococcus aureus* are stubborn and notorious, because of their wide variations in antibiotic resistance patterns. They have consistently developed chromosomal resistances to penicillins and cephalosporins, as well as have frequently shown resistances to the wide range of antibiotics commonly prescribed in the hospitals( Pavillard *et al*.,1982).The resistance pattern of prevalent MRSA strains are tantamount to continuous changes over a period of time, as a result of the changes in antibiotic prescription patterns, as well as other factors like control measures and awareness among health care workers (Arunava *et al*., 2013). Antibiotics pressure continue to increase in various hospitals, this invariably leads the emergence of new strains with higher antibiotics resistance replacing the previous strains. MRSA might be a serious worldwide pathogen, studies are largely restricted to affluent regions of the world, giving rise to very limited information regarding the frequency and characteristics of MRSA in developing countries.

Coagulase negative staphylococci (CoNS) previously considered as non- pathogenic have been identified as the etiological agents in most hospital acquired infections, and have been frequently isolated from such infections ( Cunha *et al*.,2006). Apart from *Staphylococcus epidermidis* that is commonly isolated in wounds, other species have been implicated to cause infections. *Staphylococcus xylosus, Staphylococcus haemolyticus* and *Staphylococcus lugdunensis* have been reported to cause infections ranging from urinary tract infections(UTI), osteomyelitis to sepsis respectively (Venkatesh *et al.,* 2006). *Staphylococcus epidermidis* has emerged in recent years as a pathogen in a growing number of other serious nosocormial infections, such as in neonatal intensive care units (NICUS), most especially bloodstream infections (Hall, 1991; Gaynes *et al*., 1996). *S.epidermidis* has also been widely recognized as an etiologic agent of bacteremia, prosthetic and natural valvular endocarditis, osteomyelitis and urinary tract infections, and is found to bein frequent association with the

colonization of intravascular catheters and orthopaedic devices (Brumfitt and Hamilton - Miller, 1989; Gaynes, 1996). *Staphylococcus saprophyticus* has been documented to be the second most frequently encountered microorganism after *Escherichia coli* in acute UTI (Hall, 1991; Gupta *et al.*, 1999). Several other species of coagulase-negative staphylococci have been implicated at low incidence in a variety of infections (Javid *et al.*, 2006). One of the factors that favours the CoNS to wreak havoc in their host is their ability to produce enzymes such as lipases, proteases and other exo- enzymes, which have made them to be persistent, and possibly degrade host tissues (Otto, 2004).

Several species of CoNS have been reported to produce haemolysins which made it possible for them to bind to susceptible host cells, and causing lysis of the red blood cells for the release of free iron which is eventually utilized by the bacteria (Azukah and Idahosa ,2013). The major reservoirs of coagulase-negative staphylococci in hospitals are colonized or infected in-patients and colonized hospital workers, with carriers at risk for developing endogenous infection or transmitting infection to health care workers and patients (Harbarth *et al*., 2001). Transient hand carriage of these bacteria on the hand of health care workers account for the major mechanism for patient to patient transmission (Jarvid *et al.*, 2006). Fourteen species of CoNS are currently recognized as human isolates (Kloos and Bannerman, 1995).

Owing to the frequent and indiscriminate use of antibiotics by individuals to cure several infections earlier mentioned caused by the CoNS, they have become resistant to these antibiotics so used. However, some antibiotics are still effective against the CoNS isolates.

*Staphylococcus aureus* and coagulase-negative staphylococci are commonly found in skin and soft tissue infections, and have therefore become pathogens of clinical interest (Azukah and Idahosa, 2013) .The resistance phenotypes that exist in these bacteria, when found in

chronic skin ulcers, become an important factor, because of the pathogenicity involved, as well as the many virulence factors affecting the therapeutic process(Gordon and Lowly, 2008).The possibility of multi-drug resistant strains arising among these bacteria is a growing problem. Results from different research studies across the globe indicate a growing prevalence of these bacteria (Alvarez *et al*., 2010; Zurita *et al.*, 2010).Within the context of these pathogens, emphasis is given to methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (MRCoNS) (Martins *et al*., 2012).

The resistance of Staphylococci to most antibiotics is on the increase worldwide, most especially among hospital acquired pathogens (Duran *et al.,* 2012). Multi-drug resistant staphylococci are a threat to human health. This is because they cause hard to treat infections, such as chronic skin ulcers, as a result of their resistance to beta- lactam, aminoglycosides, and cephalosporin antibiotics among others. The most important mechanism of antibiotic resistance by the staphylococci, particularly resistance to penicillin, is associated with penicillin binding protein 2a (PBP2a), encoded by *mecA* (Zapun, 2008). Another important gene that is linked to penicillin resistance in staphylococci is *blaZ* , this gene encodes for β-lactamase (Zapun, 2008).

The accurate and rapid diagnosis of antibiotic resistance genes in the treatment of infections caused by staphylococci is vital and a panacea in preventing the spread of such infections. Techniques have been developed to identify the *mecA* genetic determinant that codes for this protein, because intrinsic resistance of staphylococci appears to be due to PBP2a production (Daniel *et al*., 1994). Polymerase chain reaction (PCR) based molecular methods are preferred for the determination of antibiotic resistance genes (Woodford and Sundsfjord, 2005). Polymerase chain reaction techniques show a high degree of correlation among susceptibility tests and allow accurate classification of both highly resistant and borderline-resistant strains (Gerberding *et al.*, 1991).

## 1.1 Statement of Research problem

*Staphylococcus aureus* has been described as the most frequent agent that is found in diabetic foot infections,this microorganism could be associated with changes in wound healing periods (Anacassia *et al*., 2011). The complication that is usually noticed in wounds is as a result of infection of such wounds which definitely led to increase in costs, morbidity, and mortality rates (Mohammed*et al*., 2013). In a retrospective study of incidences of wound infection and Antibiotic sensitivity pattern in patients attending Aminu Kano Teaching Hospital, Kano, Nigeria, 77.9% of the wound sites were contaminated with isolates of bacteria, with *S. aureus* ranking highest in the order of frequency (Mohammed*et al*., 2013).

Methicillin- resistant *Staphylococcus aureus* (MRSA) has been identified as a major cause of morbidity and mortality in skin lesions worldwide and has significant economic consequences (Shurland *et al*., 2007). Recurring problems of Diabetic foot infecions has been the isolation of multi- drug resistant organisms from such infections, of which MRSA is implicated to be the predominant resistant pathogen (Djahmi*et al*., 2013).

In western developed countries, it has also been reported that not too severe infections in patients that are acquired from the community are caused mainly by *S. aureus*, or coagulase-negative staphylococci, and that ulcer depth is linked directly with the presence of *S. aureus*(Gardner *et al*., 2013).

Venous ulcers, or stasis ulcers, account for 80 percent of lower extremity ulcerations (O'Meara *et al*., 2008).Less common etiologies for lower extremity ulcerations include arterial insufficiency; prolonged pressure; diabetic neuropathy; and systemic illness such as rheumatoid arthritis, vasculitis, osteomyelitis, and skin malignancy (Araujo *et al.,* 2003). The overall prevalence of venous ulcers in the United States is approximately 1 percent (O'Meara *et al*., 2008).Venous ulcers are more common in women and older persons(Callam *et al*.,1985

;Bergovist *et al*.,1999;Abbade and Lastoria,2005;Ravaghi *et al*.,2006). The primary risk factors are older age, obesity, previous leg injuries, deep venous thrombosis, and phlebitis (Nelson *et al*., 2000).

Venous ulcers are often recurrent, and open ulcers can persist from weeks to many years (Samson and Showalter, 1996; Nelzen *et al.*, 1997; Briggs and Nelson, 2003). Severe complications include cellulitis, osteomyelitis, and malignant change (Abbade and Lastoria, 2005). Although the overall prevalence is relatively low, the refractory nature of these ulcers increase the risk of morbidity and mortality, and have a significant impact on patient quality of life (Callam *et al*., 1985; Ruckley, 1997). The financial burden of venous ulcers is estimated to be $2 billion per year in the United States (Valencia *et al.*, 2001; Etufugh and Philips, 2007).

**1.2 Justification for the research study**

Chronic skin, open, non-healing ulcers pose a continual challenge in medicine (Emmanuela *et al*., 2000), coupled with resistance to management with antibiotics which has also become problematic over the years, most impoartantly with rise of epidemic strains of *Staphylococcus aureus* (Collier, 2003). The progress made with respect to infection control and wound management notwithstanding, wound infection is still a serious and significant clinical challenge, most especially in developing nations like Nigeria where wound site infections have become a major source of post – operative illness, causing high mortality among patients (De Macedo and Santos, 2005), and accounts for almost a quarter of all nosocomial infections(Nichols, 2001).Chronic skin wound infections is also a major source of increased trauma in patients, prolonged hospitalization and increased hospital cost.

The control and management of infection is an important aspect of wound care. The importance of antibiotics in the treatment and in prophylaxis to prevent infection cannot be

ruled out, however, the timing of administration , choice of antibiotics, durations of administration, are critical factors that could play a role in defining the value of antibiotics in reducing wound infection ( Nichols, 2001).Since wound infection is still a major concern among health care practitioners, most especially in developing nations like Nigeria, where health care cost is on the high side, human and financial resources which are also needed to run health care facilities are not enough, there is therefore the need for frequent surveillance of pathogens which are responsible for infections, as well as the susceptibility of these pathogens to antibiotics, and a frequent appraisal of possible risk or socio-demographic factors of wound infection to be able to update infection control measures.It is also partinent to note that a regular bacteriological review of infected chronic skin ulcers is necessary for the affected patients to receive qualitative health care.

### 1.3 Aim of study

To identify some antibiotic resistance genes of staphylococci isolated from some chronic skin ulcer patientsinKaduna state.

### 1.4 Specific objectives

1. To isolate and characterize staphylococci from chronic skin ulcers.

2. To determine antibiotic resistance patterns of the staphylococcal isolates.

3. To identify possible MRSA and MRCoNS isolates from the chronic skin ulcers.

4. To determine the minimum inhibitory and bactericidal concentrations (MIC and MBC) of the antibiotics.

5. To identify some genes encoding antibiotic resistance of staphylococcal isolates using PCR

6. To examine the socio-demographic factors associated with staphylococcal colonized chronic skin ulcers.

## 1.5 Research questions

i. Are staphylococci species present in chronic skin ulcers?

ii. Are the isolatedstaphylococci resistant to some antibiotics?

iii. Is there any Antibiotic resistant gene present among the staphylococci isolated?

iv. Is there any socio-demographic factor responsible for the chronic skin ulcer in the patients?

# CHAPTER TWO

# 2.0LITERATURE REVIEW

## 2.1 The Genus *Staphylococcus*

Staphylococci are Gram-positive spherical bacteria that occur in microscopic clusters resembling grapes. Bacteriological culture of the nose and skin of normal humans invariably yields staphylococci. In 1884, Rosenbach described the two pigmented colony types of staphylococci and proposed the appropriate nomenclature: *Staphylococcus aureus* (yellow) and *Staphylococcus albus* (white). The latter species is now named *Staphylococcus epidermidis. S. aureus* colonizes mainly the nasal passages, but it may be found regularly in most other anatomical locales, including the skin, oral cavity and gastrointestinal tract. *S epidermidis* is an inhabitant of the skin.

Taxonomically, the genus *Staphylococcus* is in the Bacterial family Staphylococcaceae, which includes three lesser known genera, Gamella, Macrococcus and Salinicoccus. The best-known of its nearby phylogenetic relatives are the members of the genus *Bacillus* in the family Bacillaceae, which is on the same level as the family Staphylococcaceae. *Staphylococcus aureus* forms a fairly large yellow colony on rich medium; *S. epidermidis* has a relatively small white colony. *Staphylococcus aureus* is often hemolytic on blood agar; *S. epidermidis* is non hemolytic. Staphylococci are facultative anaerobes that grow by aerobic respiration or by fermentation that yields principally lactic acid. The bacteria are catalase-positive and oxidase-negative. *Staphylococcus aureus* can grow at a temperature range of 15 to 45 degrees and at NaCl concentrations as high as 15 percent. Majority of*S. aureus* strains produce the enzyme coagulase, while strains of *S. epidermidis* lack this enzyme. *Staphylococcus aureus* should always be considered a potential pathogen; most strains of *S. epidermidis* are nonpathogenic and may even play a protective role in humans as normal

flora. *Staphylococcus epidermidis* may be a pathogen in the hospital environment. Staphylococci are perfectly spherical cells about 1 micrometer in diameter. The staphylococci grow in clusters because the cells divide successively in three perpendicular planes with the sister cells remaining attached to one another following each successive division. Since the exact point of attachment of sister cells may not be within the divisional plane and the cells may change position slightly while remaining attached, the result is formation of an irregular cluster of cells (Gardner et *al.*, 2001).

The shape and configuration of the Gram-positive cocci helps to distinguish staphylococci from streptococci. Streptococci are slightly oblong cells that usually grow in chains because they divide in one plane only, similar to a bacillus. Without a microscope, the catalase test is important in distinguishing streptococci (catalase-negative) from staphylococci, which are vigorous catalase-producers. The test is performed by adding 3% hydrogen peroxide to a colony on an agar plate or slant. Catalase-positive cultures produce $O_2$ and bubble at once. The test should not be done on blood agar because blood itself contains catalase. Coagulase-negative staphylococci are present on the skin of all humans and are the most frequent constituent of the normal flora at this site (Becker and Kahl, 2009). Once considered relatively avirulent and usually a contaminant when isolated from a clinical specimen, these organisms have become increasingly recognized as agents of clinically-significant nosocomial bloodstream infections.

Patients at risk include those with prosthetic valves, pacemakers, intravascular catheters or other foreign bodies in place, and immunocompromised hosts. Coagulase-negative staphylococci also account for significant morbidity and mortality in patients with native valve endocarditis(Foster, 2004). These infections are inherently difficult to treat given the frequent presence of foreign material and the often multiple drug resistant nature of the organisms. There are 31 species currently recognized in the genus Staphylococcus, which are

members of the Micrococcaceae family (Bowler and Davies, 1999). Staphylococci are aerobic and facultatively anaerobic gram-positive cocci that have a tendency to form irregular clusters, produce catalase, are not motile, and do not form spores. *Staphylococcus aureus* and *S. intermedius* are the only coagulase-positive staphylococcal species.

Although at least 18 species have been isolated from human skin (Hamory *et al*., 1987), *Staphylococcus epidermidis* accounts for more than one-half of resident staphylococci (Schultz *et al.*,2003)) with extensive distribution over the body surface. In terms of clinical isolates, *S. epidermidis* is clearly predominant, comprising more than 75 percent of coagulase-negative staphylococci in clinical specimens (Meka *et al*.,2004)) this is perhaps due to its sheer numbers on the skin surface, although it may possess virulence determinants that other coagulase-negative staphylococci lack(Hamory *et al*., 1987). Other clinically-significant species include *S. saprophyticus,* which causes urinary tract infections in young adult women (Jeffcoates and Harding, 2003), whereas *S. hominis, S. haemolyticus, S. warneri, and S. simulans* have been more rarely isolated as pathogens (Douglas and Sampson, 1995).S. lugdunensis has increasingly been recognized as a cause of invasive infections that include endocarditis, osteomyelitis, and sepsis (Bradley,1999)

*Staphylococcus lugdunensis* is a coagulase-negative staphylococcus (CNS). Like other CNS, *S. lugdunensis* in humans ranges from a harmless skin commensal to a life-threatening pathogen (as with infective endocarditis). Unlike other CNS, however, *S. lugdunensis* can cause severe disease reminiscent of the virulent infections frequently attributable to *S. aureus*(Jeffcoates and Harding, 2003). In addition, most *S. lugdunensis* isolates remain susceptible to a large number of antimicrobial agents.

*Staphylococcus lugdunensis* was first described in 1988 and was distinguished from other coagulase-negative staphylococcal species via DNA relatedness studies based on 11 clinical

strains. The new species was named after Lyon, the French city where the organism was first isolated (Lugdunum, the Latin name of Lyon) (Laupland *et al.,* 2008). *S. lugdunensis* is unique among CoNS because of its propensity for causing aggressive native valve infective endocarditis (IE) and its susceptibility to a vast array of antimicrobial agents. *Staphylococcus aureus* and coagulase-negative staphylococci have been the predominant organisms isolated from both prospective, purpose collected samples and retrospective analysis of clinical investigations. *S. aureus* has been reported in 43.0% of infected leg ulcers (Bowler and Davies, 1999), whereas *Staphylococcus epidermidis* has been reported in 14.0% of venous ulcer specimens (Brook and Fraizer, 1998) and 20.6% of diabetic foot ulcers (Urbancic–Rovan and Gubina, 1997). *Staphylococcus aureus* also has a diverse array of virulence factors that facilitate adherence to host tissues, immune system evasion, and destruction of host cells and tissues; including coagulase, protein A, leucocidins, hemolysin S, and super antigens (Foster, 2004). Resistance to methicillin in *Staphylococcus aureus,* and more recently emergence of resistance to glycopeptides, also complicate the treatment of burns wound infections and sepsis caused by these highly virulent organisms (Meka *et al.,* 2004) The interaction between ulcer and bacteria can be stratified into four levels; contamination, colonization, critical colonization and infection (Schultz *et al.,* 2003). Whilst, contamination and colonization by microbes are not believed to inhibit healing, the line between colonization and infection can be difficult to define. The term 'critical colonization' has been used to describe the stage at which bacteria begin to adversely affect wound healing (Schultz *et al*., 2003). Moreover, the underlying pathogenesis of chronic wounds may result in wounds of different aetiologies being differently affected by bacteria (Jeffcoates and Harding, 2003).

A range of clinical criteria have been used to define infection in chronic wounds. The consensus development conference on diabetic foot wound care (American Diabetes

Association, 1999) agreed that a Diabetic Foot Ulcer ( DFU) should be considered infected when there are purulent secretions or the presence of two or more signs of inflammation (erythema, warmth, tenderness, heat and induration). Guidelines for the management of chronic venous leg ulcers produced by the British Association of Dermatologists and the Royal College of Physicians (Douglas and Simpson, 1995) recommended that infection should be considered if one of the following is present: pyrexia, increased pain, increasing erythema of surrounding skin, lymphangitis or rapid increase in ulcer size. It is accepted that chronic wounds by their very nature may not always display the classic symptoms of infection (pain, erythema, oedema, heat and purulence) and it has been suggested that an expanded list, including signs specific to secondary wounds (such as serious exudates plus concurrent inflammation, delayed healing, discoloration of granulation tissue, friable granulation tissue, foul odour and wound breakdown be employed to identify infection (Gardner *et al.,* 2001).

**2.2 Antibiotic resistance in staphylococci**

Staphylococci are inherently susceptible to most antibiotics; they however remain frequent causes of morbidity and mortality, having developed resistance, both by mutation and by DNA transfer (Livermore, 2000). *Staphylococcus aureus* is a classical pathogen which causes infections at many sites (Waldvogel, 1995). Skin and soft tissue infections are frequent and range from minor eruptions through infected ulcers and cellulitis to severe impetigo. *S. aureus* has also been known to be a frequent invader of surgical and other wounds, sometimes causing to sepsis. In England and Wales,*S. aureus* is the second most common cause of bacteraemias after *Escherichia coli*, accounting for about 20% of cases (Reacher *et al*., 2000). Staphylococcalbone and joint infections can arise through contaminationin orthopaedic surgery, and *S*. *aureus* isalso themost common pathogen in this setting.

*Staphylococcusaureus*has been identifiedas one of the morecommon causes of prosthetic valve endocarditis and anoccasional agent of post-neurosurgical meningitis; nativevalve endocarditis also can arise, mostly amongintravenous narcotic abusers. *S. aureus* is an infrequent but serious cause of pneumonia, mostly in debilitated patients on ventilators, or following influenza (Waldvogel, 1995). Some strains produce one or more enterotoxin, which cause severe diarrhoea if contaminated food is eaten. Despite its pathogenicity, *S. aureus* is also carried on the moist skin in the nose(Solberg, 1965). It survives well on drier skin and inanimate surfaces facilitating cross-colonisation and -infection. Coagulase-negative staphylococci (CoNS) include *Staphylococcus epidermidis*, *S. haemolyticus*, *S. saprophyticus*, and a number of other species. Most are normal skin commensals, and all are much less pathogenic than *S. aureus*. Coagulase-negative staphylococci are important as causes of line-associated infections in the immunosuppressed and account for many of the bacteraemic episodes in neutropenic patients (Oppenheim, 1998). Overall, CoNS account for about 7–9% of bacteraemias reported to the Public Health Laboratory Service (Reacher *et al*., 2000) and isimportant as causes of prosthetic valve endocarditis, being more frequent than *S. aureus* in this setting. They are also a frequent cause of peritonitis in continuous ambulatory peritoneal dialysis, and *S. saprophyticus* is a frequent cause of urinary tract infections (Hamory *et al*., 1987).

The spread of antibioticresistance among strains of *S. aureus* is a major concern in the treatment of staphylococcal infections. It is well known that the organism acquires resistance soon after the introduction of new antibiotics (Lyon and Skurray, 1987). Penicillin-resistant *S. aureus* was reported within 4 years of the introduction of penicillin G into clinical use in 1941. Other antibiotics such as erythromycin, tetracycline, and aminoglycosides were used for the treatment of patients infected by penicillinase-producing *S. aureus* only to result in the appearance of multi-drug resistant *S. aureus* by the 1950s. Methicillin was developed in

1960 for the treatment of such multi-drug resistant *S. aureus*. However, in the same year, Jevons discovered methicillin-resistant *S. aureus*(MRSA), which by 1970s became spread all over the world (Jevons, 1961).

The mechanisms of acquisition of resistance in *S. aureus* are classified into two main categories: mutation of abacterial gene on the chromosome and acquisition of a resistancegene from other organisms by some form of geneticexchange (conjugation, transduction, or transformation).Resistance to quinolone antibiotics, for example, is caused by mutations in DNA gyrase (*gyr*) and/or topoisomerase IV (*grl*) gene, while resistance to rifampicin is caused by a mutation in RNA polymerase (*rpoB*) gene (Aubry-Damon et al., 1998; Hooper and Wolfson, 1993). In the case of resistance acquisition, exogenous antibiotic resistance genes are found on some mobile genetic elements (plasmids, insertion sequences (IS), transposons, or genomic islands (GIs)) of resistant bacteria.

Various mobile genetic elements carrying antibiotic resistance genes in staphylococci have been investigated extensively (Novick, 1990; Projan, 2000). Completion of whole genome sequences of three MRSA strains has provided us a certain bird's-eye view of the distribution of the mobile genetic elements in the bacterial chromosome that encode antibiotic resistance as well as pathogenicity of *S.aureus*. Here we consider the nature of *S. aureus* antibiotic resistance as viewed from the whole genome perspective with special reference to a unique GI, staphylococcal cassette chromosome *mec* (SCC*mec*) that encodes methicillin resistance and a putative transposon Tn*5801* that encodes resistance to tetracyclines.

### 2.3 Methicillin-resistant *Staphylococcus aureus* (MRSA)

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans. MRSA is not a "super bug." While *Staphylococcus aureus* itself is a virulent pathogen, methicillin resistant strains are NOT more virulent than

methicillin sensitive strains (Kazakova *et al.*, 2005). Many health care workers (HCWs) incorrectly assume that MRSA strains are more virulent because of the special isolation precautions implemented. MRSA is of special concern because it is often multi-drug resistant, thus limiting treatment options (Straubaugh *et al*., 1996). MRSA is any strain of *Staphylococcus aureus* that has developed, through the process of natural selection, resistance to beta-lactam antibiotics, which include the penicillins (methicillin, dicloxacillin, nafcillin, oxacillin, among others) and the cephalosporins. Strains unable to resist these antibiotics are classified as Methicillin-Sensitive *Staphylococcus aureus*, or MSSA. The evolution of such resistance does not cause the organism to be more intrinsically virulent than strains of *Staphylococcus aureus* that have no antibiotic resistance, but resistance does make MRSA infection more difficult to treat with standard types of antibiotics and thus more dangerous.

MRSA is identified by a bacterial culture and antibiotic sensitivity of the suspected site of Infection or colonization (e.g., blood, sputum, urine, wound, exudate, pressure ulcer material). Two criteria are necessary for the organism to be identified as MRSA. First, the organism is identified as *Staphylococcus aureus* or coagulase-positive *Staphylococcus* species. Second, the antibiotic sensitivity test will show that the organism is resistant to oxacillin, methicillin, nafcillin, cephalosporins, imipenem, and/or other beta-lactam antibiotics (Hanselman *et al*., 2006.)

MRSA is especially troublesome in hospitals, prisons, schools, and nursing homes, where patients with open wounds, invasive devices, and weakened immune systems are at greater risk of infection than the general public. *S. aureus* most commonly colonizes the anterior nares (the nostrils). The rest of the respiratory tract, open wounds, intravenouscatheters, and the urinary tract are also potential sites for infection. Healthy individuals may carry MRSA asymptomatically for periods ranging from a few weeks to many years. Patients with compromisedimmune systems are at a significantly greater risk of symptomatic secondary

infection. In most patients, MRSA can be detected by swabbing the nostrils and isolating the bacteria found inside. Combined with extra sanitary measures for those in contact with infected patients, screening patients admitted to hospitals has been found to be effective in minimizing the spread of MRSA in hospitals in the United States, Denmark, Finland, and the Netherlands(Brook and Fraizer, 1998). MRSA may progress substantially within 24–48 hours of initial topical symptoms. After 72 hours, MRSA can take hold in human tissues and eventually become resistant to treatment. The initial presentation of MRSA is small red bumps that resemble pimples, spider bites, or boils; they may be accompanied by fever and, occasionally, rashes. Within a few days, the bumps become larger and more painful; they eventually open into deep, pus-filled boils. About 75 percent of community-acquired (CA) MRSA infections are localized to skin and soft tissue and usually can be treated effectively. But some CA-MRSA strains display enhanced virulence, spreading more rapidly and causing illness much more severe than traditional hospital-acquired (HA) MRSA infections, and they can affect vital organs and lead to widespread infection (sepsis), toxic shock syndrome, and necrotizing ("flesh-eating") pneumonia. This is thought to be due to toxins carried by CA-MRSA strains, such as PVL and PSM, though PVL was recently found not to be a factor in a study by the National Institute of Allergy and Infectious Diseases (NIAID) at the NIH. It is not known why some healthy people develop CA-MRSA skin infections that are treatable while others infected with the same strain develop severe infections or die.

The most common manifestations of CA-MRSA are skin infections, such as necrotizing fasciitis and pyomyositis (most commonly found in the tropics), necrotizing pneumonia, infective endocarditis (which affects the valves of the heart), and bone and joint infections(Meka *et al.*,2004) CA-MRSA often results in abscess formation that requires incision and drainage. Before the spread of MRSA into the community, abscesses were not considered contagious, because it was assumed that infection required violation of skin

integrity and the introduction of staphylococci from normal skin colonization. However, newly emerging CA-MRSA is transmissible (similar, but with very important differences) from Hospital-Associated MRSA. CA-MRSA is less likely than other forms of MRSA to cause cellulitis.

### 2.3.1 Epidemiology and transmission of MRSA

The normal bacterial flora of humans often includes *S. aureus.* It has been estimated that nasal colonization in then general adult population is 20% to 40% and that carriage will be intermittent in 30% and prolonged in 50% of the nasal carriers (Becker and Kahl, 2009). A study of colonization stratified by multidrug resistance in a nationally representative survey conducted from 2001 through 2004 as part of the National Health and Nutrition Examination Survey found that the prevalence of colonization with *S. aureus* decreased from 32.4% in 2001-2002 to 28.6% in 2003-2004, however the prevalence of colonization with MRSA increased from 0.8% to 1.5%. In this study, colonization with MRSA was independently associated with healthcare exposure in males, age > or =60 years, diabetes, and poverty in females. In a subset of colonized people in 2003-2004, a total of 19.7% of MRSA-colonized persons carried a PFGE type associated with community transmission (Kazakova *et al*.,2005).*S. aureus* from a nasal colonization can be transferred to skin and other body areas. The main mode of transmission of MRSA is person to person via hands, usually of HCWs (CDC, 1999).Colonization of hands of personnel may be either transient, such as a single day, or of longer duration, such as several weeks. Colonization of the HCW may occur if proper handwashing and barriers (such as gowns and gloves) are not used appropriately.

MRSA may be aerosolized in the droplet nuclei from a coughing resident or from a ventilator exhaust port of an intubated resident who has MRSA in his or her sputum. The organism may also be aerosolized during the irrigation of a wound containing MRSA. However, the role

ofaerosolization in the transmission of MRSA is not known (Kazakova *et al*., 2005). Although MRSA has been isolated from environmental surfaces, transmission to residents isthought to be minimal, except in burn units (Boyce*et al*., 1994).When an infection occurs after a breach of the body's defenses of the skin, the pathogen is often endogenous. Therefore the presence of endogenous *S. aureus*, especially MRSA, is a risk factor for infection, which has been well characterized in bloodstream infections (Von Eiff *et al*., 2001; Laupland*et al*., 2008). Colonization with MRSA often precedes infection by MRSA. The connection between transmission of MRSA from an exogenous (outside of the body) source via hands, equipment, and the hospital environment and subsequent endogenous carriage of MRSA is the primary infection prevention and control consideration for the elimination of MRSA transmission in hospital setting. MRSA has a history of being frequently associated with healthcare, and conventional wisdom had categorized MRSA as a hospital problem until the late 1990s. But during that decade, data from the Canadian MRSA surveillance system showed that 5–7% of reported MRSA infections occurred in individuals with no known healthcare-associated risk factors for acquisition (Kazakova *et al*.,2005).

Concurrently, reports were being received by the CDC regarding MRSA infections infull-term newborn infants(Herold *et al*.,1998; Eckhandt *et al*.,2003), Children(CDCP,1999), Military personnel(Kallen *et al*.,2000) ,Prisoners(CDCP,2001), Athletes(Kazakova *et al*.,2005),that were both phenotypically and genotypically characterized as community-associated strains. Research from the veterinary community on MRSA infection and colonization of animals and pets has identified yet another reservoir of MRSA that is transmissible to humans (Weese *et al.,* 2006; Hanselman *et al.,* 2006). Amplification of community reservoirs of MRSA provides another incentive for aggressive action to eliminate transmission of MRSA in healthcare settings.

Numerous studies conducted in acute hospitals have identified admission from nursing homes as a major risk factor for MRSA carriage and vice versa (Bradley, 1999). Despite these studies, the epidemiology of MRSA within nursing homes has received limited attention. The available data show prevalence rates of MRSA colonization varying between 0% to over 40% (Bradley, 2002). MRSA prevalence in nursing homes shows a wide geographic variation and, within confined geographic areas, significant differences of MRSA prevalence between nursing homes have been observed (McNeil *et al*., 2002). It is not clear why MRSA is endemic or epidemic in some nursing homes while not in others. When colonized residents have been compared with noncarriers, increased age, underlying chronic disease, decreased mobility, impaired cognitive status, presence of intravenous, urinary, or enteral feeding devices, presence of wounds, recent use of antibiotics and recent hospital stay were frequently associated with MRSA carriage (McNeil *et al*., 2002) . MRSA can be acquired de novo under the selective pressure of antibiotic use.

Transferring patients between hospitals and nursing homes is common and some studies suggest that most nursing home residents acquire their MRSA carriage in a hospital rather than in the nursing home, creating a two-way flow of MRSA (Hoefnagels-Schuermans *et al*., 2002). Little is known about the extent of transmission of MRSA within nursing homes. Transmission of MRSA seems rather uncommon in nursing homes, except in the case were MRSA is endemic or epidemic. This hypothesis is supported by the findings of a recent study in which the distribution of antibiograms of MRSA strains in different nursing homes was compared. It was found that in high prevalence institutions, the proportion of isolated MRSA strains showing the same antibiogram was higher when compared with low prevalence nursing homes (Suetens *et al*., 2006). It is assumed that indirect transmission from the hands of staff members presents the major mode of spread of MRSA within a nursing home. Direct transmission from resident to resident has been described, but seems rather uncommon.

Although one study showed that the likelihood of MRSA carriage for a patient sharing a room with an MRSA-positive person was almost five times higher when compared with residents with an MRSAnegative roommate (Suetens *et al*., 2006).

Likewise, the environment has been noted to be an uncommon source for transmission of MRSA within the setting of nursing homes (Bradley, 1999). Although it commonly causes only asymptomatic colonization, *Staphylococcus aureus* is a highly pathogenic organism with the potential tocause serious infections, such as blood-stream infections, pneumonia, endocarditis, skin and soft tissue infections, and bone and joint infections, often associated with significant morbidity and mortality(Bradley, 2002) While there is no evidence for MRSA being more virulent than Methicillin-susceptible *Staphylococcus aureus,* MRSA infections are significantly more difficult and costly to treat (Lee*et al*.,1994). Much remains to be elucidated about the natural history of MRSA colonization in residents of nursing homes. Colonization with MRSA doesn't necessarily translate into a higher risk for MRSA infection. One study showed that the incidence of MRSA infections among residents with MRSA colonization was comparable with the incidence among residents not colonized with MRSA (Lee *et al.,* 1994). A 3-year follow-up study of Belgian nursing home residents noted no excess hospitalizations or mortality among MRSA carriers, except in nursing home residents with severe disorientation in time and space (Suetens *et al*., 2006). It seems, based on the scarce data, that MRSA colonization as such is not harmful to residents in relatively good health.

In most community acquired MRSA (CA-MRSA) strains in the United States, methicillin resistance is encoded in a novel genetic elements, staphylococcal cassette chromosome *mec* type IV. Many of these strains have been resistant only to B-lactams and macrolides (eg. erythromycin) and retain susceptibility to many non-Blactam antimicrobial agents such as

lincomycins (eg. clindamycin), fluoroquinolones, rifampin, trimethoprim- sulfamethoxazole, aminoglycosides and tetracyclines. CA-MRSA also produces several toxins not commonly found in healthcare associated strains, notably Panton-Valentine leukocidin, which causes leukocyte destruction and tissue necrosis. The predominant molecular genotypes that cause CA-MRSA infections are USA300 and USA400. The USA300 clone has emerged as the predominant cause of staphylococcal skin and soft tissue infections. In the majority of healthcare associated MRSA (HA-MRSA) strains in the United States, methicillin resistance is encoded in staphylococcal cassette chromosome *mec* type II. HA-MRSA are frequently resistant to many other classes of antibiotics and the Panton-Valentine leukocidin is rarely found. The predominant molecular genotypes that cause HA-MRSA infection are USA100 and USA200 (Jernigan*et al.*, 2006; Klevens *et al*., 2007).

The boundaries between HA-MRSA and CA-MRSA are becoming blurred due to the movement of patients and infections between hospitals and the community (Otter and French, 2006). Nosocomial outbreaks of CA-MRSA following the admission of colonized or infected patients have occurred (Miller *et al*., 2007). In the USA, where CA-MRSA is now common, it is becoming increasingly difficult to distinguish between CA- and HA-MRSA on clinical and epidemiological grounds (Miller *et al*., 2007; Neapolitano, 2008). Since HA-MRSA and CA-MRSA strains are often genotypically and phenotypically different, the microbiological characteristics of the isolates may help to distinguish between the two types of infection (King *et al*., 2006; Dryden, 2008). A recent study identified 12% of MRSA isolates as being community-associated, and skin and soft tissue infections were more common among community-associated cases compared with those acquired in hospital or in healthcare associated institutions (Naimi *et al.*, 2003). In a study carried out in the mid 1990s in Dublin, 8.6% of residents of six nursing home were positive for MRSA and 24% of environmental samples were also positive(Dryden, 2008). Risk factors associated with MRSA amongst these

patients included male sex, age greater than 80 years, resident in the nursing home for less than 6 months, hospitalisation during the previous 6 months, antibiotic therapy during the previous 3 months and poor mental test score(King *et al*., 2006). In the 1999 North/South study, 3.9% of cases were identified by general practitioners and/or midwives and 2.2% were in nursing homes (Naimi *et al.*, 2003).

**2.3.2 Prevention and control of MRSAInfection.**

Good infection control practices must be instituted for all patients, and not just for those known to be colonised or infected with MRSA. Patients with MRSA colonisation return safely to their own homes or to residential accommodation, without significant risk to the community. Simple hygienic precautions usually suffice. Good communications between hospitals discharging patients' home with MRSA i.e. to the carers or family members, community nurses and General Practitioners (GPs), and between hospital and community hospitals or long-stay residential units, are essential in minimising spread. Likewise, the patient's MRSA status should be communicated to the receiving hospital or admitting doctor when the patient requires admission to hospital. Routine measures to control MRSA in nursing homes include adherence to the universal precautions and a rational use of antibiotics (Bradley, 1999). Surveillance cultures to identify MRSA carriers are not warranted. Patients colonized with MRSA should not be excluded from activities or isolated, as long as the colonized site can be covered and the patients are capable of performing good hygiene (McNeil *et al*., 2002).When cultures are obtained for clinical purposes, infection and colonization rates seem to increase and an outbreak is possible, thus more intensive infection control measures should be implemented. In the setting of an outbreak or high endemicity, survey of staff and residents for the presence of asymptomatic carriage and decolonization of asymptomatic carriers should be considered.

## 2.4 Antibiotic resistance genes of staphylococci

Staphylococci have become one of the most common causes of nosocomial infections. Multidrug-resistant staphylococci pose a growing problem for human health. The rise of drug-resistant virulent strains of *Staphylococcus aureus*, particularly methicillin-resistant *S. aureus* (MRSA) is a serious problem in the treatment and control of staphylococcal infections (Livermore, 2000; Zapun, 2008) Multidrug-resistant staphylococci pose a growing problem for human health. The rise of drug-resistant virulent strains of *Staphylococcus aureus*, particularly methicillin-resistant *S. aureus* (MRSA).Methicillin-resistant staphylococci (MRS) cause hard-to-treat infections because these are resistant to most of the antibiotics such as beta-lactams, aminoglycosides, and macrolides. The most important mechanism of resistance to penicillin is production of beta-lactamase which inactivates penicillin by hydrolysis of its beta-lactam ring. Another mechanism is associated with penicillin-binding protein 2a (PBP2a), encoded by *mecA*2. Another gene involved in penicillin resistance in staphylococci is *blaZ* which encodes β-lactamase (Zapun, 2008).

Aminoglycoside modifying enzymes (AMEs) are major factors responsible for resistance to aminoglycoside in staphylococci. Until now, three classes of AMEs have been identified: acetyltransferase (AAC), aminoglycoside phosphotransferase (APH), and aminoglycoside nucleotidyltransferase (ANT) (Choi *et al.,* 2003) The most important mechanism of aminoglycoside resistance in staphylococci is drug inactivation by AMEs like aminoglycoside nucleotidyltransferases (APHs). AMEs can be plasmid or chromosome encoded. In staphylococcal strains, the most commonly found AME is *aac(6')/aph(2'')*. The bifunctional enzyme *aac (6')/aph (2'')* is encoded by the *aac(6')/aph(2'')* gene. In addition, APH(3')-III is encoded by *aph(3')- IIIa* gene and the ANT(4')-I by *ant(4')-Ia* gene, are also found in staphylococcal isolates (Yadegar *et al.*,2009).

The *mecA* gene responsible for methicillin resistance is part of a mobile genetic element found in all MRSA strains. It was demonstrated that *mecA* is part of a genomic island designated staphylococcal cassette chro-mosome *mec* (SCC*mec*) (Katayama *et al*., 2000). Staphylococcal resistance to penicillin is mediated by *blaZ*, the gene that encodes β-lactamase. This predominant extracellular enzyme, synthesized when staphylococci are exposed to β-lactam antibiotics, hydrolyzes the β-lactam ring, rendering the β-lactam inactive. *blaZ* is under the control of two adjacent regulatory genes, the antirepressor *blaR1* and the repressor *blaI* (Kernodle, 2000). Recent studies have demonstrated that the signaling pathway responsible for β-lactamase synthesis requires sequential cleavage of the regulatory proteins BlaR1 and BlaI. Following exposure to β-lactams, *BlaR*1, a transmembrane sensor-transducer, cleaves itself (Gregory *et al.,* 1997; Zhang *et al.,* 2001). Zhang *et al.* (2001) hypothesized that the cleaved protein functions as a protease that cleaves the repressor *Bla*I, directly or indirectly (an additional protein, BlaR2, may be involved in this pathway) and allows *blaZ* to synthesize enzyme.

Methicillin-resistant *S. aureus* (MRSA) was considered as one of the most difficult bacteria to treat in patients. The difficulty in treating MRSA infections is compounded by the fact that many strains also possess efflux pumps, which export certain tetracyclines, macrolides, genes which confer resistance to antibiotics and antiseptics (Marshall and Piddock, 1997). In staphylococci, three genes (*ermA, ermB* and *ermC*) encoding methyl transferases are responsible for resistance to macrolide by modification of the ribosomal target site in 23S rRNA (Trzcinski *et al.,* 2000; Fluit *et al.,* 2001). It is known that in MRSA, the *msrA* gene confer drug resistance by efflux mechanism (Ross *et al.,* 1995). Several studies concerning the epidemiological distribution of genes encoding erythromycin ribosomal methylases and efflux pumps have been performed by dot blot or Southern hybridization (Eady *et al.*, 1993),

and detection of erythromycin resistance determinants by PCR has been performed with staphylococci and streptococci (Sutcliffe *et al.*, 1996).

**2.5 Antibiotic resistance and chronicwounds**

The relationship of microorganisms with antibiotic resistance is an important public health issue which has yet to be fully investigated. The combination of increasing numbers of the population who are at risk of developing chronic wounds, together with the increasing prevalence of antibiotic resistance, makes this a highly pertinent issue. The polymicrobial nature of chronic wounds is likely to provide an appropriate environment for genetic exchange between bacteria. Indeed, the first two cases of vancomycin resistant *S. aureus* in the United States were both isolated from chronic wound patients (CDC, 2002). It is hardly surprising that antibiotic-resistant organisms have been found to colonize and infect chronic wounds. Colsky *et al*.(1998), found as many as half of all *S. aureus* isolates from hospitalized dermatology patients with leg ulcers to be methicillin-resistant *S. aureus* (MRSA) and more than one-third of *P. aeruginosa* isolates to be resistant to ciprofloxacin. A study by Tentolouris *et al.* (1999), in a diabetic foot clinic found 40% of *S. aureus* isolated from non-limb-threatening infected foot ulcers to be MRSA; giving MRSA a prevalence of 15% in all DFU patients with infected ulcers. Furthermore, there were significantly more MRSA isolates from patients who had received prior antibiotic therapy, compared with those that had not. A follow-up study, in the same clinic, identified a similar proportion of methicillin resistance in the *S. aureus* isolates, but showed that the prevalence of MRSA in foot ulcers had almost doubled over a 3 year period to 30% of all DFU patients with ulcer infection(Davies,2003).

Ge *et al.* (2002), investigated resistance in bacterial isolates from infected DFUs, from patients who had not received antibiotics during the previous fortnight, and found 12% of *S. aureus*, 46% of *S. epidermidis* and 45% of *S. haemolyticus* to be methicillinresistant. They also found high levels of resistance to erythromycin in most species of Gram-positive

organisms. The previously mentioned Swedish audit of all chronic wounds by Tammelin *et al*. (1998), also found 12.5% of *S. aureus* isolates and 21.7% of *Pseudomonas* species isolates to be resistant to a clinically relevant antibiotic. Different populations of wound patients can show wide variation in the level of antibiotic resistance encountered. For example, a prospective study of uninfected chronic venous leg ulcers from 66 patients who had received no antibiotics in the previous month identified very low levels of antibiotic resistance; only two patients were found to have MRSA [7.7% of those patients colonized with S. aureus (n = 26)](Davies,2003). In contrast, a separate, retrospective investigation of leg and foot ulcer swabs sent for analysis at the PHLS in Cardiff, from wounds presumed to be infected or having prolonged non-healing, demonstrated much higher levels of MRSA: 36% of patients with *S. aureus*. The underlying reason for these differences are unknown and could be multi-factorial, including such factors as prior antibiotic therapy and the level of contact with healthcare institutions.

Chronic wound patients are clearly a high-risk group for the acquisition, carriage and dissemination of antibiotic-resistant organisms. Day & Armstrong (1997) reviewed the limited evidence on risk factors for the carriage of MRSA in diabetic foot wounds. While they found no studies that had directly addressed this issue, suggested risks include cross-contamination of wounds from the patients themselves, inanimate objects or health care personnel, long-term use of antibiotics, prior hospitalization and severity of illness (which may increase exposure to MRSA endemic environments, such as hospitals and nursing homes). The risk that wound patients carrying antibiotic-resistant organisms pose to others is also unknown. However, dressing changes alone have been shown to disperse significant numbers of bacteria into the air (Lawrence *et al.*, 1992). The extent of this dispersal varies according to the type of dressing involved and is slow to decline (Lawrence *et al*., 1992).

Wound patients are also clearly a group of patients who have a high level of contact with health care staff and could themselves act as a reservoir for cross-contamination. High prevalence of antibiotic resistance, especially MRSA, affects treatment decisions concerning wounds and raises the question of whether and when empirical regimens should cover these resistant organisms (Armstrong *et al.*,2004). Whilst the additional impact of antibiotic-resistant organisms on wound healing is not known, overall, the morbidity, mortality and cost associated with infections in hospital patients caused by antibiotic-resistant organisms has been shown to be 1.3- to 2-fold higher than infections caused by antibiotic sensitive organisms ( Cosgrove and Cameli,2003).

It is clear from the literature that expert opinion suggests that antibiotics have an important role to play in the treatment of clinically infected chronic wounds. However, there are no conclusive scientific studies to support antibiotic use, let alone those that might definitively guide antibiotic choice, dose and duration. The use of antibiotics is not risk-free for the individual with both the immediate risk associated with anaphylactic reactions (Wilson, 2000), and the longer term prospect of antibiotic use making co-morbidities more difficult to treat. For example, the use of macrolides and metronidazole up to 10 years previously have, respectively, been associated with clarithromycin and \ metronidazole resistance in *Helicobacter pylori* isolates (McMahon *et al.*, 2003). In addition, antibiotic resistance in the general population is a continuing and growing concern. The contribution made to the development, maintenance and dissemination of resistance by those antibiotics issued for chronic wounds is not yet known, although there is reason to believe that the chronic wound patient population may be of importance due to the high levels of antibiotic prescribing to these patients, the degree of microbial load associated with their lesions and the potential they provide for dissemination of resistant organisms to others. MRSA and other resistant organisms have been isolated from both infected and colonized chronic wounds, however, the

true prevalence and impact on the wider community are, again, not known. Research needs to be undertaken to elicit the interactions between microbes, antibiotics and antibiotic resistance in chronic wounds for the benefit of both chronic wound patients and the population in general.

**2.6 Ulcer**

An **ulcer** is a sore on the skin or a mucous membrane, accompanied by the disintegration of tissue. Ulcers can result in complete loss of the epidermis and often portions of the dermis and even subcutaneous fat. Ulcers are most common on the skin of the lower extremities and in the gastrointestinal tract. An ulcer that appears on the skin is often visible as an inflamed tissue with an area of reddened skin. A skin ulcer is often visible in the event of exposure to heat or cold, irritation, or a problem with blood circulation. They can also be caused due to a lack of mobility, which causes prolonged pressure on the tissues. This stress in the blood circulation is transformed to a skin ulcer, commonly known as bedsores or decubitus ulcers. (Taylor *et al*., 2005) Ulcers often become infected, and pus forms.

**2.7Chronic skin ulcer**

A chronic ulcer is a wound that does not heal in an orderly set of stages and in a predictable amount of time the way most wounds do; wounds that do not heal within three months are often considered chronic (Taylor *et al.,* 2005). Chronic wounds seem to be detained in one or more of the phases of wound healing. For example, chronic wounds often remain in the inflammatory stage for too long (Snyder,2005;Taylor *et al*.,2005) In acute wounds, there is a precise balance between production and degradation of molecules such as collagen; in chronic wounds this balance is lost and degradation plays too large a role (Edwards *et al*.,2004;Schonfelder *et al*., 2005) Chronic wounds may never heal or may take years to do so. These wounds cause patients severe emotional and physical stress and create a significant

financial burden on patients and the whole healthcare system (Augustin and Maier, 2003).Acute and chronic wounds are at opposite ends of a spectrum of wound healing types that progress toward being healed at different rates (Moreo,2005).

### 2.7.1 Types of Chronic skin ulcer

2.7.1.1*Mouth ulcer*

A mouth ulcer is an open sore inside the oral cavity. The types of oral ulcers are diverse with a multitude of associated causes including physical or chemical trauma, infection from microorganisms or viruses, medical conditions or medications, cancerous and sometimes non specific processes. The oral ulcer appears as a white or yellow oval with an inflamed red border. Sometimes, a white circle or halo around the lesion can be observed. The grey, white or yellow coloured area within the red boundary is due to the formation of layers of fibrin; a protein involved in the clotting of blood. The ulcer may be accompanied by a painful swelling of the lymph nodes below the jaw, which can be mistaken for toothache. Ulceration of the oral tissues can be caused by an overreaction by the body's own immune system. Factors that provoke mouth ulcers include stress, fatigue, illness, hormonal changes, menstruation, and deficiencies in vitamin $B_{12,}$ (Edward and Harding, 2004))

2.7.7.2 *Venous Leg ulcers*

V**e**nous Leg ulcers are the most common ulcers of the lower leg. Persons with venous ulcers often have lower leg edema and a history of previous phlebitis, tired aching legs, and a minor traumatic event. Most venous ulcers are located on the medial malleolus and the skin surrounding the wound is hyper- pigmented (Hill *et al.*, 2003)

2.7.7.3 *Genital Ulcers*

These may be penile, vulval or labial. Most often, it is due to sexually transmitted diseases. In the United States, the majority of young sexually active patients who have genital ulcers have genital herpes, syphilis or chancroid. All three of these diseases have been associated with an increased risk for HIV infection. Malignancy could set in, resulting into genital cancer. Cervical cancer is the commonest genital cancer in women in Nigeria (Davies, 2003)

2.7.7.4 *Corneal Ulcer*

This is an inflammatory or infective condition of the cornea involving disruption of its epithelial layer with involvement of the corneal stroma. In developing countries, a corneal ulcer is the cause of great morbidity as well as economic loss to the person and family. Children affected by vitamin A deficiency are at high risk for corneal ulcer and may become blind in both eyes (Bowler *et al.*, 2001)

2.7.7.5 *Pressure (Decubitus) ulcer*

Pressure ulcers are a localized injury to the skin and underlying tissue, usually over a bony prominence as a result of pressure or pressure in combination with friction and shear. They usually occur in patients who are immobilized either temporarily or permanent especially with concomitant impaired sensation. Pressure ulcers are also called decubitus ulcers, bed sores, or pressure sores. (Davies, 2003).

2.7.7.6 *Diabetic Ulcers*

This is frequently used to describe ulcers that occur at the bottom of the feet in persons with diabetes mellitus. The direct cause of these ulcers is pressure. Peripheral neuropathy, structural foot deformities, or limited range of motion in the foot may increase pressure and contribute to the development of these ulcers. Diabetic often have ischemia as well, and the

term “neuro-ischemic foot” is often used. The neuropathic foot is charactized by dry atopic skin, deformity, and limited range of motion. The sensory function is often impaired and leaves the foot susceptible to injury. (Davies *et al.,* 2004)

2.7.7.7 *Neuropathic Ulcers*

The most common cause of neuropathy is diabetes. However, other causes include alcohol or drug abuse, hereditary defects, and some metabolic diseases or malnutrition. The frequency of neuropathy increases with age and is not uncommon in the age group of 80+ years (Davies *et al.*, 2004)

2.7.7.8 *Traumatic Ulcers*

Although almost all other ulcers have a history of minor trauma, the term traumatic ulcer is used for ulcers that occur in patients who do not have a prior history of predisposing factors such as diabetes, venous or arterial vascular disorder, or immune- incompetence. Normally traumatic ulcers heal uneventfully, but the size, necrotic tissue, contamination, or local edema may disturb healing. This may also include skin tears which are common in elderly persons. Skin tears may provide an ideal site for biofilm formation (Bowler *et al*., 2001)

2.7.7.9 *Arterial Ulcers*

Peripheral vascular occlusive disease, which often presents as intermittent claudication, can also lead to slow- to- heal wounds. Many of the risk factors are similar to those for coronary artery disease. As with venous ulcers, the direct cause of an arterial ulcer is often a minor bump or bruise. Arterial ulcers often have a “punched out” appearance and there are signs of impaired tissue perfusion, such as pale skin and diminished or absent pedal pulses. However, arterial wounds often heal after successful revascularization or bypass of the arterial blockage (Hill *et al.,* 2003)

## 2.8 Predisposing factors to chronic skin ulcer

Multiple factors can lead to impaired wound healing. In general terms, the factors that influence repair can be categorized into local and systemic. Local factors are those that directly influence the characteristics of the wound itself, while systemic factors are the overall health or disease state of the individual that affect his or her ability to heal. Many of these factors are related, and the systemic factors act through the local effects affecting wound healing (Guo and Dipietro, 2010).

### 2.8.1Local factors that influence healing

2.8.1.1 *Oxygenation*

Oxygen is important for cell metabolism, especially energy production by means of ATP, and is critical for nearly all. It prevents wounds from infection, induces angiogenesis, increases keratinocyte differentiation, migration, and re epithelialization, enhances fibroblast proliferation and collagen synthesis, and promotes wound contraction (Bishop, 2008; Rodriguez *et al*., 2008). In addition, the level of superoxide production (a key factor for oxidative killing pathogens) by polymorphonuclear leukocytes is critically dependent on oxygen levels. Due to vascular disruption and high oxygen consumption by metabolically active cells, the microenvironment of the early wound is depleted of oxygen and is quite hypoxic.

Several systemic conditions, including advancing age and diabetes, can create impaired vascular flow, thus setting the stage for poor tissue oxygenation. In the context of healing, this overlay of poor perfusion creates a hypoxic wound. Chronic wounds are notably hypoxic; tissue oxygen tensions have been measured transcutaneously in chronic wounds from 5 to 20 mm Hg, in contrast to control tissue values of 30 to 50 mm Hg (Tandara and Mustoe, 2004). In wounds where oxygenation is not restored, healing is impaired. Temporary hypoxia after

injury triggers wound healing, but prolonged or chronic hypoxia delays wound healing (Bishop, 2008; Rodriguez *et al*., 2008). In acute wounds, hypoxia serves as a signal that stimulates many aspects of the wound-healing process. Hypoxia can induce cytokine and growth factor production from macrophages, keratinocytes, and fibroblasts. Cytokines that are produced in response to hypoxia include PDGF, TGF-β, VEGF, tumor necrosis factor-α (TNF-α), and endothelin-1, and are crucial promoters of cell proliferation, migration and chemotaxis, and angiogenesis in wound healing (Rodriguez *et al*., 2008).

In normally healing wounds, ROS such as hydrogen peroxide ($H_2O_2$) and superoxide ($O_2$) are thought to act as cellular messengers to stimulate key processes associated with wound healing, including cell motility, cytokine action (including PDGF signal transduction), and angiogenesis. Both hypoxia and hyperoxia increase ROS production, but an increased level of ROS transcends the beneficial effect and causes additional tissue damage (Rodriguez *et al*., 2008). In summary, the proper oxygen level is crucial for optimum wound healing. Hypoxia stimulates wound healing such as the release of growth factors and angiogenesis, while oxygen is needed to sustain the healing process (Bishop, 2008). One therapeutic option that can sometimes overcome the influence of tissue hypoxia is hyperbaric oxygen therapy (HBOT; Rodriguez *et al*., 2008). While HBOT can be an effective treatment for hypoxic wounds, its availability is limited.

2.8.1.2 *Infections*

Once skin is injured, micro-organisms that are normally sequestered at the skin surface obtain access to the underlying tissues. The state of infection and replication status of the microorganisms determines whether the wound is classified as having contamination, colonization, local infection/critical colonization, and/or spreading invasive infection. Contamination is the presence of non-replicating organisms on a wound, while colonization is defined as the presence of replicating microorganisms on the wound without tissue

damage. Local infection/ critical colonization is an intermediate stage, with microorganism replication and the beginning of local tissue responses. Invasive infection is defined as the presence of replicating organisms within a wound with subsequent host injury (Edwards and Harding, 2004).

Inflammation is a normal part of the wound-healing process, and is important to the removal of contaminating micro-organisms. In the absence of effective decontamination, however, inflammation may be prolonged, since microbial clearance is incomplete. Both bacteria and endotoxins can lead to the prolonged elevation of pro-inflammatory cytokines such as interleukin-1 (IL-1) and TNF-α and elongate the inflammatory phase. If this continues, the wound may enter a chronic state and fail to heal. This prolonged inflammation also leads to an increased level of matrix metalloproteases (MMPs), a family of proteases that can degrade the ECM. In tandem with the increased protease content, a decreased level of the naturally occurring protease inhibitors occurs. This shift in protease balance can cause growth factors that appears in chronic wounds to be rapidly degraded (Edwards and Harding, 2004; Menke *et al*., 2007). Similar to other infective processes, the bacteria in infected wounds occur in the form of biofilms, which are complex communities of aggregated bacteria embedded in a selfsecreted extracellular polysaccharide matrix (EPS)(Edwards and Harding, 2004).

Mature biofilms develop protected microenvironments and are more resistant to conventional antibiotic treatment. *Staphylococcus aureus* (*S. aureus*), *Pseudomonasaeruginosa* (*P. aeruginosa*), and β-hemolytic *streptococci* are common bacteria in infected and clinically non-infected wounds (Edwards and Harding, 2004; Davis *et al*., 2008). *Pseudomonas aeruginosa* and *Staphylococcus aureus*appear to play an important role in bacterial infection in wounds. Many chronic ulcers probably do not heal because of the presence of biofilms containing *P. aeruginosa*, thus shielding the bacteria from the

phagocytic activity of invading polymorphonuclear neutrophils (PMNs). This mechanism may explain the failure of antibiotics as a remedy for chronic wounds (Bjarnsholt *et al*., 2008).

### 2.8.2 Systemic factors that influence healing

2.8.2.1 *Age*

The elderly population (people over 60 years of age) is growing faster than any other age group (World Health Organization [WHO, www.who.int/topics/ageing]), and increased age is a major risk factor for impaired wound healing. Many clinical and animal studies at the cellular and molecular level have examined age-related changes and delays in wound healing. It is commonly recognized that, in healthy older adults, the effect of aging causes a temporal delay in wound healing, but not an actual impairment in terms of the quality of healing (Gosain and DiPietro, 2004; Keylock *et al*., 2008). Delayed wound healing in the aged is associated with an altered inflammatory response, such as delayed T-cell infiltration into the wound area with alterations in chemokine production and reduced macrophage phagocytic capacity (Swift *et al.*, 2001). Delayed re-epithelialization, collagen synthesis, and angiogenesis have also been observed in aged mice as compared with young mice (Swift *et al.*, 1999). Overall, there are global differences in wound healing between young and aged individuals. A review of the age-related changes in healing capacity demonstrates that every phase of healing undergoes characteristic age-related changes, including enhanced platelet aggregation, increased secretion of inflammatory mediators, delayed infiltration of macrophages and lymphocytes, impaired macrophage function, decreased secretion of growth factors, delayed re-epithelialization, delayed angiogenesis and collagen deposition, reduced collagen turnover and remodeling, and decreased wound strength (Gosain and DiPietro, 2004). Several treatments to reduce the age-related impairment of healing have been studied. Interestingly, exercise has been reported to improve cutaneous wound healing in older adults

as well as aged mice, and the improvement is associated with decreased levels of pro-inflammatory cytokines in the wound tissue. The improved healing response may be due to an exercise-induced anti-inflammatory response in the wound (Emery *et al*., 2005; Keylock *et al*., 2008).

2.8.2.2 *Sex Hormones in aged individuals*

Sex hormones play a role in age-related wound-healing deficits. Compared with aged females, aged males have been shown to have delayed healing of acute wounds. A partial explanation for this is that the female estrogens (estrone and 17β-estradiol), male androgens (testosterone and 5α-dihydrotestosterone, DHT), and their steroid precursor dehydroepiandrosterone (DHEA) appear to have significant effects on the wound-healing process (Gilliver *et al*., 2007). It was recently found that the differences in gene expression between elderly male and young human wounds are almost exclusively estrogen-regulated (Hardman and Ashcroft, 2008). Estrogen affects wound healing by regulating a variety of genes associated with regeneration, matrix production, protease inhibition, epidermal function, and the genes primarily associated with inflammation (Hardman and Ashcroft, 2008). Studies indicate that estrogen can improve the age-related impairment in healing in both men and women, while androgens regulate cutaneous wound healing negatively (Gilliver *et al*., 2007).

2.8.2.3 *Stress*

Stress has a great impact on human health and social behavior. Many diseases—such as cardiovascular disease, cancer, compromised wound healing and diabetes—are associated with stress. Numerous studies have confirmed that stress-induced disruption of neuroendocrine immune equilibrium is consequential to health (Glaser and Kiecolt-Glaser, 2005; Vileikyte, 2007). The pathophysiology of stress results in the deregulation of the immune system, mediated primarily through the hypothalamic-pituitaryadrenal (HPA) and

sympathetic-adrenal medullary axes or sympathetic nervous system (SNS; Godbout and Glaser, 2006; Boyapati and Wang, 2007). Studies in both humans and animals have demonstrated that psychological stress causes a substantial delay in wound healing. Caregivers of persons with Alzheimer's and students undergoing academic stress during examinations demonstrated delayed wound healing (Kiecolt-Glaser *et al*., 1995; Marucha *et al*., 1998). The hypothalamic-pituitary-adrenal and the sympathetic- adrenal medullary axes regulate the release of pituitary and adrenal hormones. These hormones include the adrenocorticotrophic hormones, cortisol and prolactin, and catecholamines (epinephrine and norepinephrine). Stress up-regulates glucocorticoids (GCs) and reduces the levels of the proinflammatory cytokines IL-1β, IL-6, and TNF-α at the wound site. Stress also reduces the expression of IL-1α and IL-8 at wound sites—both chemoattractants that are necessary for the initial inflammatory phase of wound healing (Godbout and Glaser, 2006; Boyapati and Wang, 2007). Furthermore, GCs influence immune cells by suppressing differentiation and proliferation, regulating gene transcription, and reducing expression of cell adhesion molecules that are involved in immune cell trafficking (Sternberg, 2006). The GC cortisol functions as an anti-inflammatory agent and modulates the Th1-mediated immune responses that are essential for the initial phase of healing. Thus, psychological stress impairs normal cell mediated immunity at the wound site, causing a significant delay in the healing process (Godbout and Glaser, 2006). Stressors can lead to negative emotional states, such as anxiety and depression, which may in turn have an impact on physiologic processes and/or behavioral patterns that influence health outcomes. In addition to the direct influences of anxiety and depression on endocrine and immune function, stressed individuals are more likely to have unhealthy habits, which include poor sleep patterns, inadequate nutrition, less exercise, and a greater propensity for abuse of alcohol, cigarettes, and other drugs. All of these factors may come into play in negatively modulating the healing response.

2.8.2.4 *Diabetes*

Diabetes affects hundreds ofmillions of people worldwide.Diabetic individuals exhibit a documented impairment in the healing of acute wounds. Moreover, this population is prone to develop chronic non-healing diabetic foot ulcers (DFUs), which are estimated to occur in 15% of all persons with diabetes. DFUs are a serious complication of diabetes, and precede 84% of all diabetesrelated lower leg amputations (Brem and Tomic-Canic, 2007). The impaired healing of both DFUs and acute cutaneous wounds in persons with diabetes involves multiple complex pathophysiological mechanisms. DFUs, like venous stasis disease and pressure-related chronic non-healing wounds, are always accompanied by hypoxia (Tandara and Mustoe, 2004). A situation of prolonged hypoxia, which may be derived from both insufficient perfusion and insufficient angiogenesis, is detrimental for wound healing. Hypoxia can amplify the early inflammatory response, thereby prolonging injury by increasing the levels of oxygen radicals (Mathieu *et al*., 2006; Woo *et al*., 2007). Hyperglycemia can also add to the oxidative stress when the production of ROS exceeds the anti-oxidant capacity (Vincent *et al*., 2004). The formation of advanced glycation end-products (AGEs) under hyperglycemia and the interaction with their receptors (RAGE) are associated with impaired wound healing in diabetic mice as well (Woo *et al*., 2007). High levels of metalloproteases are a feature of diabetic foot ulcers, and the MMP levels in chronic wound fluid are almost 60 times higher than those in acute wounds. This increased protease activity supports tissue destruction and inhibits normal repair processes (Woo *et al*., 2007; Sibbald and Woo, 2008).

Several dysregulated cellular functions are involved in diabetic wounds, such as defective T-cell immunity, defects in leukocyte chemotaxis, phagocytosis, and bactericidal capacity, and dysfunctions of fibroblasts and epidermal cells. These defects are responsible for inadequate bacterial clearance and delayed or impaired repair in individuals with diabetes (Loots *et al*.,

1998; Sibbald and Woo, 2008). As mentioned above, hypoxia contributes to the compromised healing of DFUs, and diabetic wounds exhibit inadequate angiogenesis. Several studies that have investigated the mechanisms behind the decreased restoration of vasculature in diabetic wounds have implied that EPC mobilization and homing are impaired, and that the level of VEGF, the primary proangiogenic factor in wounds, is decreased in the diabetic state (Brem and Tomic-Canic, 2007; Gallagher *et al*., 2007; Quattrini *et al*., 2008).

Stem-cell-based therapies aimed at inducing EPCs or BM-MSCs have shown a promising outcome in diabetic nonhealing wounds, both in animals and in clinical trials (Wu *et al*., 2007; Liu and Velazquez, 2008; Rea *et al*., 2009). In animal studies, therapeutic restoration of VEGF has been shown to improve repair outcomes significantly (Kirchner *et al*., 2003; Wu*et al*., 2007). The neuropathy that occurs in diabetic individuals probably also contributes to impaired wound healing. Neuropeptides such as nerve growth factor, substance P, and calcitonin gene related peptide are relevant to wound healing, because they promote cell chemotaxis, induce growth factor production, and stimulate the proliferation of cells. A decrease in neuropeptides has been associated with DFU formation. In addition, sensory nerves play a role in modulating immune defense mechanisms, with denervated skin exhibiting reduced leukocyte infiltration (Galkowska *et al*., 2006; Sibbald and Woo, 2008). In summary, the impaired healing that occurs in individuals with diabetes involves hypoxia, dysfunction in fibroblasts and epidermal cells, impaired angiogenesis and neovascularization, high levels of metalloproteases, damage from ROS and AGEs, decreased host immune resistance, and neuropathy.

2.8.2.5 *Medications*

Many medications, such as those which interfere with clot formation or platelet function, or inflammatory responses and cell proliferation have the capacity to affect wound healing. Here we review only the commonly used medications that have a significant impact on healing,

including glucocorticoid steroids, non-steroidal anti-inflammatory drugs, and chemotherapeutic drugs.

*Glucocorticoid Steroids*

Systemic glucocorticoids (GC), which are frequently used as anti-inflammatory agents, are well known to inhibit wound repair *via* global anti-inflammatory effects and suppression of cellular wound responses, including fibroblast proliferation and collagen synthesis. Systemic steroids cause wounds to heal with incomplete granulation tissue and reduced wound contraction (Franz *et al*., 2007). Glucocorticoids also inhibit production of hypoxia-inducible factor-1 (HIF-1), a key transcriptional factor in healing wounds (Wagner *et al*., 2008). Beyond effects on repair itself, systemic corticosteroids may increase the risk of wound infection. While systemic corticosteroids inhibit wound repair, topical application produces quite different effects. Topical low-dosage corticosteroid treatment of chronic wounds has been found to accelerate wound healing, reduce pain and exudate, and suppress hypergranulation tissue formation in 79% of cases. While these positive effects are striking, careful monitoring is necessary to avoid a potential increased risk of infection with prolonged use (Hofman *et al*., 2007).

*Non-steroidal Anti-inflammatory Drugs*

Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen are widely used for the treatment of inflammation and rheumatoid arthritis and for pain management. Low-dosage aspirin, due to its anti-platelet function, is commonly used as a preventive therapeutic for cardiovascular disease, but not as an anti-inflammatory drug (Pieringer *et al*., 2007). There are few data to suggest that short-term NSAIDs have a negative impact on healing. However, the question of whether long-term NSAIDs interfere with wound healing remains open. In animal models, systemic use of ibuprofen has demonstrated an anti-proliferative effect on wound healing, resulting in decreased numbers of fibroblasts, weakened breaking strength,

reduced wound contraction, delayed epithelialization (Dong *et al*., 1993), and impaired angiogenesis (Jones *et al*., 1999). The effects of low-dose aspirin on healing are not completely clear. Clinical recommendations suggest that, to avoid anti-platelet effects, individualsshoulddiscontinue NSAIDs for a time period equal to 4 to 5 times the half life of drugs before surgery. Thus, the majority of surgical patients do not have significant NSAID activity at the time of wound repair. The exception may be those cardiac patients who must be maintained on low-dose aspirin due to severe risk of cardiovascular events (Pieringer *et al*., 2007). In terms of the topical application of NSAIDs on the surfaces of chronic wounds, the local use of ibuprofen- foam provides moist wound healing, reduces persistent and temporary wound pain, and benefits chronic venous leg ulcer healing (Price *et al*., 2007).

*Chemotherapeutic Drugs*

Most chemotherapeutic drugs are designed to inhibit cellular metabolism, rapid cell division, and angiogenesis and thus inhibit many of the pathways that are critical to appropriate wound repair. These medications inhibit DNA, RNA, or protein synthesis, resulting in decreased fibroplasia and neovascularization of wounds (Waldron and Zimmerman-Pope, 2003; Franz *et al*., 2007). Chemotherapeutic drugs delay cell migration into the wound, decrease early wound matrix formation, lower collagen production, impair proliferation of fibroblasts, and inhibit contraction of wounds (Franz *et al*., 2007). In addition, these agents weaken the immune functions of the patients, and thereby impede the inflammatory phase of healing and increase the risk of wound infection. Chemotherapy induces neutropenia, anemia, and thrombocytopenia, thus leaving wounds vulnerable to infection, causing less oxygen delivery to the wound, and also making patients vulnerable to excessive bleeding at the wound site. Impaired wound healing due to chemotherapeutic drugs such as adriamycin is most common when the drugs are administered pre-operatively or within 3 weeks post-operatively (Lawrence *et al*., 1986).

Additionally, low post-operative albumin levels, low post-operative hemoglobin, advanced stage of disease, and electrocautery use have all been reported as risk factors for the development of wound complications (Kolb *et al*., 1992). A newer generation of tumor chemotherapeutics is the angiogenesis inhibitors, such as bevacizumab, which is an antibody fragment that neutralizes VEGF. These therapies work in conjunction with traditional chemotherapeutics to limit the blood supply to tumors, reducing their ability to grow. Wound-healing complications, including an increase in wound dehiscence, have been described in patients on angiogenesis inhibitors (Lemmens *et al*., 2008). A caveat is that most patients on angiogenesis inhibitors are also on traditional chemotherapeutics, making it difficult to sort out whether angiogenesis inhibitors alone would perturb repair (Scappaticci *et al*., 2005; Scott, 2007). Nevertheless, current recommendations include discontinuation of angiogenesis inhibitors well in advance of any surgical procedures.

2.8.2.6 *Obesity*

The prevalence of obesity continues to increase among adults, children, and adolescents in the United States, with more than 30% of adults and 15% of children and adolescents classified as obese in a recent survey (Centers for Disease Control and Prevention, CDC). Obesity is well known to increase the risk of many diseases and health conditions, which include coronary heart disease, type 2 diabetes, cancer, hypertension, dyslipidemia, stroke, sleep apnea, respiratory problems, and impaired wound healing. Obese individuals frequently face wound complications; including skin wound infection, dehiscence, hematoma and seroma formation, pressure ulcers, and venous ulcers (Wilson and Clark, 2004).

An increased frequency of wound complications has been reported for obese individuals undergoing both bariatric and non-bariatric operations (Greco *et al*., 2008; Momeni *et al*., 2009). In particular, a higher rate of surgical site infection occurs in obese patients. Many of these complications may be a result of a relative hypoperfusion and ischemia that occurs in

subcutaneous adipose tissue. This situation may be caused by a decreased delivery of antibiotics as well. In surgical wounds, the increased tension on the wound edges that is frequently seen in obese patients also contributes to wound dehiscence. Wound tension increases tissue pressure, reducing microperfusion and the availability of oxygen to the wound (Wilson and Clark, 2004).

The increase in pressure ulcers or pressure-related injuries in obese individuals is also influenced by hypovascularity, since poor perfusion makes tissue more susceptible to this type of injury. In addition, the difficulty or inability of obese individuals to reposition themselves further increases the risk of pressurerelated injuries. Moreover, skin folds harbor micro-organisms that thrive in moist areas and contribute to infection and tissue breakdown. The friction caused by skin-on-skin contact invites ulceration. Together, these factors predispose obese individuals to the development of impaired wound healing (Wilson and Clark, 2004; Greco *et al*., 2008). In addition to local conditions, systemic factors also play an important role in impaired wound healing and wound complications in obese patients. Obesity can be connected to stress, anxiety, and depression, all situations which can cause an impaired immune response (Wilson and Clark, 2004). The function of adipose tissue used to be considered as primarily caloric storage. However, more recent findings have documented that adipose tissue secretes a large variety of bioactive substances that are collectively named adipokines. Both adipocytes themselves as well as macrophages inside the adipose tissue are known to produce bioactive molecules including cytokines, chemokines, and hormone-like factors such as leptin, adiponectin, and resistin. Adipokines have a profound impact on the immune and inflammatory response (Juge-Aubry *et al*., 2005; Greco *et al*., 2008; Wozniak *et al*., 2009).

The negative influence of adipokines on the systemic immune response seems likely to influence the healing process, although direct proof for this is lacking. Impaired peripheral blood mononuclear cell function, decreased lymphocyte proliferation, and altered peripheral cytokine levels have been reported in obesity. Importantly, though, many of the obesity related changes in peripheral immune function are improved by weight loss (Nieman *et al*., 1999; Fontana *et al*., 2007; de Mello *et al*., 2008).

2.8.2.7 *Alcohol Consumption*

Clinical evidence and animal experiments have shown that exposure to alcohol impairs wound healing and increases the incidence of infection (Gentilello *et al*., 1993; Szabo and Mandrekar, 2009). The effect of alcohol on repair is quite clinically relevant, since over half of all emergency room trauma cases involve either acute or chronic alcohol exposure (Rivara *et al*., 1993; Madan *et al*., 1999). Alcohol exposure diminishes host resistance, and ethanol intoxication at the time of injury is a risk factor for increased susceptibility to infection in the wound (Choudhry and Chaudry, 2006). Studies have demonstrated profound effects of alcohol on host defense mechanisms, although the precise effects are dependent upon the pattern of alcohol exposure (*i.e*., chronic *vs*. acute alcohol exposure, amount consumed, duration of consumption, time from alcohol exposure, and alcohol withdrawal).

A recent review on alcohol-induced alterations on host defense after traumatic injury suggested that, in general, short-term acute alcohol exposure results in suppressed pro-inflammatory cytokine release in response to an inflammatory challenge. The higher rate of post-injury infection correlates with decreased neutrophil recruitment and phagocytic function in acute alcohol exposure (Greiffenstein and Molina, 2008). Beyond the increased incidence of infection, exposure to ethanol also seems to influence the proliferative phase of healing. In murine models, exposure to a single dose of alcohol that caused a blood alcohol level of 100 mg/dL (just above the legal limit in most states in the US) perturbed re-

epithelialization, angiogenesis, collagen production, and wound closure (Radek *et al*., 2005, 2007, 2008; Fitzgerald *et al*., 2007). The most significant impairment seems to be in wound angiogenesis, which is reduced by up to 61% following a single ethanol exposure. This decrease in angiogenic capacity involves both decreased expression of VEGF receptors and reduced nuclear expression of HIF-1alpha in endothelial cells (Radek *et al*., 2005, 2008). The ethanol-mediated decrease in wound vascularity causes increased wound hypoxia and oxidative stress (Radek *et al*., 2008).

Connective tissue restoration is also influenced by acute ethanol exposure, and results in decreased collagen production and alterations in the protease balance at the wound site (Radek *et al*., 2007). In summary, acute ethanol exposure can lead to impaired wound healing by impairing the early inflammatory response, inhibiting wound closure, angiogenesis, and collagen production, and altering the protease balance at the wound site. As mentioned previously, the host response to chronic alcohol exposure appears to be different from that of acute alcohol exposure. Analysis of clinical data indicates that chronic alcohol exposure causes impaired wound healing and enhanced host susceptibility to infections, but the detailed mechanisms that explain this effect need more investigation.

2.8.2.8 *Smoking*

It is well-known that smoking increases the risk of heart and vascular disease, stroke, chronic lung disease, and many kinds of cancers. Similarly, the negative effects of smoking on wound healing outcomes have been known for a long time (Siana *et al*., 1989; Jensen *et al*., 1991). Post-operatively, patients who smoke show a delay in wound healing and an increase in a variety of complications such as infection, wound rupture, anastomotic leakage, wound and flap necrosis, epidermolysis, and a decrease in the tensile strength of wounds (Chan *et al*., 2006; Ahn *et al*., 2008). In the realm of oral surgery, impaired healing in smokers has been noticed both in routine oral surgery and in the placement of dental implants (Levin and

Schwartz-Arad, 2005; Balaji, 2008). Cosmetic outcomes also appear to be worse in smokers, and plastic and reconstructive surgeons are often reluctant to perform cosmetic surgeries on individuals who refuse to quit smoking (Siana *et al*., 1989; Goldminz and Bennett, 1991).

Approximately over 4000 substances in tobacco smoke have been identified, and some have been shown to have a negative impact on healing (Ahn *et al*., 2008). Most studies have focused on the effects of nicotine, carbon monoxide, and hydrogen cyanide from smoke. Nicotine probably interferes with oxygen supply by inducing tissue ischemia, since nicotine can cause decreased tissue blood flow *via* vasoconstrictive effects (Ahn *et al*., 2008; Sorensen *et al*., 2009). Nicotine stimulates sympathetic nervous activity, resulting in the release of epinephrine, which causes peripheral vasoconstriction and decreased tissue blood perfusion. Nicotine also increases blood viscosity caused by decreasing fibrinolytic activity and augmentation of platelet adhesiveness. In addition to the effects of nicotine, carbon monoxide in cigarette smoke also causes tissue hypoxia. Carbon monoxide aggressively binds to hemoglobin with an affinity 200 times greater than that of oxygen, resulting in a decreased fraction of oxygenated hemoglobin in the bloodstream. Hydrogen cyanide, another wellstudied component of cigarette smoke, impairs cellular oxygen metabolism, leading to compromised oxygen consumption in the tissues. Beyond these direct tissue effects, smoking increases the individual's risk for atherosclerosis and chronic obstructive pulmonary disease, two conditions that might also lower tissue oxygen tension (Siana *et al*., 1989; Jensen *et al*., 1991). Several cell types and processes that are important to healing have been shown to be adversely affected by tobacco smoke. In the inflammatory phase, smoking causes impaired white blood cell migration, resulting in lower numbers of monocytes and macrophages in the wound site, and reduces neutrophil bactericidal activity. Lymphocyte function, cytotoxicity of natural killer cells, and production of IL-1 are all depressed, and macrophage-sensing of Gram-negative bacteria is inhibited (McMaster *et al*., 2008). These effects result in poor

wound healing and an increased risk of opportunistic wound infection. During the proliferative phase of wound healing, exposure to smoke yields decreased fibroblast migration and proliferation, reduced wound contraction, hindered epithelial regeneration, decreased extracellular matrix production, and upset in the balance of proteases (Siana *et al*., 1989).

Pharmacologically, the influence of smoking on wound healing is complicated, and neither neither nicotine alone nor any other singlecomponent can explain all of the effects of smoking on wounds. What is certain is that smoking cessation leads to improved repair and reduces wound infection (Sorensen *et al*., 2003; Lauerman, 2008). For surgery patients who find it difficult to forego smoking, the use of a transdermal patch during the pre-operative period might be beneficial. A study has shown that the use of a transdermal nicotine patch as a nicotine replacement for smoking cessation therapy can increase type I collagen synthesis in wounds (Sorensen *et al*., 2006). Despite the overall negative effects of smoking, some recent studies have suggested that low doses of nicotine enhance angiogenesis and actually improve healing (Jacobi *et al*., 2002; Morimoto *et al*., 2008).

2.8.2.9 *Nutrition*

For more than 100 years, nutrition has been recognized as a very important factor that affects wound healing. Most obvious is that malnutrition or specific nutrient deficiencies can have a profound impact on wound healing after trauma and surgery. Patients with chronic or non-healing wounds and experiencing nutrition deficiency often require special nutrients. Energy, carbohydrate, protein, fat, vitamin, and mineral metabolism all can affect the healing process (Arnold and Barbul, 2006).

*Carbohydrates, Protein, and Amino Acids*

Together with fats, carbohydrates are the primary source of energy in the wound-healing process. Glucose is the major source of fuel used to create the cellular ATP that provides

energy for angiogenesis and deposition of the new tissues (Shepherd, 2003). The use of glucose as a source for ATP synthesis is essential in preventing the depletion of other amino acid and protein substrates (Arnold and Barbul, 2006). Protein is one of the most important nutrient factors affecting wound healing. A deficiency of protein can impair capillary formation, fibroblast proliferation, proteoglycan synthesis, collagen synthesis, and wound remodeling. A deficiency of protein also affects the immune system, with resultant decreased leukocyte phagocytosis and increased susceptibility to infection (Arnold and Barbul, 2006). Collagen is the major protein component of connective tissue and is composed primarily of glycine, proline, and hydroxyproline. Collagen synthesis requires hydroxylation of lysine and proline, and co-factors such as ferrous iron and vitamin C. Impaired wound healing results from deficiencies in any of these co-factors (Campos *et al*., 2008).

Arginine is a semi-essential amino acid that is required during periods of maximal growth, severe stress, and injury. Arginine has many effects in the body, including modulation of immune function, wound healing, hormone secretion, vascular tone, and endothelial function. Arginine is also a precursor to proline, and, as such, sufficient arginine levels are needed to support collagen deposition, angiogenesis, and wound contraction (Shepherd, 2003; Campos *et al*., 2008). Arginine improves immune function, and stimulates wound healing in healthy and ill individuals (Tong and Barbul, 2004). Under psychological stress situations, the metabolic demand of arginine increases, and its supplementation has been shown to be an effective adjuvant therapy in wound healing (Campos *et al*., 2008).

Glutamine is the most abundant amino acid in plasma and is a major source of metabolic energy for rapidly proliferating cells such as fibroblasts, lymphocytes, epithelial cells, and macrophages (Arnold and Barbul, 2006; Campos *et al*., 2008). The serum concentration of glutamine is reduced after major surgery, trauma, and sepsis, and supplementation of this amino acid improves nitrogen balance and diminishes immunosuppression (Campos *et al*.,

2008). Glutamine has a crucial role in stimulating the inflammatory immune response occurring early in wound healing (Arnold and Barbul, 2006). Oral glutamine supplementation has been shown to improve wound breaking strength and to increase levels of mature collagen (da Costa *et al*., 2003).

*Fatty Acids*

Lipids are used as nutritional support for surgical or critically ill patients to help mee energy demands and provide essential building blocks for wound healing and tissue repair. Polyunsaturated fatty acids (PUFAs), which cannot be synthesized *de novo* by mammals, consist mainly of two families, n-6 (omega-6, found in soybean oil) and n-3 (omega-3, found in fish oil). Fish oil has been widely touted for the health benefits of omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The effects of omega-3 fatty acids on wound healing are not conclusive. They have been reported to affect pro-inflammatory cytokine production, cell metabolism, gene expression, and angiogenesis in wound sites (McDaniel *et al*., 2008). The true benefit of omega-3 fatty acids may be in their ability to improve the systemic immune function of the host, thus reducing infectious complications and improving survival (Arnold and Barbul, 2006).

*Vitamins, Micronutrients, and Trace Elements*

Vitamins C (L-ascorbic acid), A (retinol), and E (tocopherol) show potent anti-oxidant and anti-inflammatory effects. Vitamin C has many roles in wound healing, and a deficiency in this vitamin has multiple effects on tissue repair. Vitamin C deficiencies result in impaired healing, and have been linked to decreased collagen synthesis and fibroblast proliferation, decreased angiogenesis, and increased capillary fragility. Also, vitamin C deficiency leads to an impaired immune response and increased susceptibility to wound infection (Arnold and Barbul, 2006; Campos *et al*., 2008). Similarly, vitamin A deficiency leads to impaired wound healing. The biological properties of vitamin A include anti-oxidant activity, increased

fibroblast proliferation, modulation of cellular differentiation and proliferation, increased collagen and hyaluronate synthesis, and decreased MMP mediated extracellular matrix degradation (Burgess, 2008).

Vitamin E, an anti-oxidant, maintains and stabilizes cellular membrane integrity by providing protection against destruction by oxidation. Vitamin E also has anti-inflammatory properties and has been suggested to have a role in decreasing excess scar formation in chronic wounds. Animal experiments have indicated that vitamin E supplementation is beneficial to wound healing (Arnold and Barbul, 2006; Burgess, 2008), and topical vitamin E has been widely promoted as an anti-scarring agent. However, clinical studies have not yet proved a role for topical vitamin E treatment in improving healing outcomes (Khoosal and Goldman, 2006). Several micronutrients have been shown to be important for optimal repair. Magnesium functions as a co-factor for many enzymes involved in protein and collagen synthesis, while copper is a required co-factor for cytochrome oxidase, for cytosolic anti-oxidant superoxide dismutase, and for the optimal cross-linking of collagen. Zinc is a co-factor for both RNA and DNA polymerase, and a zinc deficiency causes a significant impairment in wound healing. Iron is required for the hydroxylation of proline and lysine, and, as a result, severe iron deficiency can result in impaired collagen production (Shepherd, 2003; Arnold and Barbul, 2006; Campos *et al*., 2008). As indicated above, the nutritional needs of the wound are complex, suggesting that composite nutrition support would benefit both acute and chronic wound healing. A recent clinical research study examined the effects of a high-energy, protein-enriched supplement containing arginine, vitamin C, vitamin E, and zinc on chronic pressure ulcers and indicated that this high-energy and nutrition-enriched supplement improved overall healing of the pressure ulcer (Heyman *et al*., 2008). In summary, proteins, carbohydrates, arginine, glutamine, polyunsaturated fatty acids, vitamin A, vitamin C, vitamin E, magnesium, copper, zinc, and iron play a significant role in wound healing, and

their deficiencies affect wound healing. Additional studies will be needed to fully understand how nutrition affects the healing response.

**2.9Diagnosis of ulcers.**

To diagnose infection, microbiologic results must be evaluated in conjunction with local clinical findings, such as increase in erythema, oedema, pain, purulence and lymphadenitis, or systemic factors such as fever, leukocytosis or glucose intolerance in patients with diabetes (Hutchinson and McGuckin, 1990).

Some clinicians advocate performing biopsies of wounds that have been present for longer than three months and that have not been responded to standard therapy; others recommend performing biopsies of wounds that are older than four months (Phillips and Dover, 1991). Some clinicians routinely perform biopsy of all wounds. As a result of the uncommon nature of malignant degeneration of a chronic wound or a malignancy occurring as a chronic wound, some authors suggest that only suspicious wounds should undergo biopsy. These include wounds that have increased in size, despite appropriate treatment; malodorous wounds; painful wounds with excess granulation tissue that extends beyond the margins; wounds with irregular base or margin and wounds that experience a change in drainage, excess bleeding or exophytic growth (Franco, 2001).

Biopsies may also be performed on wounds that are not responding to standard therapy to determine, if other factors, such as inflammatory or infection causes, may be impacting wound healing. Determining etiology is a critical step in the management of venous ulcers. Characteristic differences in clinical presentation and physical examination findings can help differentiate venous ulcers from other lower extremity ulcers(Hutchinson and McGuckin, 1990). The diagnosis of venous ulcers is generally clinical; however, tests such as ankle-brachial index, color duplex ultrasonography, plethysmography, and venography may be

helpful if the diagnosis is unclear (Phillips and Dover, 1991; Scriven *et al*., 1997; McGee and Boyko, 1998; Lopez and Phillips, 1998).

Venous stasis commonly presents as a dull ache or pain in the lower extremities, swelling that subsides with elevation, eczematous changes of the surrounding skin, and varicose veins (Raju and Neglen, 2009). Venous ulcers often occur over bony prominences, particularly the gaiter area. The recurrence of an ulcer in the same area is highly suggestive of venous ulcer. On physical examination, venous ulcers are generally irregular and shallow. Granulation tissue and fibrin are often present in the ulcer base. Other findings include lower extremity varicosities; edema; venous dermatitis associated with hyperpigmentation and hemosiderosis or hemoglobin deposition in the skin; and lipodermatosclerosis associated with thickening and fibrosis of normal adipose tissue under skin. A clinical severity score based on the clinical, etiology, anatomy, and pathophysiology (CEAP) classification system can guide the assessment of chronic venous disorders. The highest CEAP severity score is applied to patients with ulcers that are active, chronic (greater than three months' duration, and especially greater than 12 months' duration), and large (larger than 6 cm in diameter) (Eklof *et al*., 2004; Raju and Neglen, 2009) Poor prognostic factors for venous ulcers include large size and prolonged duration (Stewart and leaper, 1987; Margolis *et al*., 2000).

**2.10Treatment of ulcers**

Until recently, amputation was the treatment of choice for squamous cell carcinomas that arose within chronic wounds associated with chronic osteomyelitis (Kirsner *et al.*, 2006). The rationale was the high death rate associated with primary excision. However, in some cases, wide local excision was preferred for small mobile tumors (Flemming *et al.*, 1990).

Treatment options for venous ulcers include conservative management, mechanical treatment, medications, and surgical options (Nelson*et al*., 2000; Margolis *et al*., 2000) In

general, the goals of treatment are to reduce edema, improve ulcer healing, and prevent recurrence. Although numerous treatment methods are available, they have variable effectiveness to support their use.

### 2.10.1 Conservative management

2.10.1.1 *Compression therapy*

Compression therapy is the standard of care for venous ulcers and chronic venous insufficiency (Fletcher *et al*; 1997; O'meara *et al*., 2009). Methods include inelastic, elastic, and intermittent pneumatic compression. Compression therapy reduces edema, improves venous reflux, enhances healing of ulcers, and reduces pain (Fletcher *et al*; 1997). Success rates range from 30 to 60 percent at 24 weeks, and 70 to 85 percent after one year (Margolis *et al*., 2000). After an ulcer has healed, lifelong maintenance of compression therapy may reduce the risk of recurrence (Ruckley, 1997; Phillips *et al*., 2000; Nelson *et al*., 2008). However, adherence to the therapy may be limited by pain; drainage; application difficulty; and physical limitations, including obesity and contact dermatitis (Raju and Neglen, 2009).

Contraindications to compression therapy include clinically significant arterial disease and uncompensated heart failure. Inelastic compression therapy provides high working pressure during ambulation and muscle contraction, but no resting pressure. The most common method of inelastic compression therapy is the Unna boot, a zinc oxide–impregnated, moist bandage that hardens after application. The Unna boot improves healing rates compared with placebo or hydroactive dressings (Kikta *et al*., 1998; Margolis*et al*., 2000). Unlike the Unna boot, elastic compression therapy methods conform to changes in leg size and sustain compression during both rest and activity. Stockings or bandages can be used; however, elastic wraps (e.g., Ace wraps) are not recommended because they do not provide enough pressure (O'meara *et al*., 2009). Compression stockings are graded, with the greatest pressure

at the ankle and gradually decreasing pressure toward the knee and thigh (pressure should be at least 20 to 30 mm Hg, and preferably 30 to 44 mm Hg). Compression stockings are removed at night, and should be replaced every six months because they lose pressure with regular washing. Elastic bandages (e.g., Profore) are alternatives to compression stockings.

A recent meta-analysis showed that elastic compression therapy is more effective than inelastic therapy (O'meara *et al*., 2009). In addition, high compression has been proven more effective than low compression, and multilayer bandages are more effective than single layer (Fletcher *et al*., 1997; Cullum *et al*., 2000; O'meara *et al*., 2009). The disadvantage of multilayer compression bandages is that they require skilled application in the physician's office one or two times per week, depending on drainage. Intermittent pneumatic compression therapy comprises a pump that delivers air to inflatable and deflatable sleeves that embrace extremities, providing intermittent compression (Fletcher *et al*., 1997; Nelson *et al*., 2000).The benefits of intermittent pneumatic compression are less clear than that of standard continuous compression. It also is expensive and requires immobilization of the patient; therefore, intermittent pneumatic compression is generally reserved for bedridden patients who cannot tolerate continuous compression therapy (Phillips *et al*., 2000; Robson *et al*., 2006).

2.10.1.2 *Leg elevation*

Leg elevation when used in combination with compression therapy is also considered standard of care. Leg elevation requires raising lower extremities above the level of the heart, with the aim of reducing edema, improving microcirculation and oxygen delivery, and hastening ulcer healing. In one small study, leg elevation increased the laser Doppler flux (i.e., flow within veins) by 45 percent (Abu-own et al., 1994). Although leg elevation is most effective if performed for 30 minutes, three or four times per day, this duration of treatment may be difficult for patients to follow in real-world settings.

2.10.1.3 *Dressing*

Dressings are often used under compression bandages to promote faster healing and prevent Adherence of the bandage to the ulcer. A wide range of dressings are available, including hydrocolloids (e.g., Duoderm), foams, hydrogels, pastes, and simple nonadherent dressings difference among dressing types (Palfreyman *et al*., 2007). Furthermore, the more expensive hydrocolloid dressings were not shown to have a healing benefit over the lower-cost simple nonadherent (Etufugh and Phillips, 2007; Seaman, 2002). A meta-analysis of 42 randomized controlled trials (RCTs) with a total of more than 1,000 patients showed no significant dressings. Without clear evidence to support the use of one dressing over another, the choice of dressings for venous ulcers can be guided by cost, ease of application, and patient and physician preference (Palfreyman *et al*., 2007).

**2.10.2 Mechanical treatment**

Topical negative pressure, also called vacuum-assisted closure, has been shown to help reduce wound depth and volume compared with a hydrocolloid gel and gauze regimen for wounds of any etiology (Ubbink*et al*., 2008). However, clinically meaningful outcomes, such as healing time, have not yet been adequately studied. There is currently insufficient high-quality data to support the use of topical negative pressure for venous ulcers (Ubbink *et al*.,2008) .In addition, the therapy generally has not been used in clinical practice because of the challenge in administering both topical negative pressure and a compression dressing on the affected leg.

**2.10.3 Surgical management**

Overall, acute ulcers (duration of three months or less) have a 71 to 80 percent chance of healing, whereas chronic ulcers have only a 22 percent chance of healing after six months of treatment (Nelson *et al*., 2000). Given the poor healing rates associated with chronic ulcers,

surgical evaluation and management should be considered in patients with venous ulcers that are refractory to conservative therapies (Tallman *et al*., 1997).

2.10.3.1 *Debridment*

Removal of necrotic tissue and bacterial burden through debridement has long been used in wound care to enhance healing. Debridement may be sharp (e.g., using curette or scissors), enzymatic, mechanical, biologic (i.e., using larvae), or autolytic. However, there are few high-quality studies that directly evaluate the effect of debridement versus no debridement or the superiority of one type of debridement on the rate of venous ulcer healing (Dumville *et al*., 2009; Soares *et al*., 2009) 36-40 In addition, most wounds with significant necrotic tissue should be evaluated for arterial insufficiency because purely venous ulcers rarely need much debridement.

2.10.3.2 *Skin grafting*

Human skin grafting may be used for patients with large or refractory venous ulcers. It is performed with autograft (skin or cells taken from another site on the same patient), allograft (skin or cells taken from another person), or artificial skin (human skin equivalent) (Douglas and Simpson, 1995; Jones and Nelson, 2007). However, skin grafting generally is not effective if there is persistent edema, which is common with venous insufficiency, and the underlying venous disease is not addressed. A recent Cochrane review found few high-quality studies to support the use of human skin grafting for the treatment of venous ulcers (Jones and Nelson, 2007).

**2.11 Antibiotic treatment of ulcers**

Numerous recommendations exist regarding the use or avoidance of antibiotics for chronic skin wounds, and these differ according to ulcer etiology. Importantly, there is a much lower tolerance due to the risk of amputation and subsequent morbidity and mortality. Thus, the early use of antibiotics at signs of infection is generally advocated (Jeffcoate *etal.*, 2003), with

some even advocating their use in uninfected ulcers (Edmond, 1999). In contrast, recommendation with regard to venous ulcers advocates antibiotic use solely in the presence of clinical signs or symptoms of infection (Scottish Intercollegiate Guidelines Network, 1998).

Highlighting the difficulties for the clinician, the International Working Group on Diabetic Foot (IWGDF) recommends a complex antibiotic strategy which involves intravenous and/or possibly oral use of empirical broad - spectrum antibiotics in the presence of deep foot infections. The list of regimes suggested includes ampicillin/sulbactam, ticarcillin/clavulanate, co-amoxiclav, clindamycin and a quinolone, second or third generation cephalosporin and a quinolone, and metronidazole with a quinolone. (International Working Group on Diabetic Foot, 1999). These guidelines are clearly difficult to interpret and implement in practice. The clinica1 guidelines on Type 2 diabetes by Hutchinson *etal.* (2000) recommended that only ulcers with extensive cellulitis and / or osteomyelitis should be treated with intensive, systemic antibiotics. They commented that the polymicrobial nature of diabetic foot wound would suggest use of a broad spectrum antibiotic, but conclude that there is insufficient evidence to distinguish between the relative effectiveness of different antibiotic regimes.

An update of these guidelines, by the UK National Institute for Clinical Excellence (NICE) in 2004, is no more specific, recommending only that patients with non healing or progressive ulcers with clinical signs of active infection receive intensive; systemic antibiotics (National Institute for Clinical Excellence, 2004).

The Scottish Intercollegiate Guidelines Network (SIGN) guidelines for chronic leg ulcers are equally general, again recommending that systemic antibiotics only be

instituted when there is clinical evidence of infection. Guidelines from the British Association of Dermatologist and the Royal College of Physicians on the management of chronic venous leg ulcer recommend treatment with systemic penicillin upon ulcer infection with beta haemolytic streptococci, whereas cellulitis caused by other organisms should be treated according to bacteriological sensitivity (Douglas and Simpson, 1995*)*.Despite the scarcity of evidence supporting the effectiveness of antibiotics, they are still widely used in the treatment of chronic wounds. A Swedish audit showed 26.6% of patients with chronic wounds (leg and foot ulcers; pressure ulcers, post operative and traumatic wounds, which had not healed in six weeks) were receiving systemic antibiotics at the time of the study, had done so in the previous six months. In total, therefore, 60.1% of patients with chronic wounds had received at least one antibiotic in the six months period (Tammelin *et al.*, 1998).

# CHAPTER THREE

# 3.0 MATERIALS AND METHODS

## 3.1 Materials

The materials used in the research study include:

**Glasswares**- Petri-dishes, Measuring cylinder, Glass slides, MacCartney bootles, pippetes, eppendouf bottles, micro-pippetes etc.

**Media**- Mannitol salt agar, Blood agar, Chocolate agar, Mueller-hinton agar, Brain- heart infusion broth.

**Equipments**- Microscope, Autoclave, Microgen Staph ID Kits, Incubator, Oven, Refrigerator, Thermacycler, Centrifuge, Gel dock, Electrophoresis tank, etc.

**Other reagents**- Sets of Primers, Phosphate buffer, Lysis buffer, Proteinase K, Phenol-chlorofom, Sodium acetate, Ethanol, Nuclease free water, Agarose powder, Ethidium bromide, Tris acetate EDTA.

### 3.1.1 Study area

The study was carried out in Kaduna state, Nigeria.Kaduna state occupies the central portion of Northern Nigeria, and lies between Latitude 90 and 140 North of the equator with a time of one hour ahead of Greenwich Mean Time. Kaduna state has 23 Local government areas with a population of Six million, sixty-six thousand, five hundred and sixty-two people (6,066,562). It is divided into Three (3) senatorial zones as zones 1, 2 and 3, with the headquarters located at Zaria, Kaduna metropolis and Kafanchan respectively. Below is the map of Kaduna state showing the three senatorial zones 1, 2 and 3 as depicted by the pink, yellow and sky-blue colours respectively on the map.


**Fig. 3.1: Map of Kaduna State showing the Senatorial Districts (Nigerian Chamber of Commerce USA, 2014).**

The study was done across four hospitals; one hospital each at least from the three (3) senatorial zones, where chronic skin ulcer cases are reported. The hospitals are: Barau Dikko Specialist Hospital, Kaduna, General Hospital, Kafanchan, Hajiya Gambo Sawaba Genaral Hospital, Zaria and Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, a tertiary health care hospital.

**3.1.2Ethical approval.**

Ethical approval was obtained from the authorities of the ABUTH, Zaria and from the Ministry of Health, Kaduna state, before the commencement of sample collection.

**3.1.3 Study design**

The research is a cross sectional study that entailed sample collection from both male and female patients. The sample size of 292 was arrived at, using the formula below:

$$n = \frac{Z^2 PQ}{L^2}$$

$$n = \frac{(1.96)^2(0.25)(0.75)}{(0.05)^2}$$

= 288 samples

n = Sample size
Z = 95% confidence Interval (1.96)
P = known prevalence of the infection = 25% (0.25) (Shittu *et al.*, 2002)
Q = 1-P = 1-0.25 = 0.75
L = allowable error = 0.05

**3.1.4 Study participants**.

These comprise patients with clinically diagnosed cases of chronic skin ulcer. Informed consent was however obtained from the patients concerned.

***Inclusion criteria*:** Patients with chronic skin ulcer, presenting for the first time in the clinics and/or on a routine visitation to the clinics at the various hospitals under study. The patients were registered in the various hospitals, and did consent to be included in the study.

***Exclusion criteria*:** Patients who are on admission in the various wards,and those that did not consent to be included in the study were excluded.

**3.1.5Sampling plan.**

Patients, from whom the samples were collected, were recruited consecutively from the four hospitals earlier mentioned.

**3.2 Methodology**

**3.2.1 Sample collection.**

Two hundred and ninety-two (292) swab samples were collected from clinically diagnosed chronic skin ulcer patients between January 2012 to January, 2013. The swab samples were collected from Surgical Out- patients' Department (SOPD) clinics, General Out-patients' Department (GOPD) clinics, Orthopedic and Dental clinics across the four hospitals. The samples were collected from the deep parts of the wound and placed in Brain Heart Infusion broth immediately after collection, and were cultured in the laboratory within 24 hours.

**3.2.2Isolation of staphylococci from chronic skin ulcer**.

The samples were cultured on Blood Agar and Mannitol Salt Agar (MSA) plates using the streak method.The plates were incubated at $37^{o}$C, the Blood Agar plates were observed after 24 hours, while the Mannitol Salt Agar plateswere observed after 48 hours to allow for visible growth in the case of slow fermenters (Cheesbrough, 2002). Sub- culturing was done from the agar plates in order to obtain pure cultures of the isolates. Thereafter, distinct well-separated yellow colonies and creamy white colonies on MSA and Blood agar respectively were picked aseptically and stored on agar slants, which were used for further characterization.

### 3.2.3Biochemical tests usedfor the confirmation of stapylococci isolated.

The isolates were subjected tothe following prelimnary tests to confirm if they are staphylococci before further biotyping to specie level using the Microgen Staph ID Kit (UK).

3.2.3.1 *Gram stain*:

To the heat- fixed smear of the bacterial isolate on the slide, crystal violet stain was added for 30-60 secs, the stain was rapidly washed off under running, clean water, and dried, after which Lugol's iodine was added to the smear for 30-60 secs and rinsed off with clean, running water. Dilute acetone was used to decolorize the complex on the slide for few secs, which was also washed away with clean water, before counter staining with safranine for 30 secs. The smear was washed under running water, and air dried, before examination under oil immersion objective.

3.2.3.2*Catalase test***:**

A drop of 6% hydrogen peroxide was placed on a glass slide and part of the test isolates wasremoved with a nichrome wire loop and emulsified in hydrogen peroxide.

3.2.3.3*Coagulase test:*

Part of the test isolates were emulsified in about 1 ml of physiological normal saline in a clean test tube. Two millliitres of human citrated plasma was added to the test tubes, and the tubes incubated at 37°C. The tubes were checked after four hours of incubation for coagulation or complete clotting of the plasma. Where there was no coagulation, the tubes were left overnight in the incubator.

### 3.2.4Biochemical characterization of the isolates using the microgen staph ID system.

Staphylococcal isolates confirmed via the prelimnary biochemical tests above were cultured onto fresh Nutrient agar plates and incubated at 37$^{o}$C for24 hours. Single

colonies of the isolates were emulsified in the suspending medium supplied in the Microgen kit (UK) to conform to prepared 0.5 Macfarland standards.Using sterile needle and syringe, 4 drops of the bacterial suspension were added to each well of the strip. After inoculation, wells 10 and 11 of the test strips were overlayed with 4 drops of mineral oil supplied in the kit. The top of the microwell test strips were then sealed with the adhesive tape removed earlier, and then incubated at 37$^{o}$C for 24 hours.The adhesive tape was removed for the results to be read using the colour chart and the substrate reference table in the booklets provided. However a drop of PYR reagent (supplied) was added to well 12 and the result read after 10 minutes. A drop each of Nirate A and Nitrate B reagents (supplied) were added to well 9,and the result read after 60 seconds.The results were all recorded on the forms provided to arrive at an Octal Code which was used to determine the identity of the isolate to specie level from the Microgen identification System Software provided.

**3.2.5Antibiotic susceptibility test.**

Before the Antibiotic Susceptibility test was carried out, the isolates were sub- cultured onto fresh nutrient agar slants and incubated at 37$^{O}$C for 24 hours. Suspensions of the sub-cultured isolates were prepared in clean, sterilized tubes tocorrespond to the turbidity of 0.5 McFarland's standard. *S. aureus* ATCC 25923 was used as positive control strain.Sterile, molten Mueller-Hinton agar was poured into sterile petri dishes, which were allowed to cool for solidification prior to inoculation. The isolates were then tested for their susceptibility to 9 different antibiotics: Trimethoprim+sulphamethoxazole (25µg), Gentamicin (10µg), Cefoxitin (30µg), Tetracycline (30µg), Ciprofloxacin (5µg), Chloramphenicol (30µg), Erythromycin (15µg), Vancomycin (30 µg) and Amoxicillin-clavulanic acid (30 µg).

The antibiotic discs (Liofilchem, Italy) were gently pressed to make sure they were in

contact with surface of the the inoculated Mueller - Hinton agar, and the plates were incubated at 37°C for 24 hours. After incubation, the diameters of the zones of growth inhibition were measured with a metre rule to the nearest millimeter. The interpretation of the zones of inhibition was done using the chart adapted from Clinical and Laboratory Standards Institute, CLSI, (2012).

**3.2.6Minimum inhibitory concentrations (MIC) of the antibiotics.**

The method employed was the broth tube dilution.The stock solution of each antibacterial agent was first prepared by weighing 16mg of each antibioticinto the required volume of Mueller-Hinton broth, depending on the potency of the antibiotics, to make the concentration of the stock solution to be 32mg/L or 32µg/ml. The injectables were diluted with the Mueller- Hinton broth to achieve the 32mg/L or 32µg/mlstock solutionThe sterile capped test tubes were numbered 1-9. Exactly 2.0 mls of the antibiotic stock solution (32µg/ml) was placed into the first tube, while 1.0ml of sterile Mueller-Hinton broth was added to all other tubes. Exactly 1.0ml was transferred from the first tube that contains the antibiotic and the broth, to the second tube. The content of the second tube was mixed, using another syringe and needle, 1.0ml was again transfered to the third tube.The dilutions continued to tube 8, using a new syringe and needle at each stage of dilution. Exactly1.0ml was removed from tube 8 and discarded, leaving tube 9 without an antibiotic which serves as the negative control.To all the tubes, 0.1ml of the bacterial suspension (inoculum) that conforms with to 0.5 Mcfarland standard was added. Another test tube was set aside that contains the broth and antibiotic, this serves as the positive control.All the tubes were incubated at $37^{o}$C for 24 hrs.The tubes were examined for visible growth (turbidity).The tube with the highest dilution without growth was taken as the Minimum Inhibitory

Concentration (MIC) of the antibiotic.

### 3.2.7Minimum bactericidal concentrations (MBC)

The minimum bactericidal concentration was determined by sub- culturing the contents of the tubes that showed no growth in MIC onto antibiotic –free Mueller-hinton agar plates, incubated at 37$^{o}$C for 48 hrs, and then examined for bacterial growth The Mueller- Hinton agar plate with the highest dilution of antibiotic (lowest concentration) that showed no growth was taken as the Minimum Bactericidal Concentration (MBC) of the antibiotic.

### 3.2.8 Polymerase Chain Reaction (PCR) method of detecting staphylococci resistancegenes.

3.2.8.1 ***Extraction of DNA***:

Exactly 5ml ofPhospate buffer solution was added into the Eppendorf tubes prior to the addition of the isolatesinto the tubes.The isolates from which the DNA were extracted include, *Staphylococcus aureus,Staphylococcus epidermidis, Staphylococcus heamolyticus, Staphylococcus chromogenes,Styaphylococcus xylosus* and *Staphylococcus hyicus*.Five (5) isolates of *Staphylococcus aureus* and one (1) isolate each of the coagulase –negative staphylococci,totaling ten (10) isolates were used in the study. The isolates were selected based on the antibiotic susceptibility test results, the isolates were picked from the four hospitals under the study area. The content of the tubes were vortexed and then centrifuged at 13000 rpm for 5 minutes .Lysis buffer were added to the pellets in the tubes after aspirating off the supernatants, this was followed by the addition of 20µl Proteinase K.The content of the tubes were then incubated at 65$^{o}$C for 30 minutes. Phenol-chloroform solutionwas added to the tubes, followed by another round of centrifugation at 13000 rpm for 5 minutes, that led to the separation of layers in the tubes.The supernatants in the tubes were aspirated into new separate tubes for eventual DNA isolation.Exactly 50µl of 3M sodium acetate was first added to the supernatants, followed by the addition of 700 µl of 100% ethanol. The supernatants

were placed in the freezer overnight at -20$^{o}$C.The supernatants in the tubes were subjected to centrifugation at 12000 rpm for 20 minutes, and was maintained at 4$^{o}$C for the DNA to sediment.After centrifugation, the DNA sediments were recovered by aspirating off the supernatants in the tubes. Exactly 400µl of 70% ethanol was added to the DNA sediments in the tubes, and then centrifuged at 12000 rpm, maintained at 4$^{o}$C for 10 minutes.The ethanol in the tubes was aspirated off after centrifugation to leave only the DNA pellets in the tubes. Exactly30µl of Nuclease free water was added to the DNA pellets in the tubes.The tubes containing the DNA pellets were placed in the freezer at -20$^{o}$C prior to PCR (Duran *et al*., 2012)

3.2.8.2 ***Polymerase Chain Reaction (PCR)*:** The primers for the detection of *mecA, blaZ* and*tetMgenes*were selected based on the phenotypic resistance of the staphylococcal isolates to methicillin, amoxicillin-clavulanic acid and tetracycline antibiotics used in this study. The primers were dissolved and diluted in ratio 10µl to 90 µl (1:9) volume to ultra pure water (Quality Biological Inc, USA). Approximately 2µl of the DNA of each sample was added to the 4µl of the primers, and 14µl of the Ultra pure water (Quality Biological Inc, USA) was added to these to make up to 20 µl, this was added to the Pre-mix in the reaction component tubes.The reaction component tubes that contains both the Master-mix and the Pre-mix were placed in the thermal cycler. The thermo-cycler was set as follows:

Pre-denaturation = * 1 cycle at 94$^{o}$C for 5 minutes.

Denaturation = 94$^{o}$C for 1 minute *30 cycles

Annealiing temperature =47$^{o}$C for 1 minute *30 cycles

Extension = 72$^{o}$C for 1 minute * 30 cycles

Final extension = * 1 cycle at 72$^{o}$C for 5 minutes.

The PCR Products from the thermo-cycler were loaded on to the lanes of the gel after the final extension using the micropipette for electrophoresis. The agarose gel was prepared by

dissolving 1g agarose powder and 100ml of 1% TAE ( Tris acetate EDTA), dissolving this in micro wave oven, and was allowed to cool to 60$^{o}$C,10µl of 10mg/ml of Ethidium bromide was added. The ladder, known as the molecular weight marker was also loaded to the sides of the PCR products on the gel. The two electrodes:Positive (+ve) and Negative (-ve) were plugged to the electrophoresis tank from the light source. The gel was set at 60 volts for 90 minutes for the electrophoresis. However, a negative control lane(primers, water, pre-mix, no DNA) was also loaded alongside the ten samples on the gel.After the expiration of 90 minutes, the gel was taken to the gel dock containing UV light at the platform, and the camera on top, for the bands to be captured via beaming from the UV light and photographed.The gel dock was conncted to the electronic device system for the bands to be saved. *Staphylococcus epidermidis*(ATCC 27626) was used as a positive control strain to the isolates used for PCR, to guide the likely positive bands on the gel. The ladder amplicon size ranges from 100bp – 1kb.The primer sequences used for the PCR assay were obtained from Duran *et al*. (2012), other specifications of the primers are as shown in table 1.

### 3.2.9 Administration of structured questionnaire

Clinical data for each patient was collected, such as the age, gender, educational and occupational status, using structured questionnaire, to identify the possible socio-demographic factors that assessed a patient's hospital and community risk for staphylococcal acquisition.

### 3.2.10 Statistical analysis

The chi ($\square^2$) square test of association was used to identify if association exists between the occurrence of staphylococci in the chronic skin ulcer and socio- demographic factors. Data was analysed using Statistical Package for Social Sciences (SPSS). Descriptive statistics was presented as percentages and frequencies.

**Table 1: *mecA* (methicillin), *blaZ* (beta-lactam) and *tetM* (tetracycline) resistance genes primers**

| **Primers** | **Nucleotide Sequences (5' – 3')** | **Expected Amplicon Size (bp)** |
|---|---|---|
| *mecA*– F | CCTAGTAAAGCTCCGGAA | 314 |
| *mecA*– R | CTAGTCCATTCGGTCCA | |
| *blaZ* - F | ACTTCAACACCTGCTGCTTTC | 173 |
| *blaZ* - R | TGACCACTTTTATCAGCAACC | |
| *tetM*– F | AGTGGAGCGATTACAGAA | 158 |
| *tetM* -R | CATATGTCCTGGCGTGTCTA | |

Key: F- Forward

R- Reverse

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1Species of Staphylococci isolated from chronic skin ulcer patients in Kaduna state.

The Different species of staphylococci associated with chronic skin ulcer in the study areawere:*Staphylococcus aureus,* 18 (34.0%), *Staphylococcus epidermidis,* 10(18.9%), *Staphylococcus haemolyticus,* 8(15.1%), *Staphylococcus chromogenes,* 7(13.2%), *Staphylococcus xylosus,* 5(9.4%), *Staphylococcus hyicus,* 4(7.5%) and *Staphylococcus intermedius,* 1(1.9%). *S. aureus* ranked highest, while the least specie isolated was *S. intermedius.* (Table 2).

### 4. 2SiteDistribution of Staphylococci species isolated from patients with chronic Skin ulcers.

The predominant specie from most of the sites sampled from was *Staphylococcus aureus,* closely followed by*Staphylococcus epidermidis.Staphylococcus intermedius*was found only on the leg, with one (1) isolate. The highest number of the staphylococcal isolates was found among the leg ulcer, with a total of 28 isolates, representing 52.8% of the total isolates from the different sites of ulcer, while the least number of isolate, 01, representing 1.9% was found on the breast. (Table 3)

### 4.3Resistance pattern of *Staphylococcus aureus* to some antibiotics.

The commonest resistance pattern demonstrated by the *Staphylococcus aureus* isolates to some of the antibiotics is in the order Cefoxitin ( methicillin) (FOX), Amoxicillin- clavulanic acid (AUG) which was exhibited by 72% of the isolates. The other resistance patterns demonstrated by the isolates that closely follow this are in the order Cefoxitin ( methicillin) (FOX), Amoxicillin- clavulanic acid (AUG) , Tetracycline (TE) ; Amoxicillin- clavulanic acid (AUG) , Tetracycline (TE), Trimethoprim + sulphamethoxazole ( SXT) (Table 4).

**Table 2: Species of Staphylococci isolated from chronic skin ulcer patients in Kaduna state.**

| Isolate | Total Number of isolates | Percentage |
|---|---|---|
| *Staphylococcus aureus* | 18 | 34.0 |
| *Staphylococcus epidermidis* | 10 | 18.9 |
| *Staphylococcus haemolyticus* | 8 | 15.1 |
| *Staphylococcus chromogenes* | 7 | 13.2 |
| *Staphylococcus xylosus* | 5 | 9.4 |
| *Staphylococcus hyicus* | 4 | 7.5 |
| *Staphylococcus intermedius* | 1 | 1.9 |
| Total | 53 | 100 |

**Table 3: Site Distribution of Staphylococci species isolated from patients with chronic Skin ulcers.**

| Isolate (n= 53) | Breast | Leg | Hand | MR | AR | Ear | BT | Total |
|---|---|---|---|---|---|---|---|---|
| *Staphylococcus aureus* | 00 | 10 | 02 | 03 | 01 | 02 | 00 | 18 |
| *Staphylococcus epidermidis* | 01 | 06 | 01 | 00 | 01 | 00 | 01 | 10 |
| *Staphylococcus haemolyticus* | 00 | 06 | 00 | 02 | 00 | 00 | 00 | 08 |
| *Staphylococcus chromogenes* | 00 | 03 | 01 | 00 | 01 | 00 | 02 | 07 |
| *Staphylococcus xylosus* | 00 | 02 | 01 | 01 | 01 | 00 | 00 | 05 |
| *Staphylococcus hyicus* | 00 | 00 | 03 | 00 | 00 | 01 | 00 | 04 |
| *Staphylococcus intermedius* | 00 | 01 | 00 | 00 | 00 | 00 | 00 | 01 |
| Total (%) | 1(1.9%) | 28(52.8%) | 8(15.1%) | 6(11.3%) | 4(7.5%) | 3(5.7%) | 3(5.7%) | 53(100%) |

Key:

n = Number of isolates

MR = Mouth Region

AR = Abdominal Region

BT = Buttocks

**Table 4: Resistance pattern of *Staphylococcus aureus* to some antibiotics.**

| Antibiotic pattern | Number of isolates | Percentage demonstrating pattern |
|---|---|---|
| FOX, AUG | 13 | 72.2 |
| AUG, TE, SXT | 08 | 44.4 |
| FOX, AUG, TE | 07 | 38.9 |
| FOX, AUG, SXT | 03 | 16.7 |
| SXT, TE, C | 02 | 11.1 |
| VA, TE, AUG | 01 | 5.6 |
| FOX, AUG, TE, SXT | 07 | 38.9 |
| FOX, AUG, TE, SXT, C | 02 | 11.1 |

Total number of isolates = 18

**Key:**

FOX – Cefoxitin (30µg)
VA – Vancomycin (30µg)
SXT – Trimethoprim- Sulfamethoxazole (25µg)
TE – Tetracycline (30µg)
CIP – Ciprofloxacin (5µg)
C – Chloramphenicol (30µg)
E- Erythromycin (15µg)
AUG – Amoxicillin- Clavulanic acid (30µg)
CN- Gentamicin (10µg)

### 4.4 Antibiotic resistance pattern of isolated coagulase- negative staphylococci from chronic skin ulcer patients.

The resistance pattern of coagulase – negative staphylococci (CoNS) isolates to the antibiotics follows the same trend as in *Staphylococcus aureus* isolates where 60% of the CoNS showed resistance pattern of Cefoxitin ( methicillin) (FOX), Amoxicillin- clavulanic acid (AUG), where as 40% of the isolates exhibited resistance pattern of the order Cefoxitin ( methicillin) (FOX), Amoxicillin- clavulanic acid (AUG) , Tetracycline (TE) (Table 5).

### 4.5 Antibiotic susceptibility pattern of *Staphylococcus aureus* isolated from chronic ulcer patients.

The susceptibility pattern of *Staphylococcus aureus* to the antibiotics indicated that over 80% of the isolates demonstrated susceptibilty to the antibiotics in the order Vancomycin (VA), Gentamicin (CN), 55% of the isolates exhibited susceptibility pattern in the order Erythromycin (E), Ciprofoxacin (CIP), Chloramphednicol (C), while 33% of the *Staphylococcus aureus* isolates showed susceptibility pattern order of Erythromycin ( E),Trimethoprim + sulphamethoxazole ( SXT) (Table 6).

### 4.6 Antibiotic susceptibility pattern of isolated coagulase- negative staphylococci from chronic ulcer patients

The demonstrating pattern of coagulase – negative staphylococci (CoNS) susceptibility to the antibiotics is similar to that of *Staphylococcus aureus* ,where 83% of the isolates equally exhibited susceptibility pattern to the antibiotics in the order Vancomycin (VA), Gentamicin (CN), while 57% of the CoNS isolated demonstrated susceptibility pattern in the order Erythromycin (E), Ciprofoxacin (CIP), Chloramphednicol (C) .Another striking pattern of susceptibility of CoNS to the antibiotics is the 77% demonstrating pattern of the isolates to Vancomycin (VA), Gentamicin (CN), Ciprofoxacin (CIP) (Table 7).

**Table 5: Antibiotic resistance pattern of isolated coagulase- negative staphylococci from chronic skin ulcer patients.**

| Antibiotic pattern | Number of isolates | Percentage demonstrating pattern |
|---|---|---|
| FOX, AUG | 21 | 60.0 |
| AUG, TE | 01 | 2.9 |
| FOX, TE | 01 | 2.9 |
| VA, E | 01 | 2.9 |
| FOX, AUG, TE | 14 | 40.0 |
| FOX, AUG, SXT | 12 | 34.3 |
| AUG, TE, SXT | 11 | 31.4 |
| SXT, TE, C | 03 | 8.6 |
| FOX, AUG, TE, SXT | 11 | 31.4 |
| FOX, AUG, TE, SXT, C | 03 | 8.6 |

Total number of isolates = 35

**Key:**

FOX – Cefoxitin (30µg)
VA – Vancomycin (30µg)
SXT – Trimethoprim- Sulfamethoxazole (25µg)
TE – Tetracycline (30µg)
CIP – Ciprofloxacin (5µg)
C – Chloramphenicol (30µg)
E- Erythromycin (15µg)
AUG – Amoxicillin- Clavulanic acid (30µg)
CN- Gentamicin (10µg)

**Table 6: Antibiotic susceptibility pattern of *Staphylococcus aureus*isolated from chronic skin ulcer patients.**

| Antibiotic pattern | Number of isolates | Percentage demonstrating pattern |
|---|---|---|
| VA, CN | 15 | 83.3 |
| E, SXT | 06 | 33.3 |
| SXT, C | 04 | 22.2 |
| CIP, C | 01 | 5.6 |
| FOX, CN | 01 | 5.6 |
| E, CIP, C | 10 | 55.6 |
| CIP, SXT, C | 04 | 22.2 |
| E, SXT, C | 04 | 22.2 |
| FOX, VA, CN | 03 | 16.7 |
| VA, CN, E, CIP, C | 07 | 38.9 |

Total number of isolates = 18

**Key:**

FOX – Cefoxitin (30µg)
VA – Vancomycin (30µg)
SXT – Trimethoprim- Sulfamethoxazole (25µg)
TE – Tetracycline (30µg)
CIP – Ciprofloxacin (5µg)
C – Chloramphenicol (30µg)
E- Erythromycin (15µg)
AUG – Amoxicillin- Clavulanic acid (30µg)
CN- Gentamicin (10µg)

**Table 7: Antibiotic susceptibility pattern of isolated coagulase- negative staphylococci from chronic skin ulcer patients.**

| Antibiotic pattern | Number of isolates | Percentage demonstrating pattern |
|---|---|---|
| VA, CN, | 29 | 82.9 |
| SXT, C | 15 | 42.9 |
| FOX, CN | 01 | 2.9 |
| CIP, C | 01 | 2.9 |
| FOX, AUG | 01 | 2.9 |
| E, CIP, C | 20 | 57.1 |
| CIP, SXT, C | 14 | 40.0 |
| VA, CN, CIP | 27 | 77.1 |
| E, SXT, C | 11 | 31.4 |
| FOX, VA, CN | 06 | 17.1 |
| FOX, AUG, VA | 06 | 17.1 |
| FOX, AUG, VA, TE | 05 | 14.3 |
| TE,CIP, SXT, C | 09 | 25.7 |
| VA, CN, E, CIP, C | 17 | 48.6 |

Total number of isolates = 35

**Key:**

FOX – Cefoxitin (30µg)
VA – Vancomycin (30µg)
SXT – Trimethoprim- Sulfamethoxazole (25µg)
TE – Tetracycline (30µg)
CIP – Ciprofloxacin (5µg)
C – Chloramphenicol (30µg)
E- Erythromycin (15µg)
AUG – Amoxicillin- Clavulanic acid (30µg)
CN- Gentamicin (10µg)

### 4.7 Resistance of Isolated *Staphylococcus aureus* from skin ulcer patients to antibiotics

Vancomycin is the most active antibiotic against *Staphylococcus aureus*, with the percentage susceptibility of *Staphylococcus aureus* to Vancomycin as 93 %. However, *Staphylococcus aureus* exhibited 100% resistance to Amoxicillin - Clavulanic acid. Percentage resistance bars are indicated by red colour, while percentage susceptibility bars are indicated by blue colur (Fig 1).

### 4.8 Percentage antibiotic resistance of isolated coagulase negative staphylococci from chronic skin ulcer patients.

Percentage resistance of coagulase – negative staphylococci(CoNS) indicated that, on the average, all the coagulase - negative staphylococci, except *Staphylococcus intermedius* showed 79% resistance to Cefoxitin (methicillin), whileEighteen (18) (51%) of the Coagulase-negative staphylococci isolates showed 100% susceptibility to Gentamicin on the average basis. The percentage susceptibility of the CoNS isolates to the antibiotics is indicated in parenthesis (Table 8).

### 4.9Minimum inhibitory concentrations (MICs) of some antibiotics on the *Staphylococcus aureus* isolates.

The Minimum Inhibitory Concentrations (MICs) of the antibiotics on *S.aurues* isolates showed that 12(67%) of the isolates became susceptible to Amoxicillin - Clavulanic acid at the concentration of 32.0µg/ml (Table 9).

### 4.10 Minimum inhibitory concentrations (MICs) of some antibiotics on the coagulase-negative staphylococci isolates

The same pattern was observed for the coagulase –negative staphylococci, where 13(52%) of the isolates were also susceptible to Amoxicillin - clavulanic acid at the same concentration of 32.0µg/ml. Only 4(22%) and 7(28%) isolates of *S.aureus* and coagulase – negative staphylococci respectively were susceptible to some of the antibiotics at the concentration of 8.0µg/ml (Table 10).

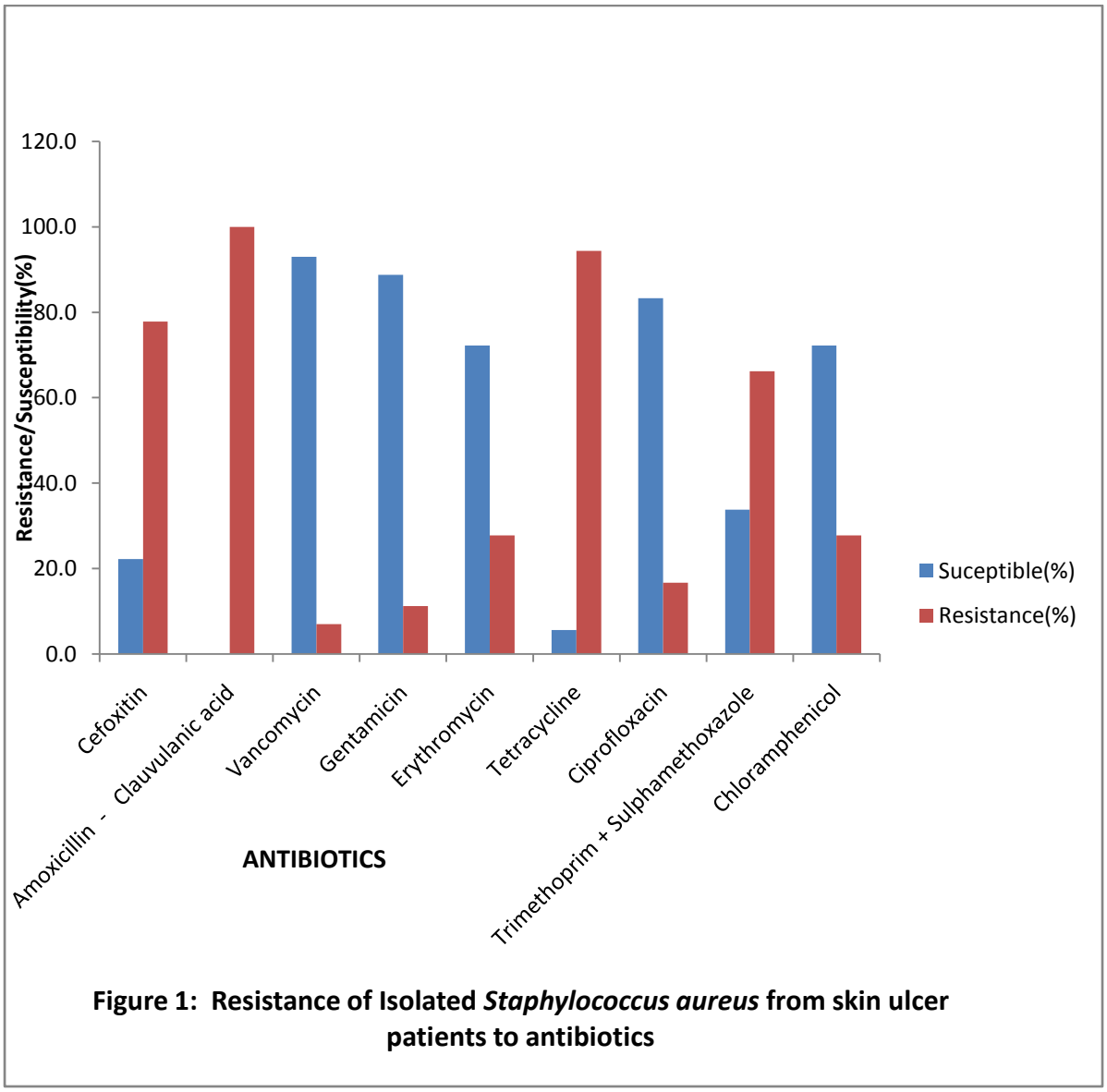


**Figure 1: Resistance of Isolated *Staphylococcus aureus* from skin ulcer patients to antibiotics**

**Table 8: Percentageantibiotic resistance of isolated coagulase negative staphylococci from chronic skin ulcer patients.**

| ANTIBIOTICS | *Staphylococcus epidermidis* n = 10 | *S.haemolyticus* n = 8 | *S. hyicus* n =4 | *S.xylosus* n = 5 | *S.chromogenes* n = 7 | *S.intermedius* n = 1 |
|---|---|---|---|---|---|---|
| Cefoxitin(30µg) | 60(40) | 100(0) | 100(0) | 80(20) | 57(43) | 0(100) |
| Amoxicillin - Clavulanic acid(30µg) | 50(50) | 88(12) | 100(0) | 60(40) | 57(43) | 100(0) |
| Vancomycin(30µg) | 2(98) | 3(97) | 0(100) | 0(100) | 2(98) | 0(100) |
| Gentamicin(10 µg) | 10(90) | 0(100) | 0(100) | 0(100) | 14(86) | 0(100) |
| Erythromycin(15 | 30(70) | 13(87) | 0(100) | 20(80) | 29(71) | 0(100) |
| Tetracycline(30µg) | 50(50) | 50(50) | 75(25) | 40(60) | 29(71) | 0(100) |
| Ciprofloxacin(5 µg) | 10(90) | 0(100) | 0(100) | 0(100) | 0(100) | 0(100) |
| Trimethoprim+sulphamethoxazole(25 µg) | 30(70) | 38(62) | 75(25) | 40(60) | 29(71) | 0(100) |
| Chloramphenicol(30µg) | 10(90) | 25(75) | 0(100) | 20(100) | 14(86) | 0(100) |

n = no of isolates tested

**Table 9: Minimum inhibitory concentrations (MICs) of some antibiotics on the *Staphylococcus aureus* isolates.**

| Antibiotics | Concentrations in µg/ml | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.25 | 0.5 | 1.0 | 2.0 | 4.0 | 8.0 | 16.0 | 32.0 |
| Number of | Isolates inhibited at different concentrations. | | | | | | | |
| Amoxicillin - Clavulanic acid(30µg) | - | - | - | - | - | - | 6 | 12 |
| Trimethoprim+ sulphamethoxazole(25µg) | - | - | - | - | - | 2 | 8 | 2 |
| Tetracycline(30µg) | - | - | - | - | - | - | 2 | 8 |
| Ciprofloxacin(5µg) | - | - | - | - | - | 1 | - | - |
| Chloramphenicol(30µg) | - | - | - | - | - | - | - | 4 |
| Erythromycin(15µg) | - | - | - | - | - | - | 2 | - |
| Gentamicin(10µg) | - | - | - | - | - | 1 | - | - |

**Table 10: Minimum inhibitory concentrations (MICs) of some antibiotics on the coagulase- negative staphylococci isolates.**

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| Antibiotics | Concentrations in µg/ml | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.25 | 0.5 | 1.0 | 2.0 | 4.0 | 8.0 | 16.0 | 32.0 |
| Number of | Isolates inhibited at different concentrations. | | | | | | | |
| Amoxicillin - Clavulanic acid(30µg) | - | - | - | - | - | - | 12 | 13 |
| Trimethoprim+ sulphamethoxazole(25µg) | - | - | - | - | - | 3 | 4 | 6 |
| Tetracycline(30µg) | - | - | - | - | - | - | 5 | 8 |
| Ciprofloxacin(5µg) | - | - | - | - | - | 1 | - | - |
| Chloramphenicol(30µg) | - | - | - | - | - | - | - | 4 |
| Erythromycin(15µg) | - | - | - | - | - | 2 | 5 | - |
| Gentamicin(10µg) | - | - | - | - | - | 1 | 1 | - |

### 4.11Minimum bactericidal concentrations (MBCs) of some antibiotics on the *Staphylococcusaureus* isolates and coagulase negative staphylococci isolates.

For the minimum bactericidal concentrations (MBCs), all the antibacterial agents, with the exception of ciprofloxacin have a cidal effect on 50% of the isolates of *S.aureus* and coagulase – negative staphylococci at the highest concentration of 32.0µg/ml. However, ciprofloxacin has a cidal effect on each isolate of *S.aureus* and Coagulase – negative staphylococci at the concentration of 8.0µg/ml (Tables 11 and 12).

### 4.12 Gel electrophoresis of the amplicons showing some antibiotic resistance genes of staphylococci

The result of the Ethidium bromide –stained multiplex PCR products after gel electrophoresis indicated that bands were visible at different base pairs along the DNA marker, which was used as the molecular size standard. The base pairs at which the bands appeared corresponds to the base pairs of the three primer sequences coding for the respective resistance genes under investigation in the isolates.These genes include, *blaZ* (173bp), *tetM* (158bp) and *mecA*(314bp) (Plate I).

### 4.13 Statistical Analysis of Socio-Demographic Factors In Relation to Chronic Skin Ulcer Infection in the Patients.

For the statistical analysis, Chi square test of association was used to ascertain if association exists between certain variables and the occurrence of chronic skin ulcer in the patients.There is a significant difference ($P< 0.05$) in the trend of the age distribution of the patients (Fig 3). Similarly, there is a significant difference ($P<0.05$) in the trend of the distribution of the sex and and educational status of the patients (Fig 4) and (Fig 5). In the case of type of urbanization as a avariable, the graph shows that there is no significant difference ($P>0.05$) in the pattern of the distribution (Fig 6).There is also a significant difference ($P<0.05$) in the pattern of distribution of the occupation, cause of ulcer and site of ulcer of the patients, as evident in the upper and lower limits of the curves (Figs 7-9). The overall analysis of the

variables considered is that there is association between all the variables, except area of domicile and the occurrence of chronic skin ulcer in the patients.

**Table 11: Minimum bactericidal concentrations (MBCs) of some antibiotics on the *Staphylococcusaureus* isolates.**

| Antibiotics | Concentrations in µg/ml | | | |
|---|---|---|---|---|
| | 4.0 | 8.0 | 16.0 | 32.0 |
| | Number of isolates killed by the antibiotics | | | |
| Amoxicillin - Clavulanic acid(30µg) | - | - | - | 18 |
| Trimethoprim+ sulphamethoxazole(25µg) | - | - | - | 12 |
| Tetracycline(30µg) | - | - | - | 10 |
| Ciprofloxacin(5µg) | - | 1 | - | - |
| Chloramphenicol(30µg) | - | - | - | 4 |
| Erythromycin(15µg) | - | - | 2 | - |
| Gentamicin(10µg) | - | - | 1 | - |

**Table 12: Minimum bactericidal concentrations (MBCs) of some antibiotics on the coagulase negative staphylococci isolates.**

| Antibiotics | Concentrations in µg/ml | | | |
|---|---|---|---|---|
| | 4.0 | 8.0 | 16.0 | 32.0 |
| | Number of isolates killed by the antibiotics | | | |
| Amoxicillin -Clavulanic acid(30µg) | - | - | - | 25 |
| Trimethoprim+ sulphamethoxazole(25µg) | - | - | - | 13 |
| Tetracycline(30µg) | - | - | - | 13 |
| Ciprofloxacin(5µg) | - | 1 | - | - |
| Chloramphenicol(30µg) | - | - | - | 4 |
| Erythromycin(15µg) | - | - | 7 | - |
| Gentamicin(10µg) | - | - | 2 | - |

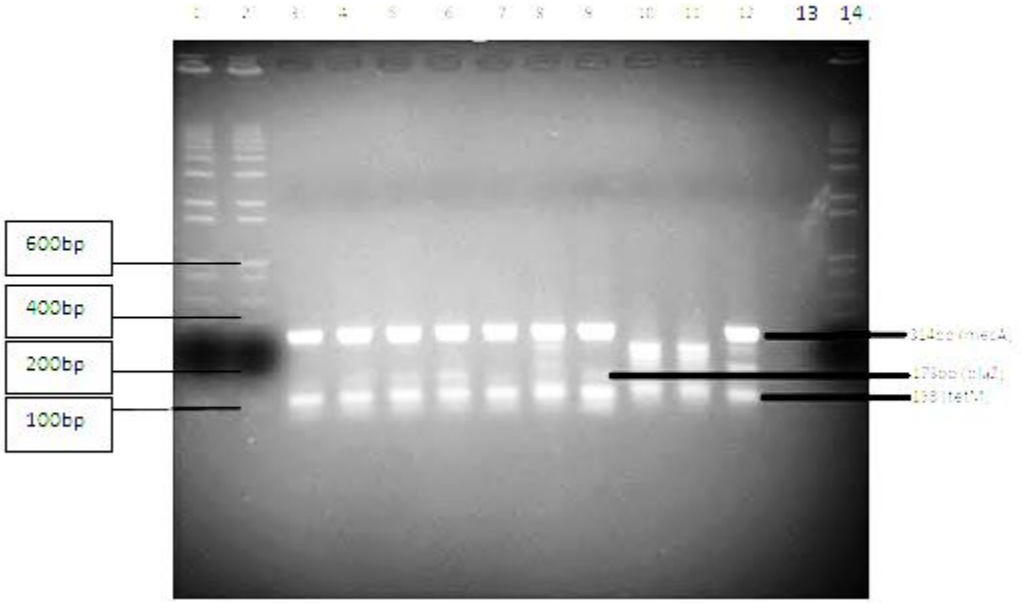
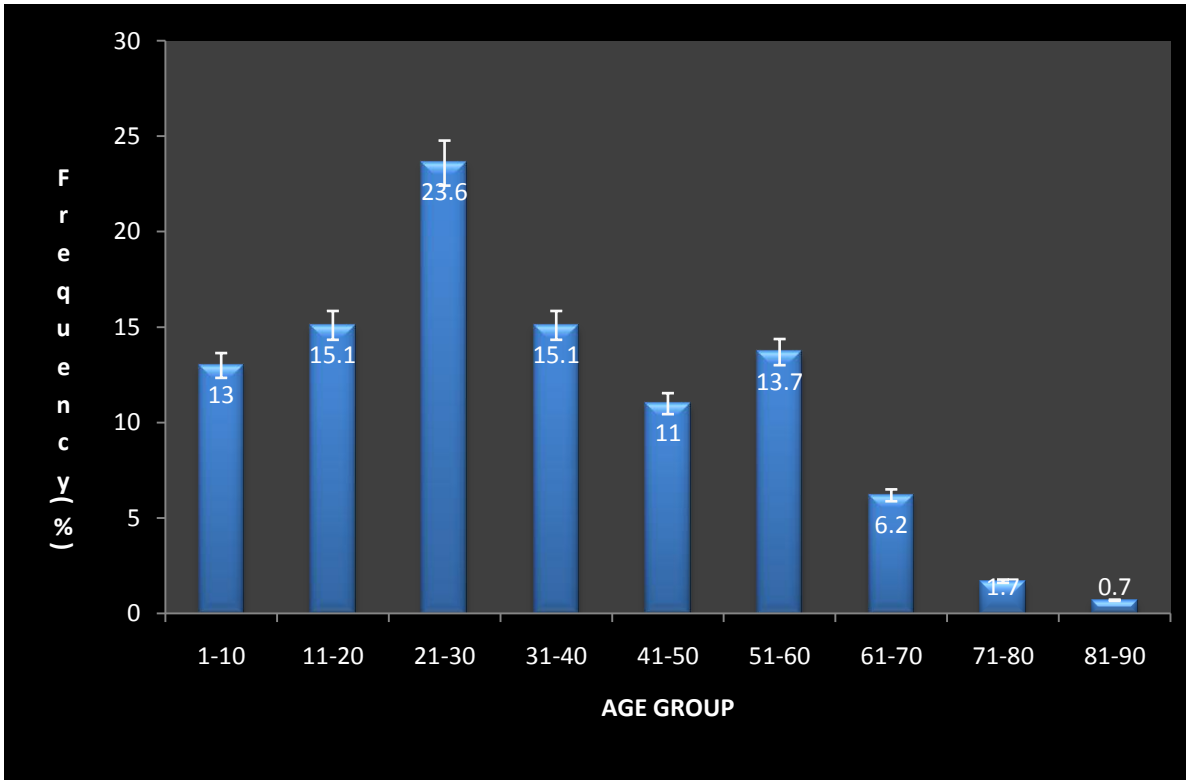



**Plate I: Gel electrophoresis of the amplicons showing some antibiotic resistance genes of staphylococci**

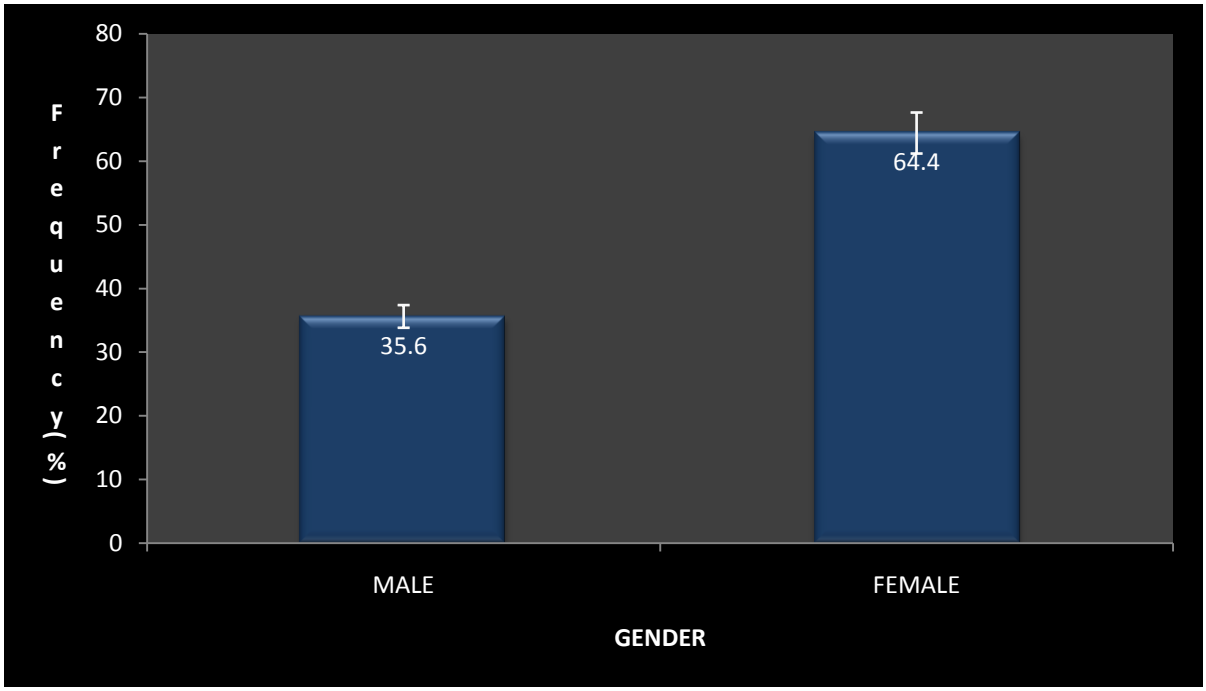
Ethidium bromide- stained multiplex PCR products after gel electrophoresis for the *blaZ* (173bp), *tetM* (158bp), and *mecA* (314bp) genes. DNA molecular size marker (1Kb) was used. From left, Lanes 1 and 2: DNA marker (molecular size standard); Lanes 3-5,7-9, 12: *mecA*; Lanes 5, 9,12: *mecA* and *blaZ*; Lanes 4-5,8,9, 12: *mecA, blaZ* and *tetM*;Lane 6:positive control, Lane 13:negative control,Lane 14: DNA marker (molecular size standard).




**( $\chi^2$=0.000; P<0.05)** **(LOS=**)**

**FIG 4.2: Age Distribution of Chronic Skin ulcer patients.**

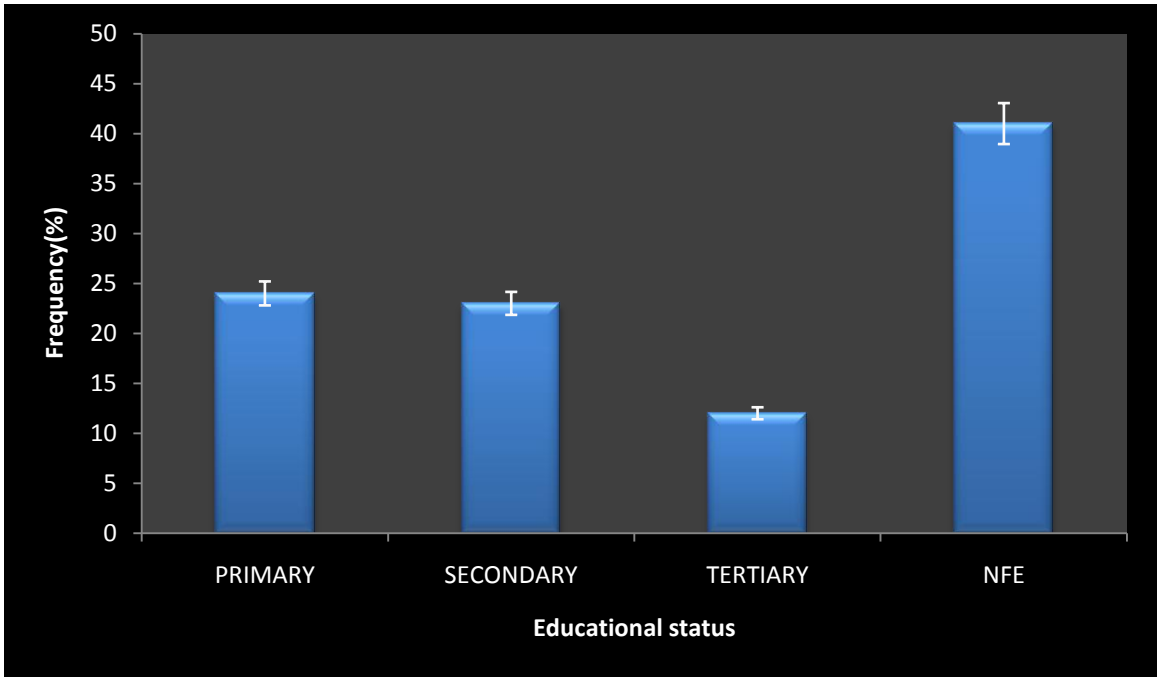
LOS= Level of significance, indicating high level of significance




**( $\square^2$=0.000; P<0.05)** **(LOS=**)**

**FIG 4.3: Sex Distribution of patients with Chronic Skin ulcer.**

LOS= Level of significance, indicating high level of significance



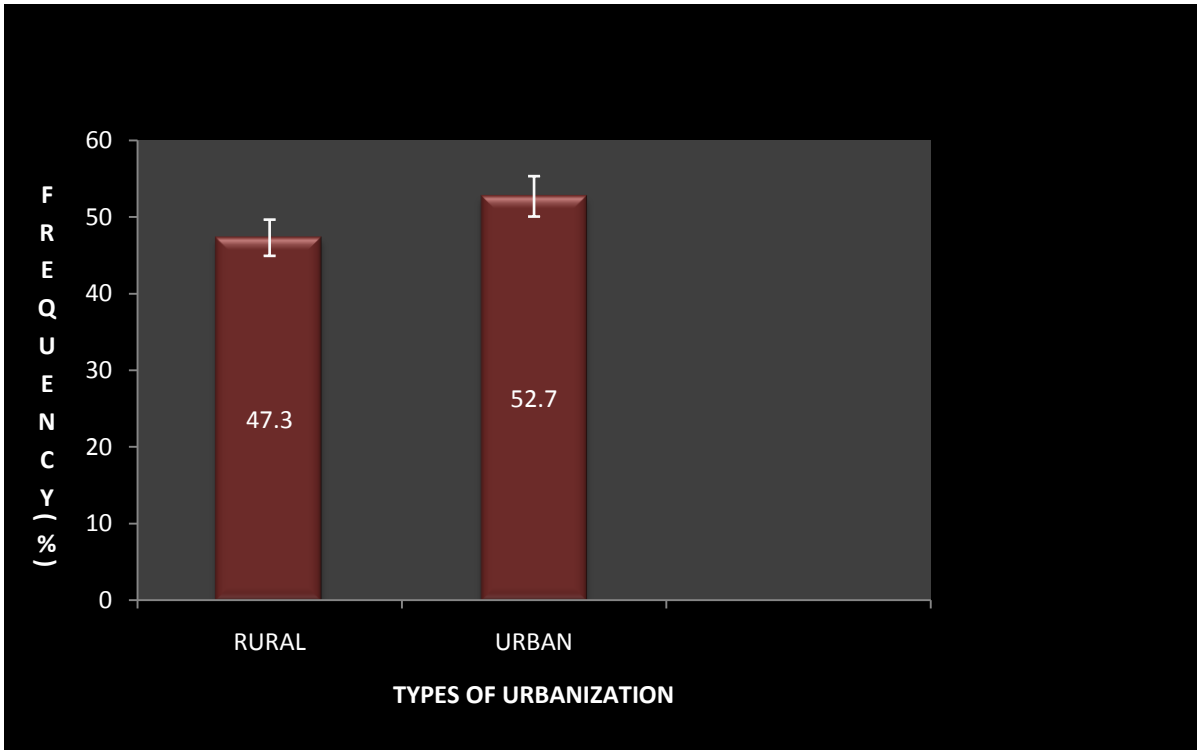

**( $\square^2$=0.000; P<0.05)** **(LOS=**)**

Key:

NFE = Non formal Education.

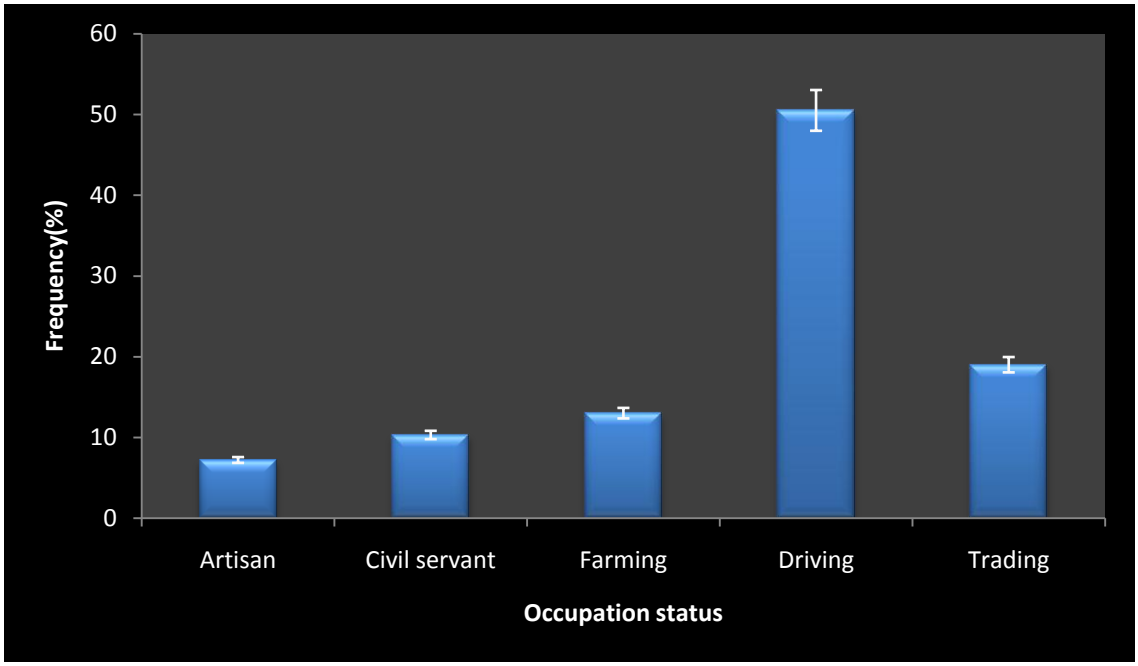
LOS= Level of significance, indicating high level of significance

**FIG 4.4**: **Distribution of chronic skin ulcer Patients by Educational status.**




**( $\square^2$=0.242; P>0.05)**

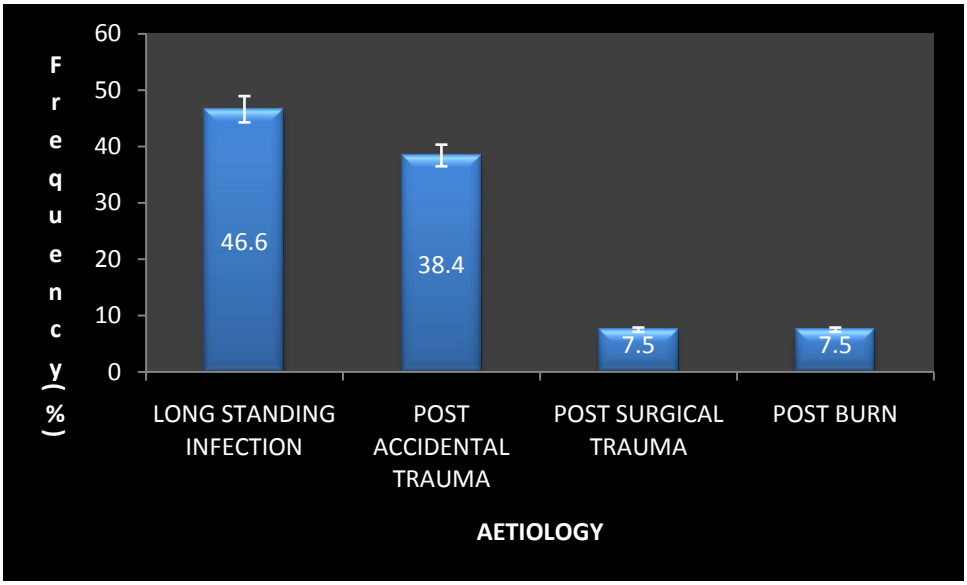
**FIG 4.5: Distribution of chronic skin ulcer Patients by Urbanization type.**




**( $\chi^2$=0.000; P<0.05)** **(LOS=**)**

**FIG4.6: Distribution of chronic skin ulcer Patients by occupation status.**

LOS= Level of significance, indicating high level of significance




**( $\square^2$=0.000; P<0.05)** **(LOS=**)**

**FIG 4.7:Distribution of chronic skin ulcer Patients by aetiology.**

LOS= Level of significance, indicating high level of significance



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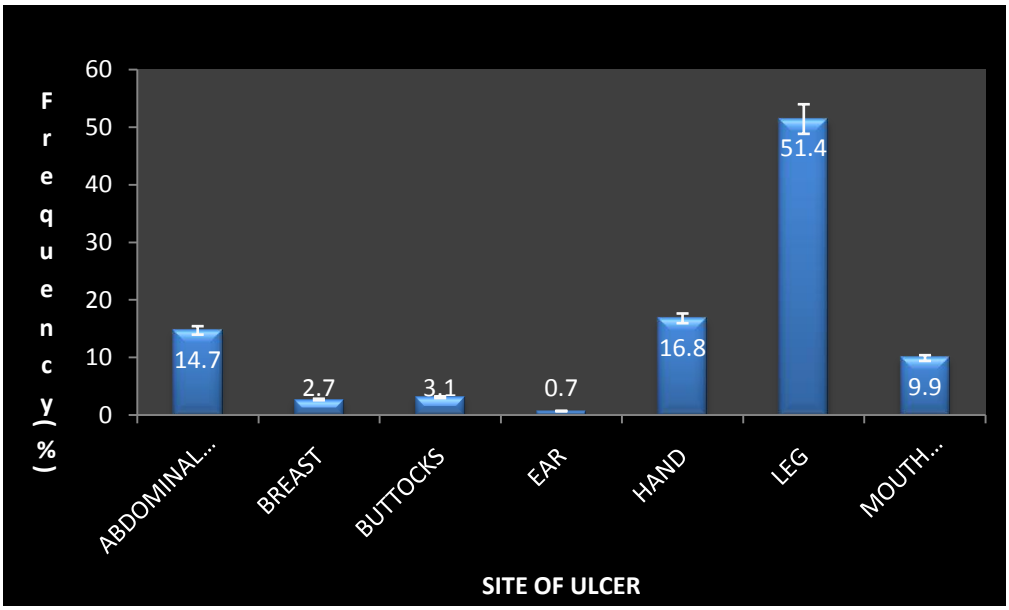


**( $\square^2$=0.000; P<0.05)** **(LOS=**)**

**FIG 4.8: Site Distribution of chronic skin ulcer patients.**

LOS= Level of significance, indicating high level of significance



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# CHAPTER FIVE

## 5.0DISCUSSION

*Staphylococcus aureus* is the most numerous of the staphylococci isolated from the chronic skin ulcer in this study. This is in conformity with the work of Gj□dsb□l *et al*. (2006), which affirmed that*S. aureus* is frequently isolated from chronic leg ulcers. Gjadsbal *et al*.(2013) also reported that *S. aureus* was found in 13 out of 16 ulcers.This work also confirmed the results reported by Zmudzinska *et al*. (2005) on *S. aureus* as the predominant microorganism cultured from leg ulcer swabs. This study agrees with the work of Shao- Hua *et al*. (2010), who reported that *S. aureus* was the most common Gram-positivepathogen isolated from ulcers.From the work of Negar *et al*.(2012), it was also affirmed that *S.aureus* is a bacterium that can cause broad spectrum of diseases, and a major cause of hospital acquired infections worldwide.

Various reports on bacterial flora of leg ulcers in patients admitted to the hospital in a research study carried by Zmudzinska *et al*. (2005), indicated that 80 to 100% of chronic ulcers are at some point colonized by *Staphylococcus aureus*, making the bacterium the most predominant microorganism isolated from leg ulcer swabs.*Staphylococcus aureus* has also been described as one of the most frequent bacterial pathogens in humans that causes skin infections, osteo-arthritis and respiratory tract infections in the community, as well as post-operative and catheter – related infections in hospitals (Didier *et al*.,2004). The results from this study across the four hospitals showed that 14(78%) isolates of *Staphylococcus aureus* recovered from the chronic skin ulcer cases were to Methicillin Resistant *Staphylococcus aureus* (MRSA).

Methicillin Resistant *Staphylococcus aureus* (MRSA) has been reported to be a major public health problem worldwide (Jarvis *et al.,* 2007). This burden of MRSA has been on the

increase, Nimmo *et al*. (2006), reported a growth rate of 14% of all *S.aureus* strains from clinically significant samples in New South Wales, Australia. The emergence of MRSA in the hospitals, particularly in this present study is not suprising.This is because there is the probability that the MRSA isolates from the patients are community- acquired. In other words, MRSA could find its way to the hospital environment via community – hospital cross – infection and vice versa.The rising colonization rate of MRSA leads to the increase of infection rates in the community and in hospitals (Al-Ruaily and Khalil, 2011).

The patients infected with MRSA risk significantly higher mortality and morbidity rates (Ladise and Mckinnon, 2005). It has been reported that within United States (U.S.) hospitals, nearly 60% of nosocomial *S. aureus* infections acquired in intensive care units are methicillin resistant (NNIS, 2004). There is the probability that the health care workers may carry MRSA on their hands, and/ or clothes, since they are always in contact with the asymptomatic carriers or patients who have clinical infection. Through this means, the unsuspecting health workers may transmit MRSA to other patients. Apart from this, the source of MRSA infection could equally be from the contaminated environmental surfaces.Baird and Hawly (2000) suggested that the main reason for the increased risk of mortality in patients acquiring MRSA is that these patients are already compromised by other disease processes, and are also often immunosuppressed. It therefore becomes a necessity for considerable emphasis to be placed on identifying effective measures to control the spread of MRSA wihin hospitals.Studies have shown that, once the MRSA is introduced into a hospital, it becomes difficult to be eradicated (Nettleman *et al.*, 1991).

Methicillin sensitive *Staphylococcus aureus* (MSSA) discovered in the present study amounts to a relatively non- significant proportion (n=4, 22%). Methicillin resistant *Staphylococcus aureus*has been reported to be capable of producing serious diseases such as osteomyelitis, pneumonia, enterocolitis and septicaemia (Kucers *et al.*, 1997). Incidentally, one quarter of

the patients from whom swab samples were taken in the present study were diagnosed with a co-morbid chronic osteomyelitis.Another category of patients from whom wound swabs were collected in the present study were patients of clinically diagnosed diabetic foot ulcer. Methicillin resistant *Staphylococcus aureus*(MRSA) was also found to be isolated from such cases in the present study. Suzanne (2002) also reported that MRSA has emerged as a serious and commonly occurring problem in diabetic patients with foot ulcers.This must have prompted the increase in the rate at which MRSA is constantly been isolated from diabetic foot ulcers (Wang*et al*., 2010).This as is again supported by the work of Shankar *et al*.(2005), stating that several studies have found the emergence of MRSA in as many as 15-30% of diabetic wounds.

There has been recent concern that MRSA strains had risen in the community independent of hospital strains, and that these community derived strains do not represent dissemination of a single clone, are not related genetically to hospital strains and are often susceptible to class of antibiotics other than the β- lactams (Suzanne, 2002).Most infections with these unique community strains had occurred in healthy children (Cookson, 2000). Thirteen (13) (93%) out of the 14(100%) MRSA isolated in the present study were Vancomycin susceptible, leaving only 1(7%) MRSA isolates as Vancomycin reduced susceptible*Staphylococcus aureus* (VRSSA).This suggests that vancomycin might be the antibiotic of last resort to treat most of the chronic skin ulcer infections. In an earlier study on vancomycin sensitivity of *Staphylococcus aureus* isolates, Denis *et al*. (2002),also affirmed that Vancomycin remains one of the few (and in some cases only) antibiotic to treat MRSA. Walsh and How (2000) , reported the description of strains of *S.aureus* that are Vancomycin susceptible by conventional testing, but have a sub-population of resistant cells.As found in the present study, MRSA isolates that were equally VRSA had been documented in a series of research studies. Appelbaum (2006), reported the identification of clinical isolates of VRSA from a

patient that had been treated for chronic foot ulceration, and had also been the recipient of multiple courses of antimicrobial therapy, including Vancomycin.

*Staphylococcus haemolyticus* is the second most predominant coagulase-negative Staphylococci isolated in the present study (n= 8, 23%). The isolation of *S. haemolyticus* and other CoNS in this study may be due to the fact that mostof the patients that participated in the study had implanted devices in them, where these CoNS cling, produced slime, formed biofilm and eventually dropped into the wounds and thereby degenerating the wounds further making it chronic. Similar to this finding was Bouchami *et al*. (2011) who reported that *S. haemolyticus* was one of the commonest species of coagulase negative staphylococci (CoNS) isolated from clinical samples in their previous study. This also agreed with the reports of Cuevas *et al*. (2004)and Secchi *et al*. (2008). Akpaka *et al*. (2006), who reported that infection with *S. epidermidis*, and less commonly with *S.haemolyticus* usually involves implantation of medical devices such as catheter, to support the earlier submission that a lot of the patients from which swab samples were taken had implanted medical devices, like the catheter.

The isolation of *Staphylococcus .epidermidis* as the predominant CoNS from the chronic skin ulcer of patients in this study was not suprising, since the bacterium is gradually transiting from being a contaminant to a pathogen. Similar to this, is the report by Javier *et al*. (2010), that *S.epidermidis* is now being considered a pathogen, owing to the frequent isolation of this bacterium in a series using bone biopsies. To further butress the isolation of *S.epidermidis* from chronic skin ulcer cases in this study, most especially from chronic osteomyelitis, Campolini and Harding (2010) reported *S.epidermidis* to be responsible for the majority of chronic osteomyelitis associated with orthopaedic implants.A study conducted by Rahman *et al*. (2012) also stressed the importance of *S. epidermidis* as a pathogen in foot ulcers with

protruding bone, that suggested underlying osteomyelitis, which led to the proposal that *S. epidermidis*should be considered as a nosocomial pathogen. The other CoNS isolated in this study includes, *Staphylococcus xylosus, Staphylococcus hyicus, Staphylococcus chromogenes and Staphylococcus intermedius*. The importance of these bacteria in chronic skin ulcer is supported by the research study conducted by Rahman *et al*. (2012), where *S.xylosus, S. hyicus, S, chromogenes* were isolated from surgical wounds alongside *S. epidermidis* and *S.heamolyticus*.

An important finding among the staphylococci isolated in the present study is *S. intermedius*.The name of this specie reflects the fact that while the organism possesses some phenotypic properties of *S.aureus,* it also exhibits some properties of *S. epidermidis* (Sudha *et al.*, 2004). The organism was previously recognized as an invasive zoonotic pathogen that had been isolated from 18% of canine – inflicted wounds (Lee, 1994). Sudha *et al*. (2004), also reported two cases of *S. intermedius* wound infection from two separate hospitals which occurred over an 8- month period.Sixty-six percent (66%) of the coagulase negative staphylococci isolated in the presentstudy were also Methicillin resistant, with a percentage resistance of 79% to cefoxitin. It is worthy to note that cefoxitin antibiotic is used as a surrogate antibiotic for prediction of *mecA* mediated resistance in staphylococci (CLSI, 2012). In a study conducted by Saxena *et al*. (2013), it was reported that all strains of *S.epidermidis* and *S.haemolyticus* isolated from neonatal intensive care units (NICU's) of tertiary hospitals were resistant to methicillin and gentamicin.

The result of antibiotic susceptibility studies on the phenotypically characterized isolates in the present study indicated that *S. aureus* isolates were susceptible to Gentamicin, Ciprofloxacin, Erythromycin, Vancomycin and Chloramphenicol. The percentage susceptibility of *S. aureus* isolates to gentamicin was 88.8%. This clearly indicated the

efficacy of the antibiotic against the bacteria. Al-Ruaily and Khalil (2011) reported 67% susceptibility of *S.aureus* isolates from hospital to Gentamicin, to nearly support the result of this study. This finding is similar to the report of Gjadsbal *et al.* (2013), where *S. aureus* strains isolated from from chronic leg ulcer were susceptible to Gentamicin by 100%, similar to what was reported by Ruhe *et al.* (2007). In a related development, a research conducted on variability of antibiotic susceptibility and toxin production of *S. aureus* strains from skin, soft tissue and bone related infections revealed that this strains had 95% susceptibility to Gentamicin (Sina *et al*., 2013), which is similar to the finding in the present study. However, there are contrary reports to this, for instance, Wang *et al*.(2010), reported only 24% susceptibility of hospital acquired MRSA (HA-MRSA) from infected foot ulcers in diabetic patients to gentamicin.A similar report from a study on the antibiotic susceptibility patterns of staphylococci isolates from various clinical samples of patients showed 38% of *S. aureus* susceptibility to Gentamicin.

Another antibiotic that was discovered to be active against *S. aureus*isolates in the present study was ciprofloxacin, which showed 83% potency towards the *S. aureus* isolates. Ruhe *et al*. (2007), earlier reported 100% efficacy of Ciprofloxacin against *S. aureus*. In the same vein, the susceptibility of Gram- positive bacteria, particularly *S. aureus* to Ciprofloxacin has been documented in an earlier research as reported by Manjula *et al.* (2005),all these similar to the findings in this study.

Erythromycin is another antibiotic that had high activity against *S. aureus as* reported in the present study, with a potency of 72%. A research study conducted on the isolation of *S. aureus* strains from skin and soft tissue infections, showcasing the susceptibility of these strains to various antibiotics revealed that the strains were well above 90% susceptibility to erythromycin (Sina *et al.*, 2013), corroborating the findings in this study.In a related

development,*S. aureus strains* from chronic leg ulcer, were 90% susceptible to erythromycin to again support the findings in this study. Vancomycin is another antibiotic that can be used as last resort antibiotic to control the spread of *S. aureus* infection of chronic skin ulcers. The outcome of a research study conducted by Ruhe *et al.* (2007) on the antibiotic susceptibility profile of community-onset MRSA again corroborated the findings of this study, since all the MRSA isolates showed 100% susceptibility to Vancomycin.In a similar report, MRSA isolated from foot ulcers in diabetic patients were found to be 100% susceptible to Vancomycin (Wang *et al.*, 2010).

The last of the antibiotic that was found to be effective against *S.aureus* isolates in the present study was chloramphenicol.The percentage susceptibility of *S.aureus* isolates to this antibiotic as found in this study was 72%. Similarly,a research on the evaluation of secondary bacterial infection of skin diseases and sensitivity of bacteria to antibiotics revealed that *S.aureus* isolated showed 85% sensitivity to chloramphenicol (Abdallah*et al.*, 2007), which agrees with the findings of the present study.*Staphylococcusaureus* isolates that were however resistant to other antibiotics were resistant to a β-lactam antibiotics, Amoxicillin-clavulanic acid (Augmentin), Cefoxitin, Tetracycline and Trimethroprim +Sulphamethoxazole. The efficacy of Amoxicillin -clavulanic acid against the *S. aureus* isolates was found to be 0%, meaning that *S. aureus* isolates showed 100% resistance toAmoxicillin -clavulanic acid. This was similar to the findings of Abdallah*et al.* (2007) whoreported Amoxicillin- clavulanic acid to have only 15% efficacy against *S. aureus* isolates from clinical specimens, in other words, the isolates were confirmed to have resisted this antibiotic by as much as 85%. Cefoxitin antibiotic which is currently used as a surrogate antibiotic to methicillin was also found to be resisted by the*S.aureus* isolates by 78%. It is worthy to note that, it is on the basis of *S.aureus resistance* to this antibiotic that *S. aureus* has been classified as MRSA and MSSA, as discovered in the present study.Several research

findings have also reported the resistance and susceptibilitry of *S.aureus* to Cefoxitin.This result is similar to the findings of Martins *et al.* (2012) and Gjadsbal *et al.*(2013) who reported 100% resistance of *S.aureus* isolates from venous ulcer and chronic leg ulcer respectively to cefoxitin. In Contrasthowever to the report of this study is the work of Sina *et al*. (2013) that *S.aureus* isolates from skin, soft tissue infections were 82 % susceptible to cefoxitin.

*Staphylococcus aureus* isolates from chronic skin ulcer patients in this sudy showed moderate resistance of 50% to tetracycline. To support this finding, Abdallah*et al.* (2007) and Sina *et al.* (2013) reported 79% and 60% *S.aureus* resistance to tetracycline respectively.Ruhe *et al.* (2007)also reported 93% susceptibility of *S.aureus,* particularly MRSA from skin and soft tissue to tetracycline. Trimethoprim+sulphamethoxazole (co-trimoxazole) was found to be another antibiotic that *S.aureus*isolates from chronic skin ulcer in this study resisted by 67%.This result is similar to the report ofSina *et al*. (2013) that *S.aureus* strains from skin and soft tissue infections showed 60% resistance to Trimethoprim+sulphamethoxazole The report of Duran*et al.* (2012) is however contrary to the findings in this study, it showed that *S.aureus* isolates from clinical samples displayed susceptibility to Trimethoprim+sulphamethoxazole.

The CoNS that were isolated in the present study include *S. epidermidis, S. heamolyticus, S.hyicus, S.xylosus, S.chromogenes* and *S.intermedius* .The CoNS were also found to be susceptible to Gentamicin, Ciprofloxacin, Chloramphenicol, Trimethoprim+sulphameth-oxazole and Vancomycin, as reported in this our study.The susceptibility of CoNS isolates from chronic skin ulcer to Gentamicin in this study is supported by the reports of Duran *et al*.(2012) and Saxena *et al*.(2013) that CoNS isolates from clinical samples were 74% and 77% susceptibility to Gentamicin respectively. In contrast to this submission is the report of

Martins *et al*. (2012) which showed that CoNS isolates were resistant to gentamicin by 71%. Another antibiotic of high activity against CoNS isolates in the present study was Ciprofloxacin. Saxena *et al*. (2013) reported that Ciprofloxacin has high activity of 83% against the CoNS isolates.The submission of Martins *et al*. (2012) however contradicted the submission of the present study, he reportedthat the CoNS isolates of venous ulcers showed resistance to Ciprofloxacin by 71%.

In this study, Chloramphenicol also proved to be effective against CoNS isolates from chronic skin ulcer of patients, this corroborates Bouchami*et al*.(2011) that reported 85% susceptibility of the CoNS isolates to chloramphenicol.The susceptibility of the CoNS isolates from chronic skin ulcer of patients in this study to both Trimethoprim+sulphamethoxazlole and Vancomycin had been reported. Bouchami *et al*. (2011) and Duran *et al*. (2012) reported the susceptibility of CoNS to Trimethoprim+sulphamethoxazlole by as much as 95%. On the other hand, the efficacy of Vancomycin on the CoNS isolates as reported in the present study conform with the findings on the susceptibility pattern of CoNS,where Vancomycin was found to be 100% active against CoNS (Duran *et al*.,2012;Saxena *et al*.,2013).

Methicillin resistant coagulase negative staphylococci (MRCoNS) isolates were also found to be present in chronic skin ulcers of the patients sampled across the hospitals in the study areaMethicillin resistant coagulase negative staphylococci were reported on the basis of their resistance to cefoxitin. Most of the CoNS isolates were resistant to cefoxitin. This finding is in accordance with the report of Martins *et al*. (2012). Another antibiotic of interest that was resisted by the CoNS is Amoxicillin- clavulanic acid. The reason for this may not be far fetched, since CoNS, primarily *S.epidermidis* and *S.heamolyticus* are often resistant to multiple antibiotics, and glycopeptides are the likely drugs to be used in order to manage the infection caused by CoNS (Cunningham, 1992; Menichetti, 1992).

The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the antibiotics on the isolates were determined for the *S.aureus* and CoNS.In both cases, the MICs ranged from 8.0µg-32.0 µg for all the antibiotics. Several studies reporting the MICs for both the *Staphylococcus aureus* and CoNS involving similar antibiotics indicated MIC values with a range similar to what was obtained in the present study.There are however exceptions to some antibiotics where the MIC values on the isolates differed (Duran *et al*.,2012; Martins *et al*.,2013). The minimum bactericidal concentrations (MBCs) of the antibiotics on the isolates of *S.aureus* and CoNS were further investigated.The values of MBC ranged from 8.0 µg-32.0 µg, depending on the antibiotic.This were the concentrations at which the isolates were totally eliminated or said to have become static or killed.

Some isolates of *S.aureus* and CoNS were selected based on their resistance to the antibiotics for Polymerase chain reaction (PCR), to be able to identify possible antibiotic resistance genes in them.These isolates were then subjected to PCR analysis after successfully isolating the DNA from them.From the gel electrophoresis, it was observed that bands appeared on all the lanes of the gel where the DNA from the isolates was placed. These bands occurred at the base pair that corresponded to the base pairs of the primer sequences coding for the resistance genes under investigation in the isolates,these genes included*mecA, blaZ, and tetM.* It was observed that three (3) out of the *S.aureus* isolates screened by gel electrophoresis had*mecA* genes, two (2) had*blaZ* gene and all the five (5) isolates had*tetM* gene. While four(4) out of the CoNS isolates also had*mecA,blaZ* and *tetM* genes, one(1) isolate of the CoNS had both *mecA* and *tetM* genes. The resistance of these *S.aureus* and CoNS isolates to the various antibiotics could be due to the presence of *mecA, blaZ* and *tetM* genes in them.Methicillin resistance in staphylococci had been reported to have an association with the presence of penicillin binding proteins, PBP2' (PBP2a) which is encoded by the *mecA* gene (Zapun *et al*., 2008; Yadegar *et al*., 2009). In support of the findings in this study, a research study showed

that 15(15%) of *S.aureus* isolates that were classified as MRSA based on Methicillin susceptibility testing , 13/15 (87%) of the MRSA isolates expressed *mecA* gene by PCR typing in addition to beta lactamase enzyme production(Al-Ruaily and Khalil,2011).Polymerase chain reaction (PCR) analysis to determine antibiotic resistance genes in staphylococci by Duran *et al*. (2012) showed that *blaZ* gene was widely spread among both *S.aureus* and CoNS. This work is in agreement with their submission findings.Duran *et al*. (2012) did report the phenotypic resistance to tetracycline in *S. aureus* and CoNS isolates, where he observed that 42 % of the*S.aureus* carried *tetK* and *tetM* genes and 39% of CoNS were also found to carry these genes.The outcome of the findings in this study corroborated the report of Bouchami *et al*. (2011) that the isolates of CoNS were *mecA* positive.

Ch-square analysis revealed that the age bracket of 21-30 years and 81-90 years were the upper and lower limits respectively in the age variable of the patients. This indicates that the patients in the ages from 21 -30 years (23.6%) had more chronic skin ulcer than the others. This could be attributed to the fact that human activity is more pronounced in this age bracket, which could make the patients more vulnerable to injury and subsequently staphylococci colonized chronic skin ulcer. This agrees with the work of Gordon and Lowly (2008) where they stressed the importance of human activity as a factor that could pre-dispose an individual to physical injury. The site of ulcer with the highest occurrence of chronic skin ulcer among the patients was the Leg, with 51.4%. The reason for this may not be far from the fact that most of the wounds sampled during the study were diabetic foot ulcer. The category of the patients that belonged to non-formal education in the educational status variable had the highest percentage of 41.4%. Patients in this category were those with non- formal education that could probably present wounds late to the hospital or abuse drugs, leading to wound degeneration. This agrees with the findings of Duran *et al.* (2012). Long

lasting infection as one of the causes of ulcer in the patients has upper limit of 46.6%. Patients in this category could have underlying disease, such as diabetes, attributing to the non- healing of such wounds (Martins *et al*., 2012). In the sex variable, the female had the highest percentage of 64.4% with chronic skin ulcer. This could also be due to an underlying disease among the females. Type of urbanization had no association with the occurrence of chronic skin ulcer in the patients, judging by the significance difference. However, the patients in the urban center had more chronic skin ulcer with 52.7%. The category of patients that fell among driving in the Occupation variable had the highest percentage of chronic skin ulcer with 44.9%. Patients in this category may be those that also had underlying disease, such as diabetes, or those that would present wounds late to the hospital or could possibly abuse or mis-use drugs.

# CHAPTER SIX

# 6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

## 6.1 Summary

From this research study, eighteen isolates of *Staphylococcus aureus*was discovered to have colonized the chronic skin ulcer of the patients in Kaduna state. The *Staphylococcus aureus*cut across the chronic skin ulcer of the patients from the three (3) senatorial zones of Kaduna state.

Most importantly, methicillin resistant *Staphylococcus aureus* (MRSA) isolates accounts for 14 (78%) of the total *Staphylococcus aureus* isolated. This was based on the antibiotic susceptibility test conducted on the isolates, as 14(78%) were resistant to cefoxitin, a surrogate antibiotic to methicillin. The MRSA isolates are equally distributed across the chronic skin ulcer patients from the three (3) senatorial districts of Kaduna state.

Different species of Coagulase- negative staphylococci (CoNS) were also isolated from the chronic skin ulcer patients from the three (3) senatorial districts of Kaduna state. The total number of these CoNS totalled 35. Eighteen (18)(52%) of the CoNS isolates are also methicillin resistant coagulase- negative staphylococci( MRCoNS), based on antibiotic susceptibility test result.

Leg was found to be the highest site colonized by staphylococci species, the lowest site colonized was breast.

Staphylococci isolates from the chronic skin ulcer patients across the three (3) senatorial districts of Kaduna state showed resistance to other antibiotics like amoxicillin- clavulanic and tetracycline. The resistance of the staphylococci isolates from chronic skin ulcer patients to methicillin, amoxicillin- clavulanic acid and tetracycline was investigated to know if there are genes encoding this antibiotics resistance. The Polymerase chain reaction (PCR) analysis

confirmed this through gel electrophoresis. The PCR result showed that all the staphylococci isolates from the chronic skin ulcer patients from the three (3) senatorial zones of Kaduna state has *mecA, blaZ* and *tetM* genes with their respective base pairs (bp) of 314, 173 and 158 on the gel.

Chi- square (□$^2$) testof association between the occurrences of chronic skin ulcer in the patients and some socio- demographic factors indicated that Age, Gender, Educational status, Site of ulcer, Aetiology and Occupational status are statistically significant as shown by the P- value ( $P<0.05$) and the Chi-square value. The type of urbanization was however not statistically significant ($P> 0.05$).

**6.2 Conclusion**

The presence of *Staphylococcus aureus* and different species of Coagulase – negative staphylococci isolates in the chronic skin ulcers of patients could be responsible for the delay in the healing of these ulcers. The presence of these isolates could lead to the degeneration of the patient's wounds, as these isolates possesessurface proteins with which they could attach to host tissues and other accessory toxins such as α-toxins and fatty acid modifying enzymes (FAME) of *Staphylococcus aureus* and Coagulase – negative staphylococci respectively which these isolates used in destroying host tissues.

The various ways of antibiotic resistance deployed by the isolates, most especially the methicillin- resistant *Staphylococcus aureus* (MRSA) and methicillin- resistant coagulase – negative staphylococci (MRCoNS) includes the production of distorted penicillin binding protein (PBP2a) on the surface of the isolates, which allows MRSA and MRCoNS to bind the β- lactam antibiotics in the wrong orientation and thereby causing the isolates to resist all β-lactam antibiotics and therefore wrecking a lot of havoc in the patients wounds.

The presence of the resistance genes and the fact that the isolates could carry Resistance plasmid (R- plasmid) that could habour resistance genes might be responsible for the antibiotic resistance in the isolates, making them to evade antibiotics, thereby causing degeneration of the patients' chronic skin ulcer.

Some socio-demographic factors such as age, gender, educational staus etc. are responsible for the occurrence of staphylococci colonized chronic skin ulcer in the patients.

### 6.3 Recommendations

1. Patients should be advised to report wounds to the hospital early enough to avoid wound degeneration.

2. Health care practitioners should at all times wash their hands before and after administering treatment to the patients, even when they are to use gloves.This would prevent transmission of MRSA, MRCONS, and other microbes from the health care handlers to the patients and vice versa.

3. Public awareness should be embarked upon, particularly on the use of drugs. Many patients have developed the idea of indiscriminate use of drugs, even when such drugs have not been recommended by the physician.This would eventually lead to drug abuse. The microorganism would also eventually developed resistance to such drugs.

4. Rapid and accurate detection of methicillin resistance in *Staphylococcus aureus* is necessary to be able to know the exact antimicrobial therapy and to enable the control of hospital spread of MRSA strains.

5. Surveillance culture of high risk patients should be developed. More attention should also be focused much more on the out- patients that are infected with MRSA, to be able to eradicate the infection in order to prevent Methicillin resistant *Staphylococcus aureus* spread through the community.

6.The molecular technique method should be used in detecting *Staphylococcus aureus* at both genus and specie level.This would help to detect MRSA with 100% accuracy.

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## APPENDIXI

**STRUCTURED QUESTIONNAIRE USED IN CROSS-SECTIONAL STUDY OF STAPHYLOCOCCI ISOLATED FROM CHRONIC SKIN ULCER PATIENTS IN KADUNA STATE**

Date:

Serial no:

Hospital no:

Age:

Gender: Male { } Female{ }

Educational status: Primary { } Secondary{ } Tertiary { } Others { }

Area of domicile: Urban { } Rural { }

Occupation: Civil Servant { } Farmer { } Welder { } Mechanic { }

Painter { } Housewife { } Others { }

Primary Diagnosis:

Cause of Ulcer: Post-trauma { } Post burn { } Long standing infection { }

Size of Ulcer:

Site of Ulcer: Breast { }, Skin { }, Leg { }, Mouth { }

Antibiotic Usage: Less than a month { } between 1-2 months { } Greater than 2 months { }

If yes, which antibiotics? _____

## APPENDIX II

## LABORATORY CHARACTERISTICS OF STAPHYLOCOCCI ISOLATED FROM PATIENTS WITH CHRONIC SKIN ULCERS

| CODE OF THE ISOLATE | COLONY MORPHOLOGY | SHAPE | GRAM STAIN REACTION | CATALASE TEST | COAGULASE TEST |
|---|---|---|---|---|---|
| 256MS | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |
| 106MK | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |
| 063MB | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |
| 206MS | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |
| 116MK | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |
| 121MK | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |
| 037MB | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |
| 240MG | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |
| 090MK | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |
| 110MK | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |
| 268MS | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |
| 174MS | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |

| | | | | | |
|---|---|---|---|---|---|
| 001MB | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |
| 011MB | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |
| 010FB | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |
| 173MS | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |
| 270MS | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |
| 027FB | ROUND,CONVEX | COCCI IN CLUSTERS | + | + | + |
| 116MK(2) | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 260MG | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 260MG(2) | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 117FK | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 031FB | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 125FK | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 132MK | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 049MB | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 253FG | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 272MG | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 271MS | ROUND | COCCI IN | + | + | _ |

| | | | | | |
|---|---|---|---|---|---|
| | | CLUSTERS | | | |
| 227MS | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 112FK | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 245FS | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 090MK(2) | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 108MK | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 011MB(2) | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 001MB(2) | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 120MK | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 106MK(2) | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 242MS | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 187FS | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 191MS | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 089MK | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 149MK | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 187FS(2) | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 066(FB | ROUND | COCCI IN CLUSTERS | + | + | _ |

| | | | | | |
|---|---|---|---|---|---|
| 218MG | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 212MS | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 007MB | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 158MG | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 155MK | ROUND | COCCI IN CLUSTERS | + | + | _ |
| 001MB(3) | CONVEX | COCCI IN CLUSTERS | + | + | _ |
| 087MK | CONVEX | COCCI IN CLUSTERS | + | + | _ |
| 224MS | CONVEX | COCCI IN CLUSTERS | + | + | _ |

## APPENDIX III

## CHARACTERIZATION OF THE ISOLATES USING THE MICROGEN STAPH IDENTIFICATION KIT

| CODE | LAT | CPG | NIT | SUC | TRE | MAN | NAG | MNS | TUR | PHO | βGL | NIT | URE | ARG | PYR | STAPH ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 031FB | - | - | + | + | - | - | - | - | - | + | - | - | + | + | - | *Staphylococcus epidermidis* |
| 089MK | - | + | + | - | + | + | + | - | + | + | + | + | + | + | + | *Staphylococcus chromogenes* |
| 149FK | - | + | + | + | + | + | + | + | - | + | + | - | + | + | + | *Staphylococcus chromogenes* |
| 187FS(2) | - | - | + | + | + | - | + | - | + | + | + | + | + | + | - | *Staphylococcus chromogenes* |
| 125FK | - | - | + | + | - | - | - | - | - | + | - | - | + | + | - | *Staphylococcus epidermidis* |
| 256MS | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | *Staphylococcus aureus* |
| 158MG | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *Staphylococcus xylosus* |
| 132MK | - | - | + | + | - | - | - | - | - | + | - | - | + | + | - | *Staphylococcus epidermidis* |
| 158MG | - | + | + | + | + | + | + | + | - | + | + | + | + | + | + | *Staphylococcus xylosus* |
| 187FS | + | + | + | + | - | + | + | - | - | - | - | - | + | + | + | *Staphylococcus haemolyticus* |
| 116MK(2) | + | - | + | + | + | - | + | - | - | + | + | + | + | + | + | *Staphylococcus hyicus* |
| 260MG | + | + | + | - | + | - | + | - | - | + | + | + | + | - | - | *Staphylococcus hyicus* |
| 260MG(2) | + | - | + | + | + | + | + | + | - | + | + | + | + | + | - | *Staphylococcus hyicus* |
| 049MB | - | - | + | + | - | - | - | - | - | + | - | - | + | + | + | *Staphylococcus epidermidis* |
| 090MK(2) | + | + | + | + | - | + | + | - | - | - | - | - | + | + | + | *Staphylococcus haemolyticus* |
| 271MS | - | - | + | + | - | - | - | - | - | + | - | - | + | + | - | *Staphylococcus epidermidis* |
| 240MG | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | *Staphylococcus aureus* |
| 212MS | - | + | + | + | + | + | - | + | + | + | + | - | + | + | - | *Staphylococcus chromogenes* |
| 090MK | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | *Staphylococcus aureus* |
| 087MK | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | *Staphylococcus xylosus* |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 011MB(2) | + | + | + | + | - | + | + | - | - | - | - | - | + | + | + | *Staphylococcus haemolyticus* |
| 001MB(2) | + | + | + | + | - | + | + | - | - | - | - | - | + | + | + | *Staphylococcus haemolyticus* |
| 173MS | + | + | - | + | + | - | - | + | + | + | + | - | + | + | + | *Staphylococcus aureus* |
| 242MS | + | + | + | + | - | + | + | - | - | - | - | - | + | + | + | *Staphylococcus haemolyticus* |
| 007MB | - | + | + | + | + | + | + | + | - | + | + | + | + | + | - | *Staphylococcus chromogenes* |
| 270MS | + | + | + | + | + | - | + | - | - | + | + | - | + | + | - | *Staphylococcus aureus* |
| 191MS | - | - | + | + | + | + | + | - | - | + | - | - | + | + | - | *Staphylococcus intermidius* |
| 027FB | + | + | + | + | + | + | + | + | - | + | + | - | + | + | - | *Staphylococcus aureus* |
| 112FK | - | - | + | + | - | - | - | - | - | + | - | - | + | + | - | *Staphylococcus epidermidis* |
| 117FK | + | - | - | + | + | + | + | + | - | + | + | + | + | + | - | *Staphylococcus hyicus* |
| 224MS | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | *Staphylococcus xylosus* |
| 010FB | + | - | + | + | + | + | + | + | + | + | + | + | + | + | - | *Staphylococcus aureus* |
| 245FS | - | - | + | + | - | - | - | - | - | + | - | - | + | + | - | *Staphylococcus epidermidis* |
| 106MK | + | + | + | + | + | + | - | + | - | + | + | + | + | - | - | *Staphylococcus aureus* |
| 253FG | - | - | + | + | - | - | - | - | - | + | - | - | + | + | - | *Staphylococcus epidermidis* |
| 108MK | + | + | + | + | - | + | + | - | - | - | - | - | + | + | + | *Staphylococcus haemolyticus* |
| 063MB | + | + | + | + | + | - | + | + | - | + | + | - | + | + | - | *Staphylococcus aureus* |
| 110MK | + | + | + | + | + | + | + | + | - | + | + | - | + | + | - | *Staphylococcus aureus* |
| 227MS | - | - | + | + | - | - | - | - | - | + | - | - | + | + | - | *Staphylococcus epidermidis* |
| 268MS | + | + | + | + | + | + | + | + | - | + | + | - | + | + | - | *Staphylococcus aureus* |
| 120MK | + | + | + | + | - | + | + | - | - | - | - | - | + | + | + | *Staphylococcus haemolyticus* |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 174MS | + | + | + | - | - | - | + | - | - | + | + | - | + | + | - | *Staphylococcus aureus* |
| 106MK(2) | - | + | + | + | - | + | + | - | - | - | - | - | + | + | + | *Staphylococcus haemolyticus* |
| *001MB* | + | + | + | + | + | + | + | + | - | + | + | - | + | - | - | *Staphylococcus aureus* |
| *011MB* | + | + | + | + | + | + | + | + | - | + | + | - | + | + | - | *Staphylococcus aureus* |
| *001MB(3)* | + | + | + | + | + | + | + | + | - | + | + | + | + | - | + | *Staphylococcus xylosus* |
| *206MS* | + | + | + | + | + | + | + | + | + | + | + | - | + | + | - | *Staphylococcus aureus* |
| *272MG* | - | - | + | + | - | - | - | - | - | + | - | - | + | + | - | *Staphylococcus epidermidis* |
| *116MK* | + | + | - | + | + | - | + | + | + | + | + | - | + | + | - | *Staphylococcus aureus* |
| *121MK* | + | + | + | + | + | - | + | + | - | + | + | - | + | + | - | *Staphylococcus aureus* |
| *066FB* | - | + | + | + | + | + | + | + | + | + | + | - | + | + | + | *Staphylococcus chromogenes* |
| *218MG* | - | + | + | + | + | + | + | + | + | + | + | - | + | + | + | *Staphylococcus chromogenes* |
| *037MB* | + | + | + | + | + | + | + | + | - | + | + | - | + | + | - | *Staphylococcus aureus* |

## APPENDIX IV

## BIO- DATA AND CASE PROFILE OF PATIENTS WITH CHRONIC SKIN ULCERS IN THE VARIOUS HOSPITALS IN KADUNA STATE

| S/NO | AGE(YRS) | GENDER | ED.ST | AOD | OCCUP | COU | SOU | PRE. OF STAPHY | DOS |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 41-50 | MALE | OTHERS | RURAL | OTHERS | LSI | MOUTH REGION | (3)POSITIVE(+) | BDSH, KADUNA |
| 2 | 51-60 | FEMALE | OTHERS | URBAN | HOUSE WIFE | LSI | BUTTOCKS | | |
| 3 | 51-60 | FEMALE | OTHERS | URBAN | HOUSE WIFE | LSI | LEG | | |
| 4 | 41-50 | FEMALE | SECONDARY | URBAN | CIVIL SERVANT | LSI | LEG | | |
| 5 | 51-60 | MALE | TERTIARY | RURAL | CIVIL SERVANT | LSI | LEG | | |
| 6 | 51-60 | MALE | PRIMARY | RURAL | FARMING | POST- ACCIDENTAL TRAUMA | LEG | | |
| 7 | 31-40 | MALE | SECONDARY | URBAN | OTHERS | LSI | BUTTOCKS | POSITIVE(+) | BDSH. KADUNA |
| 8 | 41-50 | FEMALE | PRIMARY | URBAN | OTHERS | LSI | LEG | | |
| 9 | 31-40 | MALE | OTHERS | RURAL | FARMING | POST- ACCIDENTAL TRAUMA | HAND | | |
| 10 | 11-20 | FEMALE | SECONDARY | URBAN | OTHERS | LSI | MOUTH REGION | POSITIVE(+) | BDSH, KADUNA |
| 11 | 61-70 | MALE | SECONDARY | URBAN | CIVIL SERVANT | LSI | MOUTH REGION | (2)POSITIVE(+) | BDSH, KADUNA |
| 12 | 51-60 | FEMALE | SECONDARY | URBAN | CIVIL SERVANT | LSI | MOUTH REGION | | |
| 13 | 21-30 | MALE | PRIMARY | URBAN | OTHERS | LSI | LEG | | |
| 14 | 41-50 | FEMALE | PRIMARY | RURAL | HOUSE WIFE | POST- ACCIDENTAL TRAUMA | LEG | | |
| 15 | 41-50 | FEMALE | OTHERS | URBAN | HOUSE WIFE | LSI | LEG | | |
| 16 | 21-30 | FEMALE | PRIMARY | URBAN | HOUSE WIFE | POST-BURN | LEG | | |
| 17 | 21-30 | FEMALE | OTHERS | RURAL | HOUSE WIFE | LSI | ABDOM REGION | | |
| 18 | 21-30 | FEMALE | PRIMARY | URBAN | OTHERS | POST-BURN | LEG | | |
| 19 | 1-10 | MALE | PRIMARY | URBAN | OTHERS | LSI | HAND | | |
| 20 | 21-30 | FEMALE | PRIMARY | RURAL | HOUSE WIFE | POST-ACCIDENTAL TRAUMA | LEG | | |
| 21 | 1-10 | FEMALE | PRIMARY | URBAN | OTHERS | POST-BURN | MOUTH REGION | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 22 | 1-10 | MALE | PRIMARY | RURAL | OTHERS | LSI | LEG | | | |
| 23 | 1-10 | FEMALE | PRIMARY | RURAL | OTHERS | POST-BURN | LEG | | | |
| 24 | 1-10 | FEMALE | PRIMARY | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 25 | 11-20 | FEMALE | SECONDARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 26 | 21-30 | MALE | OTHERS | URBAN | OTHERS | LSI | MOUTH REGION | | | |
| 27 | 11-20 | FEMALE | OTHERS | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | POSITIVE(+) | BDSH, KADUNA | |
| 28 | 71-80 | MALE | OTHERS | RURAL | OTHERS | LSI | LEG | | | |
| 29 | 21-30 | FEMALE | SECONDARY | URBAN | ARTISAN | LSI | HAND | | | |
| 30 | 21-30 | MALE | SECONDARY | URBAN | ARTISAN | LSI | HAND | | | |
| 31 | 41-50 | FEMALE | SECONDARY | URBAN | HOUSE WIFE | LSI | LEG | POSITIVE(+) | BDSH, KADUNA | |
| 32 | 1-10 | MALE | PRIMARY | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | HAND | | | |
| 33 | 11-20 | MALE | SECONDARY | RURAL | ARTISAN | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 34 | 11-20 | MALE | OTHERS | RURAL | FARMING | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 35 | 1-10 | MALE | OTHERS | URBAN | OTHERS | LSI | LEG | | | |
| 36 | 21-30 | FEMALE | OTHERS | URBAN | HOUSE WIFE | LSI | LEG | | | |
| 37 | 21-30 | MALE | TERTIARY | URBAN | OTHERS | LSI | LEG | POSITIVE(+) | BDSH, KADUNA | |
| 38 | 41-50 | MALE | TERTIARY | RURAL | CIVIL SERVANT | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 39 | 1-10 | MALE | PRIMARY | RURAL | OTHERS | LSI | LEG | | | |
| 40 | 1-10 | MALE | PRIMARY | RURAL | OTHERS | LSI | LEG | | | |
| 41 | 51-60 | FEMALE | PRIMARY | URBAN | HOUSE WIFE | LSI | MOUTH REGION | | | |
| 42 | 1-10 | MALE | OTHERS | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | HAND | | | |
| 43 | 41-50 | MALE | TERTIARY | URBAN | CIVIL SERVANT | LSI | LEG | | | |
| 44 | 41-50 | MALE | TERTIARY | URBAN | CIVIL SERVANT | LSI | LEG | | | |
| 45 | 81-90 | MALE | PRIMARY | RURAL | OTHERS | LSI | LEG | | | |
| 46 | 71-80 | MALE | OTHERS | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 47 | 1-10 | MALE | PRIMARY | URBAN | OTHERS | POST-BURN | HAND | | | |
| 48 | 51-60 | FEMALE | PRIMARY | URBAN | HOUSE WIFE | POST-ACCIDENTAL TRAUMA | LEG | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 49 | 31-40 | MALE | SECONDARY | URBAN | CIVIL SERVANT | POST-ACCIDENTAL TRAUMA | LEG | POSITIVE(+) | BDSH, KADUNA |
| 50 | 41-50 | FEMALE | PRIMARY | RURAL | HOUSE WIFE | LSI | LEG | | |
| 51 | 11-20 | MALE | SECONDARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | |
| 52 | 41-50 | MALE | OTHERS | URBAN | FARMING | POST-ACCIDENTAL TRAUMA | LEG | | |
| 53 | 51-60 | MALE | OTHERS | URBAN | FARMING | LSI | LEG | | |
| 54 | 51-60 | MALE | OTHERS | RURAL | FARMING | LSI | ABDOMINAL REGION | | |
| 55 | 61-70 | MALE | TERTIARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | |
| 56 | 51-60 | MALE | OTHERS | RURAL | OTHERS | LSI | LEG | | |
| 57 | 11-20 | MALE | SECONDARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | |
| 58 | 61-70 | MALE | OTHERS | URBAN | OTHERS | LSI | ABDOMINAL REGION | | |
| 59 | 1-10 | MALE | PRIMARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | HAND | | |
| 60 | 71-80 | FEMALE | PRIMARY | RURAL | HOUSE WIFE | LSI | MOUTH REGION | | |
| 61 | 21-30 | MALE | SECONDARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | |
| 62 | 11-20 | FEMALE | SECONDARY | URBAN | OTHERS | LSI | LEG | | |
| 63 | 21-30 | MALE | SECONDARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | POSITIVE(+) | BDSH, KADUNA |
| 64 | 51-60 | FEMALE | PRIMARY | URBAN | HOUSE WIFE | LSI | LEG | | |
| 65 | 21-30 | MALE | SECONDARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | |
| 66 | 51-60 | FEMALE | SECONDARY | URBAN | HOUSE WIFE | LSI | LEG | POSITIVE(+) | BDSH, KADUNA |
| 67 | 31-40 | FEMALE | OTHERS | URBAN | HOUSE WIFE | POST-ACCIDENTAL TRAUMA | HAND | | |
| 68 | 11-20 | MALE | PRIMARY | URBAN | FARMING | POST-ACCIDENTAL TRAUMA | HAND | | |
| 69 | 41-50 | FEMALE | OTHERS | RURAL | HOUSE WIFE | POST-ACCIDENTAL TRAUMA | LEG | | |
| 70 | 31-40 | FEMALE | OTHERS | URBAN | HOUSE WIFE | LSI | LEG | | |
| 71 | 51-60 | FEMALE | OTHERS | URBAN | HOUSE WIFE | LSI | HAND | | |
| 72 | 11-20 | MALE | SECONDARY | URBAN | OTHERS | LSI | LEG | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 73 | 11-20 | FEMALE | SECONDARY | URBAN | OTHERS | LSI | LEG | | | |
| 74 | 11-20 | FEMALE | OTHERS | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 75 | 21-30 | MALE | SECONDARY | RURAL | ARTISAN | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 76 | 21-30 | MALE | TERTIARY | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 77 | 31-40 | MALE | OTHERS | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 78 | 21-30 | FEMALE | OTHERS | URBAN | HOUSE WIFE | LSI | LEG | | | |
| 79 | 21-30 | MALE | TERTIARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | HAND | | | |
| 80 | 51-60 | MALE | OTHERS | URBAN | OTHERS | LSI | LEG | | | |
| 81 | 1-10 | MALE | OTHERS | URBAN | OTHERS | LSI | HAND | | | |
| 82 | 31-40 | MALE | SECONDARY | URBAN | CIVIL SERVANT | POST-ACCIDENTAL TRAUMA | HAND | | | |
| 83 | 61-70 | FEMALE | OTHERS | RURAL | HOUSE WIFE | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 84 | 21-30 | MALE | TERTIARY | URBAN | CIVIL SERVANT | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 85 | 21-30 | MALE | SECONDARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 86 | 61-70 | MALE | PRIMARY | RURAL | OTHERS | LSI | LEG | | | |
| 87 | 41-50 | MALE | OTHERS | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | POSITIVE(+) | GH, KAFANCHAN | |
| 88 | 21-30 | MALE | SECONDARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 89 | 11-20 | MALE | SECONDARY | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | HAND | POSITIVE(+) | GH, KAFANCHAN | |
| 90 | 21-30 | MALE | SECONDARY | RURAL | OTHERS | LSI | LEG | (2)POSITIVE(+) | | GH, KAFANCHAN |
| 91 | 11-20 | MALE | SECONDARY | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | ABDOMINAL REGION | | | |
| 92 | 21-30 | MALE | TERTIARY | RURAL | OTHERS | LSI | ABDOMINAL REGION | | | |
| 93 | 21-30 | FEMALE | PRIMARY | RURAL | HOUSE WIFE | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 94 | 31-40 | MALE | SECONDARY | URBAN | CIVIL SERVANT | LSI | LEG | | | |
| 95 | 21-30 | MALE | TERTIARY | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 96 | 41-50 | MALE | SECONDARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | HAND | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 97 | 11-20 | MALE | SECONDARY | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 98 | 51-60 | MALE | TERTIARY | URBAN | CIVIL SERVANT | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 99 | 31-40 | MALE | OTHERS | RURAL | FARMING | POST-ACCIDENTAL TRAUMA | MOUTH REGION | | | |
| 100 | 31-40 | MALE | OTHERS | RURAL | FARMING | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 101 | 51-60 | FEMALE | TERTIARY | RURAL | CIVIL SERVANT | POST-BURN | MOUTH REGION | | | |
| 102 | 21-30 | MALE | SECONDARY | URBAN | ARTISAN | POST-ACCIDENTAL TRAUMA | MOUTH REGION | | | |
| 103 | 1-10 | MALE | OTHERS | URBAN | OTHERS | LSI | HAND | | | |
| 104 | 1-10 | MALE | PRIMARY | RURAL | OTHERS | LSI | LEG | | | |
| 105 | 41-50 | FEMALE | OTHERS | RURAL | HOUSE WIFE | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 106 | 1-10 | MALE | OTHERS | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | (2)POSITIVE(+) | | GH, KAFANCHAN |
| 107 | 11-20 | FEMALE | OTHERS | RURAL | HOUSE WIFE | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 108 | 21-30 | MALE | SECONDARY | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | POSITIVE(+) | GH, KAFANCHAN | |
| 109 | 1-10 | MALE | OTHERS | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | MOUTH REGION | | | |
| 110 | 51-60 | MALE | PRIMARY | URBAN | CIVIL SERVANT | LSI | ABDOMINAL REGION | POSITIVE(+) | GH, KAFANCHAN | |
| 111 | 11-20 | MALE | SECONDARY | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | MOUTH REGION | | | |
| 112 | 21-30 | FEMALE | TERTIARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | POSITIVE(+) | GH, KAFANCHAN | |
| 113 | 11-20 | MALE | PRIMARY | RURAL | FARMING | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 114 | 61-70 | FEMALE | OTHERS | RURAL | HOUSE WIFE | LSI | LEG | | | |
| 115 | 21-30 | FEMALE | OTHERS | RURAL | HOUSE WIFE | LSI | LEG | | | |
| 116 | 21-30 | MALE | SECONDARY | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | EAR | (2)POSITIVE(+) | | GH, KAFANCHAN |
| 117 | 11-20 | FEMALE | SECONDARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | HAND | POSITIVE(+) | GH, KAFANCHAN | |
| 118 | 31-40 | MALE | TERTIARY | RURAL | CIVIL SERVANT | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 119 | 1-10 | MALE | OTHERS | RURAL | OTHERS | LSI | BUTTOCKS | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 120 | 1-10 | MALE | PRIMARY | URBAN | OTHERS | LSI | ABDOMINAL REGION | POSITIVE(+) | GH, KAFANCHAN | |
| 121 | 1-10 | MALE | PRIMARY | URBAN | ARTISAN | POST-BURN | ABDOMINAL REGION | POSITIVE(+) | GH, KAFANCHAN | |
| 122 | 1-10 | FEMALE | PRIMARY | URBAN | OTHERS | LSI | MOUTH REGION | | | |
| 123 | 31-40 | MALE | PRIMARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | HAND | | | |
| 124 | 41-50 | MALE | TERTIARY | RURAL | CIVIL SERVANT | LSI | LEG | | | |
| 125 | 31-40 | FEMALE | OTHERS | RURAL | HOUSE WIFE | POST-ACCIDENTAL TRAUMA | LEG | POSITIVE(+) | GH, KAFANCHAN | |
| 126 | 61-70 | MALE | SECONDARY | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 127 | 31-40 | MALE | PRIMARY | RURAL | FARMING | LSI | MOUTH REGION | | | |
| 128 | 21-30 | MALE | PRIMARY | URBAN | ARTISAN | POST-ACCIDENTAL TRAUMA | HAND | | | |
| 129 | 11-20 | FEMALE | SECONDARY | RURAL | HOUSE WIFE | LSI | LEG | | | |
| 130 | 11-20 | MALE | SECONDARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 131 | 61-70 | MALE | PRIMARY | RURAL | OTHERS | LSI | LEG | | | |
| 132 | 1-10 | MALE | PRIMARY | RURAL | OTHERS | LSI | LEG | POSITIVE(+) | GH, KAFANCHAN | |
| 133 | 11-20 | MALE | SECONDARY | RURAL | FARMING | LSI | HAND | | | |
| 134 | 61-70 | MALE | OTHERS | RURAL | FARMING | POST-ACCIDENTAL TRAUMA | MOUTH REGION | | | |
| 135 | 41-50 | FEMALE | SECONDARY | RURAL | FARMING | POST-ACCIDENTAL TRAUMA | HAND | | | |
| 136 | 31-40 | FEMALE | SECONDARY | URBAN | HOUSE WIFE | POST-SURGICAL TRAUMA | ABDOMINAL REGION | | | |
| 137 | 1-10 | MALE | PRIMARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 138 | 11-20 | MALE | PRIMARY | RURAL | ARTISAN | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 139 | 41-50 | FEMALE | OTHERS | RURAL | HOUSE WIFE | LSI | BREAST | | | |
| 140 | 41-50 | MALE | OTHERS | RURAL | FARMING | LSI | MOUTH REGION | | | |
| 141 | 31-40 | MALE | TERTIARY | URBAN | OTHERS | LSI | EAR | | | |
| 142 | 1-10 | FEMALE | PRIMARY | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | HAND | | | |
| 143 | 71-80 | MALE | OTHERS | RURAL | FARMING | LSI | ABDOMINAL REGION | | | |
| 144 | 11-20 | FEMALE | SECONDARY | RURAL | OTHERS | LSI | ABDOMINAL REGION | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 145 | 51-60 | FEMALE | OTHERS | RURAL | HOUSE WIFE | POST-ACCIDENTAL TRAUMA | LEG | | |
| 146 | 61-70 | FEMALE | OTHERS | RURAL | HOUSE WIFE | LSI | LEG | | |
| 147 | 31-40 | FEMALE | OTHERS | RURAL | HOUSE WIFE | LSI | BUTTOCKS | | |
| 148 | 31-40 | FEMALE | OTHERS | RURAL | HOUSE WIFE | POST-ACCIDENTAL TRAUMA | ABDOMINAL REGION | | |
| 149 | 21-30 | FEMALE | OTHERS | RURAL | HOUSE WIFE | LSI | ABDOMINAL REGION | POSITIVE(+) | GH, KAFANCHAN |
| 150 | 21-30 | FEMALE | OTHERS | RURAL | HOUSE WIFE | LSI | ABDOMINAL REGION | | |
| 151 | 11-20 | MALE | SECONDARY | URBAN | OTHERS | POST-SURGICAL TRAUMA | ABDOMINAL REGION | | |
| 152 | 21-30 | MALE | SECONDARY | RURAL | OTHERS | POST-SURGICAL TRAUMA | LEG | | |
| 153 | 21-30 | FEMALE | TERTIARY | URBAN | CIVIL SERVANT | LSI | ABDOMINAL REGION | | |
| 154 | 21-30 | MALE | TERTIARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | MOUTH REGION | | |
| 155 | 1-10 | MALE | PRIMARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | HAND | POSTIVE(+) | GH, KAFANCHAN |
| 156 | 21-30 | MALE | SECONDARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | |
| 157 | 21-30 | MALE | PRIMARY | URBAN | ARTISAN | LSI | LEG | | |
| 158 | 51-60 | MALE | OTHERS | URBAN | OTHERS | POST-SURGICAL TRAUMA | ABDOMINAL REGION | POSITIVE(+) | HGSGH, ZARIA |
| 159 | 21-30 | MALE | PRIMARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | HAND | | |
| 160 | 1-10 | MALE | OTHERS | URBAN | OTHERS | LSI | ABDOMINAL REGION | | |
| 161 | 1-10 | MALE | PRIMARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | |
| 162 | 11-20 | MALE | PRIMARY | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | HAND | | |
| 163 | 51-60 | MALE | OTHERS | RURAL | FARMING | POST-SURGICAL TRAUMA | ABDOMINAL REGION | | |
| 164 | 11-20 | FEMALE | OTHERS | RURAL | HOUSE WIFE | POST-SURGICAL TRAUMA | ABDOMINAL REGION | | |
| 165 | 11-20 | MALE | PRIMARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | |
| 166 | 1-10 | FEMALE | OTHERS | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | |
| 167 | 81-90 | MALE | OTHERS | URBAN | OTHERS | POST-SURGICAL TRAUMA | ABDOMINAL REGION | | |
| 168 | 41-50 | FEMALE | OTHERS | URBAN | HOUSE WIFE | POST-SURGICAL TRAUMA | ABDOMINAL REGION | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 169 | 21-30 | MALE | SECONDARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | HAND | | | |
| 170 | 61-70 | MALE | TERTIARY | URBAN | OTHERS | LSI | LEG | | | |
| 171 | 51-60 | MALE | OTHERS | URBAN | FARMING | POST-ACCIDENTAL TRAUMA | HAND | | | |
| 172 | 11-20 | FEMALE | SECONDARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 173 | 31-40 | MALE | PRIMARY | URBAN | OTHERS | LSI | LEG | POSITIVE(+) | ABUTH, ZARIA | |
| 174 | 31-40 | MALE | TERTIARY | URBAN | CIVIL SERVANT | POST-ACCIDENTAL TRAUMA | LEG | POSITIVE(+) | ABUTH, ZARIA | |
| 175 | 11-20 | FEMALE | PRIMARY | RURAL | HOUSE WIFE | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 176 | 11-20 | MALE | SECONDARY | URBAN | OTHERS | LSI | ABDOMINAL REGION | | | |
| 177 | 1-10 | MALE | PRIMARY | URBAN | OTHERS | LSI | LEG | | | |
| 178 | 11-20 | FEMALE | PRIMARY | URBAN | HOUSE WIFE | POST-BURN | HAND | | | |
| 179 | 51-60 | FEMALE | OTHERS | RURAL | HOUSE WIFE | POST-ACCIDENTAL TRAUMA | ABDOMINAL REGION | | | |
| 180 | 31-40 | MALE | SECONDARY | RURAL | OTHERS | LSI | LEG | | | |
| 181 | 21-30 | FEMALE | OTHERS | RURAL | HOUSE WIFE | LSI | MOUTH REGION | | | |
| 182 | 51-60 | FEMALE | PRIMARY | RURAL | CIVIL SERVANT | LSI | BREAST | | | |
| 183 | 51-60 | FEMALE | TERTIARY | URBAN | OTHERS | LSI | LEG | | | |
| 184 | 31-40 | MALE | PRIMARY | URBAN | ARTISAN | LSI | LEG | | | |
| 185 | 41-50 | MALE | TERTIARY | URBAN | CIVIL SERVANT | LSI | LEG | | | |
| 186 | 51-60 | MALE | OTHERS | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 187 | 1-10 | FEMALE | OTHERS | RURAL | OTHERS | POST-BURN | LEG | (2)POSITIVE(+) | | ABUTH, ZARIA |
| 188 | 1-10 | MALE | OTHERS | URBAN | OTHERS | LSI | LEG | | | |
| 189 | 1-10 | FEMALE | OTHERS | URBAN | OTHERS | LSI | ABDOMINAL REGION | | | |
| 190 | 51-60 | MALE | TERTIARY | URBAN | CIVIL SERVANT | POST-ACCIDENTAL TRAUMA | LEG | | | |
| 191 | 51-60 | MALE | TERTIARY | URBAN | CIVIL SERVANT | POST-BURN | LEG | POSITIVE(+) | ABUTH, ZARIA | |
| 192 | 31-40 | MALE | OTHERS | URBAN | FARMING | LSI | LEG | | | |
| 193 | 21-30 | FEMALE | OTHERS | RURAL | HOUSE WIFE | LSI | HAND | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 194 | 11-20 | MALE | PRIMARY | RURAL | OTHERS | LSI | ABDOMINAL REGION | | |
| 195 | 11-20 | MALE | OTHERS | RURAL | ARTISAN | POST-BURN | LEG | | |
| 196 | 41-50 | FEMALE | OTHERS | RURAL | HOUSE WIFE | POST-ACCIDENTAL TRAUMA | MOUTH REGION | | |
| 197 | 51-60 | MALE | OTHERS | RURAL | FARMING | LSI | LEG | | |
| 198 | 11-20 | MALE | PRIMARY | RURAL | FARMING | LSI | ABDOMINAL REGION | | |
| 199 | 61-70 | MALE | OTHERS | URBAN | ARTISAN | POST-SURGICAL TRAUMA | ABDOMINAL REGION | | |
| 200 | 21-30 | FEMALE | OTHERS | RURAL | HOUSE WIFE | POST-BURN | ABDOMINAL REGION | | |
| 201 | 11-20 | MALE | OTHERS | RURAL | OTHERS | LSI | ABDOMINAL REGION | | |
| 202 | 51-60 | FEMALE | OTHERS | RURAL | HOUSE WIFE | LSI | HAND | | |
| 203 | 71-80 | FEMALE | OTHERS | RURAL | HOUSE WIFE | LSI | LEG | | |
| 204 | 61-70 | FEMALE | PRIMARY | URBAN | HOUSE WIFE | POST-SURGICAL TRAUMA | BREAST | | |
| 205 | 11-20 | MALE | TERTIARY | URBAN | OTHERS | POST-BURN | HAND | | |
| 206 | 51-60 | MALE | TERTIARY | RURAL | CIVIL SERVANT | LSI | LEG | POSITIVE(+) | ABUTH, ZARIA |
| 207 | 21-30 | FEMALE | PRIMARY | RURAL | HOUSE WIFE | POST-SURGICAL TRAUMA | ABDOMINAL REGION | | |
| 208 | 11-20 | MALE | SECONDARY | RURAL | OTHERS | LSI | ABDOMINAL REGION | | |
| 209 | 11-20 | MALE | SECONDARY | URBAN | OTHERS | POST-BURN | HAND | | |
| 210 | 41-50 | MALE | OTHERS | RURAL | FARMING | LSI | ABDOMINAL REGION | | |
| 211 | 21-30 | MALE | SECONDARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | MOUTH REGION | | |
| 212 | 31-40 | MALE | SECONDARY | URBAN | FARMING | POST-BURN | LEG | POSITIVE(+) | ABUTH, ZARIA |
| 213 | 21-30 | MALE | PRIMARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | |
| 214 | 21-30 | MALE | OTHERS | RURAL | OTHERS | LSI | HAND | | |
| 215 | 31-40 | MALE | OTHERS | RURAL | FARMING | POST-ACCIDENTAL TRAUMA | LEG | | |
| 216 | 61-70 | FEMALE | OTHERS | RURAL | HOUSE WIFE | POST-SURGICAL TRAUMA | BREAST | | |
| 217 | 21-30 | FEMALE | TERTIARY | URBAN | CIVIL SERVANT | LSI | LEG | | |
| 218 | 31-40 | MALE | OTHERS | RURAL | FARMING | LSI | LEG | POSITIVE(+) | HGSGH, ZARIA |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 219 | 61-70 | MALE | OTHERS | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | BUTTOCKS | | |
| 220 | 51-60 | FEMALE | OTHERS | RURAL | HOUSE WIFE | LSI | BUTTOCKS | | |
| 221 | 31-40 | FEMALE | PRIMARY | RURAL | HOUSE WIFE | POST-SURGICAL TRAUMA | ABDOMINAL REGION | | |
| 222 | 31-40 | MALE | SECONDARY | URBAN | CIVIL SERVANT | POST-BURN | MOUTH REGION | | |
| 223 | 11-20 | FEMALE | OTHERS | RURAL | HOUSE WIFE | LSI | LEG | | |
| 224 | 11-20 | MALE | SECONDARY | URBAN | OTHERS | POST-SURGICAL TRAUMA | LEG | POSITIVE(+) | ABUTH, ZARIA |
| 225 | 21-30 | FEMALE | OTHERS | URBAN | OTHERS | POST-BURN | ABDOMINAL REGION | | |
| 226 | 31-40 | MALE | TERTIARY | URBAN | OTHERS | LSI | LEG | | |
| 227 | 31-40 | MALE | OTHERS | RURAL | FARMING | LSI | LEG | POSITIVE(+) | ABUTH, ZARIA |
| 228 | 21-30 | MALE | TERTIARY | URBAN | OTHERS | LSI | LEG | POSITIVE(+) | ABUTH, ZARIA |
| 229 | 21-30 | MALE | OTHERS | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | ABDOMINAL REGION | | |
| 230 | 21-30 | FEMALE | OTHERS | RURAL | HOUSE WIFE | POST-BURN | HAND | | |
| 231 | 31-40 | MALE | OTHERS | URBAN | ARTISAN | POST-ACCIDENTAL TRAUMA | LEG | | |
| 232 | 41-50 | MALE | OTHERS | RURAL | FARMING | LSI | HAND | | |
| 233 | 21-30 | MALE | PRIMARY | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | |
| 234 | 61-70 | MALE | OTHERS | RURAL | FARMING | LSI | LEG | | |
| 235 | 21-30 | MALE | SECONDARY | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | | |
| 236 | 31-40 | MALE | OTHERS | RURAL | FARMING | POST-ACCIDENTAL TRAUMA | MOUTH REGION | | |
| 237 | 11-20 | MALE | PRIMARY | URBAN | OTHERS | POST-SURGICAL TRAUMA | ABDOMINAL REGION | | |
| 238 | 1-10 | FEMALE | OTHERS | RURAL | OTHERS | POST-SURGICAL TRAUMA | BUTTOCKS | | |
| 239 | 21-30 | FEMALE | SECONDARY | URBAN | HOUSE WIFE | LSI | LEG | | |
| 240 | 1-10 | MALE | PRIMARY | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | HAND | POSITIVE(+) | HGSGH, ZARIA |
| 241 | 51-60 | MALE | OTHERS | URBAN | OTHERS | LSI | LEG | | |
| 242 | 21-30 | MALE | TERTIARY | RURAL | OTHERS | POST-ACCIDENTAL TRAUMA | LEG | POSITIVE(+) | ABUTH, ZARIA |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 243 | 41-50 | FEMALE | OTHERS | URBAN | HOUSE WIFE | LSI | BREAST | | |
| 244 | 51-60 | FEMALE | OTHERS | URBAN | HOUSE WIFE | POST-ACCIDENTAL TRAUMA | LEG | | |
| 245 | 51-60 | FEMALE | OTHERS | RURAL | HOUSE WIFE | LSI | BREAST | POSITIVE(+) | ABUTH, ZARIA |
| 246 | 31-40 | FEMALE | OTHERS | RURAL | HOUSE WIFE | LSI | BREAST | | |
| 247 | 41-50 | MALE | TERTIARY | URBAN | CIVIL SERVANT | LSI | BUTTOCKS | | |
| 248 | 11-20 | MALE | SECONDARY | URBAN | OTHERS | POST-SURGICAL TRAUMA | LEG | | |
| 249 | 31-40 | MALE | SECONDARY | URBAN | OTHERS | LSI | HAND | | |
| 250 | 21-30 | MALE | SECONDARY | URBAN | CIVIL SERVANT | LSI | HAND | | |
| 251 | 31-40 | MALE | SECONDARY | URBAN | CIVIL SERVANT | POST-ACCIDENTAL TRAUMA | HAND | | |
| 252 | 41-50 | FEMALE | OTHERS | URBAN | HOUSE WIFE | POST-SURGICAL TRAUMA | LEG | | |
| 253 | 51-60 | FEMALE | OTHERS | URBAN | HOUSE WIFE | LSI | BUTTOCKS | POSITIVE(+) | HGSGH, ZARIA |
| 254 | 11-20 | MALE | PRIMARY | URBAN | ARTISAN | POST-SURGICAL TRAUMA | HAND | | |
| 255 | 51-60 | MALE | OTHERS | URBAN | ARTISAN | LSI | ABDOMINAL REGION | | |
| 256 | 61-70 | MALE | TERTIARY | URBAN | CIVIL SERVANT | POST-ACCIDENTAL TRAUMA | HAND | POSITIVE(+) | ABUTH, ZARIA |
| 257 | 21-30 | MALE | TERTIARY | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | MOUTH REGION | | |
| 258 | 41-50 | MALE | OTHERS | RURAL | FARMING | LSI | MOUTH REGION | | |
| 259 | 31-40 | FEMALE | OTHERS | RURAL | HOUSE WIFE | LSI | BREAST | | |
| 260 | 41-50 | MALE | OTHERS | URBAN | OTHERS | POST-ACCIDENTAL TRAUMA | HAND | POSITIVE(+) | HGSGH, ZARIA |
| 261 | 41-50 | FEMALE | OTHERS | RURAL | HOUSE WIFE | POST-SURGICAL TRAUMA | ABDOMINAL REGION | | |
| 262 | 31-40 | MALE | SECONDARY | URBAN | OTHERS | LSI | ABDOMINAL REGION | | |
| 263 | 21-30 | FEMALE | OTHERS | RURAL | HOUSE WIFE | POST-SURGICAL TRAUMA | HAND | | |
| 264 | 21-30 | FEMALE | PRIMARY | RURAL | HOUSE WIFE | POST-SURGICAL TRAUMA | ABDOMINAL REGION | | |
| 265 | 11-20 | MALE | OTHERS | URBAN | ARTISAN | POST-SURGICAL TRAUMA | LEG | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 266 | 41-50 | FEMALE | OTHERS | RURAL | HOUSE WIFE | POST-SURGICAL TRAUMA | MOUTH REGION | | |
| 267 | 21-30 | MALE | PRIMARY | RURAL | OTHERS | POST-BURN | LEG | | |
| 268 | 51-60 | MALE | OTHERS | RURAL | ARTISAN | LSI | LEG | POSITIVE(+) | ABUTH, ZARIA |

## APPENDIX V

## ZONES OF INHIBITION (mm) OF ANTIBIOTICS ON STAPHYLOCOCCUS AUREUS ISOLATED FROM CHRONIC SKIN ULCERS

| S/NO | CODE | ISOLATES | Cefoxitin (30µg) | Augmentin (30µg) | Vancomycin (30µg) | Gentamicin(10µg) | Erythromycin(15µg) | Tetracycline(30µg) | Ciprofloxacin(5µg) | Co-trimoxazole(30µg) | Chloramphenicol(30µg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 256MS | *Staphylococcus aureus* | 24 | 16 | 16 | 26 | 32 | 08 | 30 | R | 29 |
| 2 | 106MK | *Staphylococcus aureus* | 21 | R | 24 | 19 | 18 | 16 | 22 | R | 22 |
| 3 | 063MB | *Staphylococcus aureus* | 12 | R | 16 | 14 | 24 | 18 | 28 | 20 | 12 |
| 4 | 206MS | *Staphylococcus aureus* | 24 | R | 22 | 30 | 32 | 18 | 30 | 24 | 18 |
| 5 | 116MK | *Staphylococcus aureus* | 14 | 11 | 18 | 20 | 26 | 20 | 36 | 16 | 30 |
| 6 | 121MK | *Staphylococcus aureus* | 12 | R | 16 | 24 | 28 | 12 | 32 | R | 28 |
| 7 | 037MB | *Staphylococcus aureus* | 10 | R | 18 | 26 | 26 | 08 | 32 | R | 22 |
| 8 | 240MG | *Staphylococcus aureus* | 26 | 12 | 10 | 26 | 26 | R | 26 | 24 | 22 |
| 9 | 090MK | *Staphylococcus aureus* | 26 | 14 | 18 | 24 | 30 | 16 | 36 | 34 | 14 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 110MK | *Staphylococcus aureus* | 16 | R | 15 | 24 | 28 | 14 | 30 | R | 20 |
| 11 | 268MS | *Staphylococcus aureus* | 12 | R | 20 | 32 | 28 | 18 | 30 | 18 | 28 |
| 12 | 174MS | Staphylococcus *aureus* | R | R | 18 | 22 | 20 | 12 | 32 | R | 32 |
| 13 | 001MB | *Staphylococcus aureus* | R | R | 15 | 15 | R | R | 16 | R | R |
| 14 | 011MB | *Staphylococcus aureus* | R | R | 17 | 12 | R | R | R | R | **13** |
| 15 | 010FB | *Staphylococcus aureus* | 16 | R | 18 | 24 | R | 16 | R | R | 18 |
| 16 | 173MS | *Staphylococcus aureus* | R | R | 20 | 20 | 14 | 09 | 27 | R | 12 |
| 17 | 270MS | *Staphylococcus aureus* | R | R | R | 22 | 25 | 16 | 32 | R | 30 |
| 18 | 027FB | *Staphylococcus aureus* | 14 | 08 | 18 | 28 | 32 | 14 | 30 | R | 26 |
| | | % Susceptible | 22.2 | 0 | 93.0 | 88.8 | 72.2 | 5.6 | 83.3 | 33.3 | 72.2 |
| | | % Resistance | 77.8 | 100 | 7.0 | 5.6 | 16.7 | 50.0 | 11.1 | 66.7 | 16.7 |
| | | % Intertermidiate | 0 | 0 | 0 | 5.6 | 11.1 | 44.4 | 5.6 | 0 | 11.1 |

| S/NO | CODE | ISOLATES | Cefoxitin (30µg) | Augmentin (30µg) | Vancomycin (30µg) | Gentamicin | Erythromycin(15µg) | Tetracycline(30µg) | Ciprofloxacin(5µg) | Co-trimoxazole(30µg) | Chloramphenicol(30µg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 116MK(2) | *Staphylococcus hyicus* | R | R | 17 | 26 | 22 | 20 | 32 | 10 | 30 |
| 2 | 260MG | *Staphylococcus hyicus* | 14 | R | 15 | 26 | 27 | R | 30 | R | 18 |
| 3 | 260MG(2) | *Staphylococcus hyicus* | 14 | R | 16 | 24 | 26 | 14 | 28 | 14 | 17 |
| 4 | 117FK | *Staphylococcus hyicus* | 12 | R | 16 | 24 | 26 | 08 | 30 | R | 16 |
| | | % Susceptible | 0 | 0 | 100 | 100 | 100 | 25 | 100 | 0 | 50 |
| | | % Resistance | 100 | 100 | 0 | 0 | 0 | 75 | 0 | 75 | 0 |
| | | % Intertermidiate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 50 |

| S/NO | CODE | ISOLATES | Cefoxitin (30µg) | Augmentin (30µg) | Vancomycin (30µg) | Gentamicin(10µg) | Erythromycin(15µg) | Tetracycline(30µg) | Ciprofloxacin(5µg) | Co-trimoxazole(30µg) | Chloramphenicol(30µg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 031FB | *Staphylococcus epidermidis* | 10 | R | 16 | 24 | 28 | 08 | 26 | R | 22 |
| 2 | 125FK | *Staphylococcus epidermidis* | 18 | R | 19 | 25 | 30 | 16 | 26 | 18 | 20 |
| 3 | 132MK | *Staphylococcus epidermidis* | R | 22 | 18 | 26 | 24 | 12 | 34 | 30 | 22 |
| 4 | 049MB | *Staphylococcus epidermidis* | 26 | 22 | 20 | 22 | R | 18 | 40 | 24 | 24 |
| 5 | 253FG | *Staphylococcus epidermidis* | 26 | 30 | 18 | 32 | 22 | 22 | 36 | 44 | 22 |
| 6 | 272MG | *Staphylococcus epidermidis* | 30 | 22 | 20 | 32 | 30 | 26 | 38 | 38 | 20 |
| 7 | 271MS | *Staphylococcus epidermidis* | R | R | 18 | 24 | 22 | 06 | 30 | R | 18 |
| 8 | 227MS | *Staphylococcus epidermidis* | 10 | R | R | 28 | R | R | 28 | 20 | 14 |
| 9 | 112FK | *Staphylococcus epidermidis* | 24 | 26 | 26 | 32 | 32 | 24 | 22 | 40 | 22 |
| 10 | 245FS | *Staphylococcus epidermidis* | 17 | R | R | R | R | R | R | R | 10 |
| | | % Susceptible | 40 | 50 | 98 | 90 | 50 | 30 | 90 | 70 | 80 |
| | | % Resistance | 60 | 50 | 02 | 10 | 30 | 50 | 10 | 30 | 10 |
| | | % Intertermidiate | 10 | 0 | 0 | 0 | 20 | 20 | 0 | 0 | 10 |
| **1** | 191MS | *Staphylococcus intermedius* | 23 | 18 | 24 | 21 | 20 | 22 | 22 | 24 | 22 |

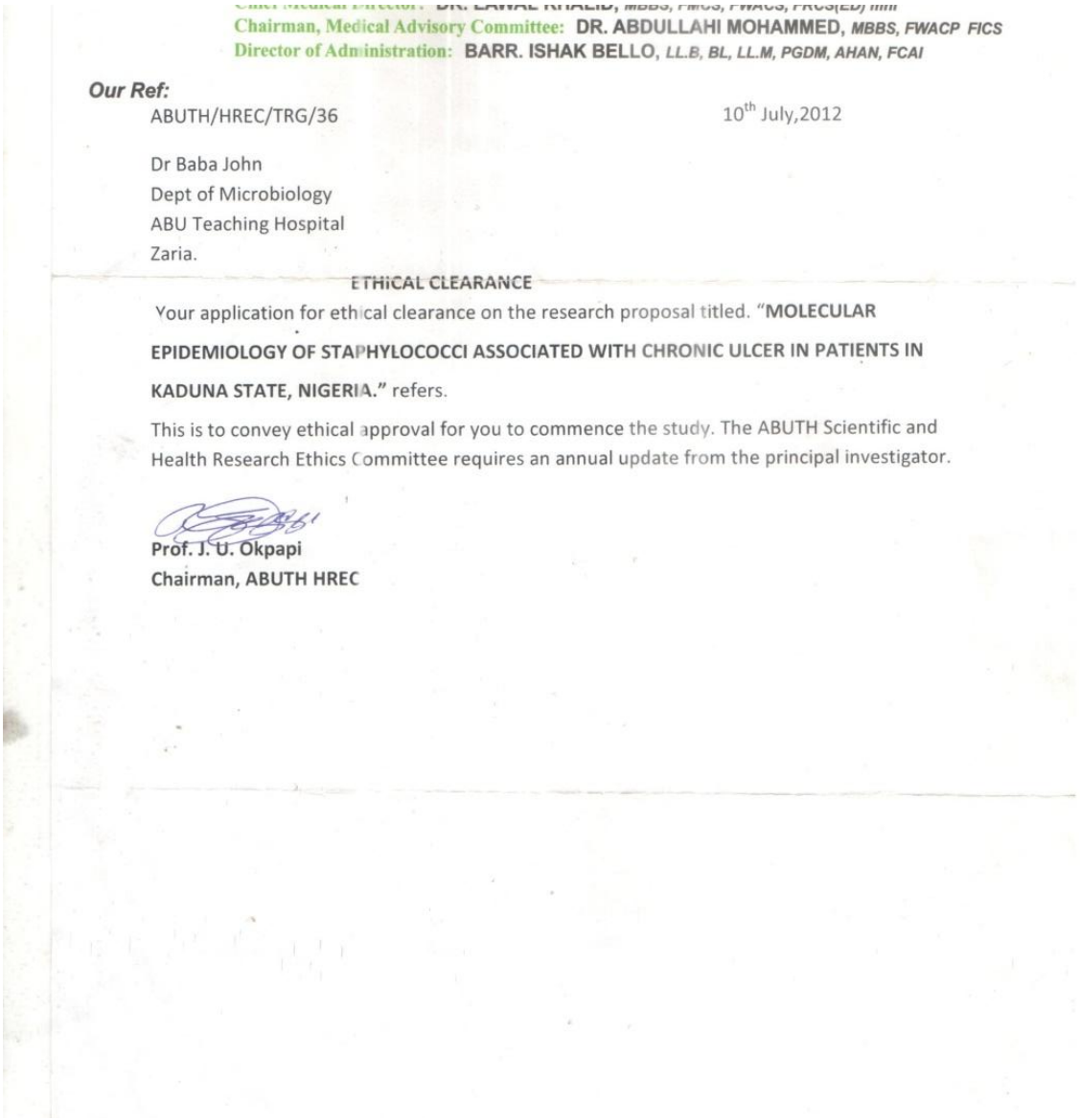
| S/NO | CODE | ISOLATES | Cefoxitin (30µg) | Augmentin (30µg) | Vancomycin (30µg) | Gentamicin(10µg) | Erythromycin(15µg) | Tetracycline(30µg) | Ciprofloxacin(5µg) | Co-trimoxazole(30µg) | Chloramphenicol(30µg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % Susceptible | 100 | 0 | 100 | 100 | 0 | 100 | 100 | 100 | 100 |
| | | % Resistance | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | | % Intertermidiate | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 090MK(2) | *Staphylococcus haemolyticus* | R | R | R | 22 | 28 | R | 28 | 16 | 22 |
| 2 | 108MK | *Staphylococcus haemolyticus* | R | R | 17 | 27 | 14 | 26 | 36 | 40 | 11 |
| 3 | 011MB(2) | *Staphylococcus haemolyticus* | 14 | 10 | 16 | 16 | 28 | 14 | 28 | R | 26 |
| 4 | 001MB(2) | *Staphylococcus haemolyticus* | R | R | 18 | 28 | 32 | 18 | 28 | 20 | 16 |
| 5 | 120MK | *Staphylococcus haemolyticus* | 12 | 16 | 20 | 30 | 28 | 24 | 36 | 24 | 18 |
| 6 | 106MK(2) | *Staphylococcus haemolyticus* | 14 | R | 16 | 24 | 26 | 08 | 26 | R | 24 |
| 7 | 242MS | *Staphylococcus haemolyticus* | R | R | 18 | 16 | R | R | 16 | R | R |
| 8 | 187FS | *Staphylococcus haemolyticus* | 12 | 22 | 20 | 30 | 32 | 24 | 34 | 40 | 22 |
| | | % Susceptible | 0 | 12.5 | 97.5 | 100 | 75 | 37.5 | 87.5 | 62.5 | 62.5 |
| | | % Resistance | 100 | 87.5 | 02.5 | 0 | 12.5 | 50 | 0 | 37.5 | 25 |
| | | % Intertermidiate | 0 | 0 | 0 | 0 | 12.5 | 12.5 | 12.5 | 0 | 12.5 |
| 1 | 158MG | *Staphylococcus xylosus* | 12 | R | 17 | 24 | 28 | 11 | 26 | R | 18 |
| 2 | 155MK | *Staphylococcus xylosus* | 16 | R | 18 | 23 | R | 08 | 28 | R | R |
| 3 | 001MB(3) | *Staphylococcus xylosus* | 27 | 30 | 24 | 20 | 36 | 36 | 27 | 26 | 36 |
| 4 | 087MK | *Staphylococcus xylosus* | 18 | R | 20 | 24 | 30 | 16 | 28 | 18 | 22 |
| 5 | 224MS | *Staphylococcus xylosus* | 20 | 20 | 20 | 26 | 30 | 26 | 26 | 44 | 22 |
| | | % Susceptible | 20 | 40 | 100 | 100 | 80 | 40 | 100 | 60 | 80 |
| | | % Resistance | 80 | 60 | 0 | 0 | 20 | 40 | 0 | 40 | 20 |
| | | % Intertermidiate | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 |
| 1 | 089MK | *Staphylococcus chromogenes* | 12 | R | 20 | 30 | 20 | 21 | 28 | 32 | 09 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 149FK | *Staphylococcus chromogenes* | 23 | 20 | 24 | 24 | 14 | 18 | 20 | 19 | 16 |
| 3 | 187FS(2) | *Staphylococcus chromogenes* | 28 | 20 | R | R | R | 10 | 38 | R | 26 |
| 4 | 066FB | *Staphylococcus chromogenes* | 14 | R | 20 | 28 | R | 16 | 30 | 22 | 20 |
| 5 | 218MG | *Staphylococcus chromogenes* | 24 | 26 | 30 | 14 | 30 | 28 | 38 | 18 | 18 |
| 6 | 212MS | *Staphylococcus chromogenes* | 12 | 12 | 16 | 22 | 34 | 28 | 28 | 24 | 34 |
| 7 | 007MB | *Staphylococcus chromogenes* | 10 | R | 18 | 28 | 28 | 10 | 30 | R | 24 |
| | | % Susceptible | 42.9 | 42.9 | 98.5 | 71.4 | 42.8 | 42.8 | 85.7 | 71.4 | 71.4 |
| | | % Resistance | 57.1 | 57.1 | 01.5 | 14.3 | 28.6 | 28.6 | 0 | 28.6 | 14.3 |
| | | % Intertermidiate | 0 | 0 | 0 | 14.3 | 28.6 | 28.6 | 14.3 | 0 | 14.3 |

## APPENDIX VI

## ETHICAL CLEARANCE APPROVAL FROM ABUTH, ZARIA



Chief Medical Director: DR. LAWAL KHALID, MBBS, FMCS, FWACS, FRCS(ED) IIIIII
Chairman, Medical Advisory Committee: DR. ABDULLAHI MOHAMMED, MBBS, FWACP FICS
Director of Administration: BARR. ISHAK BELLO, LL.B, BL, LL.M, PGDM, AHAN, FCAI

**Our Ref:**
ABUTH/HREC/TRG/36

10th July,2012

Dr Baba John
Dept of Microbiology
ABU Teaching Hospital
Zaria.

**ETHICAL CLEARANCE**

Your application for ethical clearance on the research proposal titled. "**MOLECULAR EPIDEMIOLOGY OF STAPHYLOCOCCI ASSOCIATED WITH CHRONIC ULCER IN PATIENTS IN KADUNA STATE, NIGERIA.**" refers.

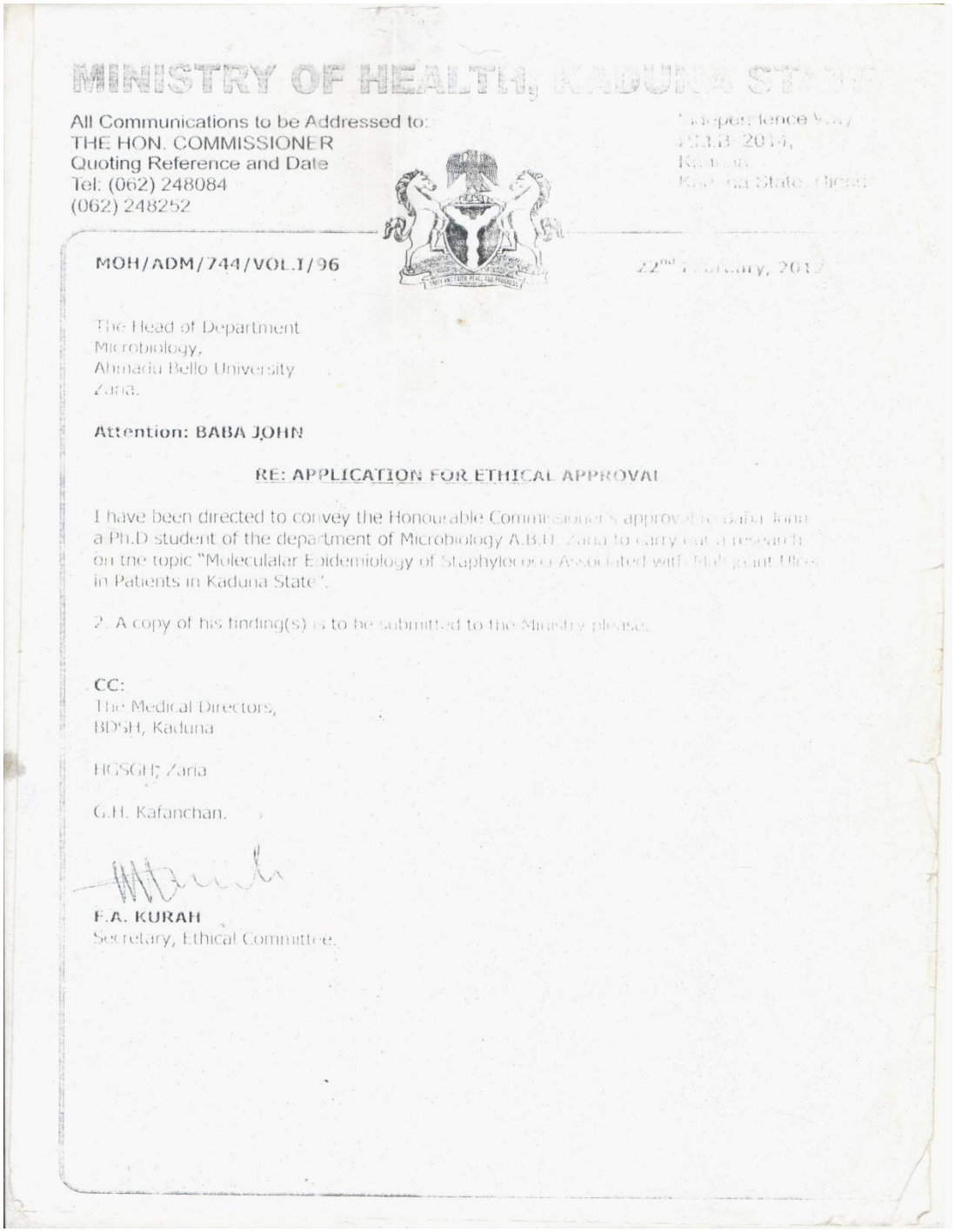
This is to convey ethical approval for you to commence the study. The ABUTH Scientific and Health Research Ethics Committee requires an annual update from the principal investigator.

**Prof. J. U. Okpapi**
**Chairman, ABUTH HREC**



## APP[illegible] VII

## ETHICAL CLEARANCE APPROVAL FROM MINISTRY OF HEALTH, KADUNA



MINISTRY OF HEALTH, KADUNA STATE

All Communications to be Addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Tel: (062) 248084
(062) 248252

Independence Way,
P.M.B. 2013,
Kaduna.
Kaduna State, Nigeria.

MOH/ADM/744/VOL.I/96

22nd February, 2012

The Head of Department
Microbiology,
Ahmadu Bello University
Zaria.

**Attention: BABA JOHN**

**RE: APPLICATION FOR ETHICAL APPROVAL**

I have been directed to convey the Honourable Commissioner's approval to Baba John a Ph.D student of the department of Microbiology A.B.U. Zaria to carry out a research on the topic "Molecular Epidemiology of Staphylococci Associated with Skin and Ulcers in Patients in Kaduna State".

2. A copy of his finding(s) is to be submitted to the Ministry please.

CC:
The Medical Directors,
BDSH, Kaduna

HGSGH, Zaria

G.H. Kafanchan.

F.A. KURAH
Secretary, Ethical Committee.



## APPI VIII

## PATIENT'S CONSENT FORM IN ENGLISH

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My name is Baba john, a student of the Microbiology Department, Ahmadu Bello University, and Zaria, Nigeria. I am undertaking a study on the Molecular Epidemiology of Staphylococci associated with chronic ulcer in patients in Kaduna State. This research study has been reviewed and granted approval by ethics committee of the Ahmadu Bello University Teaching Hospital, Zaria.

I would indeed appreciate your participation in this study. The information to be acquired from your participation will assist the government to plan her health services, most especially by providing drugs such as antibiotics that would enable you to attain the necessary rapid healing. Such information will also be kept confidential, and will not be shown to other persons.

As part of the study, you will be asked some questions about your life style and wound swab samples shall be collected from you. All your responses to the questions that would be asked shall be taken confidential. Your participation in the study is however voluntary. If you come across any question you do not want to answer, you can leave it and go to the next or you may wish to stop answering questions at any time.

I do hope you will participate in the study, the information you will so give will be of immense help to the success of the study.

_____ _____

**Signature of Respondent**. **Date**

**APPENDIX IX**

**PATIENT'S CONSENT FORM IN HAUSA**

# TAKADAR AMINCEWA

Suna na Baba john, dalibi a Department of Microbiology, Jami'ar Ahmadu Bello, Zaria, Nigeria. Ina Gudanar da bincike akan yanayin halittar kwayar cutar dake jawo dadewar gyambo bai warke ba a Jihar Kaduna. Shi wannan bincike ya samu amincewar kwamitin tantance ayyukan bincincike na Asibitin koyarwa Jami'ar Ahmadu Bello dake Zaria.

Zan yi farin cikin amincewar ka da bada gudumawa a kan wan nan bincike. Bayanin da za ka ba ni zai taimaka wa gwamnati wajen gudanar da ayyukan kiwon lafiya, musamman samar da magungunan da za su warkar da ciwukan da gaggawa. Kuma ina baka tabbacin cewa duk bayanen da ka bani zan adana su a matsayin sirri.

Ina bukatar bayani akan yadda ka ke gudanar da rayawar ka da kuma daukar samfurin da za muyi gwaji da ga gyambo da ke jikin ka. Ka na da zabin shiga wannan bincike, idan ka ga dama, ka na kuma iya cewa ba zaka shiga ba. Sannan idan ka shiga shima kanna iya kin amsa kowace tanbaya da baka son amsawa.

Ina fata za ka/ki shiga ka bada gudumawar, domin yin hakan zai matukar taimakawa samun nasara a binciken.

Na gode

_____ _____

**Suna** **wata**

## APPENDIX X

## AGE DISTRIBUTION OF CHRONIC SKIN ULCER PATIENTS IN KADUNA STATE

| AGE | FREQUENCY | PERCENTAGE |
|---|---|---|
| 1-10 | 38 | 13.0 |
| 11-20 | 44 | 15.1 |
| 21-30 | 69 | 23.6 |
| 31-40 | 44 | 15.1 |
| 41-50 | 32 | 11.0 |
| 51-60 | 40 | 13.7 |
| 61-70 | 18 | 6.2 |
| 71-80 | 5 | 1.7 |
| 81-90 | 2 | 0.7 |
| **TOTAL** | **292** | **100** |

## APPENDIX: XI

## SEX DISTRIBUTION OF PATIENTS WITH CHRONIC SKIN ULCER IN KADUNA STATE.

| Sex | Frequency | Percentage |
|---|---|---|
| Male | 104 | 35.6 |
| Female | 188 | 64.4 |
| **Total** | **292** | **100** |

## APPENDIX XII

**DISTRIBUTION OF CHRONIC SKIN ULCER PATIENTS BY EDUCATIONAL STATUS IN KADUNA STATE**

| Educational Status | Frequency | Percentage |
|---|---|---|
| Primary | 71 | 24.3 |
| Secondary | 66 | 22.6 |
| Tertiary | 34 | 11.6 |
| Others | 121 | 41.4 |
| **Total** | **292** | **100** |

## APPENDIX XIII

**DISTRIBUTION OF CHRONIC SKIN ULCERPATIENTS BY URBANIZATIONTYPE IN KADUNA STATE**

| Area Of Domicile | Frequency | Percent |
|---|---|---|
| Rural | 138 | 47.3 |
| Urban | 154 | 52.7 |
| **Total** | **292** | **100.0** |

## APPENDIX XIV

**DISTRIBUTION OF CHRONIC SKIN ULCER PATIENTS BY OCCUPATION STATUS IN KADUNA STATE**

| Occupation | Frequency | Percent |
|---|---|---|
| Artisan | 21 | 7.2 |
| Civil Servant | 30 | 10.3 |
| Farming | 38 | 13.0 |
| House Wife | 72 | 24.7 |
| Others | 131 | 44.9 |
| **Total** | **292** | **100.0** |

## APPENDIX XV

**DISTRIBUTION OF CHRONIC SKIN ULCER PATIENTS BY AETIOLOGY IN KADUNA STATE**

| Cause Of Ulcer | Frequency | Percent |
|---|---|---|
| Long Standing Infection | 136 | 46.6 |
| Post Accidental Trauma | 112 | 38.3 |
| Post Surgical Trauma | 22 | 7.5 |
| Post-Burn | 22 | 7.5 |
| **Total** | **292** | **100.0** |

## APPENDIX XVI

**DISTRIBUTION OF CHRONIC SKIN ULCER PATIENTSBY SITE IN KADUNA STATE**

| Site of Ulcer | Frequency | Percent |
|---|---|---|
| Abdominal Region | 43 | 14.7 |
| Breast | 8 | 2.7 |
| Buttocks | 9 | 3.1 |
| Ear | 2 | .7 |
| Hand | 49 | 16.8 |
| Leg | 150 | 51.4 |
| Mouth Region | 29 | 9.9 |
| **Total** | **292** | **100.0** |

# APPENDIX XVII
# PUBLICATIONS FROM THE THESIS

*IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*
*e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 14, Issue 2 Ver. IV (Feb. 2015), PP 79-83*
*www.iosrjournals.org*

## Phenotypic Characterization and Antibiotic Susceptibility Studies of Coagulase- Negative Staphylococci (Cons) Isolated From Chronic Skin Ulcer of Patients In Kaduna State.

J. BABA[1]*, H.I. INABO[2], V.J. UMOH[4], and A.T. OLAYINKA[3]
[1]. *Department of microbiology, Ibrahim Badamasi Babangida University, Lapai, Nigeria.*
[2]. *Department of microbiology, Ahmadu Bello University, Zaria, Nigeria.*
[3]. *Department of medical microbiology, Ahmadu Bello University Teaching Hospital, Zaria.*
[4]. *Department of microbiology,Akwa ibom State University,Ikot-ekpene,Nigeria.*
* Corresponding author. E-mail:babajohn200133@vahoo.co.uk

***Abstract:*** *The study was aimed at characterizing the different Coagulase- negative staphylococci (CoNS) isolated from chronic skin ulcer out-patients attending four different hospitals in Kaduna State, Nigeria. A total of 292 swab samples were collected from January 2012 to January 2013. The samples were cultured using Chocolate and Blood agar media. Phenotypic characterization of the CoNS revealed the presence of Staphylococcus epidermidis (n=10, 28.5%), Staphylococcus haemolyticus (n=8, 22.9%), Staphylococcus chromogenes (n=7, 20.0%) Staphylococcus hyicus (n=4, 11.4%), Staphylococcus xylosus (n=5, 14.3%) and Staphylococcus intermedius (n=1, 2.9%). The different sites of chronic skin ulcer from where the CoNS were recovered revealed that the leg harbored the highest number of CoNS isolates of (n= 1, 51.4%), while the breast has the least isolate of (n= 1, 2.9%). The CoNS isolates were found mostly to be susceptible to Gentamicin, Ciprofloxacin, Trimethoprim-sulphamethoxazole, Chloramphenicol, Vancomycin and Erythromycin. However, they showed resistance mostly to Cefoxitin. Amoxicillin-clavulanic acid and tetracycline.*
***Key words:*** *Phenotypic, CoNS, Antibiotic, Susceptibility, ulcer.*

### I. Introduction

The skin becomes broken when there is a cut or abrasion on the skin, and there is every likelihood that their protective defense mechanism becomes obstructed. When this happens, the environment becomes conducive for bacteria to contaminate the skin, increase in number and possibly cause an infection. The bacteria contaminating wounds are from the environment, through dust particles, bacteria on hands, clothing and equipment. Different kinds of wounds exist, ranging from superficial burns, bite wounds and surgical wounds (Bowler, 2001). The activities of microorganism in the wound could cause delayed healing in such wounds, thereby making the wound to be chronic. The most common type of chronic wound is an ulcer, which occurs usually in the lower leg of individual having underlying diabetes. The healings of such ulcer would delay, even when less pathogenic microorganisms are present (Williams et al., 2004).Coagulase negative staphylococci (CONS).previously considered as non- pathogenic have been identified as the etiological agents in most hospital acquired infections, and have been frequently isolated from such infections ( Cunha et al.,2006). Apart from Staphylococcus epidermidis that is commonly isolated in wounds, other species have been implicated to cause infections. Staphylococcus, xylosus, Staphylococcus, haemolyticus and Staphylococcus lugdunensis have been reported to cause infection ranging from urinary tract infections(UTI), osteomyelitis and sepsis respectively (Venkatesh et al., 2006). Staphylococcus epidermidis has emerged in recent years as a pathogen in a growing number of other serious nosocormial infections, such as in neonatal intensive care units (NICUS), most especially bloodstream infections (Hall, 1991; Gaynes et al., 1996). S.epidermidis has also been widely recognized as an etiologic agent of bacteremia, prosthetic and natural valvular endocarditis, osteomyelitis and urinary tract infections, however in a frequent association with the colonization of intravascular catheters and orthopaedic devices (Sheagren, 1984; Brumfitt and Hamilton -Miller, 1989). Staphylococcus saprophyticus has been documented to be the second most frequently encountered microorganism after Escherichia coli in acute UTI (McTaggart et al., 1990; Gupta et al., 1999). Several other species of coagulase-negative Staphylococci have been implicated at low incidence in a variety of infections (Javid et al., 2006). One of the factors that favours the CoNS to wreak havoc in their host is their ability to produce enzymes such as lipases, proteases and other exo- enzymes, which have made them to be persistent, and possibly degrade host tissues (Otto, 2004).Several species of CoNS have been reported to produce haemolysins which made it possible for them to bind to susceptible host cells, and causing lysis of the red blood cells for the release of free iron which is eventually utilized by the bacteria (Azukah,2013). The major reservoirs of coagulase-negative Staphylococci in hospitals are colonized or infected in-patients and colonized hospitals workers, with carriers at risk for

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*(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219*
*www.iosrphr.org Volume 5, Issue 2 (February 2015), PP. 07-12*

# Antibiotic resistance patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from chronic skin ulcer of patients in Kaduna state, Nigeria.

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[1.] *Department of microbiology, Ibrahim Badamasi Babangida University, Lapai, Nigeria.*
[2.] *Department of microbiology, Ahmadu Bello University, Zaria, Nigeria.*
[3.] *Department of medical microbiology, Ahmadu Bello University Teaching Hospital, Zaria.*
[4.] *Department of microbiology,Akwa ibom State University,Ikot-ekpene,Nigeria.*

***ABSTRACT:*** *The aim of this study is to investigate the antibiotic resistance patterns of methicillin-resistant Staphylococcus aureus (MRSA) isolated from out - patients with chronic skin ulcer in four different hospitals in Kaduna state. A total number of 292 swab samples were collected from January 2012 to January 2013 and analyzed. MRSA isolates recovered were 14 (78 %) of the total number of Staphylococcus aureus found on the different sites of infection sampled. The highest number of MRSA isolates of 08 (57 %) were recovered from the leg (lower extremity ulcer), while 02 (14 %) isolates each were recovered from hand, abdominal region and mouth (buccal cavity) sites of infection. No MRSA isolates was recovered from the other parts of the skin. From the susceptibility studies of the isolates, it was found that all the MRSA isolates showed 100 % resistance to Amoxacillin + clavulanic acid (Augmentin), and least resistance of 07% to gentamicin. The antibiotic resistance pattern common to all the MRSA isolates is in the order of cefoxitin, amoxicillin + clavulanic acid, Trimethoprim + sulphamethoxazole.*

***Keywords:*** *Antibiotic, resistance, methicillin, Staphylococcus aureus, ulcer*

## I. INTRODUCTION

A wound can be defined as any injury that damage the skin and therefore compromise its protective function. However, a wound is said to be chronic when healing is not achieved within three months (Siddiqui and Bernstein, 2010). Ulcer appears to be the commonest type of chronic wound. Every open skin wounds are colonized by bacteria, this does not mean that all wounds are infected. Many factors determine the progression of a wound from contamination to infection, which may include bacterial load, the types of bacteria present in such wounds, their synergistic effect including their virulent nature (Edwards and Harding, 2004; Siddiqui and Bernstein, 2010). The initial colonizers of the skin are those bacteria that live symbiotically on the skin. The non-healing condition of a wound over time exposes it to different pathogenic bacteria.

Ulcerations of the lower leg of venous and diabetic origin are the most frequent of all chronic wounds. The delays of these wounds could be the disturbance in the supply of nutrients and removal of metabolic products, caused by the pathology of blood vessels (Bowler, 1998). Generally, infection may be caused by pathogenic bacteria originating from the external environment, as well as bacteria forming physiological microflora of the skin (Schmidt *et al.*, 2000).

*Staphylococcus aureus* has been reported to be a major cause of several infection that includes, bacteremia, skin and soft tissue infections and osteomyelitis (Diekema *et al.*, 2001; Alam *et al.*, 2002). The root of most pyogenic local and systemic infections in both hospitals and community has been linked to *Staphylococcus aureus* (Arunara *et al.*, 2013). Methicillin resistant *S. aureus* (MRSA) is often acquired when there is an individual exposure to hospitals, and other health care facilities, the consequence of this is a different serious healthcare- associated infections (Guidelines, 2008). MRSA isolates that were first recognized in the 1960's, and were found to be largely limited to those patients with certain health care exposures, have however been also recognized among previously healthy members of the community that lack health care exposures (Herold *et al.*, 1998). Therefore, community acquired MRSA (CA-MRSA) emerged throughout the world in the late 1990's (Otto, 2007).