METHICILLIN RESISTANCE PROFILE OF STAPHYLOCOCCUSAUREUS ISOLATED FROM BURN WOUND PATIENTS IN SELECTED HOSPITALS IN ZARIA, NIGERIA

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FACULTY OF LIFE SCIENCES

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN MICROBIOLOGY

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DECLARATION

I declare that the work in this dissertation entitled "METHICILLIN RESISTANCE PROFILE OFSTPHYLOCOCCUSAUREUS ISOLATED FROM BURN WOUND PATIENTS IN SELECTED HOSPITALS IN ZARIA, 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 at this or any other Institution.

Muhammad BATUREDate

(P13SCMC8006)

CERTIFICATION

This dissertation entitled "METHICILLIN RESISTANCE PROFILE OFSTAPHYLOCOCCUSAUREUS ISOLATED FROM BURN WOUND PATIENTS IN SELECTED HOSPITALS IN ZARIA, NIGERIA" by Muhammad BATURE (P13SCMC8006) meets the requirement for the award of Master of Science Degree in Microbiology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my parents Barr M. B.Garba and Haj. Aishatu Muhammad.

ABSTRACT

Antimicrobial resistance is a persistent problem where antibiotics are abused or misused. The work determined the methicillin resistance profile of *Staphylococcus aureus* isolated from burn wound patients in selected hospitals in Zaria, Nigeria. A total of 160 burn wound swabs were obtained from patients and inoculated on Mannitol Salt Agar (MSA) medium. Twelve (12) *Staphylococcus aureus*isolates were biochemically characterized. Antibiotic susceptibility testing was carried out on all the isolates by disk diffusion method. All the isolates were resistant to at least 2 out of 8 antibiotics tested. The resistancepattern of the isolates was:Cefoxitin(100%),Oxacillin(83.33%),Tetracycline(75%), Vancomycin(75%),Ciprofloxacin(66.7%),Erythromycin(66.7), Gentamicin(41.7%) andChloramphenicol(25%). The Multiple Antibiotic Resistance Index was between 0.25-1.00. Out of the 12 *Staphylococcus aureus* isolates 9 were methicillin resistant as detected using PBP latex agglutination test kit. It is important that hospitals should monitor *Staphylococcusaureus* infection in patients with burn wound so as to facilitate management and treatment of the burn wound.

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ABBREVIATION AND SYMBOLS

AgrAccessory Gene Regulator

CbpCollagen-binding Protein

CHIPS Chemotaxis Inhibitory Protein of *S. aureus*

ClfClumping Factor

ECM ExtracellulerMatrix

ET Exfoliative Toxin

FLIPrFormyl Peptide Receptor-like-1 Inhibitory Protein

FnbFibronectin-binding Protein

IgImmunoglobulin

MRSA Methicillin Resistant *Staphylococcusaurus*

MSCRAMM Microbial Surface Components Recognizing Adhesive Matrix Molecule

PBPs Penicillin Binding Proteins

PHAC Public Health Agency Canada

PTSAgsPyrogenic Toxin Superantigen

PVL Panton-Valentine Leukocidin

SAK Staphylokinase

SCC*mec*Staphylococcal Cassette Chromosome *mec*

SCIN Staphylococcal Complement Inhibitor

SE Staphylococcal Enterotoxin

SP Staphylococcal Protein

SSSS Staphylococcal Scalded Skin Syndrome

TSSST Toxic Shock Syndrome Toxin

β Beta

χ^2 Chi Square

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Burn is an injury to the skin or other organic tissue primarily caused by heat or due to radiation, radioactivity, electricity, friction or contact with chemical (WHO, 2017).The skin provides primary protection against infection by acting as a physical barrier. When this barrier is damaged, pathogen can directly infiltrate the body, resulting in an infection (Fonseca and Chandrasker, 2016).

Burn infection remains a major complication in burn patients after initial period of shock and the chance of infection persist until complete healing (Zulfiqar *et al*., 2007).Infection is the most common cause of morbidity and mortality in burn patients, with almost 61% of death being caused by infection (Fonseca and Chandrasekar, 2016).

Staphylococcus aureus develop resistance very quickly and successfully to different antimicrobials over a period of time (Salau *et al*., 2015). Methicillin resistant *Staphylococcusareus*(MRSA) is a type of *Staphylococcus aureus* that is resistant to several antibiotics (CDC,2016).It is also called oxacillin-resistant *Staphylococcus aureus*. 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 (McDougal*et al*., 2003).

Microbial mechanisms of overcoming the activities of antimicrobial agents include the production of structure-altering or inactivating enzymes (β-lactamase or aminoglycoside-modifying enzymes), alteration of penicillin-binding proteins or other cell-wall target sites, altered DNA gyrase targets, permeability mutations, active efflux and ribosomal modification (Akinjogunla and Enabulele, 2010;Gad *et al*., 2010).

Plasmids, which are extra-chromosomal bodies, allow the movement of genetic material, including antimicrobial resistance genes between bacterial species and genera. It may contain resistance genes for single or multiple antimicrobial agents, which have been reported among staphylococci to several therapeutically useful antibiotics including penicillin family, streptomycin, rifampicin, fusidic acid and novobiocin and fluoroquinolones (Akinjogunla and Enabulele, 2010).

Methicillin resistance in *Staphylococcusaureus* result from the acquisition of *mecA* gene carried on a genomic island called staphylococcal cassette chromosome *mec* (*SSCmec*) by a methicillin susceptible strain (methicillin susceptible *S. aureus*, MSSA). *mecA* encodes a modified penicillin binding protein (PBP2a) that retain transpeptidase activity in the presence of concentrations of methicillin that inhibits the native penicillin binding proteins (Ryback *etal*., 2005).

In healthcare settings, MRSA infections occur most frequently among patients who undergo invasive medical procedures (such as surgery), have invasive devices (like catheters or ventilators), and who have weakened immune systems. MRSA in healthcare settings commonly causes serious and potentially life threatening infections, such as bloodstream infections, surgical site infections, or pneumonia. About 40-60% of nosocomial infections with *Staphylococcus aureus* are resistant to oxacillin (Tiemersma, *et* *al*., 2004). It is assumed that infected people transmit at a much higher rate owing to increased attention from, and contact with health care workers. (Massey *et al.*, 2006).

1.2 Statement of Research Problem

Staphylococcusaureus has a growing problem of resistance to many of the current antibioticsbeing used to treat its infection, thereby further complicating its management in the hospital(Raygada and Levine, 2009).

Methicillin resistant *Staphylococcusaureus* in healthcare settings commonly causes serious and potentially life threatening infections, such as bloodstream infections, surgical site infections, or pneumonia. About 40-60% of nosocomial infections with *Staphylococcus aureus* are resistant to oxacillin (Tiemerma*et al*., 2004).

Patients with burn injuries are prone to microbial colonization due to disruption of the skin, thereby providing a favorable environment to microorganism ranging from the normal flora of the patients, healthcare personnel and the hospital environment (Church *etal*., 2006).

Infection is the most common cause of morbidity and mortality in burn patients,with almost 61%of death being caused by infection (Fonseca and Chandrasekar, 2016).

1.3 Justification of the Study

Burn units are high risk units, where patients are on multiple antibiotics and have a long stay in hospital. Thus, prospective studies are needed in order to determine if the burn wounds are infected or not. Improved outcomes for severely burn patients have been attributed due to medical advances in fluid resuscitation, nutritional support, pulmonary care, burned wound care and infection control practices.

Accurate and up to date data are vital for the successful management of an infection with *Staphylococcus aureus*. However, there is still paucity of current information on methicillin resistant *Staphylococcusaureus*(MRSA) in patients in Africa and treatments options available compared to the developed world.

1.4 Aim

The aim of the research was to determine the Methicillin Resistance profile of *Staphylococcus aureus* isolated from burn wound patients in selected hospitals in Zaria, Nigeria.

1.5 Objectives

The specific objectives were to:

1. isolateand characterize *Staphylococcusaureus* from patients with burn wounds.
2. assessthe relationship between the prevalence of *S.aureus* and socio-demographic factors.
3. determinethe antibiotic susceptibility patterns of *S. aureus* by disc diffusion technique.
4. determinethe methicillin resistance of *S. aureus* using Penicillin binding protein latex agglutination test kit.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Staphylococcusaureus*

Staphylococcus aureus is a Gram-positive, catalase positive and coagulase positive coccus belonging to the family *Staphylococcaceae*(Becker *etal*., 2004). It is approximately 0.5-1.5 µm in diameter, nonmotile, non-spore-forming, facultative anaerobe that usually form clusters (PHAC, 2011). *Staphylococcus aureus* is an opportunistic pathogen that can cause a variety of self-limiting to life-threatening diseases in humans (Becker *etal*., 2004). In normal healthy and immunocompentent person, *S. aureus* colonization of the skin, intestinal tract, or nasopharynx does not lead to any symptoms or disease. In animals and humans that are immunocompromised or immunodeficient, this bacteria may be life threatening. It may lead to pyogenic (abscess) infections of the skin, eyes and genital tract (Mandal, 2012).

2.2 Classification

2.2.1 Scientific classification

Domain: Bacteria

Kingdom: Eubacteria

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: *Staphylococcaceae*

Genus: *Staphylococcus*

Species: S. *aureus*

2.2.2 Binomial name

Staphylococcus aureus (Rosenbach, 1884) Adapted from ITIS (2017).

2.3 Colonization

Staphylococcus aureus acquired from an external source could be the cause of an infection when inoculated into an open wound (George, 2009). More commonly, the human host is infected by bacteria that colonize his or her skin or mucosal surface (Wertheim *etal*., 2004). The mucosal surfaces that harbor *S. aureus* include the nose, throat, vaginal wall, and gastrointestinal tract (George, 2009). Nasal carriage is probably most important because nose-picking could effectively disseminate the bacterium to other body surfaces and other hosts (Wertheim *etal*., 2006). Remarkably, 20% of individuals are persistently colonized in the anterior nares (nostrils) and 30% are transiently colonized (George, 2009).

For *S. aureus*, colonization of the human nostrils presents a significant challenge that requires not only adherence to nasal epithelial cells, but also an ability to cope with host defense and competing resident microorganisms (George, 2009). *Staphylococcus aureus* adheres and invades host epithelial cells using a variety of molecules that are collectively termed Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMM). A number of bacterial products (including MSCRAMM) have been suggested to be important for adhesion and attachment to nasal epithelial cells, but two factors (clumping factor B and wall-associated teichoic acid) have so far proven roles in nasal colonization of humans and rats (Wertheim *etal*., 2008).

Host immune deterrents for bacterial nasal colonization include antimicrobial peptides, lysozyme, lactoferrin, and IgA (Wertheim *etal*., 2005). However, little is known of the critical host defenses against *S. aureus* colonization. A study in mice has identified the cystic fibrosis transmembrane conductance regulator and toll like receptor 2, but not toll like receptor 4 as important factors controlling *S. aureus* carriage (Gozalez-zom *etal*., 2005).

Resident nasal flora presents an equally formidable challenge for *S. aureus*. Studies of *S. aureus* carriers and non-carriers have shown that presence of certain bacteria, such as *Corynebacterium*, *S.epidermidis* or *S. pneumoniae*, could preclude carriage of *S. aureus* (Bogaert*etal*., 2004;Cohen*etal*., 2008).

Once colonization is established, *S. aureus* is positioned in close proximity to the throat, ears, mouth and sinus; yet, surprisingly nasal carriage rarely leads to overt infection of these sites (George, 2009). Studies of *S. aureus* regulation suggest that during colonization, many *S. aureus* virulence genes may be downregulated (Novick, 2003). Among the genes that control *S. aureus* colonization and virulence, the best known global regulator is the accessory gene regulator *agr* which has been described in details in many excellent reviews (Novick, 2003). Briefly, *agr* is a quorum sensing locus, which directly controls expression of a number of virulence and colonizing factors (George, 2009). Downregulation of *agr* is associated with colonization and activation of *agr* with host invasion (George, 2009).

2.4 Pathogenesis

The broad range of infections caused by *S. aureus* are related to a number of virulence factors that allow it to adhere to surface, invade or avoid the immune system, and cause harmful toxic effects to the host (Holmes *etal*., 2005).

2.4.1 Adherence factors (Adhesins)

The attachment of *S. aureus* to the host cell surface initiating the colonization process is mediated by several adhesins (Bien *etal*., 2011). One major class of *S. aureus* adhesins comprises proteins covalently anchored to cell peptidoglycans (via the threonine residue in the sorting signal motif at their C-terminus), which specifically attach to the plasma or Extracellular Matrix (ECM) components and collectively are termed the Microbial Surface Component Recognizing Adhesive Matrix Molecules (MSCRAMMs) (Maresso and Scneewind, 2008; Speziale *etal*., 2009). These molecules recognize the most prominent components of the ECM or blood plasma, including fibrinogen, fibronectin, and collagens. Typical members of the MSCRAMM family are staphylococcal protein A (SpA), fibronectin-binding proteins A and B (FnbpA and FnbpB), collagen-binding protein, and clumping factor (Clf) A and B proteins (Ghasemian *etal*., 2015).

2.4.2 *Staphylococcus aureus* exoproteins

Nearly all strains of *S. aureus* secrete a group of exoproteins such as exotoxins and enzymes, including; nucleases, proteases, lipases, hyaluronidase, and collagenase.The purpose of secreting these enzymes is to aid their pathogenesis, to enable them spread through tissues and cause disease.

Staphylococcus aureus produces exotoxins that possess cytolytic activity. Cytolytic toxins form β-barrel pores in the plasma membrane and cause leakage of the cell contents and lysis of the target cell (Foster, 2005). The secreted cytolytic toxins include α-hemolysin, β-hemolysin, γ-hemolysin, leukocidin, and Panton-Valentine leukocidin (PVL) (Kaneko and Kamio, 2004). Alpha-hemolysinis inserted into the eukaryotic membrane and oligomerizes into a β-barrel toform pore which causes osmotic cytolysis and is particularly cytolytic toward human platelets and monocytes (Menestrine, 2001). Panton-Valentine leukocidin(PVL) is classified as a bi component cytolysin (LukF-PV and LukS-PV) that insert itself into the host's plasma membrane and hetero-oligomerize to form a pore. Panton-Valentine leukocidin exhibits a high affinity toward leukocytes, while other bicomponent toxins, γ-hemolysin and leukocidin, are cytotoxic toward erythrocytes and leukocytes, respectively (Kaneko and Kamio, 2004).

Staphylococcus aureus produces additional group of exotoxins, which include the toxic shock syndrome toxin-1 (TSST-1), the staphylococcal enterotoxins (SEA, SEB, SECn, SED, SEE, SEG, SEH, and SEI) and the exfoliative toxins (ETA and ETB). Among them, TSST-1 and the staphylococcal enterotoxins belong to the group of toxins known as pyrogenic toxin superantigens (PTSAgs) (Holtfreter and Boker, 2005). The best characterized property of this group is super antigenicity, which refers to the ability of this toxin to stimulate proliferation of T-lymphocytes. These toxins cause toxic shock syndrome and food poisoning (Bien *etal*., 2011). Exfoliative toxin A and B are involved in staphylococcal scalded skin syndrome (SSSS) (Mishra *etal*., 2016).

Staphylococcus aureus has also other specific proteins that have profound impact on the innate and adaptive immune system. Examples of such kind of proteins are the staphylococcal complement inhibitor (SCIN), chemotaxis inhibitory protein of *S. aureus* (CHIPS), staphylokinase (SAK), extracellular fibrinogen binding protein (Efb), extracellular adherence protein (Eap), and formyl peptide receptor-like-1 inhibitory protein (FLIPr). SCIN is a C3 convertase inhibitor, which blocks the formation of C3b on the surface of the bacterium and, thereby, the ability of human neutrophils to phagocytose *S. aureus* (Rooijakkers *etal*., 2005).Chemotaxis inhibitory protein of *S. aureus*(CHIPS) and formyl peptide receptor-like-1 inhibitory protein (FLIPr) block neutrophil receptors for chemoattractants (Prat *etal*., 2006), Epa blocks migration of neutrophils from blood vessels into the tissue (Chavakis *etal*., 2002), SAK binding to α-defensins abolishes their bactericidal properties (Bokarewa *etal*., 2006), while Efb inhibits both classical and alternative pathways of complement activation (Lee *etal*., 2004).

The virulence of *S. aureus* is generally considered to be multifactorial and due to the combined action of several virulence determinants. One exception is the toxinoses, such as toxic shock syndrome, SSSS, and staphylococcal food poisoning, which are caused by toxic shock syndrome toxin, exfoliative toxins A and B, and different staphylococcal enterotoxins, respectively (Bien *etal*., 2011).

2.5 Host Defense against *Staphylococcusaureus*

Despite regular contact throughout life, humans have a well-developed resistance to staphylococcal infections. Studies performed on human volunteers established that an injection of several hundred thousand staphylococcal cells into unbroken skin was not sufficient enough to cause abscess formation. Yet when sutures containing only a few hundred cells were sewn into the skin, a classic lesion rapidly formed, indicating the aggravating effect of a foreign body in infection. Specific antibodies are produced against most of the staphylococcal antigens and intracellular substances; though none of these antibodies appear to effectively immunize a person against reinfection for long period (except for the antitoxin to SSSS toxin). The most powerful defense lies in the phagocytic response by neutrophils and macrophages. When aided by the opsonic action of compliment, phagocytosis effectively disposes off staphylococci that have gained access to tissue. Inflammation and cell mediated immunity that stimulate abscess formation also help contain staphylococci and prevent further spread (Tolaro and Tolaro, 2002).

2.6 Biochemical Characterization of *Staphylococcusaureus*

Identification of *S. aureus* in clinical samples includes the following tests;

2.6.1 Coagulase test

Coagulase causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of *S. aureus*:

i. Free coagulase which convert fibrinogen to fibrin by activating a coagulase-reacting factor present in plasma. Free coagulase is detected by clotting in the tube test.
ii. Bound coagulase (clumping factor) which converts fibrinogen directly to fibrin without requiring a coagulase-reacting factor. It can be detected by the clumping of bacterial cells in the rapid slide test (Cheesbrough, 2000).

Coagulase test differentiate between coagulase positive staphylococci which are pathogenic and coagulase negative staphylococci which are low pathogenic (A. A. Odewumi personal communication, 2010).

2.6.2 DNase test

Deoxyribonuclease hydrolyses deoxyribonucleic acid (DNA). The test organism is cultured on a medium which contains DNA. After overnight incubation, the colonies are tested for DNase production by flooding the plates with a weak hydrochloric acid solution. The acid precipitates unhydrolyzed DNA. DNase-producing colonies are surrounded by clear zone due to DNA hydrolysis (Cheesbrough, 2000).

2.6.3 Lecithinase test

Lecithinase also called phospholipases is an enzyme released by bacteria that have the ability to destroy animal tissues. Lecithin is a normal component of egg yolk. Lecithinase breakdown this lecithin to an insoluble diglyceride resulting in an opaque hallow surrounding the colony when grown on the egg yolk agar medium (A. A. Odewumi personal communication, 2010).

2.6.4 Gelatin hydrolysis test

Gelatin is protein derived from animal protein collagen. It has been used as a solidifying agent in food. Gelatin hydrolysis test is a great way to highlight proteolysis by bacteria. It’s used to detect the ability of an organism to produce gelatinase which liquefy gelatin (Acharya, 2014).

2.6.5 Haemolysis test

Haemolysins are class of bacterial exotoxin that disrupts the cell membrane of red blood cells. This damage cause the red blood cell to hemolyze that is to burst and release hemoglobin pigment. Hemolysin increase pathogenicity that includes β toxins of *S. aureus* (Tolaro and Tolaro, 2002).

Other test includes sugar fermentation such as glucose fermentation, lactose fermentation and mannitol fermentation.

2.7 Epidemiology of *Staphylococcusaureus* Infection

It is surprising that a bacterium with such a great potential for virulence as *S. aureus* is a common intimate human associate. The microbe is present in most environments frequented by human and is readily isolated from fomites. Colonization of the infants begins within few hours after birth and continues throughout life. The carriage rate of normal healthy adults varies anywhere from 20% to 60%, and the pathogen tends to be harbored intermittently rather than chronically. Carriage occurs mostly in the anterior nares (nostrils) and to a lesser extent in the skin, nasopharynx and intestine. Usually this colonization is not associated with symptoms nor does it ordinarily lead to disease in carriers or their contacts. Circumstances that predispose an individual to infection include poor hygiene and nutrition, tissue injury, preexisting primary infections, *diabetes mellitus* and immunodeficiency states. Staphylococcus infection in the new born nursery and surgical wards are the third most common nosocomial infection. The so-called “hospital strains” can readily spread in an epidemic pattern within and outside the hospital (Tolaro and Tolaro, 2002).

2.8 Methicillin

Methicillin is a semisynthetic, narrow spectrum beta-lactamase-resistant penicillin antibiotic with bactericidal and beta-lactamaseresistant activity.It is a semisynthetic derivative of penicillin. Methicillin binds to specific penicillin-binding proteins (PPBs) on the bacterial cell wall, thereby preventing the cross-linkage of peptidoglycans, which are critical components of the bacterial cell wall. This leads to an interruption of the bacterial cell wall and causes bacteria lysis (DrugBank, 2007).

2.8.1 Mechanism of action

Like other beta-lactam antibiotics, methicillin acts by inhibiting the synthesis of bacterial cell wall. It inhibits cross-linkage between the linear peptidoglycan polymer chains that make up a major component of the cell wall of Gram-positive bacteria. It does this by binding to and competitively inhibiting the transpeptidase enzyme used by bacteria to cross-link the peptide (D-alanyl-alanine) used in peptidogylcan synthesis (Drug Bank, 2007).

2.8.2 Therapeutic uses

Methicillin is indicated in the treatment of bone and joint infections. It is also indicated in the treatment of bacterial endocarditis, bacterial septicemia, sinusitis, skin and soft tissue infections caused by susceptible organisms (DrugBank, 2007).

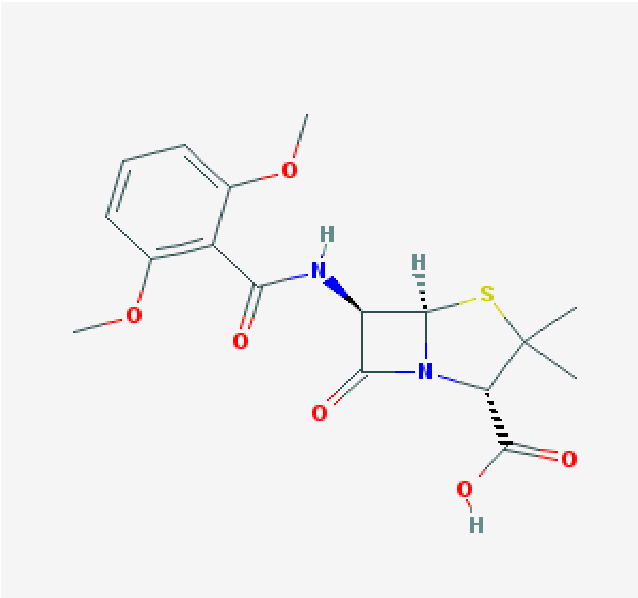

Figure 2.1: Structure of methicillin (http// pubchem.ncbi.nlm.nih.gov).

IUPAC Name

(2S,5R,6R)-6-[(2,6-dimethoxybenzoyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid

2.9 Resistance of *Staphylococcusaureus* to Methicillin

Resistance to methicillin is due to the acquisition of *mecA* gene, that encodes new protein designated PBP2a, belonging to the family of enzymes necessary in synthesizing the bacterial cell wall (Muhammad *et al*., 2014). The protein (PBP2a) has a very low affinity for βeta-lactam antibiotics and confers resistance to Methicillin and the other beta-lactams (Pantosti *et al*., 2007).

The *mecA* gene is located on a mobile genetic element, named staphylococcal cassette chromosome *mecA* (SCC*mec*) inserted in the *S. aureus* chromosome upstream *orf* X (Djoudi *etal*., 2016).

2.9.1*mecA*gene

Methicillin resistant *Staphylococcusaureus*(MRSA) differ genetically from methicillin-sensitive *S. aureus*(MSSA) isolates by the presence, in the chromosome, of a large stretch of foreign DNA (40-60 Kb), referred to as the *mec*element, and the presence of the *mecA* gene encodes the 76 KDa penicillin-binding protein, PBP2a (also referred to as PBP2′) (Paul and Peter, 2002).

The *mecA* gene has been proposed to originate from *Staphylococcus sciuri* (Wu *etal*., 2001). Although the mechanism of gene acquisition from this specie is not known, two genes, *ccrA* and *ccrB,* present on the *mec* element from one isolate, have been shown to code for recombinase proteins that are capable of excising and integrating the *mec* element into the chromosome (Katayama *etal*., 2000). Examination of a large number of MRSA isolates has led to the conclusion that the original acquisition of the *mecA* gene has occurred once and that MRSA isolates are descendants of a single clone (Kreiswirth *etal*., 1993). Although the arrangement and composition of the *mec* element may vary between isolates, the *mecA* gene itself is highly conserved (Oliveira *et al*., 2000).

2.9.2 Modified penicillin binding protein (PBP2a)

In common with other PBPs, PBP2a has the common structural motifs that are associated with penicillin binding yet its affinity for β-lactam antibiotics is greatly reduced (Paul and Peter, 2002). Consequently, at therapeutic levels of methicillin that would inhibit the transpeptidational activities of other PBPs, PBP2a remains active ensuring the cross-linking of the glycan chains in peptidoglycan. PBP2a is not able to completely compensate for the other PBPs since cells grown in the presence of methicillin exhibit a marked reduction in the degree of cross-linking. However, the limited degree of cross-linking is enough to ensure survival of the cell (Paul and Peter, 2002).

2.10 Methicillin Resistant *Staphylococcusaureus*

Methicillin Resistant *Staphylococcusaureus* 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, etc.) and the cephalosporins.

2.10.1 Epidemiology of MRSA Infection

Methicillin was developed to treat *Staphylococcus* infections, with the widespread use of the drug beginning in 1959. In 1961, a hospital in the United Kingdom, reported finding a strain of *Staphylococcus* that had developed resistance to Methicillin, this led to the emergence of Methicillin Resistant *Staphylococcus aureus* (MRSA) (Muhammad *etal*., 2014). The case reports of Methicillin Resistant *Staphylococcus aureus*infectionsin the

United Kingdom slowly increased in number, but did not reach an outbreak level. The first case of Methicillin Resistant *Staphylococcus aureus* (MRSA) infectionin the United States was reported in Boston in 1968 (Muhammad *etal*., 2014). The Methicillin Resistant *Staphylococcus aureus* (MRSA) cases were diagnosed in patients hospitalized with immunodeficiency syndrome conditions (Muhammad *etal*., 2014). The first reported case of Methicillin Resistant *Staphylococcus aureus* (MRSA) outbreak was in Eastern Australia during the 1970s and culminated in the spread of the infection to most of the European countries, infecting many of the patients hospitalized in their health care centers and hospitals, especially in Eastern Europe (CDC, 2004). Nigeria and some other African countries have been reported to have relatively high incidence of MRSAbetween 21-30% (Kesah *etal*., 2003).The prevalence of MRSA in different counties between 2008 and 2014 is summarized in Table 2.1.

2.11Definition of the term 'Burn'

A burn is an injury to the skin or other organic tissue primarily caused by heat or due to radiation, radioactivity, electricity, friction or contact with chemical (WHO, 2017).

The tissue damage incurred is classified into three categories: first, second and third-degree burns (Garcia-Espainoza*et al*., 2017). First-degree burns involve only damage to the topmost layer of the skin, the epidermis. Second-degree burns contain damage to the epidermis as well the dermis, the underlying layer of the skin. Third-degree burns refer to damage or destruction of the entire depth of the skin as well as tissues that lie beneath it (Garcia-Espainoza *et al*., 2017).

Burn wounds have three separate zones of concern. The zone of coagulation is located where the skin came in contact with the burn source, at the center of the wound. It is made up of dead, leathery tissue that forms the burn eschar (scab). The zone of stasis surrounds the zone of coagulation; tissue in this zone is alive but at a high risk of infection and necrosis (tissue death) due to decreased perfusion, a result of poor circulation, to the area. Lastly, the zone of hyperemia surrounds the zone of stasis and contains healthy skin though vasodilatation in this area is common as a result of the injury (Garcia-Espainoza *et al*., 2017).

2.12 Burn Wound Infection

Infection of burn wounds are common because the skin, which is a physical barrier against microorganisms, has been compromised. Furthermore, in moderate and severe burns the underlying vasculature of the skin has been damaged or destroyed and so immune cells such as T cells, cannot reach sites of infection. Accordingly, the risk of infection increases proportionately with the size of the burn (Fonseca and Chandrasekar, 2016).

Signs of infection include change in color of the wound, spontaneous separation of the eschar, cellulitis and graft loss. These signs may be accompanied by localized redness, heat and tenderness. Sepsis occurs when microbes or their toxins so past the wound to infect the rest of the body.This is characterized by high fevers, accelerated heart rate, increased respirations per minute and hyperglycemia (Murray and Hospenthal, 2008).

Bacteria and fungi are the most common pathogens of burn wounds. These microorganisms form multi-species biofilms on burn wounds within 48-72 hours of injury (Branski, *etal*.,2009). The organisms originate from the patient’s own skin, gut and respiratory flora, as

Table 2.1: Prevalence of MRSA in some countries.

Country	Sample size	Prevalence(%)	Source	Reference
Argentina	591	16	Hospital	Egea *etal*., 2014.
Bangladesh	49	53.1	Hospital	Afroz*etal*., 2008.
Bolivia	585	0.5	Community	Batoloni *etal*., 2013.
Cameroon	295	34.6	Hospital	Gonsu *etal*., 2014.
Chile	246	80	Hospital	Guzman-Blanco, 2009.
Colombia	538	92.4, 65.1, 43.6	Hospital	Jimenez *etal*., 2012.
Congo	-	60	Hospital	Iyamba *etal*., 2014.
Costa-Rica	674	58	Hospital	Guzman-Blanco, 2009.
Malaysia	-	26	Hospital	Norazah, 2008.
Nigeria	208	19.2	Hospital	Olowe *etal*., 2013.
Sudan	426	69.4	Hospital	Eliman *etal*., 2012.

Source: Grema *et al* (2015).

well as from contact with contaminated health care environments and workers.Gram-positive bacteria are some of the first to colonize burns, followed quickly by Gram-negative (Murray and Hospenthal, 2008).

Two bacterial species, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas areuginosas* are of the most prevalent infective agents. These two species have proven particularly difficult to treat because they possess a large number of virulence factors and antimicrobial resistance genes(Fonseca and Chandrasekar, 2016).

Fungal infection tends to occur in the later stages after the majority of bacteria have been eliminated by topical antibiotics (Capoor *etal*., 2010).

Burn wound infection is problematic because it delays healing, encourages scarring and may result in bacteremia, sepsis or multiple-organ dysfunction syndrome whereby organs from several systems are unable to maintain homeostasis on their own, requiring immediate medical attention (Chamania *etal*., 2012)

2.13 MRSA Colonization of Burn Wound

Methicillin Resistant *Staphylococcusaureus* are Gram-positive, spherical microbes and some of the earliest colonizers of burn wounds. These bacteria possess capabilities for both aerobic and anaerobic metabolism. Their genome is contained on one circular chromosome, with antibiotic resistance encoded in transposons. Methicillin Resistant *Staphylococcusaureus* dwell in the sweat glands, hair follicles and mucous membranes of humans. It can be difficult to eradicate because they often can colonize a host for a long time before causing an infection; until symptoms of it infection emerge, MRSA will remains undetected hence will be untreated (Zetola *etal*., 2005).

2.14 Treatment Options to MRSA Infections

The indiscriminate exposure of humans and animals to antibiotics created problem through acquisition and dissemination of MRSA which limit the choice of treatment (Grema *etal*., 2015). Most antibiotics used for treatment of MRSA infection have been reported to have cause resistance. Antibiotics such as trimethoprim-sulphamethoxazole, clindamycin and doxycycline are reported to be effective in the treatment of Community Aquired Methicillin Resistant *Staphylococcusaureus*(CA-MRSA)infection (Ernst, 2012). Newer drugs such as oritavancin, telavancin omadacycline, tedizolid and dalbavancin have promising impact on the treatment of MRSA infections. Other existing agents such as fosfomycin and fusidic acid are under investigation for potential use in the treatment of MRSA infection (Burke and Warren, 2014).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Zaria, Nigeria. It is a city in Kaduna State in northern Nigeria. It was a formerly known as Zazzau and is one of the original seven Hausa city-states. Zaria lies within the coordinates 11^{o}04'N 7^{o}42'E / 11.067^{o}N 7.700^{o}E (htts://en.m.wikipedia.org/wiki/zaria).The selected hospitals were Hajiya Gambo Sawaba General Hospital, New City Hospital, located at Zaria city and Zaria Clinic and Medical Center located at Tudun Wada, Zaria, Nigeria.

3.2 Ethical Approval

Ethical approval was obtained from Kaduna State Ministry of Health (Ref. No. MOH/ADM/744/VOL.1/525) (Appendix VII).

3.3 Study Population

The study population were burn wound patients in the selected hospitals.

3.4 Inclusion Criteria

Burn wound patients, who gave their consent, were recruited into the study.

3.5 Exclusion Criteria

This included non-burn wound patients and burn wound patients who did not give their consent.

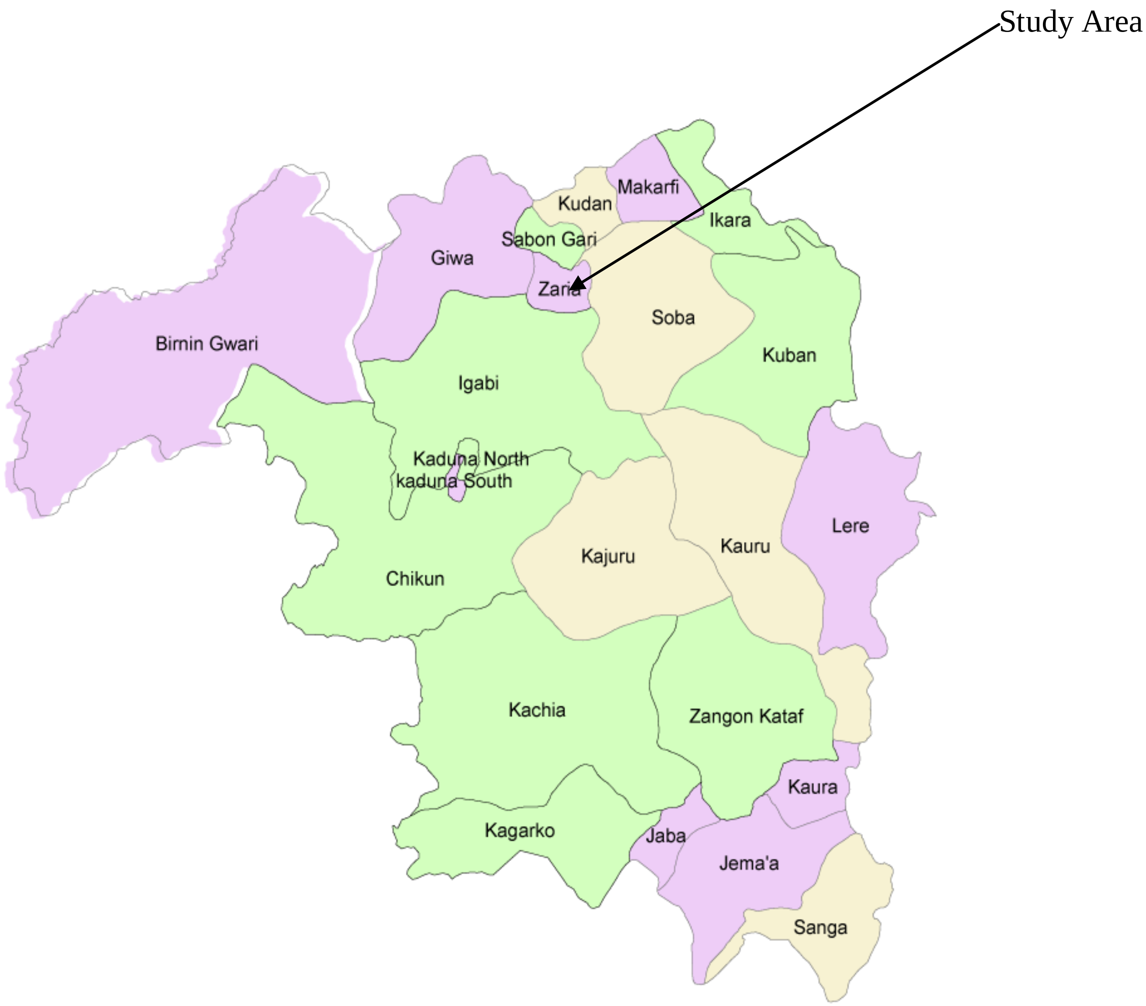

Figure 3.1: Map of Kaduna State

3.6 Sample Size Calculation

To calculate the sample size, formula described by Zubair (2012), prevalence rate of 11.5% (Kolawole *et al.*, 2013) were used.

$$n = \frac{Z^2 pq}{e^2}$$

Where n = sample size

z = standard normal distribution at 95% confidence limit = 1.96

p = prevalence rate of the infection of the previous study = 11.5%

e = absolute desired precision = 0.05

q = 1- p

Therefore;

$$n = \frac{1.96^2 \times 0.115 \times 0.885}{0.05^2} \qquad n = 156.39$$

For a reliable result, a total of 160 samples were collected and examined, irrespective of age, sex, and degree of burn.

3.7 Collection of Samples

A total of one hundred and sixty (160) burn wound swab samples were collected using convenience sampling technique from patients in the study area.

3.8 Isolation and Characterization

All swabs were inoculated onto the surface of prepared Mannitol salt agar plates, using streak plate method and the plates were incubated at 37^0C for 48 hours.The incubated plates were examined (Cheesbrough, 2000). Sub culturing was carried out from the agar plates in order to obtain pure cultures.

3.8.1 Gram staining of isolates

Distinct well separated yellow colonies were selected aseptically and smears were prepared on glass slides and air dried. Each smear was heat fixed. The fixed smear was covered with crystal violet stain for 60 seconds, before washing with distilled water. The treated slide was also covered with Gram`s iodine for 60 seconds and then washed with distilled water. Decolorization was done in few seconds with acetone and immediately washed off with distilled water. The treated smear was covered with safranin for 60 seconds and washed off with distilled water. The prepared smear was examined with oil immersion objective, (X 100 objective) (Cowan and Steel, 2003). Colonies showing Gram positive cocci were subcultured on nutrient agar slants for biochemical characterization.

3.8.2 Biochemical characterization of isolates

Pure isolates from the sub-cultured medium were confirmed by biochemical tests as described in Cheesbrough, (2000). Such tests include; Coagulase test, DNase test, Lecithinase test, Gelatin hydrolysis test. Others were Hemolysis on blood agar and sugar fermentation tests such as; Mannitol, Glucose, and Lactose fermentation tests.

3.8.2.1 Coagulase test

About 2-3 colonies were emulsified into 5ml nutrient broth and incubated at 37^0C for 24 hours. Also 0.4ml of human citrated plasma was placed into clean labeled serological test tubes. Then 1.6ml from a 24 hours culture was added to the labeled serological test tube and mixed gently. Negative control using sterile nutrientbroth was also prepared. The tubes were incubated at 37^0C and examined after 1hr, 3hrs and the following day of the preparation. Clotting of the tube contents indicated positive result for *S. aureus* (Cheesbrough, 2000).

3.8.2.2 DNase test

DNase agar was prepared from ready to use dehydrated powder (Oxoid) by dissolving 3.9g in 100ml of distilled water, and autoclaved at 121^0C for 15minutes. The prepared medium was divided into six (6) sides and labeled. A flamed wire loop was used to pick the test colonies and inoculated on the plates. The inoculated plates were incubated at 37^0C for 24 hours. The surfaces of the incubated plates were flooded with 10% HCl solution. Within five minutes clear zone around the test colonies were checked. Clearing around the colonies indicate DNase positive reaction (Cheesbrough, 2000).

3.8.2.3 Lecithinase test

A modified egg yolk medium was prepared. An egg was cleaned and immersed into 70% v/v ethanol for 10 minutes. The egg was then aseptically broken and the egg white removed. The egg yolk was aseptically transferred into cooled prepared nutrient agar and mixed well, before dispensing into sterile petri dishes. Using a flamed wire loop a sector of the egg yolk medium was inoculated with the test organism and the plate was incubated for 24 hours at 37^0C. Presence of opaque zone indicates a positive result.

3.8.2.4 Gelatin hydrolysis test

About 12.8g of nutrient gelatin was dissolved in 100ml distilled water over a Bunsen burner. Approximately 5ml of the prepared medium was dispensed into test tubes and autoclaved at 121^0C for 15 minutes, the medium was allowed to cool in an upright position. Each nutrient gelatin tube was stabbed with an inoculating needle which was used to select the colony of the test organism. The inoculated media were incubated at 37^0C for 48hours. The incubated tubes were gently removed from the incubator and put into refrigerator for 30 minutes each day. Negative test were incubated at 35^0C and checked daily for 1 week (Acharya, 2014). Partial or total liquefaction of the inoculated tubes while the uninoculated tube remains completely solidified after exposure to cold temperature of refrigerator (4°C) indicated a positive result.

3.8.2.5 Haemolysis test

Blood agar base was used to prepare blood agar and sterilized as per manufacturer`s instructions. As the prepared blood agar cooled to 45^0C, sheep blood in a ratio of 1:20 was aseptically added and mixed gently, then dispensed aseptically in 15ml amount in sterile petri dishes. A prepared blood agar plate was first incubated at 37^0C overnight to check for sterility after which it was inoculated with the test organism and incubated at 37^0C overnight. Clear zone around the colonies indicate positive result.

3.8.2.6 Glucose fermentation test

About 1g of glucose and 1.5g *andrade* peptone were dissolved into 100ml distilled water and dispensed in 5ml into bijou bottles then autoclaved at 121^0C for 15 minutes. The test isolates were inoculated into the prepared media and incubated at 37^0C overnight. Pink or yellow coloration indicate glucose utilization.

3.8.2.7 Lactose fermentation test

About 1g of lactose and 1.5g *androde* peptone were dissolved into 100ml distilled water and dispensed in 5ml into bijou bottles then autoclaved at 121^0C for 15 minutes. The test isolates were inoculated into the prepared media and incubated at 37^0C overnight. Pink or yellow coloration indicate lactose utilization.

3.8.2.8 Mannitol fermentation test

Mannitol salt agar medium was prepared by weighing of 11.1g and dissolving 100ml distilled water. The medium was prepared and sterilized by autoclaving. It was dispensed aseptically into sterile petri dishes and allowed to solidify (Cheesbrough, 2000). The test isolates were inoculated and incubated at 37^0C for 48 hours. Growth of yellow colonies indicates mannitol fermentation.

3.9 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed by disc diffusion technique using the Kirby-Bauer NCCLS modified disc diffusion technique (Cheesbrough, 2000).

Mueller-Hinton Agar (Oxoid) was prepared and sterilized according to manufacturer's instructions.

3.9.1 MacFarland turbidity standard (0.5) preparation

About 1% v/v solution of sulphuric acid and 1%w/v solution of barium chloride were prepared. Approximately 0.6ml of the prepared barium chloride solution was added to 99.4ml of the prepared sulphuric acid solution (Cheesbrough, 2000).

3.9.2 Inoculum preparation

About 3-5 well isolated 24 hours old colonies were inoculated into 4ml nutrient broth; the turbidity of inoculum was matched with 0.5 MacFarland turbidity standards (Cheesbrough, 2000).

3.9.3 Kirby-Bauer NCCLS modified disc diffusion technique

A sterile swab was used to obtain the inoculum of the test organism and inoculated on the surface of well driedprepared Mueller Hinton Agar and treaked for confluent growth. After about 5 minutes, eight commercial antibiotic discs (Oxoid) including; Oxacillin (1µg), Erythromycin (15µg), Tetracycline (30µg), Gentimicin (30µg), Cefoxitin (30µg), Ciprofloxacin (30µg) Chloramphenicol (30µg) and Vancomycin (30µg) were placed on the agar surface, using a pair of flamed forceps and incubated aerobically at 37^0C for about 24 hours. After overnight incubation the diameter of the zones of inhibition were measured in mm using ruler and interpreted according to Clinical and Laboratory Standard Institute (CLSI, 2014).

3.10 Determination of the MAR Index of the *Staphylococcus aureus* Isolates

The MAR index of the isolates was calculated using the formula below as described by Olonitola *et al*. (2007).

$$\text{MAR Index} = \frac{\text{Number of antibiotics to which the isolate is resistant}}{\text{Total number of antibiotics used}}$$

3.11 Determination of Methicillin Resistance Profile

Methicillin resistance profile of the *Staphylococcusaureus* isolated was detected using PBP latex agglutination test kit (Oxoid) as per manufacturer`s instructions.

3.11.1 PBP Latex agglutination test

PBP2a latex agglutination test detect PBP2a protein which is produced by *mecA*gene. The latex particles are sensitized with a monoclonal antibody against PBP2a which will specifically react with methicillin resistant *Staphylococci* to cause agglutination visibly. This test does not detect those strains that are hyper producer of other beta-lactamase or PBPs.

This test involved two (2) steps:

3.11.1.1Extraction procedure

Four (4) drops of the extraction reagent 1 was placed into Ependorff tube. About 3-4 well isolated colonies to be tested were picked using µl loop and placed into the Ependorff tube. The tube was placed into water bath set at95^{0}C for 3 minutes. The tube was allowedto cool at 25^{0}C. A drop of extraction reagent 2 was placed into the tube and mixed well. The whole preparation was centrifuged at 1500 rpm,for 5 minutes.Thereafter the supernatant was used for the test.

3.11.1.2 *Latex agglutination procedure*

Each circle of the slide was labeled according to the isolates to be tested, and a control circle was also labeled. The latex reagent was mixed well and control latex added to each circle. About 50µl each of the supernatant was placed on test circle and control circle. The card was rocked for three minutes and observed for agglutination under normal light condition.

3.12 Data Analysis

The data obtained were analyzed statistically using GraphPad software (www.graphpad.com). Chi square test was used to determine the statistical association between *Staphylococcus aureus*and factors such as gender and age group. $P \leq 0.05$ was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

Out of one hundred and sixty(160)burn wound swabs examined for *Staphylococcusaureus*in this study, twelve (12) isolates of *S. aureus*were obtained giving a prevalence of 7.5%.

4.1 Prevalence of *Staphylococcusaureus*infection in burn wound patients based onhospitals.

Out of 47 samples examined from patients in Hajia Gambo Sawaba General Hospital, Zaria, 2 were positive for *S. aureus* giving a prevalence of 1.25%. A prevalence of 3.75%(n= 6)was obtained for patients in New City Hospital, Zaria. While 4(p = 2.5%) out of the 49 samples examined from patients admitted in Zaria Clinic and Medical Center were positive. (Table 4.1)The difference observed was not statistically significant (χ^2 = 1.073, df = 2, *p*= 0.5848).

4.2 Prevalence of *Staphylococcusaureus*infection inburn wound patients based on patients' socio-demography.

Based on sex, *S. aureus* was isolated in both male (5.0%) and female (2.5%) patients. The difference observed was not statistically significant (*p* = 0.7247). All the age groups with the exception of ≥41 yearsshow the presence of *S. aureus* infection. The highest prevalence was recorded from age group 1-10 years (3.125%) followed by age group 11-20 years (1.875%), then age groups 21-30 years (1.25%) and 31-40 years (1.25%). The difference observed was not statistically significant (p = 0.4080). On the duration of burn wound, no statistical association (p = 0.5684) between *S. aureus* infection and the duration of the burn. Patients having 1-3 days old burn wound had the highest prevalence (4.375%). (Table 4.2)

Table 4.1:Prevalence of *Staphylococcus aureus*infection in burn wound patients based onhospital.

Hospital	Number Examined	Number Positive	Prevalence (%)
HGS	47	2	(1.25)
NCH	64	6	(3.75)
ZCMC	49	4	(2.5)
Total	**160**	**12**	**(7.5)**

χ^2 = 1.073, df =2, p =0.5848.

KEY: HGS = Hajia Gambo Sawaba General Hospital, Zaria. NCH = New City Hospital, Zaria. ZCMC = Zaria Clinic and Medical Center.

Table 4.2: Prevalence of *Staphylococcus aureus* infection in burn wound patients based on patients socio-demography.

Factor	Number Examined	Number Positive	Prevalence (%)	*p*-value
Sex: 0.7425				
Male	99	8	(5.0)	
Female	61	4	(2.5)	
Age Group: 0.4080 **(Years)**				
1-10	37	5	(3.13)	
11-20	73	3	(1.87)	
21-30	31	2	(1.25)	
31-40	17	2	(1.25)	
≥41	2	0	(0.0)	
Duration of Burn: (Days)				0.5684
1-3	103	7	(4.37)	
4-6	26	3	(1.87)	
7-9	11	1	(0.63)	

10-12	13	0	(0.0)
13-15	5	1 (0.63)	
Total	**160**	**12**	**(7.5)**

4.3: Prevalence of *Staphylococcus aureus* infection in burn wound patients based on source.

Burn wound caused by hot water and fire had prevalence of 3.13% each, while burn wound caused by hot object show prevalence of 1.25% (Table 4.3). The difference observed was not statistically significant (χ^2 = 1.718, df =2, p = 0.4236).

4.4 Antibiotic Susceptibility Pattern of *Staphylococcus aureus* Isolated from Burn Wound Patients.

Antibiotic susceptibility pattern of the isolates presented in table 4.4 indicates 100% resistance to Cefoxitin by the isolates; others are: Oxacillin (83.3%), Tetracycline and Vancomycin (75% each). There was 50%, 25%, 25%, 16.7%, 8.3% and 8.3% susceptibility of the isolates to Gentamicin, Tetracycline, Chloramphenicol, Ciprofloxacin, Erythromycin and Oxacillin respectively. Intermediary susceptibility of the isolates to the antibiotics were; Chloramphenicol (50%), Erythromycin (25%), Vancomycin (25%), Ciprofloxacin (16.7%) Gentamicin (8.3%) and Oxacillin (8.3%) (Table 4.4B)

Table 4.3: Prevalence of *Staphylococcus aureus* infection in burn wound patients based on source of burn.

Source of Burn	Number Examined	Number Positive	Prevalence (%)
Hot water	44	5	(3.13)
Hot object	26	2	(1.25)
Fire	90	5	(3.13)
Total	**160**	**12**	**(7.5)**

χ^2 = 1.718, df =2, p = 0.4236.

Table 4.4A: Interpretation table for the antibiotic susceptibility test of *S. aureus* isolates from burn wound patients in Zaria.

Antibiotic	Concentration (µg)	Zone (mm)		
		Sensitive	Intermediate	Resistant
Ciprofloxacin	30	≥21	16-20	≤15
Erythromycin	15	≥23	14-22	≤13
Tetracycline	30	≥19	15-18	≤14
Gentimicin	30	≥15	13-14	≤12
Oxacillin	1	≥22	-	≤21
Cefoxitin	30	≥22	-	≤21
Chloramphenicol	30	≥18	13-17	≤12
Vancomycin	30	≥17	15-16	≤14

Source: CLSI, (2014).

Table4. 4B: Antibiotic susceptibility pattern of *Staphylococcus aureus* isolated from burn wound patients in Zaria.

Antibiotic (disk content)	*Number (%) of isolates		
	Susceptible	Intermediate	Resistant
Ciprofloxacin (5 µg)	2(16.7)	2(16.7)	8(66.7)
Erythromycin (15 µg)	1(8.3)	3(25)	8(66.7)
Tetracycline (30 µg)	3(25)	0(0.0)	9(75)
Gentamicin (30 µg)	6(50.0)	1(8.3)	5(41.7)
Oxacillin (30 µg)	1(8.3)	1(8.3)	10 (83.3)
Cefoxitin (30 µg)	0(0.0)	0(0.0)	12 (100)
Chloramphenicol (30 µg)	3(25.0)	6(50)	3(25)
Vancomycin (30 µg)	0(0.0)	3(25)	9(75)

* Total number of isolates screened (n = 12)

4.5 Multiple antibiotic resistance (MAR) indicesand resistance patterns of *Staphylococcusaureus*isolates.

Table 4.5 shows the antibiotic resistance pattern and the Multiple Antibiotic Resistance Index of the *Staphylococcus aureus* isolates. All the isolates screened were resistant to 2 or more antibiotics. The MAR index of the isolates was between 0.25 and 1.00. Two of the 12 isolates were resistant to 3 out of the 8 antibiotics tested with a MAR index of 0.38. Also 2 of the 12 isolates screened were resistant to 4 out of the 8 antibiotics tested (MAR index = 0.50). Three of the isolates were resistant to 6 out of the 8 antibiotics tested (0.75). Two of the isolates were resistant to 7 out of the 8 antibiotics used (0.88). Finally 2 of the isolates tested were resistant to all of the antibiotics used with a MAR index 1.00. The resistance pattern observed was FOX, OX TE, VA, E, CIP, CN and C which are in order of most resisted to the least resisted drug.

4.6 Distribution of methicillin resistant*Staphylococcus aureus*among the isolates examined from burn wound patients in Zaria.

Among the 12 *Staphylococcus aureus* isolated from burn wound patients, 9 isolates were methicillin resistant *Staphylococcus aureus* (MRSA) as detected by PBP latex agglutination test. Therefore, the prevalence of MRSA is 75.0% as shown in Figure 4.1.

Table 4.5: Multiple Antibiotic Resistance Indices (MARI) and resistance patterns of *Staphylococcus aureus* isolated from burn wound samples in Zaria.

Isolate code	Number of antibiotics	Resistance pattern	MAR index
N 7	6	CIP, E, CN, OX, FOX, VA.	0.75
N 10	3	OX, FOX, VA.	0.38
N 18	4	CIP, TE, OX, FOX.	0.50
N 35	2	FOX, VA.	0.25
N 45	3	TE, CN, FOX.	0.38
N 46	6	CIP, E, TE, OX, FOX, VA.	0.75
H 3	7	CIP, E, TE, OX, FOX, C, VA.	0.88
H 12	8	CIP, E, TE, CN, OX, OX, C, VA.	1.00
Z 5	7	CIP, E, TE, CN, OX, FOX, VA.	0.88
Z 6	4	E, TE, OX, FOX.	0.50
Z 8	6	CIP, E, TE, OX, FOX, VA.	0.75
Z 10	8	CIP, E, TE, CN, OX, FOX, C, VA.	1.00

Key: CIP = Ciprofloxacin, E = Erythromycin, TE = Tetracycline, CN = Gentamicin, OX= Oxacillin, FOX = Cefoxitin, C = Chloramphenicol, VA = Vancomycin.

N = New City Hospital, H = Hajia Gambo Sawaba General Hospital and Z = Zaria Clinic and Medical Center

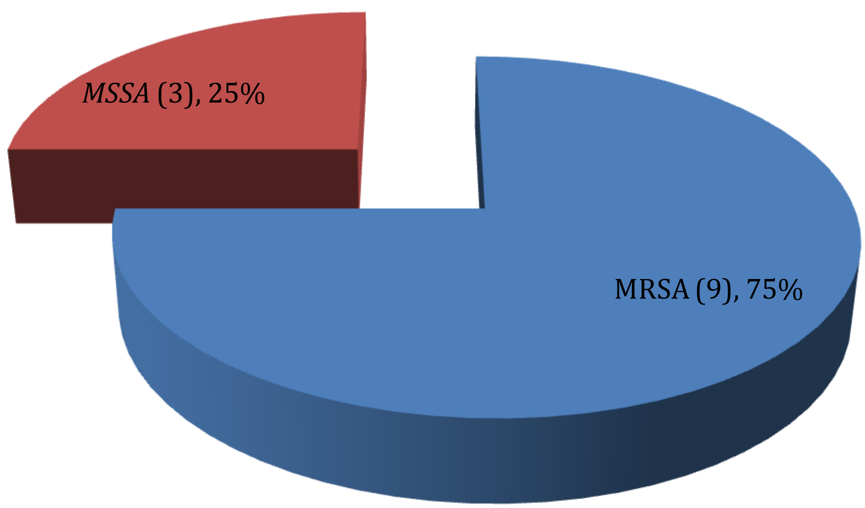

Figure 4.1: Distribution of methicillin resistant *Staphylococcusaureus* among the isolates obtained from burn wound patients.

CHAPTER FIVE

5.0 DISCUSSION

Staphylococcus aureus is highly ubiquitous in humans both as a commensal and as a pathogen. It is one of the most infectious agents with high prevalence in various communities and healthcare institutions (Akindele *et al*., 2010).

A prevalence of 7.5% for *Staphylococcus aureus* was obtained in this study. This might be due to good hygienic practices in some of the sampling locations, limited access to the study population and even the method of identification employed. This prevalence is in line with the continuous fall in the prevalence of *S. aureus* infection as reported by Oladele and Olabanji (2010), Kolawole *etal*. (2013) and Alabi *etal*. (2016) with 50%, 31.8% and 27.5% prevalence respectively.

The study covered three (3) different hospitals in Zaria; with the highest prevalence of 3.75% in New City Hospital, 2.50% in Zaria Clinic and Medical Center and the lowest prevalence of 1.25% obtained from Hajia Gambo Sawaba General Hospital. Even though there was no statistical association (p = 0.5848) between the occurrence of *S. aureus* and the sampling locations, the observed difference might be due to the differences in sample size, degree of burn and burn wound care given to the patients in each of the hospital.

A prevalence of 5.0% observed among male patients as compared to the 2.5% in female patients might be due to the higher number of samples obtained from the male patients. Behavioral practice such as hand shaking and hand hygiene may also influence *S. aureus*colonization and infection rates among the male patients than female patients as also reported by Humphreys*etal*.(2015).There was no statistical association (p = 0.7247) between the occurrence of*S. aureus* infection and sex in this study. Alsothe result of these findings are in consonance with the report of Onanuga and Temedie (2011) who reported a higher prevalence of 38.3% in males than 28.3% observed among female patients.

The highest prevalence of 3.13% recorded in age group 1-10 years might be attributed to their poor personal hygiene and low immunity, hence prone to more colonization. This agrees with the findings of Iroha *et al*. (2012).

Even though duration of a burn wound plays a great role in terms of colonization due to the length of period of exposure of the burn wound, but no statistical association (p =0.5684) between the occurrence of*S.aureus* infection and the duration of burn wounds has been established in this study. This agreed with the findings of Udeani *etal*. (2016). The implication of this result is that *S.aureus*has the ability to colonize and cause an infection inburn woundwhich may worsen the condition of the burn wound irrespective of its duration. Nevertheless, this can be attributed to the fact that *S. aureus* is predominantly presence on human skin as a normal floragiving it an opportunity to revert to pathogenic whenever it gain access to the burn wound.

The sources through which the burn wound patients got their injury varies from patients to patients. In this study, those burn wound patients as a result of hot water and fire had

3.125% prevalence of *S. aureus* infection each while those patients as a result of hot object had 1.25% prevalence. There was no statistical association between the occurrence of *S. aureus* infection and sources through which the burn wound patients got their injury. This also agrees with the findings of Udeani *etal*. (2016).

High level of resistance demonstrated by the isolates to cefoxitin (100%) and oxacillin (83.3%) could likely be attributed to the presence of modified penicillin binding protein (PBP2a) that is normally encoded by *mec A*gene, of which PBP test result of this research indicates its activity by the isolates. The 75% resistance to vancomycin and tetracycline by the isolates might be attributed to the emergence of multi-drug resistant *Staphylococcus aureus* since *S. aureus* has pottentials of acquiring resistance genes from related organisms like *Enterococcus* as reported by Onanuga and Temedie (2011);Samson and Anthony (2013). A moderate susceptibility to chloramphenicol and gentamicin (50% each) by the isolates might be due to low usage since they are less prescribed. Similarly gentamicin is administered intramuscularly or intravenously, hence it is not likely abused.

The Multiple Antibiotic Resistance (MAR) index is a tool used to analyze health risk.It is also helpful in monitoring the spread of bacterial resistance in a given population (Christopher *et al.,* 2013). The MAR indices observed in this study ranges from 0.25 to 1.00. All the isolates in this study had MAR index of not less than 0.2. These high MAR index values shown by the isolates are indicative of environment with high endemic disease potentials and many drugs are often used or abused. Isolates that are resistant to ≥3 different groups of antimicrobial agents were considered to be multidrug resistant (MDR) isolates(Tanwar *etal*., 2014). About 91.7 % of *Staphylococcus aureus* isolates in this research were resistant to not less 3 of the different antibiotics tested. Thisindicates that they are multi drug resistant *S. aureus* and thus require extra attention.

Methicillin resistance in *Staphylococcusaureus* has been reported to be a result of the expression of PBP2a which is encoded by *mecA* gene. PBP2a has significant lower affinity for βeta lactam antibiotics that inhibit transpeptidation reaction during cell wall synthesis (Peacock and Paterson, 2015).

The fact that MRSA (75%) predominate the methicillin sensitive*Staphylococcus aureus* (MSSA)isolated in this study, could be an indication of indiscriminate use of drugs or drug abuse by the patients. Most patients do not complete their prescribed dosages, possibly assuming that they have recovered from their ailments.Same prevalence in wound was reported by Udobi *et al.* (2013). The prevalence is similar to that reported by Baba *et al*. (2015) (78%) and 69% reported among healthy women in Zaria by Onanuga *et al*. (2005).The emergence of MRSA in the hospitals, particularly in this study is not surprising, this is because there is the possibilities that the MRSA so isolated from the patients are from the community where these patients live, which means MRSA could find its way to the hospital environment via community-hospital cross-infection and vice versa. There is also probability that the health care workers may carry MRSA on their hands andor clothes, since they are always in contact with the asymptomatic carriers or patients who have clinical infection.Apart from these, the source of MRSA infection could equally be from contaminated environmental surfaces.

CHAPTER SIX

6.0 CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION TO KNOWLEDGE

6.1 Conclusion

In this study, 12 *Staphylococcus aureus*isolates were isolated from 160 burn wound swabs accounting for 7.5% prevalence. All the isolates were resistant to Cefoxitin while 83.3%, were resistant to Oxacillin. There is 75% resistanceto Tetracycline and Vancomycin by the isolates. About 91.7% isolates are multi-drug resistant isolates as shown by MAR Index. Prevalence of methicillin resistant *Staphylococcusaureus* among the isolates was 75% as screened by PBP latex agglutination test.

6.2 Recommendations

1. Hospitals should monitor *Staphylococcusaureus* infection in patients with burn wound so as to facilitate management and treatment of the patients.

2. Public health awareness on the use of antimicrobial agents and self-medication should be improved in order to reduce the increasing emergence of drug resistant organisms.

3. More research on MRSA from different samples should be conducted from time to time so as to check any changes in it resistant pattern.

6.3 Contribution to knowledge

This study has revealed that:

1. The prevalence of *Staphylococcusaureus* infection in 160 burn wound patients in selected hospitals in Zaria, Nigeria was 7.5%.
2. That *Staphylococcusaureus* is persistently resistant to these antibiotics: CIP, E, TE, CN, OX, FOX and VA.
3. The prevalence of methicillin resistant *Staphylococcusaureus* among the *Staphylococcusaureus* isolates in this study was 75%.

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APPENDICES

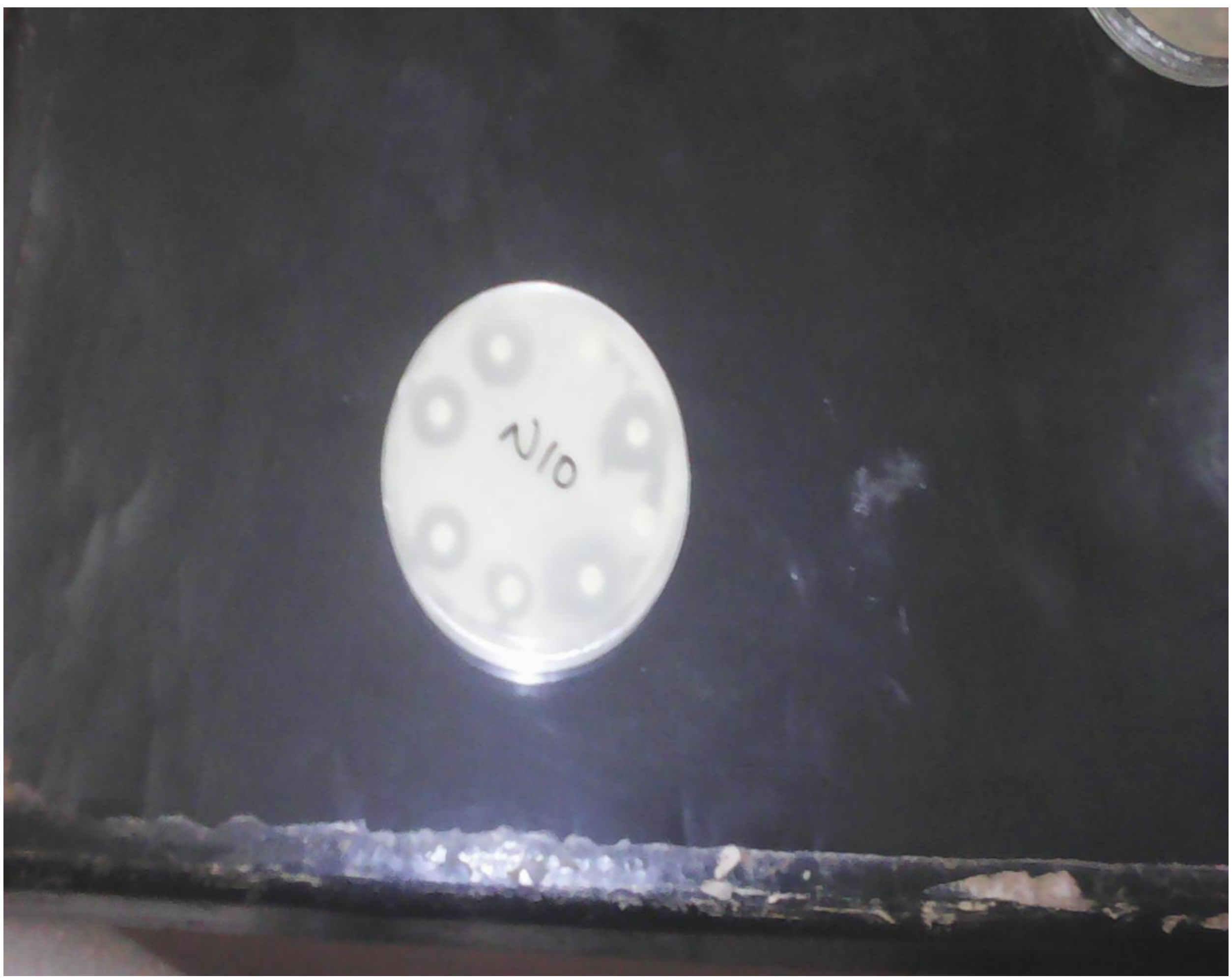
Appendix VI: Mannitol Salt Agar plates showing suspected colonies of *Staphylococcusaureus*.

Appendix VIII: Biochemical Characterization of the isolates

Isolate code	Coagulase	DNase	Lecithinase	Gelatinase	Fermentation			Hemolysis
					Glucose	Lactose	Mannitol	
N 7	+	+	+	+	+	+	+	β
N 10	+	+	+	+	+	+	+	β
N 18	+	+	+	+	+	+	+	β
N 35	+	+	+	+	+	+	+	β
N 45	+	+	+	+	+	+	+	β
N 46	+	+	+	-	+	+	+	β
H 3	+	+	+	-	+	+	+	β
H 12	+	+	+	+	+	+	+	β
Z 5	+	+	+	+	+	+	+	β
Z 6	+	+	+	+	+	+	+	β

Z 8	+	+	-	+	+	+	+	β
Z 10	+	+	+	+	+	+	+	β

Keys: N= New City Hospital, H= Hajia Gambo Sawaba General Hospital, Z = Zaria Clinic and Medical Center. Positive (+) Negative (-)



Appendix III: Antibiotic susceptibility test on Mueller Hinton agar using Kirby-Bauer disc diffusion technique

Appendix VIIIV: PBP Latex Agglutination Test result

Appendix IX: Consent form used in the study.

DEPARTMENT OF MICROBIOLOGY

SCHOOL OF POSTGRADUATE STUDIES

J BELLO UNIVERSITY, ZARIA, NIGERIA.Head of Department: Prof I.O. Abdullahi; B.Sc, M.Sc, PhD

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INFORMED CONSENT FORM (ICF)

This Informed Consent Form is for burned wound patients attending some selected hospitals in Zaria, Nigeria. We are inviting you to participate in this research work titled **"Determination of Methicillin Resistance Profile of *Staphylococcusaureus* Isolated from Burned Wound Patients in Some Selected Hospitals in Zaria, Nigeria."**

Principal Investigator: Muhammad Bature

Collaborating Investigators: Prof. H.I. Inabo

Dr. M.S. Aliyu

Name of Organization: Department of Microbiology

Ahmadu Bello University, Zaria - Nigeria

Name of Sponsor: Parents

Name of Research: Postgraduate Research work

This Informed Consent Form has two parts:

- Information Sheet (to share information about the research with you)
- Certificate of Consent (for signatures if you agree to take part)

PART I: INFORMATION SHEET

Introduction

I am Muhammad Bature, a postgraduate student of the Department of Microbiology, Ahmadu Bello University, Zaria, carrying out a research work under the supervision of Prof. H.I. Inabo andDr M. S. Aliyu.

We are conducting a research work on **"Determination of Methicillin Resistance Profile of *Staphylococcusaureus* Isolated from Burn Wound Patients in Selected Hospitals, in Zaria, Nigeria."** I will give you information and invite you to be part of this research. You do not have to decide today whether or not you will participate in the research. Before you decide, you can talk to anyone you feel comfortable with about the research.

There may be some words that you do not understand. Please ask me to pause as we go through the information and I will take time to explain. If you have questions, you can ask me, the study doctor or the staff.

Purpose of the research

The purpose of this research is to determine the methicillin resistance profiles of *S. aureus* isolated from burn wound patients in selected hospitals in Zaria.

Participant selection

We are inviting all patients with burn wound attending selected hospitals in Zaria to participate in research.

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this clinic will continue and nothing will change. If you choose not to participate in this research project, you will be offered the treatment that is routinely offered in this clinic/hospital. You may change your mind later and stop participating even if you agreed earlier.

Procedures and Protocol

When you have agreed to participate in this research, swab sample of your burn wound is going to be taken.

Risks

There is a risk that you may share some personal or confidential information by chance, or that you may feel uncomfortable talking about some of the topics. However, we do not wish for this to happen. You do not have to answer any question or take part in the research if you feel the question(s) are too personal or if talking about them makes you uncomfortable.

Benefits

If you participate in this research, you will have the following benefits:

- ✓ Your will be screen for *Staphylococcus aureus*
- ✓ *Antibiotic susceptibility testing will be carried out to determine the drug to be used.*
- ✓ *Methicillin resistance profile of the isolates will be determined.*

PART II: CERTIFICATE OF CONSENT

I have read the foregoing information, or it has been read and translated to me in a language that I understand. I have also talked it over with my health care provider to my satisfaction. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it. I therefore consent voluntarily to participate as a participant in this research.

Name of Participant..

Signature of Participant: ....

Date: ....

Day/month/year

Statement by Witness

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness: .... Thumb print of participant

Signature of witness: _____________________

Date _________________________

Day/month/year

Appendix VI: Research Questionnaire

Department of Microbiology

Faculty of Science

Ahmadu Bello University

Research Questionnaire

Topic: `DETERMINATION OF METHICILLIN RESISTANCE PROFILE OF STAPHYLOCOCCUS AUREUS ISOLATED FROM BURNED WOUND PATIENTS IN SELECTED HOSPITALS IN ZARIA, NIGERIA. `

Introduction

This questionnaire is aimed at getting some socio-demographic. Your view is of utmost importance to this research work, as it stands to be a basic background for the judgments and conclusion of my thesis.Thank you.

Instruction: please tick where appropriate and provide answers where applicable.

Section A:Personaldata

1. Hospital Location…………………………………………………………..
2. Hospital No……………………………………………………………...
3. Gender: Male { } Female { }
4. Age……………………………………………………………………….
5. Marital Status: Single { } Married { } Divorced { }

6. Place of Residents……………………………………………………….

7. Level of Education: Primary { } Secondary { } Tertiary{ } Others{ }

8. Occupation……………………………………………………………..

Section B: Epidemiological Risk factors

9. Source of Burn Wound: Hot water { } Hot object{ } Fire out break{ } Others{ }

10. Number of Days Stayed at Hospital…………………………………………………

11. Have you take any treatments at home before coming to Hospital. Yes { } No { }

12. If yes which type of treatment: Traditional { } Ointment { } Drugs { }

13. what difference did you noticed between home treatment and the hospital treatment : Better{ } Worse { } No change { }

MINISTRY OF HEALTH AND HUMAN SERVICES
KADUNA STATE, NIGERIA

MOH/ADM/744/VOL.1/525

28TH JULY, 2017

NOTICE OF APPROVAL AFTER FULL COMMITTEE REVIEW

DETERMINATION OF METHICILLIN RESISTANCE PROFILE OF STAPHYLOCOCCUS AUREUS ISOLATED FROM BURNED WOUND PATIENTS, IN SOME SELECTED HOSPITALS IN ZARIA, NIGERIA.

Name of Principal Investigator :	MUHAMMAD BATURE
Address of Ethical Approval :	Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria.
Date of receipt of Application :	12th June, 2017
Date of Ethical Approval :	26th July , 2017

This is to inform you that the Research described in the submitted Protocol, the Consent Forms, advertisements and other participant information materials have been reviewed and given full approval by the the Health Research Ethics committee (HREC).

If there is delay in starting the research or any change, inform the HREC so that the dates of approval can be adjusted accordingly.

However, Researcher is kindly requested to submit a copy of his/her findings to the State Ministry of Health, please.

SMOH
Resource Centre
Kaduna.
Date 31/7/2017

Dr. BUTAWA NN

Secretary

For: Chairman.

Independence Way, P.M.B 2014, Kaduna, Kaduna State-Nigeria
Tel: +234(0) 818 407 8693 | Website: www.kdsg.gov.ng | Email: health@kdsg.gov.ng

Appendix XII: Ethical approval from Ministry of health and human resources, Kaduna state of Nigeria.