PREVALENCE AND ANTIBIOTIIC SUSCEPTIBILTY PATTERN OF METHICILLIN - RESISTANT STAPHYLOCOCCUS AUREUSAMONG THE INTERNALLY DISPLACED PERSONS IN MAIDUGURI, NIGERIA.

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DEPARTMENT OF MICROBIOLOGY,
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MARCH, 2017.

DECLARATION

I declare that the work in this dissertation entitled "THE PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS AMONG THE INTERNALLY DISPLACED PERSONS IN MAIDUGURI, NIGERIA" has been carried out by me in the Department of Microbiology, Ahmadu Bello University, Zaria. 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 this or any other institution.

Hafsah Muhammad ABDULLAHI

Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled “PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS AMONG THE INTERNALLY DISPLACED PERSONS IN MAIDUGURI, NIGERIA” by HAFSAH MUHAMMAD ABDULLAHI, meets the regulations governing the award of the degree of Master of Science (M.Sc.) in Microbiology of the Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Antimicrobial resistance has been noticed as one of the paramount microbial threats in the twenty first century. *Staphylococcus aureus* has always been a stumbling block for antimicrobial chemotherapy and methicillin resistance of *S. aureus* remains to be a significant problem and a global threat to human race due to its multidrug resistance propensity and avalanche of diseases associated with it. Methicilin-Resistant *Staphylococcus aureus* is troublesome in homeless shelters that are crowded and confined with poor hygiene practice, (a typical of internally displaced persons camp) which may proliferate, thus putting inhabitants at increased risk of contracting MRSA. This study determined the prevalence of MRSA among the internally displaced persons (IDP) in Maiduguri, Nigeria. In this study, 400 nasal swabs were collected from IDP's in some selected camps within Maiduguri metropolis. The swabs were inoculated on blood agar and mannitol salt agar. Fifty one *S. aureus* were isolated giving a prevalence of 12.75%.The isolates were further characterised by phenotypic and molecular methods, for detection of MRSA. Out of the fifty one *S. aureus* isolates, 46 (90.20%) isolates were identified by Cefoxitin disc diffusion as MRSA. Eight representative of MRSA isolates were confirmed by PCR. Forty six (46) MRSA and five (5) MSSA isolates exhibited multi resistant pattern to the commonly used antibiotics. The antibiotic susceptibility test of *S. aureus* showed a high level of resistance to Oxacilin and Cefoxitin (90.20%) followed by Penicilin (84.31). Relatively high level of resistance was observed to Tetracycline (50.98%) but no resistance was observed to chloramphenicol and Ofloxacin. Polymerase chain reactiondetected mecA gene from the eight methicillin-resistant phenotypes which were selected randomly from the five IDP camps.The prevalence of *S. aureus* and MRSA isolated from nasal swab of IDPs was found to be 12.75% and 90.20% respectively.

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SYMBOLS AND ABBREVIATION

bp Base pair

clf Clumping factor

CA-MRSA Community acquired Methicilin-resistant *Staphylococcus aureus*

CAPD Continuous ambulatory peritoneal dialysis

dfDegree of freedom

DNA Deoxyribonucleic acid

HA-MRSA Hospital acquired Methicilin-Resistant *Staphylococcus aureus*

HIV Human Immunodeficiency Virus

IDPs Internally Displaced Persons

IS Insertion Sequence

kbKilobase pair

kDaKilodalton

MGE Mobile Genetic Elements

MISA Methicillin-Intermediate *S. aureus*

MRSA Methicillin-Resistant *S. aureus*

MSSA Methicillin-Susceptible *S. aureus*

NEMA National Emergency Management Agency

ORSA Oxacillin-Resistant *S. aureus*

PBP Penicillin Binding Protein

PVL Panton Valentine Leukocidin

RNA Ribonucleic Acid

SaPIs Staphylococcal Pathogenicity Island

Sar Staphylococcal accessory regulator

SCCmec Staphylococcal cassette chromosome

SE Staphylococcal enterotoxin

TSST Toxic Shock Syndrome Toxins

UN United Nation

βbeta

γgamma

χ^2chi square

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Staphylococcus aureus is one of the species of the genus *Staphylococcus.* It is a gram positive, non-motile, catalase positive, coagulase positive, facultative anaerobe, involved in causing a number of diseases including boils, pustules, impetigo, osteomyelitis, mastitis, septicaemia, meningitis, pneumonia and toxic shock syndrome. For humans, this organism is an important cause of food borne intoxication, pneumonia, post-operative wound infections, and nosocomial bacteremia (Umaru *et al*., 2011). *Staphylococcus aureus* is considered the most resistant of all non-spore forming pathogens, with well-developed capacities to withstand high salt concentrations (7.5 – 10%), extremes in pH and high temperatures (up to 60°C for 60minutes), it also remains viable after months of air–drying and resists the effects of many disinfectants and has overcome most of the therapeutic agents that have been developed in recent years and hence, antimicrobial chemotherapy for this species has always been empirical (Jun *et al*., 2004). Its mechanism of resistance to beta lactam and the fluoroquinolones has been documented (Kloos, 1998).

Staphylococcus aureus colonises the skin and nasal carriage occurs in about 25-30% of healthy people (Chambers, 2009). It is a versatile human pathogen responsible for nosocomial (Hospital acquired) and community acquired (CA) infection, with clinical manifestation of superficial and systemic diseases, associated with high morbidity and mortality rates. The unique characteristic of *S.aureus* is the production of virulence factors responsible for the establishment of staphylococcal diseases and propensity to develop resistance to multiple antibiotics (Tenover*et al*., 2000; Chambers, 2009).It has also been reported that *S. aureus* strains have a wide variety of multi-drug

resistant genes on plasmids, which can be exchanged and spread among different species of staphylococcus and can be transferred to new bacterial hosts by any of transduction, conjugation or transformation (Lujan*et al*., 2007). *Staphylococcus aureus* is known to be notorious in their acquisition of resistance to new drugs and continues to defy control measures (Talaro and Talaro, 2002). Many strains of *S. aureus* carry a wide variety of multi-drug resistance genes on plasmids. *Staphylococcus aureus* are frequently resistant to penicillinase-reistant penicillin's. An organism exhibiting this type of resistance is referred to as methicillin (oxacillin)-resistant *S. aureus* (MRSA). Such organisms are also frequently resistant to most of the commonly used antimicrobial agents, including the amino-glycosides, macrolides, chloramphenicol, tetracycline and fluoroquinolones (Tenover and Gaynes, 2002; Ikeagwu *et al.,* 2008;Umaru *et al.,*2011).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is major nosocomial pathogens with identifiable risk factors, which includes hospitalization, surgery, residence in chronic Healthcare facilities and injection drug users (Lowy, 1998). Consequential effect of *S. aureus* multidrug resistance includes prolonged hospitalization of patients, difficulty in patient management and treatment, and problem in infection control (Kleven, 2007).Methicillin resistance is clinically very important because a single genetic element confers resistance to the beta-lactam antibiotics which include penicillin's, cephalosporins and carbapenem's among others (Grundmann*et al.*, 2006).

The resistance to methicillin was due to a penicillin binding protein coded for by a mobile genetic element termed the methicillin resistance gene *mec*A (Diekema and Pfaller, 2000). Methicillin resistance is mainly due to the expression of the *mecA* gene, which specifies penicillin binding protein 2a (PBP2a), a transpeptidase with a low affinity for β-lactams (Garza -Gonzalez*,* 2010; Zong et *al.,* 2011). The *mecA* gene is carried by a mobile genetic element (MGE) termed the staphylococcal cassette chromosome (SCC*mec*) (Zong et *al.,* 2011). Though the SSC*mec* origin

remains unknown, it has been suggested that *mecA* may act as potential SCC*mec* donor accounting for the rise of new MRSA clones. Eleven SCC*mec* elements have been described to date. SCC*mec* I-IV (Ito *et al.*, 2012) and V-XI, but few reports exist on the detection of *mecA* gene and characterization of SCC*mec* types in Nigeria (Ghebremedhin 2009; Shittu *et al*., 2011).

Over the period of 20 to 30 years, MRSA strains have been present in hospitals and the community. The organism is often sub-categorized as hospital acquired MRSA (HA-MRSA) or community acquired MRSA (CA-MRSA)(Schwalm III *et al*., 2011). These two groups, although have similar microbiological characteristics; differ for risk factors, genetic structure, virulence determinants and antibiotic resistance (Wang *et al*., 2010). Some have defined CA-MRSA by criteria related to the patients suffering from an MRSA infection while other authors have defined CA-MRSA by genetic characteristics of the bacteria themselves (Okuma *et al*., 2002).

Community acquired-Methicillin resistant *Staphyloccus aureus* strains were first reported in the late 1990s, these cases were defined by a lack of exposure to the health care settings. Several years later, it became clear that CA-MRSA infections were caused by strains of MRSA that differed from the older and better studied health care-associated strains (Okuma *et al*., 2002). Community acquired-Methicillin resistant *staphylococcus aureus* infections occurs in otherwise healthy people without a recent history of hospitalization or clinical presentation, and are usually associated with skin and soft tissue infection. Risk factors for CA- MRSA include crowding, frequent contact, compromised skin, contaminated surfaces and shared items, and poor hygiene. Hospital acquired-Methicillin resistant *staphylococcus aureus* infections occurs most commonly in immune compromised individuals in hospitals and health care centers. Risk factors for HA- MRSA include hospitalization, surgery, dialysis, long-term care, indwelling devices, and history of previous MRSA infection. The

bulk of MRSA related clinical infections are caused by HA- MRSA, which are considered "nosocomial" (Hanselman *et al*., 2006; Klevens *et al*., 2007).

From virtually zero prevalence level in 1961, (Jevons, 1961) it has been detected worldwide with varied level influenced by geographical location, type of health institution and studied population.In Europe, MRSA prevalence ranges from over 50% in Portugal and Italy to below 2% in Switzerland and the Netherlands, where infection control measures have been shown to work (Queand Moreillon, 2010). In Asia, the prevalence lies around 50%, with extremely high rates in Hong Kong (75%) and Japan (72%) (Queand Moreillon, 2010).In Africa, MRSA prevalence varies with different countries, high in some and low in others (Bell and Turridge, 2002). Despite this epidemiological data on MRSA in some African countries, available data are still relatively limited when compared to information from developed countries, which may be attributable to high level of awareness of MRSA infections and its clinical and societal consequences.

The epidemiology of MRSA is fast changing and has become one of the established pathogen in both hospital and community. Methicillin-Resistant *Staphylococcus aureus* infection and colonization has been reported in humans in Nigeria, in both hospital and outside the hospital environment. In Nigeria, several reports of human MRSA infections have been documented. Ike (2003) reported a prevalence rate of 43% at Jos University Teaching Hospital while Onanuga *et al*. (2005; 2006) showed a prevalence rate of 71.7% and 69% from urine samples in Abuja and Zaria respectively among healthy women. The prevalence rate of 20% was also recorded in Zaria from non-hospital sources (Olonitola *et al*., 2007a). Also Taiwo *et al*. (2004), FusiNgwa *et al*. (2007), and Olowe *et al*. (2007) in separate studies reported a prevalence rate of 34.7%, 54.9% and 47.8% in Ilorin (University of Ilorin Teaching Hospital), Lagos (Paediatric Unit, Lagos University Teaching Hospital and Oshogbo (Ladoke Akintola University of Technology, College of Health

Sciences) respectively. Adelowo *et al*. (2014) reported a prevalence of 47.6 % from patients attending University of Maiduguri Teaching Hospital.

The internally displaced persons (IDPs) are persons or group of persons who have been forced or obliged to flee or to leave their homes or places of habitual residence, in particular as a result of or in order to avoid the effects of armed conflict, situations of generalized violence, violations of human-made disasters and who have not crossed an internationally recognized state border (UN, 2004; Ocha, 2015). The National Emergency Management Agency (NEMA) had reported that Boko Haram terrorists had forced residents of various communities to take refuge in different camps across northern Nigeria and more than one third of such camps were domiciled in the northeast, while more than half of them are in Maiduguri, the Borno State capital (NEMA, 2014).

1.2 Statement of the Research Problem

Staphylococcus aureus is a major component of normal flora of the skin and nostrils which probably explains its high prevalence as contaminants and can easily be discharged by several human activities like sneezing, talking and contact with moist skin (Ita and Ben, 2004). Pathogens spread among people with direct or indirect contact of hands and with animate and inanimate objects (Mathai, 2010;Tekerekoğlu *et al*., 2013).

There is increasing global concern for the spread of antibiotic resistant bacteria particularly MRSA.Associated with this concern is the use of antimicrobial agents in promoting the emergence and rise in the prevalence of these resistant pathogens (Mansori and Khaleghi, 1997; Lee, 2003; Ikeagwu *et al.,* 2008).MRSA infections are of special concern because these infections are

associated with prolonged hospital stay, increased hospital costs, and have a few therapeutic options for affected patients (Saxen *et al*., 2003).

1.3 Justification

Recent studies suggest that the infection due to MRSA is not only hospital-acquired but community acquired as well (Chandrashekhar *et al.,* 2012). Methicillin-Resistant *Staphylococcus aureus* now represent a global problem, some large outbreaks have been reported from different parts of the world, where it had caused severe infections including septicaemia, endocarditis and meningitis (WHO, 1996). A study by Dickinson (2002) in England and Wales has concluded an increase in the trend of death due to MRSA infection (Dickinson, 2002). Infections caused by MRSA can be expensive in terms of antibiotic therapy, isolation facilities and materials and length of hospital stay (Dickinson, 2002; Kumari *et al*., 2008). According to a World Health Organization literature (1996), the global financial burden because of MRSA infection has been worked out to be $20,000 to $ 114,000 for outbreaks and from $28,000 to $1, 600, 000 for endemic infections per year (Chandrashekhar *et al.,* 2012).The common sources of these infections are human patients and carriers (Collier *et al*.,1998; Chandrashekhar *et al.,* 2012).

In addition to dire consequences of infections, MRSA strains are important for their resistance to many other commonly used antibiotics and the emergence of resistance to vancomycin, the drug that has been used to treat MRSA infections for more than three decades. Reports of emergence of vancomycin resistance in *S. aureus* from India (Assadullah *et al.,* 2003; Tiwari *et al* 2008; Saha *et al*., 2008) further justify the necessity for this research.Methicillin-Resistant *Staphylococcus aureus* is especially troublesome in hospitals and nursing homes or long-term care facilities where patients

with open wounds, invasive devices, and weakened immune system are at greater risk of infection than the general public (Hardy *et al*., 2004).

Prisons, military barracks and homeless shelters can be crowded and confined and poor hygiene practices may proliferate, thus putting inhabitants at increased risk of contracting MRSA. The internally displaced are at greater risk of contracting MRSA infections because they are overcrowded and there is poor hygiene practice among most of them which can facilitate the spread of MRSA. Therefore,the results of this study showing cases of MRSA among the internally displaced persons will be of benefit to Medical Personnel handling the internally displaced persons, Infections prevention and control workers, and researchers on *Staphylococcal* infections. In north eastern Nigeria, there is still paucity of epidemiological information on MRSA (Adelowo *et al*., 2014) while information on its prevalence among the IDPs is non-existent. Therefore, this work will serve as a data base on the study of MRSA among the IDPs in Maiduguri, Nigeria.

1.4 Aim and Objectives

1.4.1 Aim

The aim of this study was to determine the prevalence and antibiotic susceptibility pattern of Methicillin-Resistant *Staphylococcus aureus* among the internally displaced persons in Maiduguri.

1.4.2 Objectives

The specific objectives were to:

1. Isolate and characterize *S. aureus*from the anterior nares of the IDP’s using conventional microbiological techniques.

2. Carry out an *in vitro* antibiotic susceptibility test of the *S. aureus*isolates.
3. Screen the isolates for methicillin resistance.
4. Detect mecA gene among the methicillin resistant phenotypes using Polymerase Chain Reaction.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Genus *Staphylococcus*

Staphylococci are aerobic or facultatively anerobic, non-motile, non-spore-forming, catalase positive, gram-positive cocci.They are arranged as single cells, pairs, tetrads, and short chains, but appear predominantly in grape-like clusters when a Gram stained smear is viewed (Que and Moreillon,2010). They often have a limited capsule. They are generally found on the skin and mucous membranes of humans and other animals (Winn*et al*., 2006; Brooks *et al*., 2007; Que and Moreillon, 2010). Staphylococci were first seen in pus by Koch in 1878 and were first cultivated in liquid medium by Pasteur in 1880 and named so by Sir Alexandar Ogston in 1881 (Gotz *et al*.,2004, Bergdoll and Lee Wong, 2006; Obajuluwa, 2014). The name Staphylococcus was derived from Greek words staphyle (bunch of grapes) and kokkos (grain or berry) (Arora, 2006).Pathogenic staphylococci are identified by their ability to produce coagulase thus clot the blood (Harris *et al*., 2002;Lok *et al*., 2014).There are approximately 40 species of staphylococci (Lindsay,2008). Among the staphylococci, the coagulase-positive species *S. aureus,* and two coagulase-negative species, *S. epidermidis* and *S. saprophyticus,* are seen frequently in human infections (Brooks *et al*.,2007; Que and Moreillon, 2010)

2.1.1 *Staphylococcusaureus*

Staphylococcus aureus is by far the most important human pathogen among the staphylococci, and is found in the external environment and in the anterior nares of 10 – 40% of the population. Itis both commensal and pathogenic (Queand Moreillon,2010).It is found as a commensal associated with skin, skin glands and mucous membranes affecting the skin, soft tissues, bloodstream and

lower respiratory tract. Other sites of colonization include intertriginous skin folds, the perineum, the axillae, and the vagina (Winn*et al*., 2006;and Moreillon,2010), it also causes severe deep-seated infections like endocarditis and osteolmyelitis (Schito, 2006; Joshi and Devkota, 2014).*Staphylococcus aureus* are spherical cocci about 0.8 to 1.0µm in diameter. They are arranged characteristically in grape-like clusters. They are Gram-positive, non-motile (non-flagellated), non-sporing and non capsulated (Arora, 2006; Obajuluwa, 2014). It is characterized as coagulase- and catalase positive, non-motile, non-spore-forming and as facultative anaerobic. It grows in yellow colonies on nutrient rich media and is referred to as the yellow staphylococci (Winn *et al*., 2006).

Staphyloccoccus aureus was first discovered in 1880 by a surgeon, Alexander Ogston, who described staphylococcal disease and its role in sepsis and abscesses (Shinefield *et al*., 2009; Nottasorn, 2012). He observed grape-like clusters of bacteria when examining a purulent discharge from patients with post-operative wounds during microscopy. He named them staphylé, the Greek expression for a bunch of grapes. In 1884, Rosenbach succeeded in isolating yellow bacterial colonies from abscesses and named them *Staphylococcus aureus*, “aureus” from the Latin word for golden (Stark,2013) . Over 100 years later, *S. aureus* remains a dangerous threat to human health and has become one of the leading causes of hospital-acquired infection worldwide (Boyce *et al.*, 2002; Pittet *et al.*, 2006; Nottasorn, 2012).

In the early 1940s, *S. aureus* infection was a fatal disease with a mortality rate for bacteremia of about 80% (Nottasorn, 2012).It is a major pathogen that is responsible for not only severe infections of the skin and skin structures but also life-threatening diseases because of its propensity to form biofilms on artificial materials, difficult-to-treat infections of catheters and other devices (Patel *et al*., 2012). *Staphylococcus aureus* has the ability to adapt to different environments and it

may colonize the human skin, nails, nares and mucus membranes and may thereby disseminate among recipient host populations via physical contact and aerosols (Lowy, 1998; Stark, 2013). Itis also considered the most resistant of all non-spore forming pathogens, with well-developed capacities to withstand high salt concentrations (7.5 – 10%), extremes in pH and high temperatures (up to 60°C for 60minutes). It also remains viable after months of air–drying, resists the effects of many disinfectants and has overcome most of the therapeutic agents that have been developed in recent years and hence, antimicrobial chemotherapy for this species has always been empirical (Jun *et al*., 2004).

2.1.2 Cellular structure of *S. aureus*

The cell walls of Gram positive bacteria exhibit a wide diversity from simple to very complex structures (Figure 2.1). Staphylococci cell walls have a rather extra ordinary type of structural design and to the most highly cross linked type but the walls of other Gram positive bacteria exhibit a much lower degree of cross linking and muropeptide fraction of these walls does not contain long oligometric chains (Galley *et al*., 1991; Patel *et al*.,2012). The cell wall envelope functions as a physical barrier that protects the bacteria from their environment and as a rigid exoskeletol element that prevents bacterial rupture in low osmolar environments such as host tissues. The cell walls of the microorganisms play an important role in the susceptibility to infections and pathogenicity (Van Heijenoort and Gutmann, 2000; Patel *et al*., 2012).

Structurally, the cell wall of *S. aureus* is composed of murein, techoic acid and wall- associated surface proteins (Tomaz, 2000; Mazmanian *et al*., 2001). Murein consist of glycan strands that are cross –linked by peptide bridges supplying the structural integrity of the sacculus. It is a distinctive feature of *staphylococci* that the observed degree of murein cross-linking which was determined as

a ratio of bridged peptides to the total amount of all peptide ends in general is extremely high of the order of 80-90% (Gally and Archibald, 1993; Chanda *et al*., 2012). The carbohydrate antigen is a techoic acid which in *S. aureus* is a polymer of N- acetylglucosamine and polyribitol phosphate. Teichoic acids function in the specific adherence of Gram-positive bacteria to mucosal surfaces. The protein component of the cell wall includes protein A which reacts with IgG of normal human serum (Lowy, 1998) and it can be released from the bacterial surface by treatment of staphylococci with lysostaphin, a glycylglycine endopeptidase that cleaves the pentaglycyl cross – bridge of the cell wall. Lysozyme, an N- acetylmuramidase that cuts the glycan strands, release protein A molecules as a spectrum of fragments with varying masses due to the presence of linked peptidoglycan fragments of different sizes (Figure 2.1)(Navarre *et al*., 1998). The glycan strands of all bacterial peptidoglycan consist of repeat disaccharide units, N acetylglucosamine-(β1-4)-N-acetylmuramic acid. Glycan chains are cross linked by short cell wall peptides and generate a three dimensional molecular network that maintains the integrity of the bacterium. Finally penicillin binding proteins catalyze the polymerization of lipid II subunits via trans-glycosylation and trans-peptidation reactions, thus generating the crosss-linked peptidoglycan that constitutes the main component of the bacterial cell wall (Perry *et al*., 2002; Chanda *et al*., 2012).

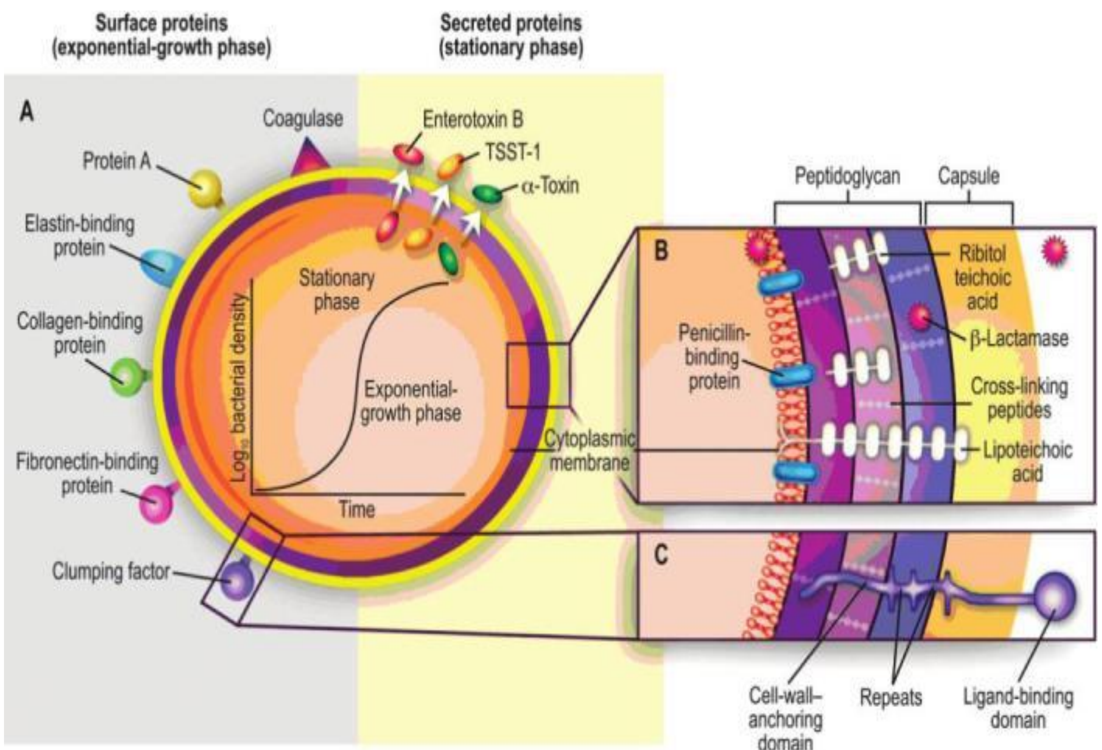


Figure2.1: Pathogenic factors of *Staphylococcus aureus*, with structural and secreted products both playing roles as virulence factors. A, Surface and secreted proteins. B and C, Crosssections of the cell envelope. TSST-1, toxic shock syndrome toxin.

Source: Gordon and Lowy (2008).

2.1.3 Biochemical characteristics

Generally, *Staphylococcus aureus* is Gram-positive cocci, appearing in clusters, catalase-positive, and oxidase-negative. *Staphylococcus aureus* ferments glucose and lactose to produce acid and gas and also ferments mannitol to produce acids. *Staphylococcus aureus* are coagulase- positive and deoxyribonuclease (DNAse) positive (Cheesbrough, 2002). The two tests often used to distinguish *Staphylococcus aureus* from other staphylococci are the coagulase test (coagulation of human or animal plasma) and the thermostable nuclease test (breakdown of deoxyribonucleic acid by nucleases that survive boiling. On a rich medium, *S. aureus* forms medium size “golden” colonies. On sheep blood agar plates, colonies of *S. aureus* often cause β-hemolysis (Ryan and Ray, 2004; Plata *et al*., 2009). The golden pigmentation of *S. aureus* colonies is caused by the presence of carotenoids and has been reported to be a virulence factor protecting the pathogen against oxidants produced by the immune system (Liu *et al*., 2005; Plata *et al*., 2009).

2.1.4 Virulence factors of *S. aureus*

The pathogenicity and virulence of *S. aureus* is associated with the capacity of this organism to produce several virulence factors. *Staphylococcus aureus* is a pathogen expressing multiple factors that mediate host colonization, invasion of damaged skin and mucosa, dissemination through the body and evasion of host defence mechanisms (Chanda *et al*., 2010).The pathogenecity and virulence of *S. aureus* infections is associated to various bacterial surface components (e.g., capsular polysaccharide and protein A) including those recognising adhesive matrix molecules e.g. clumping factor (clf), Fibronectin Bindng Protein(FBN) and to extracellular proteins e.g., coagulase, haemolysins, enterotoxins Toxic Shock Syndrome toxin, exfoliatins toxin and Panton Valentin leukocidin (Labandeira-Rey *et al*., 2007; Obajuluwa, 2014).

Virulence factors can generally be separated into three based on their function:

Adhesins: they are surface attached proteins that allow the bacteria to attach to a wide variety of human tissues. In *S. aureus* the adhesion genes which include clf and fnb that encode the fibrinogen and the fibronectin- binding proteins respectively. Fibronectin binding protein (FnBP) A and Fibronectin binding protein (FnBP) B encoded by the fnbA and fnbB genes respectively, play prominent roles in *S. aureus* colonization and attachment of host tissues or implanted biomaterials (Greene *et al*., 1995; Obajuluwa., 2014).

Toxins: are secreted proteins that cause tissue damage and generate pus in abscesses which is believed to facilitate transmission between hosts

Immunedilators: are proteins that interfere with host immunity preventing defence against infections.

2.1.4.1 Capsular polysaccharides

Some strains of *S. aureus* produce an exopolysaccharide capsule that may prevent ingestion of the organism by polymorphonuclear cells. Clinical isolates of *S. aureus* have been classified into 11 capsular serotypes based on immunotyping of the capsular polysaccharide (Peacock, 2006; Winn *et al*.,2006) and 70 to 80% of significant clinical isolates belong to capsular serotypes 5 and 8 (Fournier *et al*., 1993; Winn *et al*., 2006; Que and Moreillon,2010). Predominant numbers of *S. aureus* strains resistant to oxacillin express the serotype 5 capsular polysaccharide.

2.1.4.2 Biofilm

Staphylococcus aureus produces a slime called biofilm, which is an extracellular polysaccharidic network that gathers bacterial communities within a mechanically cohesive scaffold. This provides

the microbial community protection against host defences and antibiotics (Que and Moreillon, 2010; Morell *et al*.,2010) thus making them a major therapeutic problem (Petrelli *et al*., 2008). Biofilm-producing staphylococci were initially described mainly in coagulase-negative species, but are also formed by *S. aureus* especially in the settings of colonization and persistence on catheters and biomaterials (Gordon and Lowy, 2008; Que and Moreillon,2010).

2.1.4.3 Peptidoglycan and teichoic acid

Peptidoglycan and teichoic acid are the major components of the staphylococcal cell wall. Peptidoglycans are cross-linked polymers of N – acetyl-glucosamine and N – acetyl-muramic acid with a peptide side chain, while teichoic acid are unique ribitol (5-carbon monosaccharide) – phosphate polymers (Winn *et al*., 2006). Teichoic acid functions in the specific adherence of gram-positive bacteria to mucosal surfaces. Peptidoglycan on the other hand provides rigidity and resilience to the staphylococcal cell wall. It is also thought that they contribute to virulence due to their possessing the ability to activate complement, enhance chemotaxis of polymorphonuclear cells, elicit interleukin-1 production by monocytes, and stimulate the production of opsonic antibodies (Winn *et al*., 2006; Gordon and Lowy, 2008).

2.1.4.4 Surface adhesins

Adherence to soluble plasma components and/or extracellular matrix proteins is promoted by a range of *S. aureus* cell-wall–associated proteins. These microbial surface components reacting with adherence matrix molecules have been given the acronym MSCRAMM (Que and Moreillon,2010). These adhesins play a central role in the colonization of the host and during invasive disease. Relevant MSCRAMMs for pathogenesis include clumping factor B (ClfB) for colonization of nasal epithelia (Walsh *et al*.,2008) clumping factor A (ClfA) and fibronectin-

binding protein A (FnBPA) and B (FnBPB) in experimental endocarditis,collagen-binding protein in osteoarthritis,protein A as an antiphagocytic factor (Gordon and Lowy, 2008) and major histocompatibility complex (MHC) class II analog protein (MAP) [or the extracellular adherence protein, EAP] as an immunomodulator subverting the T-cell response in mice.

2.1.4.5 Protein A

This surface adhesin also contained in the cell wall functions as a virulence factor by interfering with opsonization and ingestion of the organisms by polymorphonuclear cells, activating complement and eliciting immediate and delayed-type hypersensitivity reactions (Winn *et al* ., 2006; Morell *et al*., 2010). It also binds the Fc portion of all human IgG subclasses except IgG_3 and von Willebrand factor (Peacock, 2006; Winn *et al* ., 2006; Gordon and Lowy, 2008).

2.1.4.6 Secreted Enzymes and Heamolysins

Staphylococcus aureus produces a number of exoenzymes and membrane active proteins (heamolysins and leukocidins) that are involved in pathogenesis of disease. *Exoenzymes* encompass proteases and lipases, which are destructive regarding host tissues and useful for getting nutrient to the invading bacterium, though their pathogenic role has not yet been fully established (Que and Moreillon,2010).

Staphylococcus aureus has a minimum of four *Heamolysins* whichhave several biologic activities (Que and Moreillon,2010). They are able to lyse erythrocytes and other eukaryotic cells. The α-hemolysin has lethal effects on a wide variety of cells including human polymorphonuclear cells and will lyses erythrocytes from several animal species. The toxin is a protein monomer that interacts on the target cell to form cylindrical heptamers with a central pore. These pores lead to osmotic swelling and rupture of the cell. On the other hand, β-hemolysin is a sphingomyelinase

that damages membranes by enzymatic alteration of their lipid content. The δ-heamolysin is also a protein which acts primarily as a surfactant, and may interact with the cell membrane to form channels, resulting in leakage of cellular content. Both γ-hemolysin and Panton-Valentine leukocidin (PVL) are bicomponent hemolysins encoded by *hlg* and *luk-PV* loci respectively (Winn *et al.,* 2006; Peacock, 2006).

It comprises one of the S-component (HlgA, HlgC or LukS-PV) combining with anyone of the F-component (HlgB or LukF-PV) to constitute six "two component" toxins or chimera complexes, PVL is a homologue of γ-hemolysin (Winn *et al.*,2006) Unlike the other hemolysins, the prevalence rate of PVL is very low (≤2%) in Methicillin susceptible *Staphylococcus aureus* (MSSA) and health-care associated MRSA, where as it is present in almost 100% of isolates of community acquired MRSA (CA-MRSA) (Gordon and Lowy 2008; Que and Moreillon,2010). Panton-Valentine Leukocidin primes neutrophils at sublytic concentrations for enhanced release of reactive oxygen species upon stimulation, thus probably contributing to pathogenesis by causing an exaggerated inflammatory response and injury to the host (Gordon and Lowy ,2008; Chambers *et al*., 2009).

2.1.4.7 Toxins

Staphylococcus aureus also cause disease by elaborating certain extracellular proteins called toxins. These include: Exfoliative or epidermolytic toxins A and B (ET-A and ET-B): they cause staphylococcal scalded skin syndrome (SSSS) (Gordon and Lowy 2008). Exfoliative Toxin-A is thermostable and chromosomal in origin while ET-B is thermolabile and of plasmid origin. They both have proteolytic activity and dissolve mucopolysaccharide matrix of the epidermis, resulting in intraepidermal blisters.

Staphylococcus aureus enterotoxins (SEs): Enterotoxins are short, extracellular proteins that are water-soluble. They are most commonly described as very stable, and are resistant to heat as well as degrading enzymes .To date, 22 SEs have been identified and designated SEA to *SElV*.

Staphylococcus aureus harbours up to 22 enterotoxins (A, B, C, D, E, G, H, I, *l*J, *l*K, *l*L, *l*M, *l*N, *l*O, *l*P, *l*Q, R, S, T, *l*U, W,*I*V)(Jenny*et al*., 2011)and they are able to produce gastrointestinal symptoms that include vomiting and diarrhea (Gordon and Lowy, 2008), although not all of them have a clear role in human disease (Que and Moreillon,2010). *Staphylococcus aureua* enterotoxin is a member of a large family of pyrogenic exotoxins called *superantigens*. These are proteins that do not activate the immune system via normal contact between antigen-presenting cells and T-lymphocytes, rather they bypass the normal highly specific interaction and attach to an external portion of the Vβ domain from large numbers of lymphocytes and directly wedge them to the MHC class II receptors of antigen-presenting cells, thus activating up to 20% of the total pool of T-cells, instead of approximately 1/10,000 during normal antigen presentation.(Que and Moreillon,2010).

Toxic shock syndrome toxin (TSST-1) causes toxic shock syndrome which is also a super antigen-induced hyper activation of the immune system, resulting in a ‘cytokine storm’ (Gordon and Lowy, 2008)

2.1.5 Regulatory Mechanism of Virulence in *S. aureus*

The pathogenicity of *S. aureus* is a complex process involving a diverse array of extracellular and cell wall components that are co-ordinately expressed during different stages of infection (i.e colonization, avoidance of host defence, growth and cell division, and bacterial spread) (Torres *et al*., 2010).

The coordinated expression of diverse virulence factors in response to environmental cues during infections (e.g., expression of adhesins early during colonization versus production of toxins late in infection to facilitate tissue spread) hints at the existence of global regulators in which a single regulatory determinant controls the expression of many unlinked target genes (Cheung *et al*., 2004; Bien *et al*., 2011). These regulators help bacteria to adapt to a hostile environment by producing factors enabling the bacteria to survive and subsequently to cause infection at the appropriate time.

Among the environmental signals, changes in nutrient availability, temperature, pH, osmolarity, and oxygen tension have the greatest potential to influence the expression of virulence factors (Torres *et al*., 2010; Bien *et al*., 2011). Production of *S. aureus* virulence determinants is controlled by several global regulatory loci, such as accessory gene regulator (agr),Staphylococcal accessory regulator (sarA), sae, sigB , arl , and number of sarA homologues (Bien *et al*.,2011). These regulators are parts of an important network modulating the expression of *S. aureus* virulence genes. One target virulence gene can be under the influence of several regulators that “cross talk” to ensure that the specific gene is expressed only when conditions are favorable. For instance, *in vitro* studies have demonstrated that agr negatively regulates the expression of spa, which encodes SpA, whereas SarS binds to the spa promoter and activates its expression. Interestingly, agr down regulates sarS expression. Thus, it has been proposed that agr down regulates spa expression by suppressing the expression of its activator, sarS. Therefore, virulence gene regulators could affect the expression of target genes directly, by binding to their promoters, or indirectly, via other regulators (Tegmark *et al*., 2000; Bien *et al*., 2011).

2.1.6 Infections caused by *S. aurues*.

Staphylococcus aureus is found as a commensal organism on the squamous epithelium of the anterior nares up to 20% of the population at any one time, however, it has been estimated that *S. aureus* can transiently colonize up to 60% of the human population (Foster, 2004; Chakraborty *et al*., 2012). Colonization significantly increases the risk of infections since it provides a reservoir of the pathogen from which bacteria are introduced when host defense is compromised (Kluytmans *et al*., 1997; Plata *et al*., 2009). *Staphylococcus aureus* can cause a wide range of infections ranging from minor skin abscesses to more serious invasive diseases. *Staphylococcus aureus* commonly causes boils, carbuncles, furuncles and impetigo, but after gaining access to the blood, may also be a major cause of endocarditis, osteomyelitis, pneumonia, toxic shock syndrome and septicaemia. *Staphylococcus aureus* is one of the main causes of hospital- acquired and community-acquired infections which can result in serious consequences (Diekema *et al*., 2001; Plata *et al*., 2009). Nosocomial *S. aureus* infections affect the bloodstream, skin, soft tissues and lower respiratory tracts. *Staphylococcus aureus* can be a cause of central venous catheter-associated bacteremia and ventilator-assisted pneumonia. It also causes serious deep-seated infections, such as endocarditis and osteomyelitis (Schito, 2006; Plata *et al*., 2009). In addition to the infections listed above, *S. aureus* is often responsible for toxin-mediated diseases, such as toxic shock syndrome, scalded skin syndrome and staphylococcal foodborne diseases (SFD). Hospitalized patients are particularly exposed to *S. aureus* infections due to their compromised immune system and frequent catheter insertions and injections (Lindsay and Holden, 2004; Plata, 2009). The major diseases by *S. aureus* are:

2.1.6.1 **Bacteremia**: The overall rate of mortality from staphylococcal bacteremia, ranges from 11 to 43%, Factors associated with increased mortality include an age of more than 50 years, non-

removable foci of infection, and serious underlying cardiac, neurologic, or respiratory disease. The frequency of complications from staphylococcal bacteremia is high, ranging from 11 to 53%.As many as 31% of patients with bacteremia, who do not have evidence of endocarditis, do have evidence of metastatic infection. An increasing percentage of bacteremic infections are related to catheterization (Sa'di, 2010).

2.1.6.2Endocarditis: The incidence of *S. aureus* endocarditis has increased and accounts for 25 to 35% of cases, it occurs in intravenous drug users, elderly patients, patients with prosthetic valves, and hospitalized patients. *Staphylococcus aureus* endocarditis is characterized by a rapid onset, high fever, frequent involvement of normal cardiac valves, and the absence of physical stigmata of the disease on initial presentation (Sa'di, 2010).

2.1.6.3 Metastatic Infections: *S. aureus* has a tendency to spread to particular sites, including the bones, joints, kidneys, and lungs. Suppurative collections at these sites serve as potential foci for recurrent infections. Patients with persistent fever despite appropriate therapy should be examined for the presence of suppurative collections(Sa'di, 2010).

2.1.6.4 Sepsis: A minority of bacteremia or local infections progress to sepsis. Risk factors for sepsis include advanced age, immunosuppression, chemotherapy, and invasive procedures. *Staphylococcus aureus* is one of the most common gram-positive pathogens in cases of sepsis (Sa'di, 2010).

2.1.6.5 Toxic Shock Syndrome: The disease is characterized by a fulminant onset, often in previously healthy persons. The diagnosis is based on clinical findings that include high fever, erythematous rash with subsequent desquamation, hypotension, and multiorgan damage (Lowy, 1998; Sa'di, 2010).

2.1.7 The *S. aureus* genome

The genome of *S. aureus* is a circular chromosome that is 2.8–2.9 Mbp in size, with a G+C content of about 33% (Crossley and Archer, 1997). The chromosome encodes approximately 2700 CDSs (protein coding sequences) as well as structural and regulatory RNAs. It has been proposed that the *S. aureus* genome is composed of the core genome, accessory component and foreign genes. The first genome sequences of *S. aureus* strains Mu50 and N315 were published in 2001 (Kuroda *et al.*, 2001). At present, complete genomic sequences of ten *S. aureus* strains are available, and the genomes of several others have been partially determined (Kuroda *et al.*, 2001; Baba *et al.*, 2002; Gill *et al.*, 2005; Diep *et al.*, 2006).

The core genes are present in more than 95% of isolates, represent 75% of any *S. aureus* genome and determine the backbone of the genome. The organization of the core component is highly conserved and the identity of individual genes between isolates is 98–100%. The majority of core genes are associated with fundamental functional categories of housekeeping functions and central metabolism. The accessory component includes genetic regions present in 1–95% of isolates and accounts for about 25% of any *S. aureus* genome. It typically consists of mobile genetic elements that have or previously had the ability of horizontal transfer between strains. These genetic elements include pathogenicity islands, genomic islands, prophages, chromosomal cassettes and transposons (Lindsay and Holden, 2004).

2.1.7.1 Pathogenicity islands

The family of staphylococcal pathogenicity islands that carry genes for super antigen toxins (SaPIs) are 15–20 kb elements located at constant positions in the chromosome. Staphylococcal

Pathogenicity Island posess certain bacteriophage-related attributes: genes coding for integrases, helicases and terminases, and flanking direct repeats (Novick, 2003). The archetype of this family, SaPI1, codes for toxic shock syndrome toxin TSST (tst) and is excised and induced to replicate as well as transduced at high frequency by phage 80α. DNA of SaPI1 is encapsulated into 80α phage-like particles for transfer (Lindsay *et al*., 1998; Ruzin *et al*., 2001). Another member of SapI family, SapI3, encodes enterotoxin B and is thought to be mobilized and encapsulated by phage 29 (Novick, 2003). Certain bovine isolates of *S. aureus* carry SaPIbov1 which encodes toxic shock syndrome toxin (tst) and can be induced by three phages: 80α, ϕ11 and ϕ147 (Ubeda *et al*., 2005). Members of the SaPI family have been found in almost all strains of *S. aureus* sequenced so far (Kuroda *et al*., 2001; Baba *et al*., 2002; Diep *et al*., 2006).

In addition to SaPIs, *S. aureus* strains contain genomic islands from the νSa family. These islands carry genes coding for about half of the *S. aureus* toxins and virulence factors, and greatly contribute to the pathogenicity of this species (Gill *et al*., 2005). They are found in all sequenced strains in the same locations and some of the genes carried by them are highly conserved (Lindsay and Holden, 2004). They encode their own integrase and usually are spontaneously excised from the host chromosome (Baba *et al*., 2002). Members of this family of genomic islands include, but are not limited to, νSa1 (carrying enterotoxin genes seb, tsst, ear), νSa2 [containing genes encoding enterotoxin (sec) and toxic shock syndrome toxine (tsst)] (Gill *et al*., 2005). Other examples include νSaα and νSaβ, which are found in all sequenced isolates and have a defective transposase gene, and therefore are not excised from the chromosome. Additionally they carry a cassette encoding a restriction-modification system and genes encoding leukocidin (lukDE) (Baba *et al*., 2002).

2.1.7.2 Genomic island

Three families of genomic island exist among the *S.aureus* strains whose genome have been sequenced (Baba *et al*.,2002; Ito *et al*.,2003; Lindsay *et al*.,2004; Gill *et al*.,2005).These genomic islands are flanked by a broken transposase gene upstream and partial restriction-modification system type one downstream. Given the composition of genomic islands (remnant transposase genes and a G+C content that differs from the core genome), a current notion is that genomic islands were once mobile genetic elements acquired by horizontal gene transfer (Dobrindt *et al*., 2004)

2.1.7.3 Prophages

Prophages of *S. aureus* can be classified into three groups based on the size of their genome. Class I includes phages with genomes of less than 20 kb, class II has a genetic material of approximately 40 kb and class III of more than 125 kb (Kwan *et al*., 2005). Prophages are thought to play an important role in evolution and pathogenicity of *S. aureus* and very often offer means for the horizontal transfer of genetic information. Each of the *S. aureus* strains sequenced so far contains between one and three prophages, most of them carry virulence determinants exemplified by enterotoxins A, G, K, exfoliative toxin, staphylokinase and Panton-Valentine Leukocidin (Kuroda *et al*., 2001; Lindsay and Holden, 2004; Diep *et al*., 2006).

2.1.7.4 Insertion sequences and transposons

Insertion sequences (IS) carry at least one gene coding for a transposase which participates in the recombination required for transposition. Most IS elements also contain short inverted terminal repeats acting as transposase binding sites (Baba *et al*., 2004). Insertion elements are randomly

scattered throughout the genome of *S. aureus*, both in coding and non-coding regions. In MRSA, *S. aureus* N315 and Mu50 strains, eight copies of IS1181 have been found (Kuroda *et al*., 2001; Gill *et al*., 2005). Transposons are larger transposable genetic elements that, in addition to a transposase gene, carry other genes which very often are antibiotic resistance determinants. *Staphylococcusaureus* is the host to more than ten transposons, the majority of which carry antibiotic resistance genes (Baba *et al*., 2004).

2.1.7.5 Plasmids

Plasmids, defined as extrachromosomal genetic elements bearing only non-essential genes which, however, may provide a benefit to the host under special environmental conditions, often encode factors determining resistance to antibiotics or heavy metals, virulence factors and proteins facilitating survival in the presence of unusual nutrients (Wegrzyn, 2005). Plasmids of *S. aureus* have been categorized into three classes. Class I plasmids are of the size of 1–5 kb and occur in high copy number (15–50 per cell). They usually carry a single antibiotic resistance determinant. The class II plasmids are of intermediate size and occur in intermediate copy number, and they usually code for β-lactamase and confer resistance to inorganic ions. The last group of staphylococcal plasmids, class III, consists of large conjugative plasmids (40–60 kb). Class III plasmids carry multiple resistance determinants, exemplified by resistance to trimethoprim, gentamycin and ethidium bromide (Novick, 1989). The plasmids often can serve as means by which antibiotics resistance is transmitted. Moreover, the conjugative plasmids encode their own conjugative horizontal transfer mechanism by tra genes that offer an advantage by which transfer of extrachromosal genetic information to other bacteria occurs (Hartleib *et al*., 2000; Kuroda *et al*., 2001; Holden *et al*., 2004 ; Gill *et al*., 2005; Diep *et al*., 2006).

2.1.8 *Staphylococcusaureus* carriage

Staphylococcus aureus can be found on the skin and mucosal surfaces of human beings and animal species, and although it can be isolated from many body sites like the perineum, the axilla, throat and hairline, the anterior nares is the most consistent site from which the organism can be isolated (Kluytmans *et al*.,1997; Peacock,2006). This is demonstrated by a loss of carriage in other sites when the nose is decolonized using topical agents (Vandenbergh*et al*., 1999; Peacock, 2006).

Colonization is the presence, growth and multiplication of the organism in one or more body sites without observable clinical symptoms or immune reaction (Sa'di, 2010); colonized patients were considered as a chief source of *S. aureus* in hospital; approximately 10% to 40% of people on admission have nasal carriage of *S. aureus* (Eiff *et al.,* 2001; Sa'di., 2010).

A "carrier" refers to an individual who is colonized with MRSA. There are three patterns of carriage.

1- Persistent carriers: individuals always carry one type of strains, and those formed about 20% of the carriers and were more common in children than adults.
2- Intermittent carriers: this pattern confirms a large proportion of the population (60%) and the strains change with varying frequency.
3- Non carriers: people who almost never carry *S. aureus* and those are minorities of people (20%) (Kluytmans *et al*, 1997; Sa'di., 2010). Three *S. aureus* carriage patterns have been described in the healthy adult population, with approximately 20% of individuals being persistent *S. aureus* carriers, who are chronically colonized with the same strain; about 50% being intermittent or transient carriers, who are colonized with varying strains for short periods of time; and 20% persistent non-carrier. Colonization is a strong risk factor for

subsequent infection, although most persons colonized with the organism do not develop clinical disease. (Peacock, 2006; Albrich *et al* ., 2008)

The mean nasal carriage of *S. aureus* in the general population is 37.2%, however the range varies widely, probably due to the quality of sampling, culture techniques, and population sampled (Vandenbergh *et al*., 1999). Individuals who have been on admission tend to have a higher carriage, and it has been found to be particularly high amongst individuals with insulin-dependent diabetes mellitus, those on hemodialysis, those on continuous ambulatory peritoneal dialysis (CAPD), intravenous drug addicts, persons with *S. aureus* skin infections, and those with human immunodeficiency virus (HIV) infection or AIDS. Nose picking was also associated with increased *S.aureus* carriage (Wertheim, 2005). In view of environmental factors, crowding in households and hospitals, and the level of hygiene are associated with risk of carriage. Conditions in prisons, public housing projects, military barracks, and daycare centers are also known as associated risk factors for acquisition (Verbrugh, 2009).

2.1.9 Pathogenesis of *S. aureus*Infections

About 35-50% of normal adults carry *S .aureus* in the anterior nares, it is also part of the normal flora of the skin. Other sites of colonization include the perineum, axillae and vagina (Arora, 2006; Obajuluwa, 2014). *Staphylococcus aureus* carriers are at a higher risk of infection and they are presumed to be an important source of spread of *S. aureus* strains among individuals. The primary mode of transmission of *S. aureus* is by direct contact: usually skin-skin contact with a colonized or infected individual or contact with contaminated objects and surfaces (Miller and Kaplan, 2009). Various host factors including loss of the normal skin barrier, presence of underlying diseases such as diabetes and acquired Immunodeficiency syndrome or defects in neutrophils

function predispose to infection. *Staphylococcus aureus* can cause a range of illness from minor skin infections such as pimples, impetigo, boils (furnicles), cellulitis, folliculitis, carbuncles, scalded skin syndrome and abscesses, to life threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS) bacteremia and sepsis. Its incidence ranges from skin, soft tissue, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections and is often the cause of post-surgical wound infections (Bowersox, 1999, Obajuluwa, 2014). *Staphylococcus aureus* also causes a painful infection of joint fluid known as septic or infective arthritis. Most serious of all are the deep seated infections such as osteomyelitis and an infection of the heart valves called endocarditis and toxin mediated diseases such as gastroenteritis, Staphylococcal scalded skin syndrome (Yoke-Kqueen *et al.*, 2006; Miller and Kaplan, 2009; Anam *et al.*, 2010).

2.1.10 Epidemiology of *S. aureus*Infection.

The primary reservoir of Staphylococci is the nares, with colonization also occurring in the axillae, vagina, pharynx, and other skin surfaces. Nasal carriage in patient admitted to the hospital is common because close contact among patients and hospital personnel is not unusual; transfer of organisms often takes place. Increased colonization in patients and hospital workers frequently occurs in hospitals. Both hospital and community-acquired infections caused by drug resistant *S. aureus* has increased in the past 20 years (Sa'di, 2010).

In healthy individuals, the carrier rate of *S. aureus* range between 15% to 35% with a risk of 38% of individuals developing infection followed by a further 3% risk of infection when colonized with methicillin susceptible *Staphylococcus aureus* (MSSA) (File, 2008). Certain groups of individuals are more susceptible to *S. aureus* colonization than others including health-care workers, nursing

home inhabitants, prison inmates, military recruits and children (Ben-David *et al.*, 2008; Ho *et al.*, 2008). In a study, conducted in 2007 by the University of the Witwatersrand and the University Hospital of Geneva, health-care workers accounted for 93% of personnel to patient transmission of methicillin resistant *S. aureus* (MRSA) (Albrich and Harbath, 2008). Previously several outbreaks have been reported in Northern-Taiwan in 1997 that suggested MRSA transmission associated with health-care workers, including surgeons (Wang *et al.*, 2001). Grundmann and colleagues (2006) reported a prevalence of > 50% in countries such as Singapore (1993-1997), Japan (1999-2000) and Colombia (2001-2002) while countries with a prevalence of 25% to 50% included South Africa (1993-1997), Brazil (2001), Australia (2003), Mexico and United States. The lowest prevalence of less than 1% was found in Norway, Sweden and Iceland (1993-1997) (Grundmann *et al.*, 2006). In 2007, a prevalence of more than 50% of MRSA strains isolated from Cyprus, Egypt, Jordan and Malta was reported by Borg and colleagues (2007). This high prevalence was attributed to overcrowding and poor hygiene practice (Borg *et al.*, 2007).

2.1.11 Mechanism of Antimicrobial Resistance in *Staphylococcus aureus*

Staphylococcus aureus, a major cause of potentially life-threatening infections acquired in healthcare and community settings, has developed resistance to most classes of antimicrobial agents. Penicillin was the first choice of antibiotics to treat staphylococcal infection. In 1944, by destroying the penicillin by penicillinase (beta-lactamase), *S. aureus* become resistant to penicillin (Kirby, 1944). Most *S. aureus* strains (≥90%) are resistant to penicillin (Neu, 1992). *Staphylococcus aureus* can exemplify better than any other human pathogen the adaptive evolution of bacteria in the antibiotic era, as it has demonstrated a unique ability to quickly respond to each new antibiotic with the development of resistance mechanisms (Sofia *et al.*, 2013), which include:

2.1.11.1 Enzymatic inactivation of the antibiotic

Mechanism of resistance by enzyme inactivation involves the hyper production of an enzyme that inactivates the antibiotic by an enzymatic cleavage or chemical modification such that they no longer interact with the target site or are no longer taken up by the organism (Powell, 2000). The most common example of this type of resistance is that mediated by β latamases which are widely distributed in both Gram-negative and Gram positive species (Hawkey, 2000).

2.1.11.2 Inactivation of β-lactam antibiotics:

The antibiotics concerned are β-lactam ring of penicillins and cephalosporins. Staphylococci are the principal bacteria producing β-lactamase, and the genes which code for the enzymes are on plasmids that are transferred by transduction. In staphylococci the enzyme is inducible, its synthesis is at a very low level in the absence of the drug. The enzyme may diffuse through the envelope and inactivate antibiotic molecules in the surrounding medium. Gram negative organisms can also produce β-lactamases, which are a significant factor in their resistance to the semisynthetic broad spectrum β-lactam antibiotics. Here the enzyme may be determined by either chromosomal genes or by plasmid genes (Obajuluwa, 2014). The enzymes are produced constitutively (i.e. they are synthesized even when the substrate is absent) and remain attached to sites in the cell wall preventing access of the drug to the membrane associated target site; they do not inactivate the drug in the surrounding medium. Many of these β-lactamases are encoded by transposons, some of which may also carry resistance determinants to several other antibiotics. Chloramphenicol inactivation is brought about by chloramphenicol acetyl transferase while inactivation of aminoglycosides may be brought about by phosphorylation, adenylation or

acetylation, and the requisite enzymes have been found in both Gram-negative and Gram-positive organisms. The resistance genes are carried on plasmids and transposons (Obajuluwa, 2014).

2.1.11.3 Alteration of the target with decreased affinity for the antibiotic

The protein on the 30S subunit of the ribosome, which is the binding site for aminoglycosides, may be altered as a result of a chromosomal mutation. A plasmid mediated alteration of the binding site protein on the 50S subunit underlies resistance to erythromycin. Some staphylococci carry an altered penicillin binding protein (PBP2a) which is coded for by a mutated chromosomal gene; it has much lower affinity for penicillins and it confers intrinsic resistance (Powell, 2000). Vancomycin resistance is another example. Under susceptible conditions, vancomycin prevents cross linking of peptidoglycan by binding to D-Ala-D-Ala dipeptide of the muramyl peptide. Most Gram positive bacteria acquire vancomycin resistance by changing D-Ala-D-Ala to D-Ala-D-lactate, which vancomycin does not bind to (Bugg *et al*., 1991). Mutations in DNA gyrase A and B subunits in quinolone resistance is another example of an alteration of the drug target. Finally, in Rifampicin resistance, there are mutations in rpoBgene encoding beta-subunit of RNA polymerase.

2.1.11.4 Increased Efflux Activity (Efflux pumps)

Efflux pumps are membrane proteins that have the function of detoxifying cells by expelling noxious molecules (Paulsen *et al*., 1996). In *staphylococcus aureus*, several specific efflux pumps have been associated with resistance to antibiotics, such as tetracycline (TetK, TetL) and macrolides (MefA, MsrA) (Poole, 2007). Also, several multidrug efflux pumps have been described that are associated with resistance to antibiotics (e.g., fluoroquinolones) (Poole, 2007). In general, specific efflux pumps can be found either in the chromosome or in plasmids, while multidrug efflux pumps are mainly located in the chromosome, (Sofia *et al*., 2013). An important

example of decreased drug accumulating is the plasmid-mediated resistance to tetracycline in both gram positive and gram negative bacteria. The resistance genes in the plasmid code for inducible resistance proteins in the membrane which promote energy dependent efflux of the tetracycline and hence resistance. This has reduced the value of tetracycline in human and veterinary medicine. Tetracycline efflux was discovered in the early 1980s; tetK serves as an example for an efflux - mediated tetracycline resistance. Under normal conditions, the efflux gene, tetK, is not expressed, due to a suppressor that is bound to the promoter region. However, in the presence of tetracycline, it binds to the repressor, relieves the suppression, and causes transcription and translation of the efflux pump, thereby leading to tetracycline resistance (Krzysztof *et al*., 2000).

2.1.12 Identification of *S. aureus*

2.1.12.1 Coagulase test

There are several methods for testing for the production of coagulase in *S. aureus.* These include:

Tube coagulase test: This tests for free coagulase, and is the standard test for routine identification of *S. aureus* (Brown *et al*.,2005).Free coagulase is secreted extracellularly, and when a suspension of coagulase producing organisms is prepared in plasma in a test-tube, it reacts with a substance in plasma called coagulase-reacting factor (CRF) to form a complex, which in turn, reacts with fibrinogen to form fibrin, all in the absence of calcium (Que and Moreillon, 2010). The recommended medium for the procedure is rabbit plasma, and the tube is examined after 4 hours of incubation at 35^0C, if no clot is observed, the tube is reincubated at room temperature and examined again after 18 - 24 hours. Some species of staphylococci, though not commonly found in human isolates could also be tube coagulase positive. These could be easily differentiated from *S.*

aureus as they do not ferment Mannitol. In addition, some *S. aureus* strains could be tube coagulase negative (Winn *et al.,*2006; Que and Moreillon,2010).

2.1.12.2 Rapid slide agglutination test

This detects the bound coagulase or clumping factor. This is bound to the cell wall and reacts directly with fibrinogen. Up to 15% of *S. aureus* strains are usually negative (Brown *et al*., 2005).

2.1.12.3 Latex agglutination test:

These use latex beads coated with plasma or fibrinogen to detect clumping factor on the surface of *S. aureus* cells (Brown *et al*., 2005).

2.1.12.4 Passive haemagglutination test:

This uses sheep red blood cells that are sensitized with fibrinogen to detect clumping factor on the surface of *S. aureus* cells (Brown *et al*., 2005).

2.1.12.5 Deoxyribonuclease (DNAse) test

Staphylococcus aureus produces both deoxyribonuclease (DNAse) and a thermostable nuclease, which hydrolyze nucleic acid. Deoxyribonuclease (DNase) media plates can also be used to screen isolates to identify *S. aureus,* but positives require additional confirmation, as some rare coagulase negative Staphylococci also produce deoxyribonuclease and the heat stable nuclease (Que and Moreillon,2010).

2.1.12.6 Mannitol fermentation

Staphylococcus aureus unlike the coagulase negative staphylococci (CoNS) is able to ferment Mannitol and produce acid. This property is exploited in epidemiological studies in screening nasal

carriers of *S. aureus.* The medium used is Mannitol Salts Agar, which contains Mannitol, NaCl, phenol red and peptones. It selectively recovers staphylococci, and *S. aureus* colonies appear yellow due to acid production from Mannitol fermentation. Mannitol positive organisms recovered on this medium has to be subcultured on to blood agar and checked for coagulase, because some other infrequently isolated staphylococcal species may also produce acid from Mannitol (Que and Moreillon,2010).

2.1.12.7 Other methods for the identification of S. aureus

Enzymes unique to *S. aureus* have been exploited as a way of identifying *S. aureus*. *S. aureus* endo-β-*N*-acetylglucosaminidase (SaG) and protein A have been used in an enzyme immunoassay to identify *S. aureus*. The test showed 100% sensitivity and specificity and was found to be helpful in identifying strains with detectable clumping factor (Que and Moreillon,2010).

Several commercial kits are available for the identification of staphylococci, all of which use modified carbohydrate fermentation tests, adaptation of standard bacteriologic identification tests (e.g., nitrate reduction, urease, Voges-Proskauer), and chromogenic enzyme substrate tests for organism identification. They come in different formats like strip with small cupulae, microtiter trays, plastic cards, etc. Some of such systems include RapiDEC staph, API Staph (an 18-24 hr identification system for both micrococci and staphylococci) (Winn *et al*., 2006) the Vitek GPI card (designed for use with the automated Vitek bacterial identification/susceptibility testing system) for identification of gram positive organisms including 11 human staphylococcal species.Others include the BBL Crystal GP identification system, the Microbact Staphylococcal12S, the Microbial Identification System (MIS), and the Biolog Microplate Identification System etc (Winn *et al*., 2006)

Molecular biology techniques such as DNA probe methods and PCR based methods have also been used to identify *S. aureus.* A range of primers and probes have been developed which amplify and detect species-specific targets, such as the nuclease (*nuc*), coagulase (*coa*), protein A (*spa*), *femA* and *femB*, *Sa442*, 16S rRNA and surface-associated fibrinogen-binding protein genes (Brown *et al.,*2005 ; Que and Moreillon,2010).

2.2 Antibiotics

2.2.1 Definition

Antibiotics are hailed as the greatest medicinal triumph of the 20th Century. Before their discovery, there was little available to help a patient once they had become infected. The earlier development of vaccination had introduced immunity to some diseases and sterilization had helped to reduce the chance of infection from surgery. With the subsequent formation of germ theory and the work identifying the role of specific bacteria in the diseases anthrax and tuberculosis, the search for a cure began (Chakraborty *et al*., 2012). In 1929, nearly 250 years after van Leuwenhoek first discovered bacteria, Fleming noted that the growths of bacteria could be inhibited by the presence of a mould, *Penicilium notatum.* This effect was caused by a metabolic product from the mould that was interacting with the staphylococcal culture (Chakraborty *et al*., 2012). Penicillin was the first of the family of β-lactam antibacterial that now form the largest share of the antibacterial market.

An antibiotic is an agent that inhibits bacterial growth or kills bacteria. The term antibiotic is used as a synonym for antibacterial used to treat bacterial infections in both people and animals (WHO, 2011). Any substance of natural, synthetic or semisynthetic origin which at low concentrations kills or inhibits the growth of microorganisms but causes little or no host damage (WHO,

2000).Today, however, with increased knowledge of the causative agents of various infectious diseases, antibiotic(s) has come to denote a broader range of antimicrobial compounds, including anti-fungal and other compounds. The term antibiotic was first used in 1942 by Selman Waksman and his collaborators in journal articles to describe any substance produce by a microorganism that is antagonistic to the growth of other microorganisms in high dilution (Waksman, 1947; Chakraborty *et al*., 2012; Obajuluwa, 2014).

2.2.2 Beta-Lactam antibiotics

β-Lactam antibiotics include penicillins, cephalosporins, and penicillinase-insensitive β-Lactams such as methicillin and oxacillin. β-Lactams are bactericidal, cell wall-active agents that target the transpeptidation step of the peptidoglycan synthesis. This is achieved by binding and inactivating the transpeptidase domain of PBPs in the cell wall (Chambers, 2004; Obajuluwa, 2014). β-Lactams are structural analogs of the natural substrate of PBPs, d-alanyl-d-alanine of the peptidoglycan stem peptide.

A. Penicillins and mecillinams

The penicillins can be categorized into the following:-

1. Naturally occurring, for example those produced by fermentation of moulds such as *Penicillin notatum* and *P. chrysogenum* e.g. benzyl penicillin (penicillin G and phenoxymethyl pencillin G) and phenoxymylpencillin (pencillin V).

2. Semisynthetic: In 1959, the penicillin nucleus: 6- aminopenicillanic acid (6APA) was isolated in which a thiazolidine is attached to a -lactam ring that carries a free amino group that can be split

by bacterial and other amidases (Russell, 2004; Brooks *et al*., 2004; Obajuluwa, 2014). Acylation of 6-APA with appropriate substances resulted in the production of new penicillins with distinct pharmacological activities. The mecillinams represented by mecillinam and pirmecillinam are essentially 6- amidino-pencillins with considerable activity against Gram-negative organisms (Russell, 2004; Obajuluwa, 2014)

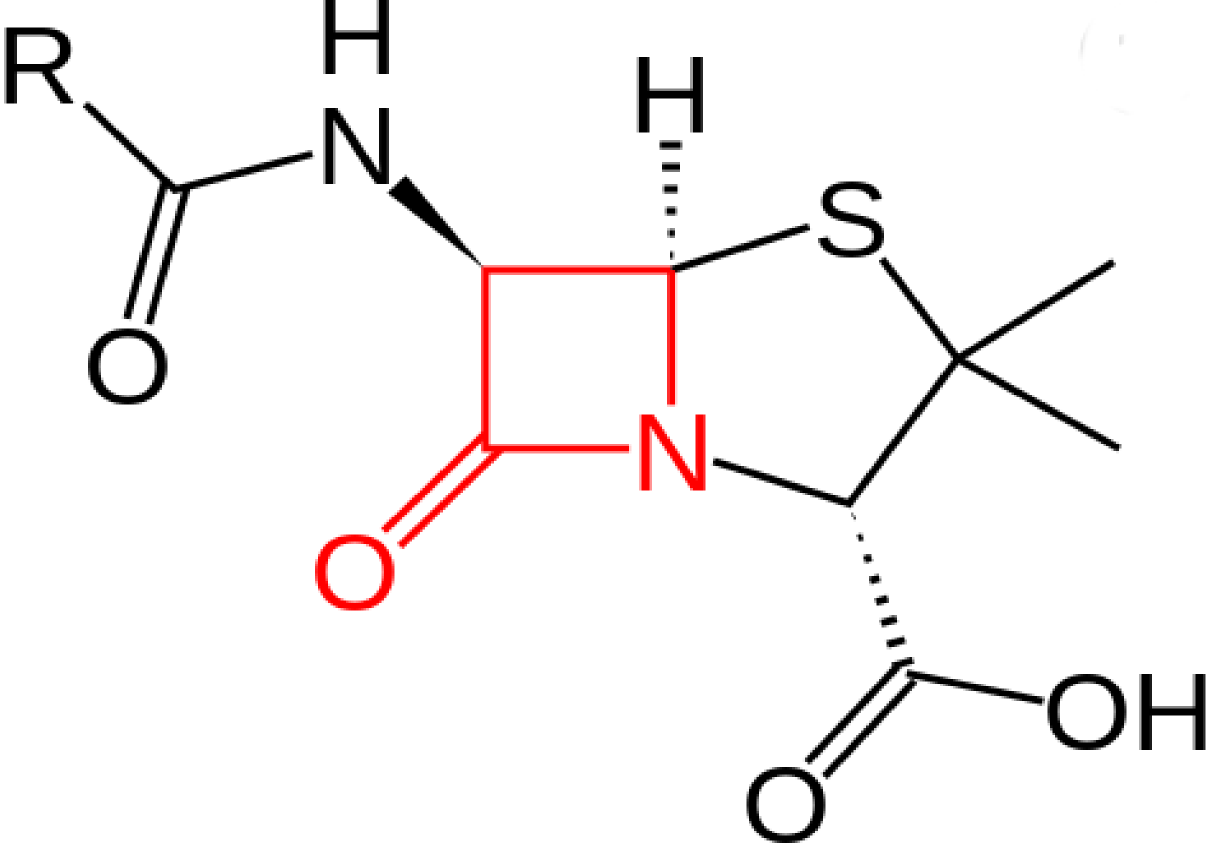


Figure 2.2: Structure of beta- lactam ring (in red).

Source: Natalia and Frank (2010).

B. Cephalosporins:

The cephalosporins are a group of beta-lactam compounds whose discovery dated back to the 1950‘s when a species of *Cephalosporium* (now *Acremonium*) was isolated near a sewage outfall off the Sardinian coast (Obajuluwa,2014). A study of this isolate revealed the production of these antibiotics:

1. An acidic antibiotic called cephahasporin P, (which is now known to have a steroid- like structure).

2. Another acidic antibiotic, called cephalosporin N which was later shown to be a penicillin since its structure was based on 6-APA.

3. Cephalosporin C, which was obtained during the purification of cephalosporin N, this is a true cephalosporin and from it 7-aminocephalosporanic acid has been obtained for new cephalosporin. Natural cephalosporins have low antibacterial activity but the introduction of various substituents have resulted in cephalosporin with varying pharmacologic properties, antimicrobial spectra and activity (Rusell, 2004, Brooks *et al*., 2004; Obajuluwa ,2014).

There are five generations of cephalosporin.These include:

a. First generation cephalosporins include cefadroxil, cefazolin cephalothin, cephalaxin

b. Second generation cephalosporin: cephaclor, cephamendole, cefoxitin, cefproxil, cefuroxime.

c. Third generation cephalosporin include cefixime, cefdimir cefotaxime, ceftazidime ceftriaxone.

d. Fourth generation cephalosporin include cefepime.

e. Fifth generation cephalosporin include ceftaroline fosamil and ceftobiprole

C. Clavams

The clavams are structurally similar to the penicillins with two major distinguishing factors. The sulphur in penicillin‘s thiazolidine ring is replaced with oxygen in the clavam oxazolidine ring. Secondly there is no side chain at position 6(Russell, 2004). Clavulanic acid, a naturally occurring clavam isolated from *Streptomyces clavuligerus* has poor antibacterial activity but is a potent inhibitor of staphylococcal -lactamase and most -lactamase produced by Gram-negative bacteria especially those with a penicillinase rather than cephalosporinase type of enzyme action (Russell, 2004). A formulation of clavulanic acid and amoxicillin (a broad spectrum but -lactamase susceptible penicillin) was introduced into clinical practice in 1981 with remarkable result (Rolinson, 1991). Combination of clavulanic acid with another -lactamase susceptible penicillin, ticarcillin extended its spectrum of activity to include *Pseudomonas aeruginosa* (Rolinson, 1998; Russell, 2004)

D. I-Carbapenems

The I-carbapenems are a family of fused -Lactam antibiotics. They are structural analogues of penicillins or clavams in which the sulphur (penicillin) or oxygen (clavam) is replaced by carbon, examples are imipenem, Doripenem, Meropenem, thienamycin (Russell, 2004).

E. Monobactams

The mono bactams are a group of monocyclic -lactam antibiotics produced by various strains of bacteria. The nucleus 3-aminomono bactamic acid (3-AMA) has been produced from naturally occurring monobactams and 6-APA. Several monobactams have been tested for antibacterial

activity and aztreonam was found to be highly active against most Gram negative bacteria (Brooks *et al*., 2004) and stable to most types of lactamases.

F. Penicillanic acid derivatives

Penicillanic acid derivatives are synthetically produced -lactamase inhibitors. Penicillanic acid sulphine (sodium salt) inhibits staphylococcal-lactamases but not all lactamases produced by Gram negative bacteria being less potent than clavulanic acid. Other examples of penicillanic acid derivatives include: bromopenicillanic acid which inhibits lactamases; tazobactam which is a penicillinanic acid sulphone derivative with comparable lactamase inhibitory activity to clavulanic acid. Tazobactam is marketed in combination with piperacillin. Sulbactam is semi-synthetic 6-desamino penicillin sulphone with structural similarity to tazobactam. Sulbactam is a potent inhibitor of lactamase it also has antibacterial activity against Gram negative organisms (Russell, 2004)

2.2.2.1 Mode of action of beta - lactam antibiotics

β-Lactams are bactericidal, cell wall-active agents that target the transpeptidation step of the peptidoglycan synthesis. This is achieved by binding and inactivating the transpeptidase domain of PBPs in the cell wall (Chambers, 2004). β-Lactams are structural analogs of the natural substrate of PBPs, d-alanyl-d-alanine of the peptidoglycan stem peptide. The peptidoglycan layer is important for cell wall structural integrity, especially in Gram-positive organisms, being the outermost and primary component of the wall. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by D-alanyl-D-alanine transpeptidases which are penicillin-binding proteins (PBPs). Penicillin binding proteins vary in their affinity for binding penicillin or other β-lactam antibiotics. The amount of PBPs varies among bacterial species. The reaction between PBP

and a β-lactam antibiotic begins with a non-covalent association between these two molecules. This intermediate can either dissociate or undergo an irreversible reaction of acylation, when the PBP covalently binds the antibiotic at its active site, cleaving the cyclic amide bond in the β-lactam ring. The natural substrate for PBP, d-alanyl-d-alanine, undergoes quick deacylation by hydrolysis that liberates the PBP for a next round of transpeptidation. However, when the substrate is a β-lactam antibiotic, the deacetylation process is very slow and the PBP is effectively inactivated. Without functional PBPs, the cell wall synthesis is inhibited and cell death occurs (Chambers, 2003). The features of β-lactams which make them attractive antimicrobial agents stem from the fact that their targets are easily accessible (localized outside of the cytoplasm membrane) and that they are specific to bacteria (lacking functional and structural equivalents in the human organism) (Wilke *et al*., 2005). If the synthesis of peptidoglycan is blocked selectively by antibiotic action the bacteria undergo a number of changes in shape and ultimately die following disruption (lysis) of the cells. Mammalian cells do not possess a cell wall and contain no other macromolecular structures resembling peptidoglycan. Consequently antibiotics which interfere with peptidoglycan have a good selective toxicity (Russell, 2004).

2.3 Mobile Genetic Elements of *Staphycoloccus aureus*

2.3.1 Definition

Mobile genetic elements (MGEs) were first described in the maize genome in the late 1940s (McClintock 1950 and McClintock 1951; Natalia and Frank, 2010) and are an important means for transfer of genetic information among prokaryotes and eukaryotes. Mobile genetic elements (MGEs) are typically identified as fragments of DNA that encode a variety of virulence and resistance determinants as well as the enzymes that mediate their own transfer and integration into

new host DNA (Frost *et al*., 2005). Mobile genetic elements demonstrate intracellular and intercellular mobility, and those within one particular cell are called a mobilome.

Among staphylococci, *S. aureus* is the most invasive species and an etiological agent of diverse human and animal maladies, including skin infections, abscesses, food poisoning, toxic shock syndrome, septicaemia, endocarditis, and pneumonia (Weems, 2001; Van Belkum, 2006; DeLeo and Chambers, 2009). Numerous putative and proven virulence factors, gene responsible directly for host adaptation and toxins, are located on *S. aureus* MGEs (Kuroda *et al*., 2001; Lindsay and Holden, 2004; Diep *et al*., 2006; Baba *et al*., 2008; Holden *et al*., 2010). *Staphylococcus aureus* contains many types of MGEs including Plasmids, Transposons, (TN), Insertion sequences (IS), Bacteriophages, Pathogenicity islands, and Staphylococcal cassette chromosomes. It is remarkable that most genes encoded by MGEs remain under the control of global regulators located within the core genome.

2.3.2 Acquisition of MGEs by *S. aureus*

This involves one or more of the following steps.

1. Incorporation of plasmids or plasmid elements into genomic DNA
2. Plasmids can be maintained as free circular DNA
3. Suicide plasmid
4. Transfer of a transposon or an insertion sequence between plasmid and genomic DNA
5. Transfer of a transposon or an insertion sequence between plasmids within the cell
6. Transfer of a transposon or an insertion sequence from genomic DNA to another plasmid

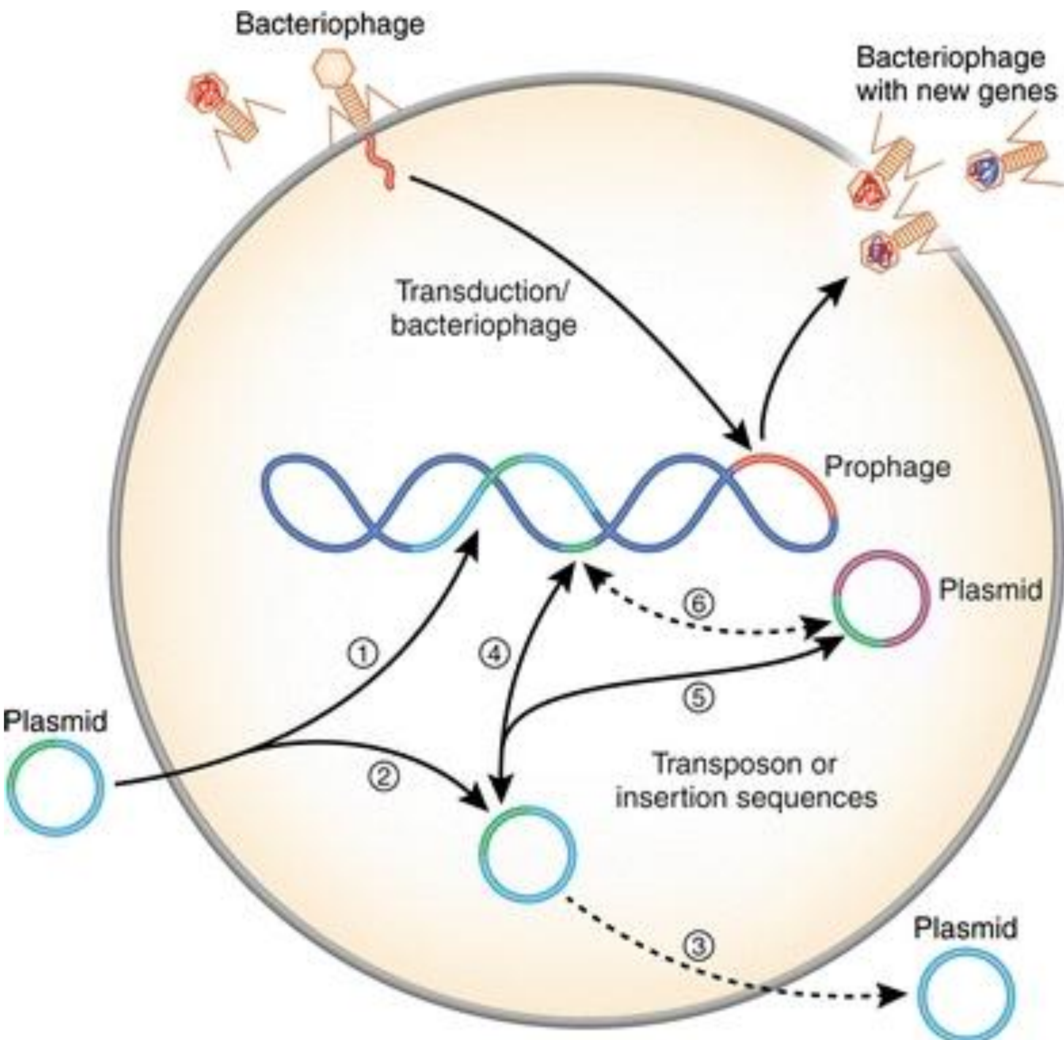


Figure2.3: Acquisition of MGEs by *S. aureus.*

Incorporation of plasmids or plasmid elements into genomic DNA. *2* Plasmids can be maintained as free circular DNA. *3* Suicide plasmid. *4* Transfer of a transposon or an insertion sequence between plasmid and genomic DNA. *5* Transfer of a transposon or an insertion sequence between plasmids within the cell. *6* Transfer of a transposon or an insertion sequence from genomic DNA to another plasmid.

Source: Natalia and Frank (2010).

As a consequence of the limited ability of *S. aureus* to acquire DNA from the environment (low natural competence) compared to bacteria such as *Escherichia coli* and *Bacillus subtilis* most of the intercellular transfer of staphylococcal plasmids occurs by transduction or conjugation (Morikawa *et al*., 2003).

2.3.4 Plasmid – encoded antibiotic resistance

Plasmids are auto-replicating DNA molecules. Staphylococci typically carry one or more plasmids per cell and these plasmids have varied gene content. Staphylococcal plasmids can be classified into one of the three following groups:

(1) Small multicopy plasmids that are cryptic or carry a single resistance determinant (Malachowa and DeLeo, 2010)

(2) Large (15 – 30 kb) low copy (4-6/cell) plasmids, which usually carry several resistance determinants: and

(3) Conjugative multiresistance plasmids (Berg *et al*., 1998).

2.3.4.1 Plasmid encoded Penicillin resistance:

Penicilin was the first antibiotic mass produced for use in humans. Although initially highly effective for treatment of *S. aureus* infection, today over 90% of human *S. aureus* strains are resistant to this antibiotic (Olsen *et al*., 2006). Penicillin resistance is conferred by β - lactamase, which hydrolyzes the β – lactam ring of penicillin there by inactivating the antibiotic, and or production of a low – affinity penicillin – binding protein(PBP2a) encoded by the *mecA* gene (Hackbath and Chambers, 1993; Olsen *et al*.,2006; Chambers and DeLeo, 2009). In *S. aureus*, β -

lactamase is encoded by the blaZ gene and the closely linked regulators genes, blaI and blaR. Aside from plasmid encoded β – lactamase*, bla* genes may be located on transposons or within chromosomal DNA (Sidhu *et al*., 2002; Olsen *et al*., 2006).

2.4.3.2 Plasmid encoded Vancomycin resistance

Staphylococcus aureus was found to acquire vancomycin resistance elements from enterococci, resulting in the emergence of vancomycin-resistance *S. aureus* (VRSA) (Wiegel *et al*., 2003; Zhu *et al*., 2008). Tn1546 encodes the vancomycin resistance gene cluster within a conjugative plasmid. This MGE was most likely transferred to methicillin – resistance *S. aureus* (MRSA) from vancomycin – resistant enterococci (VRE) during co –infection (Weigel *et al.,* 2003; Ballard *et al*., 2005; Zhu *et al*., 2008; Lindsay, 2010). There are two predicted fates of the enterococcal plasmid upon entering staphylococci: On one hand, the enterococcal plasmid could simply be maintained (Zhu *et al*., 2008; Perichon and Courvalin, 2009), alternatively, Tn1546 could be incorporated into a staphylococcal plasmid in which case the original enterococcal plasmid functions as a suicide vector (Zhu *et al*., 2008; Perichon and Courvalin, 2009). Transposon Tn1546 encodes the vanA operon, which consists of vanA, vanH, vanX, vanS, vanR, vanY, and vanZ (Weigel *et al*., 2003; Saha *et al*., 2008).

2.4 Staphylococcal Cassette Chromosome

This is a mobile genetic element that carries the central determinant for broad-spectrum beta-lactam resistance encoded by the *mecA* gene. Staphylococcal Cassette Chromosome(SCCmec) is a 21–67-kb genetic element that is found in the chromosome of methicillin-resistant *S. aureus* at a unique site designated attBscc, located near the origin of replication.The emergence of methicillin

resistant staphylococcal lineages is due to the acquisition and insertion of the SCCmec element into the chromosome of susceptible strains. The integration site of SCCmec, attBscc, contains a 15-bp sequence that, when SCCmec is inserted in the chromosome, is found at both chromosome SCCmec junctions (attL and attR). However, unlike the direct repeats found in transposons, which are created by target duplication, one of the two repeat sequences is located within SCCmec at its right end. Degenerate inverted repeats are also present at both ends of SCCmec (Ito *et al.*, 2001; Noto *et al.*, 2008). These incomplete inverted repeats are thought to be recognized by SCCmec-specific recombinase during excision and integration of this element from and to chromosome (Noto *et al.*, 2008).

Staphylococcal Cassette Chromosome elements are classified into types by the combination of the type of ccr gene complex and the class of mec gene complex. Eleven SCCmec elements are reported to Date: SCCmec I to XI (Ito, 2009; Li *et al;* 2011; Shore *et al*; 2011; Ito *et al;* 2012). Staphylococcal Cassette Chromosome elements are highly diverse in their structural organization and genetic content and have been classified into types and subtypes. The SCCmec is comprised of:

(1) mec gene complex containing the *mecA* gene, its regulatory genes and associated insertion sequences, and

(2) Cassette chromosome recombinase (ccr) gene complex (Ito, 2009; Ito *et al.*, 2012).

Six classes of mec gene complexes (A, B, C1, C2, D and E) and three ccr genes (ccrA, ccrB and ccrC) for integration and excision of the SCCmec element have been reported so far (www.sccmec.org). The variable regions of SCCmec, called J-region, contain integrated genetic

elements such as plasmids (pT181, pUB110 and p1258), transposons (Tn554) and insertion sequences (IS431, IS1272 and IS256) (Hanssen & Ericson Sollid, 2006).

The hallmark of SCCmec is the mec operon that consists of *mecA* and its regulatory genes *mecI* and mecR1. The operon is found in several variants as a component of different SCCmec elements. The variants of the mec operon are divided into two main categories: those with both *mecI* and *mecR1* genes intact and those with portions of one or both of these regulatory genes deleted. The first group of the mec complex is known as class A*mec* operon while the latter have been categorized as classes B, C, D, E. All classes of the mec operon include a copy of IS431 associated with the *mecA* gene and therefore designated IS431mec. Classes B–E contains deletions of *mecI* that may be extended to part of the mecR1 gene. Usually these deletions coincide with insertion of IS elements. Moreover, the ccr locus of SCCmec elements also exists in several variants (Komatsuzawa *et al.,* 1994; Katayama *et al*., 2001).

Among these, SCCmec types I–V are the most commonly reported (Figure 2.4). SCCmec types I–III are usually carried by hospital- associated methicillin resistant *S. aureus*

(HA-MRSA) while types IV and V are usually carried by community-associated (CA) MRSA.

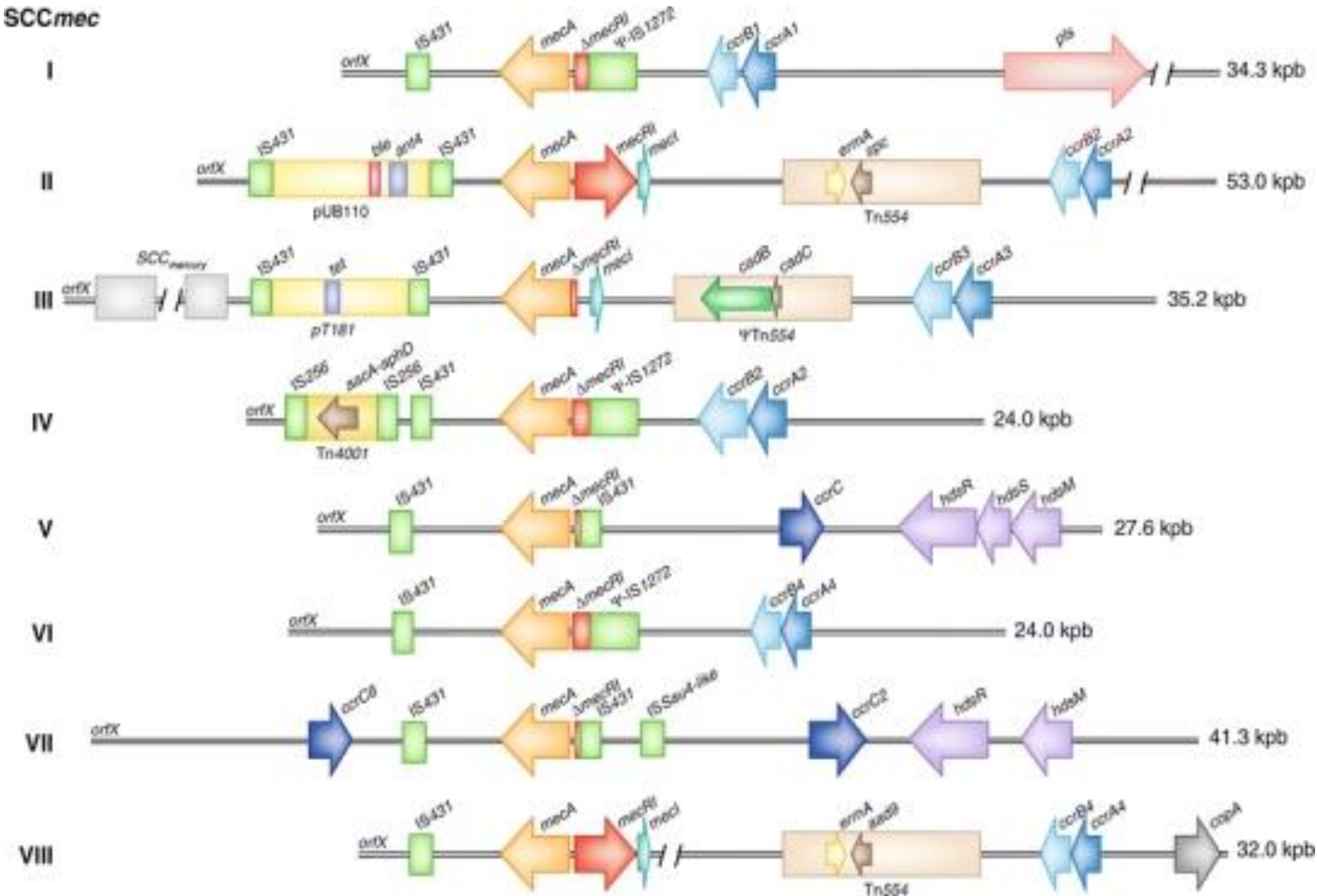


Figure2.4: Comparison of *S. aureus* SCCmec types. ClassA SCCmec contains a complete *mecA* regulon (mec1-mecR1-mecA). Class B and class C SCCmec contain regulatory genes that are disrupted by IS, IS1272ΔmecR1-mecA and IS431-ΔmecR1-mecA, respectively. Tn554 encodes erythromycin (ermA) and streptomycin/spectinomycin resistance (aad9 or spc); copA encodes a putative copper-transport ATPase; hsdR, hsdM, and hsdS encode a partial restriction-modification system (RM) type I; Tn4001 encodes an aminoglycoside resistance operon (aacA-aphD); plasmid pT181 encodes tetracycline resistance (tet); ΨTn554 encodes cadmium resistance (cadB, cadC); and plasmid pUB110 encodes bleomycin (ble) and tobramycin resistance (ant4′). pls Plasmin-sensitive surface protein

Source: Natalia and Frank (2010).

2.5 Methicillin

2.5.1 History of Methicillin

Methicillin belongs to the group of semi-synthetic penicillin's introduced following the development ofresistance to the natural penicillins due to β-lactamase/penicillinase production by bacteria (β-lactamase is an enzyme that hydrolyzes the central β-lactam ring of penicillins, making them ineffective). This group of penicillins (semi-synthetic penicillins) is β-lactamase resistant due to the presence of the *ortho*-dimethoxyphenyl side chain directly attached to the carbonyl group of the penicillin nucleus (figure 2.5). They include methicillin (figureb2.6), oxacillin, nafcillin, cloxacillin, dicloxacillin and flucloxacillin. Methicillin –resistant *Staphylococcus aureus* was first noted in 1961 (Jevons, 1961; Enright *et al.,* 2002) about two years after methicillin was initially used to treat *S. aureus* and other bacterial pathogens. Methicillin-resistant *staphylococcus aureus* (MRSA) has now reached epidemic levels worldwide. Infections caused by MRSA result in increased lengths of hospital stay, increased health care costs, and increased morbidity and mortality (Cosgrove *et al*., 2005; Zahar *et al.,*2005) compared to those caused by methicillin-sensitive strains.

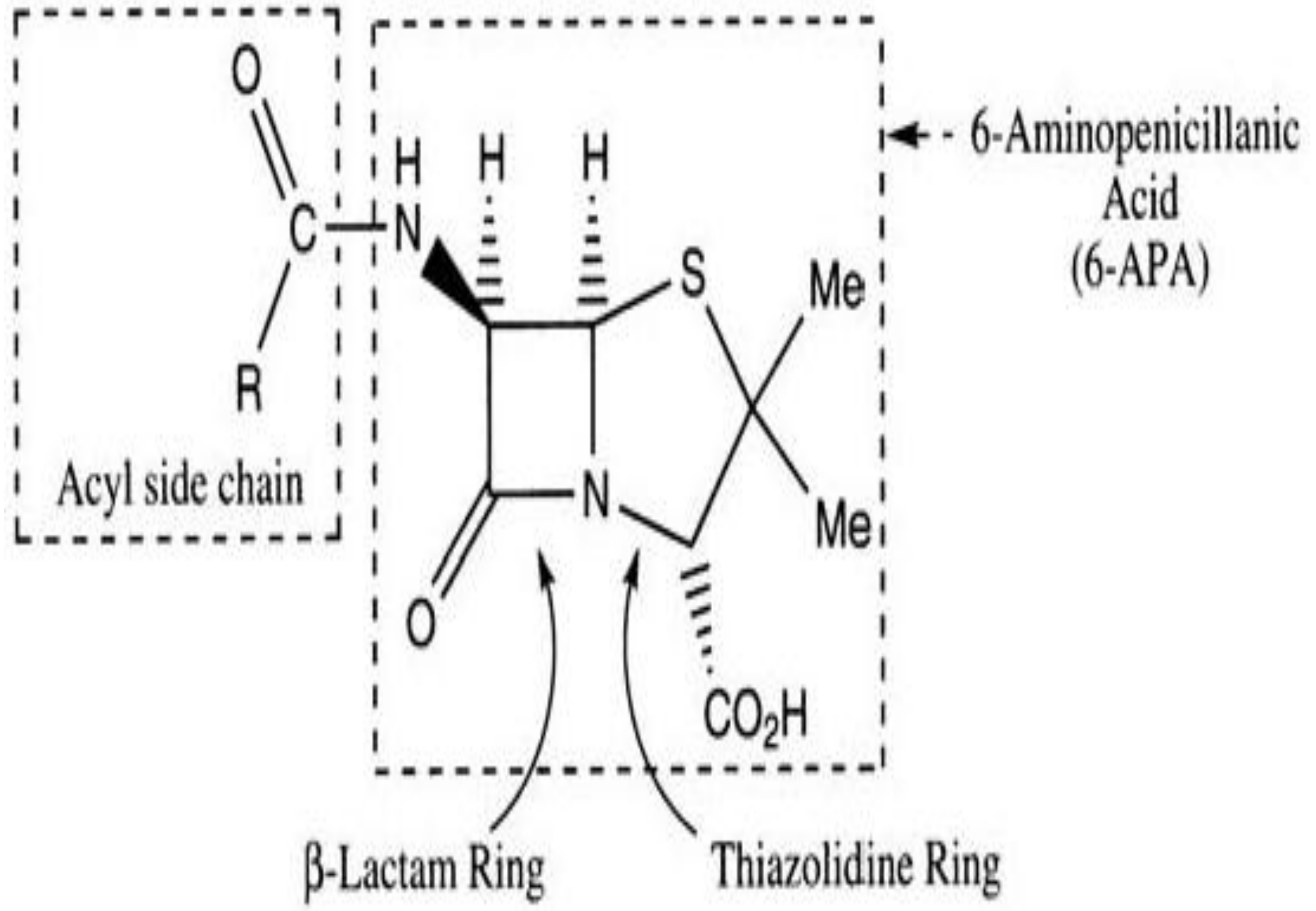


Figure 2.5: The primary structure of the penicillins (the nucleus).

Source: Natalia and Frank (2010).

Figure 2.6: The structure of Methicillin.

Source: Natalia and Frank (2010).

2.5.2 Methicillin-Resistant *S.aureus* (MRSA)

Methicillin-resistant *S.aureus* (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans. It is also called oxacillin-resistant *Staphylococcus aureus* (ORSA) (McDougal *et al.,* 2003). Methicillin-resistant *S.aureus* is any strain of *S.aureus* that has developed, through the process of natural selection, resistance to beta-lactam antibiotics, which include the penicillins, methicillin, oxacillin, nafcillin, cloxacillin, dicloxacillin , flucloxacillin and the cephalosporins (except for the fifth generation) (Morell, 2010). Strains unable to resist these antibiotics are classified as methicillin-susceptible *Staphylococcus aureus* (MSSA). The evolution of such resistance does not cause the organism to be more intrinsically virulent than strains of *S. 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).

Methicillin-resistant *S.aureus* is especially troublesome in hospitals, prisons, and nursing homes, where patients with open wounds, invasive devices, and weakened immune systems are at greater risk of nosocomial infection than the general public. Methicillin-resistant *S.aureus* began as a hospital-acquired infection, but has developed limited endemic status and is now sometimes community-acquired and livestock-acquired. In 1961, the first isolate of MRSA was reported in England (Jevons, 1961). Since then, MRSA has increasingly been isolated in various countries, and at present, it is one of the major causes of nosocomial infection throughout the world. The organism is often sub-categorized as community-acquired (Community-associated) MRSA (CA-MRSA) or hospital-associated (Health care-associated) MRSA (HA-MRSA) although this distinction is complex (Leonard and Markey, 2008).

2.5.2.1 Hospital or healthcare associated MRSA (HA-MRSA)

Hospital acquired-Methicillin resistant *Staphylococcus aureus* is typically defined as MRSA isolated from in patients that had been MRSA-negative at the beginning of hospitalization or MRSA isolated from in patients 48 hours or more after hospitalization (Brumfitt and Hamilton, 1989; Tacconelli *et al.,* 1998; Naimi *et al*., 2003; Vandenesch *et al*., 2003). Healthcare associated MRSA (HA-MRSA) was associated with traditional risk factors like recent hospitalization, or surgery, living in nursing home, carrying an indwelling catheter or device, previous antibiotic exposure, admission to an intensive care unit and exposure to an MRSA colonized patient. It was also found to be commoner in the older age group (Naimi *et al* .,2003). It produced mainly hospital-related bacteremia and pneumonia. It is usually multi resistant and clonal.

2.5.2.2 Community-Associated MRSA (CA-MRSA)

Community acquired-Methucillin resistant *Staphylococcus aureus* has been defined by criteria related to the patients suffering from an MRSA infection while other authors have defined CA-MRSA by genetic characteristics of the bacteria themselves (Okuma *et al*., 2002).Community acquired-MRSA strains were first reported in the late 1990s; these cases were defined by a lack of exposure to the health care setting. In the next several years, it became clear that CA-MRSA infections were caused by strains of MRSA that differed from the older and better studied health care-associated strains (Okuma *et al*., 2002).Community acquired-MRSA infections occur in otherwise healthy people without a recent history of hospitalization or clinical presentation, and are usually associated with skin and soft tissue infection. Risk factors for CAMRSA includes crowding, frequent contact, compromised skin, contaminated surfaces and shared items, and poor hygiene and found in populations such as high schools, colleges, prison , among men who have sex

with men, military personnel, refugees and children in daycare centers. It produced primarily skin and soft tissue infections such as necrotizing fasciitis, abscesses and fulminant, necrotizing pneumonia (Gordon and Lowy, 2008).Community acquired-MRSA is not multi resistant, but has been associated with an enhanced virulence than HA-MRSA.Community acquired-MRSA was found to be associated more with the PVL toxin than HA-MRSA, and contained mainly the SCC*mec* type IV (Naimi *et al*., 2003; Que and Moreillon, 2010).

2.6 Prevalence of MRSA

Great geographic variations exist in the worldwide prevalence of MRSA, ranging from as low as 5.7% in places like Canada (Que and Moreillon,2010) to 24.1% in Turkey(Savaş *et al*.,2005) 26.3% in Europe, 34.2% in the pacific region (Que and Moreillon,2010).There is also marked variability in prevalence even among the countries within a region, for example, in the western pacific region, prevalence ranged from 23.6% in Australia, to about 70% in Japan and Hong Kong, (Que and Moreillon,2010)while in Europe, percentages ranged from as low as 2% in the Netherlands and Switzerland to as high as 54% in Portugal and 43 – 58% in Italy(Alli *et al* ., 2007).

Figures from Africa display the same variability. In a 1997 study across eight large Hospitals in Africa, prevalence were found to be relatively high in Cameroon, Nigeria and Kenya (21-30%), and South Africa (about 50%)(Klugman, 1998)while less than 10% in Tunisia, Malta and Algeria (Kesah,2002). The Islands of Cape Verde and Somalia seems to have a very low prevalence of MRSA (Aires *et al* .,2000; Alli *et al* .,2007).

The prevalence of MRSA has also been on the increase over the years since its discovery. According to one survey of the National Nosocomial Infection Surveillance (NNIS) in the United States, the hospital prevalence of MRSA increased from 2.1% in 1975 to 35% in 1995. In the United Kingdom, prevalence increased from 33.4% in 1999 to 45.4% in 2001(Que and Moreillon,2010).The epidemiology of MRSA is fast changing and has become one of the established pathogen in both hospital and community. Methicillin-resistant *Staphylococcus aureus* infection and colonization have been reported in humans in Nigeria, in both hospital and outside the hospital environment. In Nigeria, several reports of human MRSA infections have been documented.

Ikeh (2003) observed a prevalence rate of 43% at Jos University Teaching Hospital while Onanuga *et al*. (2005; 2006) showed a prevalence rate of 71.7% and 69% from urine samples in Abuja and Zaria respectively among healthy women. The prevalence rate of 20% was also recorded in Zaria from non-hospital sources (Olonitola *et al*., 2007a). Also Taiwo *et al*. (2004), Fusi Ngwa *et al*. (2007), and Olowe *et al*. (2007) in separate studies observed a prevalence rate of 34.7%, 54.9% and 47.8% in Ilorin (University of Ilorin Teaching Hospital), Lagos (Pediatric Unit, Lagos University Teaching Hospital and Oshogbo (Ladoke Akintola University of Technology, College oh Health Sciences) respectively.

Fadeyi *et al.* (2010) worked on MRSA carriage amongst health workers of the critical care units in a Nigeria hospital. Of the 198 health worker screened, 104 had MRSA either in the nose, hand or both giving a carriage rate of 52.5%, nasal carriage (38.9%) was higher than hand (25.3%). Doctors (22.75%) or Nurses (16.7%) were the predominant carriers.Methicillin-resistant *Staphylococcus aureus* isolates were resistant to commonly available antibiotics. Only 1 (1.3%) of the nasal isolates was vancomycin resistant. In Kano, Nwanko and Nasiru (2011) studied

methicillin-resistant *S. aureus* (MRSA) and their antibiotic sensitivity pattern. Their results showed that out of the 185 *S. aureus* tested, 53 (28.6%) were found to be methicillin resistant. While 38 (62%) isolates were obtained from inpatients 15 (28%) were from out-patients, and surgical wound infection had the highest prevalence of 32 (60%) isolates.

2.7 Clinical Implications of MRSA

Methicillin-resistant *Staphylococcus aureus* is a life-threatening, multi - drug resistant bacterium, changing its resistance patterns by acquiring resistance to each new antimicrobial agent. This has made MRSA infection more difficult to treat with standard classes of antibiotics and thus more dangerous. Methicillin-resistant *Staphylococcus aureus*strains are a persistent and increasing cause of nosocomially acquired infection in the world (Narezkina *et al.*, 2006) .It has been difficult to quantify the degree of morbidity attributable to MRSA. Inpatients with *S. aureus* infection had, on average, 3 times the length of hospital stay, 3 times the total charges and 5 times the risk of in-hospital death than in - patients without this infection (Noskin *et al.*, 2005). They affect patients in high dependency units such as intensive care units, burns units and cardiothoracic units. There is also several epidermic strain circulating in milk (Finch, 2000). Clinical implications of CA-MRSA include skin and soft tissue infection (in form of skin abscess, carbuncle, furuncle impetigo); pneumonia, osteomyelitis, arthritis, endocarditis. Community-acquired Methicillin resistant *Staphylococcus aureus*can also cause wound infections (Demlin and Water house, 2007).Community-acquired Methicillin resistant *Staphylococcus aureus* has also been detected from surgical site infection, urinary tract infection (Baba-Moussa *et al.*, 2008), Infection of the eye and orbit (Blomquist, 2006), meningitis and sinusitis (Munckhof *et al.*, 2008).

2.8 Mechanism of Methicillin resistance

Methicillin resistance in *S.aureus* has been reported to arise from expression of a methicillin-hydrolysing β-lactamase (Montanari *et al*., 1996) and through the expression of an altered form of PBP2 that has a lower penicillin-binding affinity and higher rates of release of the bound drug compared to the normal PBP2 (Hackbarth *et al*., 1995).

However, the main mechanism of methicillin resistance in *S. aureus* is through the expression of a foreign PBP, PBP2a (not to be confused with PBP2), that is resistant to the action of methicillin but which can take over the transpeptidation (cross-linking) reactions of the host PBPs. Synthesis of PBP2a is regulated and normally kept at low level, but the level of synthesis can be enhanced if mutations occur in the regulatory genes (Obajuwula, 2014).

2.8.1 Altered Penicillin Binding Protein (PBP2a)

Methicillin-resistant *Staphylococcus aureus* differ genetically from methicillin-sensitive *S. aureus* 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 that encodes the 76 KDa penicillin-binding protein, PBP2a (also referred to as PBP2′). Penicillin binding protein2a confers resistance to all known β lactam antibiotics (except the fifth generation cephalosporins). The gene *mec*A is part of a 30 to 50kb of additional chromosomal DNA, *mec* found in methicillin resistant strains of S*taphylococci* together with *mecI* and *mecRI,* which are regulatory elements controlling *mecA* transcription (Chambers,1997). Penicillin binding proteins are enzymes that function in the last step of peptidoglycan (cell wall) synthesis where they bring about the cross-linking (transpeptidation) of the pentapeptide side chain which ends in d-Ala–d-Ala. They remove the

terminal d-Alanine of the pentapeptide and crosslink to a nearby peptide usually to the third amino acid on the chain. This crosslink gives the cell wall integrity and thus shape to bacteria. β-lactam antibiotics are structural analogs of the natural d-Ala-d-Ala substrate, thus they bind to the active site of the PBPs. After a β–lactam antibiotic has attached to the PBP, the transpeptidation reaction is inhibited, cell wall formation is inhibited, and the cell dies (Graumlich, 2004).

Penicillin binding protein2a is a modified PBP which has reduced affinity for β lactam antibiotics (Gordon and Lowy, 2008). So, when the normally present PBP is inactivated by a β lactam antibiotic, PBP2a takes over the function and with the β lactam antibiotic unable to bind to the PBP2a active site, the enzyme is free to carry out its transpeptidase function and hence allowing for the synthesis of a stable peptidoglycan structure and the bacteria survives.

2.8.2 Regulation of PBP2a expression

Adjacent to *mecA* on the staphylococcal chromosome are two genes, mecR1 and mecI, that are co-transcribed divergently from *mecA*. The *mecR1* gene encodes a membrane-bound signal transduction protein (*MecR1*) while *mecI* encodes a transcriptional regulator (*MecI).* Between *mecA* and *mecR*1 are the promoters for these genes and an operator region that encompasses the −10 sequence of *mecA* and the -35 sequence of *mecR1* (Sharma *et al*., 1998) (Figure 2.7A). *MecR1* and *MecI* have high protein sequence homology with the proteins, *BlaR*1 and *Bla*I, respectively that are involved in the inducible expression of the plasmid-mediated staphylococcal βlactamase gene, *blaZ*. The arrangement of the genes coding for *BlaR*1 and *Bla*I resembles the *mecA* system suggesting that *mecA* may have acquired the regulatory genes from the *blaZ* system sometime in the past (Song *et al*., 1987). The operator regions are similar enough to allow *BlaI* to regulate PBP2a expression (Gregory *et al*., 1997). Consequently, the presence of a plasmid carrying the

blaZ regulatory genes can render PBP2a expression inducible under the control of *BlaR*1 and *Bla*I, a situation that commonly occurs in clinical isolates of MRSA (Hackbarth and Chambers, 1993).

The nature of the signaling system for inducible β-lactamase expression has been elucidated (Zhang *et al*., 2001). BlaI, a DNA-binding protein, binds to the operator region as a homodimer and represses RNA transcription from both blaZ and blaR1-blaI (Figure 2.7B). Consequently, in the absence of a β-lactam antibiotic, β-lactamase is expressed at low levels. BlaR1, present in the cytoplasmic membrane, detects the presence of the β-lactam by means of an extracellular penicillin-binding domain and transmits the signal via a second intracellular zinc metalloprotease signalling domain (Figure 2.7B). Binding of a β-lactam to BlaR1 stimulates the autocatalytic conversion of the intracellular zinc metalloprotease domain of BlaR1 from an inactive proenzyme to an active protease (Zhang *et al*., 2001). The activated form of BlaR1 is thought to directly or indirectly cleave BlaI resulting in fragments that are incapable of forming dimers and binding DNA (Figure 2.7) (Gregory *et al*., 1997). Without BlaI bound to the operator site, transcription of both blaZ and blaR1-blaI can commence and β-lactam resistance can be conferred through β-lactamase synthesis (Figure 2.7C). An additional gene product, BlaR2, also regulates β-lactamase synthesis, although the role of this protein has not been elucidated. Whether there are other proteins involved in the signaling system also remains to be determined.

Unlike β-lactamase synthesis, expression of PBP2a is not strongly inducible in isolates carrying the normal regulatory genes (mecA and mecR1-mecI) and induction is much slower (15 minutes for β-lactamase expression compared to up to 48 hours for PBP2a synthesis). This is because MecI is a tight regulator of mecA transcription (Kuwahara-Arai et *al*., 1996) and most β-lactam antibiotics do not efficiently activate MecR1. Consequently, some isolates, referred to as pre-MRSA, are methicillin-sensitive despite carrying the mecA gene. However, selective pressure

through antibiotic usage has promoted *S. aureus* isolates that have mutations or deletions in mecI or the mecA promoter/ operator region giving rise to an inactive repressor and constitutive PBP2a expression (Kobayashi *et al*., 1998).

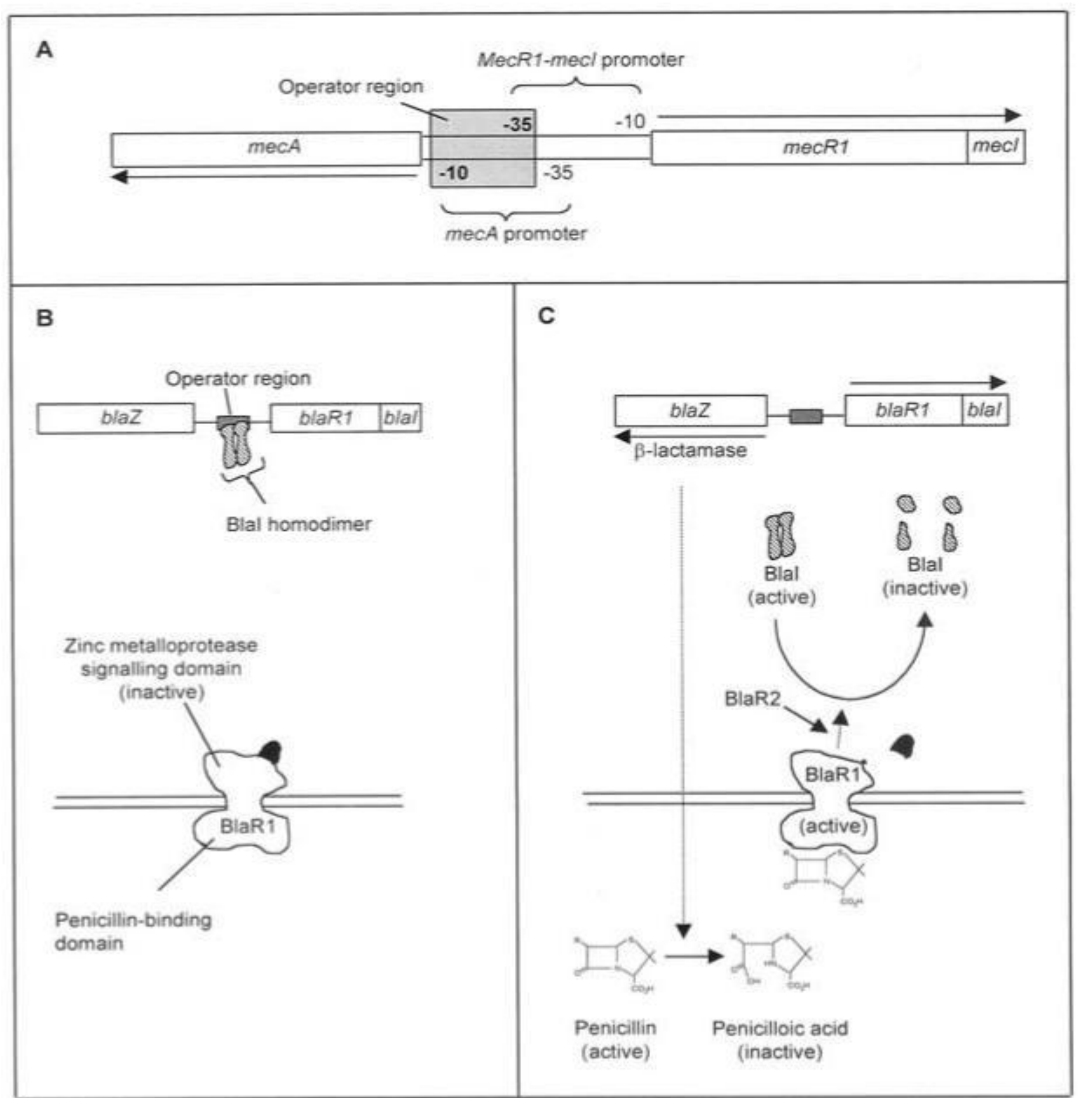


Figure 2.7: A, Schematic representation of the mecA-mecR-mecI coding region.

Arrows indicate the relative directions of transcription of the *mecA* and *mecR1-mecI* genes. *B*, Repression of *blaZ* and *blaR1-blaI* transcription by BlaI in the absence on an inducer. *C*, Induction.

Source: Natalia and frank (2010).

2.8.3 Internal factors affecting methicillin resistance

Since PBP2a is essential in conferring methicillin resistance, any factor that interferes with the expression of the mecA gene or with the activity of PBP2a will affect methicillin resistance. Genetic and biochemical studies have established that PBP2a has strict substrate requirements. Consequently, any factors that influence formation of the substrate have the potential to perturb or modulate methicillin resistance. Studies have shown that PBP2a requires: Glycan chains to be of certain lengths (Pinho *et al.*, 2001), the stem peptide to have the normal peptide configuration (Ludovice *et al.*, 1998) and the pentaglycine cross-bridge to be intact (Berger-Bächi and Tschierske 1998).

2.8.4 External factors that affect Methicillin resistance

External factors that affect methicillin resistance include among others, salt concentration, pH, medium composition, osmolarity and temperature (Matthew and Stewart, 1984). Some of these external influences are exploited in the clinical laboratory to enhance the detection of strains exhibiting heterogeneous methicillin resistance; isolates are grown in the presence of high sodium chloride concentrations (4%) and at lower temperatures (30-35°C).

2.9 Clinical Treatments for MRSA Infections

2.9.1 Current Clinical Treatments

The difficulty in identifying new compounds with suitable antibacterial activity is one of the major problems faced in the fight against resistant organisms (Payne *et al.*, 2007; Wright and Sutherland, 2007). Penicillin was the first class of the -lactams for the treatment of bacterial infections. With

the development of resistance, however, many of these -lactams became ineffective against a significant proportion of *S. aureus* clinical strains (Guignard *et al.*, 2005). The glycopepides belong to a different class of antibiotics effective against Gram-positive organisms. Vancomycin and teicoplanin are the preminent members of this class of antibiotics (Loffler and Macdougall, 2007), and vancomycin is the antibiotic most commonly used to treat MRSA infections, the inhibition of cell wall biosynthesis by the glycopetides results from their stable noncovalent binding to the D Ala-D-Ala terminal of peptidoglycan precursors. However some MRSA strains have evolved resistance to vanvomycin (Kollef, 2007).

Linezoid, quinupristin-dalfopristin, daptomycin, and tigecycline represent newer agents for the treatment of *S. aureus* infections, including those caused by non-vancomycin susceptible MRSA. Linezolid is a synthetic oxazolidinone antimicrobial agent that blocks the formation of protein synthesis initiation complexes. Quinupristin-dilfopristin is a mixture of semisyntheric streptogramin derivatives that bind to different sites of the 50S ribosomal subunit, resulting in the irreversible inhibition of bacterial protein synthesis (Llarrull *et al.*, 2009). Daptomycin is a cyclic lipopeptide that forms a calcium ion complex in the bacterial cytoplasmic membrane causing the loss of the transmembrane electrical potentaial lipoteichoic acid, and lipid biosynthesis are also observed, although it is not yet clear if these are consequences of the loss of the transmembrane electrical potential gradient or the independent effects of daptomycin.

2.9.2 New weapons in the pipeline: -lactam antibiotics that inhibit PBP2a:

Four -lactams antibiotics are being evaluated for the treatment of MRSA associated infections by targeted inhibition of PBP 2a transpeptidase activity. In a review published in 2005 by Guignard and collegues (2005), 16 novel anti MRSA -lactam antibiotics, only 1 of which (ceftobiprole)

remains in clinical development. In a later review, Page (2006) reported on a new cephalosporin (ceftaroline) also active against MRSA, two additional -lactams antibiotics ME1036 and PZ-601, have been added to the anti MRSA pipeline (Butler and Buss 2006; Abbanat *et al*., 2008; Butler, 2008; Kattan *et al*., 2008).

i. Ceftobiprole medocaril:

Ceftobiprole medocaril, a water-soluble cephalosporin prodrug, belongs to a new class of cephem antibiotics with activity against a wide range of Gram-positive organisms,including MRSA and penicillin- resistant Streptococcus pneumoniae, and Gram negative pathogenic bacteria (Bush *et al*., 2007; Page, 2007; Anderson and Gums, 2008). Rapid cleavage of ceftobiprole medocaril in plasma produces the active drug, ceftobiprole (Murthy and Schmitt-Hoffmann 2008). Ceftobiprole inactivates all four *S.aureus* PBPs and PBP 2a, as indicated by competition assays against a fluorescent lactam. Ceftobiprole medocaril, the prodrug, is converted rapidly and almost completely by typeA esterases to active ceftobiprole (Murthy and Schmitt-Hoffmann 2008). The pharmacokinetics and pharmacodynamic of ceftobiprole describe a drug that should be appropriate for the early emprirical hospital treatment of patients with infections (Murthy and Schmitt-Hoffmann, 2008). One phase 3 clinical trial for community acquired pneumonia has been completed (Abbanat *et al*, 2008), and two phase 3 trials for complicated skin and skin structure infections (cSSSIs) (Moreillon, 2008; Noel *et al*., 2008a; Noel *et al*., 2008b) are currently on going in the United States. Ceftobiprole monotherapy was as effective as vancomycin plus ceftazidime for the treatment of patients with a broad range of complicated skin and skin structure infections (cSSSIs) and infections due to Gram-positive bacteria (including MRSA) and Gram-negative bacteria (Noel *et al*., 2008a). In another phase 3 study, the cure rates for patient with MRSA infections were 92% (56/61) with ceftobiprole treatment and 90% (54/60) with vancomycin

monotherapy (Noel *et al*., 2008b). Ceftobiprole monotherapy was as effective as vancomycin monotherapy or vancomycin plus ceftazidime (Noel *et al*., 2008a).

ii. Ceftaroline fosamil.

Ceftaroline fosamil, a water soluble N-phosphono type cephalosporin prodrug, is a member of a new class of cephem antibiotics having antibacterial activity against a wide range of species including the resistant Gram positive pathogens MRSA and multi drug resistant *Streptococcus pneumoniae*, as well as common Gram-negative pathogenic bacteria. Ceftaroline fosamil, discovered by Takeda Chemical Industries (Osaka, Japan), is currently being developed by Forest Laboratories (New York, NY) for the treatment of infections, including cSSSIs and community acquired pneumonia (Obajuluwa, 2014).

Ceftaroline, the active form of ceftaroline fosamil, is a potent inhibitor of PBP 2a of MRSA (IC50= 0.16 to 0.18 g/ml) (Moisan *et al*., 2008; Villegas-Estrada, 2008), which translates into a high level of inhibitory activity (MIC for MRSA =0.25 to 0.5 g/ml) (Moisan *et al*., 2008) Ceftaroline fosamil the prodrug, undergoes rapid conversion by plama phosphates to active ceftaroline. Two phase 3 clinical trials with patients with cSSSIs have been completed, and two phase 3 clinical trials with patients with community-acquired pneumonia are currently on going in United States. In a randomized, double blinded study of the efficacy and safety of ceftaroline versus those of vancomycin plus aztreonam in patients with cSSSIs, ceftaroline monotherapy (intravenous) was as effective and well tolerated as vancomycin plus aztreonam combination therapy for the treatment of patients infected with both Gram positive and Gram-negative pathogens (Corey *et al*., 2008). The clinical cure rates were similar for ceftaroline and vancomycin

plus aztreonam in clinically evaluable subjects (91% and 93%, respectively). The clinical cure rate for MRSA infections was 95% for ceftaroline and vancomycin plus aztreonam.

Microbiological success was similar for ceftaroline and vancomycin plus aztreonam overall (92% and 93%, respectively and for MRSA in particular (95% and 92% respectively). In conclusion Ceftaroline monotherapy was as effective as vancomycin plus aztreonam (Corey *et al.*, 2008). Girish and Balakrishnan (2011) reported that the United States Food and Drug Administration has granted approval for ceftaroline fosamil (teflaro) on October 2010, to treat adults with community acquired bacterial pneumonia and acute bacterial skin and skin structure infections, including MRSA. The dose of ceftaroline fosamil recommended is 600mg intravenously, every 12 h for patients with normal renal function or mild renal dysfunction. The drug formulation of ceftaroline fosamil available is in a powder form for intravenous administration as 400mg and 600mg vials (FDA, 2011). This drug is not yet available in Nigeria.

iii. Razupenem (PZ-601)

Razupenem (PZ-601) is a new carbapenem being developed by Protez pharmaceuticals (now Novartis) that has demonstrated a high degree of potency against MRSA. Razupenem PZ-601 has in vivo efficacy against VISA and is in phase 2 clinical trials for the treatment of cSSSIs (Abbanat, 2008). In a study carried out to determine the safety and multiple dose pharmacokinetics of PZ-601 in health male volunteers PZ-601 did not cause any serious adverse events (Lo *et al.*, 2008)

The -Lactam antibiotics that inhibit PBP2a is a great progress and offers a glimmer of hope for the future treatment of MRSA infections including those caused by vancomycin-resistant and intermediate strains.

2.10 Detection of Methicillin Resistance *Staphylococcus aureus*

2.10.1 Conventional Methods

Solid media have been used in different combinations, with or without prior broth enrichmentfor the detection of MRSA in screening samples from carriers. Most screening media used usually contain an indicator system composing of a carbohydrate, usually Mannitol, and a pH indicator, usually phenol red to identify staphylococci; inhibitory substances - such as NaCl, ciprofloxacin,(Brown *et al*., 2005) tellurite, polymixin B, aztreonam and desferioxamine- to suppress the growth of other organisms; and antibiotics like oxacillin, and recently cefoxitin (Smyth *et al.,* 2005) to help select for MRSA. Most commonly used media, has been Mannitol Salts Agar and Baird-Parker medium, supplemented with various combinations of these indicators and inhibitors (Brown *et al*., 2005).

2.10.2 Screening methods

2.10.2.1 Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration [MIC] by the dilution method has traditionally been the reference method. This could be carried out either by agar dilution method, broth microdilution method or using the epsilometer test (E-test). Of the penicillinase-stable penicillins, oxacillin is preferred for *in vitro* testing. Oxacillin susceptibility test results can be applied to the other penicillinase stable penicillins, i.e., cloxacillin, dicloxacillin, flucloxacillin, methicillin and nafcillin (CSLI, 2015).

Minimum Inhibitory Concentration by agar dilution: The antimicrobial agent is incorporated into the agar medium, with each plate containing a different concentration of the agent. The inoculum

is prepared using the direct colony suspension to achieve a turbidity equivalent to a 0.5 McFarland turbidity standard. This is then inoculated on to the various agar medium (Mueller-Hinton agar with 2% NaCl), then incubated for 24 h at 35°C. According to the CLSI standard, an oxacillin MIC of ≤ 2μg/mL indicates that the strain is susceptible and ≥ 4μg/mL is resistant another method in the BSAC standard is the use of Columbia agar with 2% NaCl (CSLI, 2012).

Minimum Inhibitory Concentration by broth microdilution: The only defined method is the CLSI method, which requires the use of Mueller Hinton broth with 2% NaCl, and an inoculum of 5×10^5 cfu/mL, prepared using the direct colony suspension to achieve a turbidity equivalent to a 0.5 McFarland turbidity standard. (CSLI, 2012) The bacterial suspension is then inoculated in to each well of the microplate within 15 minutes of adjusting the turbidity of the inoculumand incubated at 33 - 35°C for 24 h. Minimum inhibitory concentrations are the same for both the agar and Microbroth dilution methods.

Minimum Inhibitory Concentration by the epsilometer test (Etest®) method: This gives an MIC result and is affected by test conditions in a similar way to other MIC and diffusion methods. The test conditions include Muellar Hinton agar with 2% NaCl, an inoculum density equivalent to 0.5–1.0 McFarland standards, application of inoculum with a swab and incubation at 35°C for 24 h. It has an advantage over other MIC methods, in that it is as easy to set up as a disc diffusion test (Brown *et al.*, 2005).

2.10.2.2 Oxacillin salt-agar screening method

This method as is recommended by the CLSI(2006) requires suspending the test organism to the density of a 0.5 McFarland standard and inoculating Muellar Hinton Agar containing 4% NaCl and 6 mμ/mL oxacillin with a spot or a streak of the organism covering an area of about 10 – 15 mm in

diameter. Plates are incubated at 33 - 35°C for 24 h and any growth other than a single colony is indicative of resistance. This method has been recommended for screening colonies isolated on routine media and for confirmation of suspect resistance seen in disc diffusion tests, especially where MIC detection or molecular methods are not available

2.10.2.3 Disc diffusion methods

Either oxacillin- or cefoxitin-based methods can be used for detection of *mecA*-mediated resistance in *Staphylococcus aureus*. Standardized methods have been defined by the BSAC and the CLSI (2015) for both, and many studies have shown that tests with cefoxitin are more reliable than with oxacillin (Fernandes *et al*., 2005;CSLI, 2009). It is a disk diffusion test with specific breakpoints for both *S. aureus* (≤21mm for resistant and ≥ 22mm for susceptible) and coagulase negative staphylococci (CoNS) (≤ 24mm for resistant and ≥25mm for susceptible) (CLSI, 2015). The test was approved for two reasons:

a) The test is highly sensitive and specific for the presence of *mecA* mediated resistance especially for CoNS. The cefoxitin disc test is more accurate than oxacillin susceptibility testing for detection of *mecA* mediated resistance.

b) The cefoxitin disc test is easier to read than the oxacillin disk diffusion test.

The zone around an oxacillin test must be read with close scrutiny and transmitted light. The cefoxitin disk test produces clearer zones and can be read with reflected light.

Oxacillin and cefoxitin are tested instead of methicillin because methicillin is no longer commercially available and that oxacillin maintains its activity during storage better than methicillin and is more likely to detect heteroresistant strains.

In the oxacillin-based method, the inoculum should be prepared by direct colony suspension, because the slowly growing, heteroresistant subpopulation may be overwhelmed by the rapidly growing susceptible cells, if the growth method is used. This is subsequently inoculated on to Muellar Hinton Agar (which is not supplemented with 2% NaCl (CSLI,2009) as in the dilution method), a 1μg oxacillin disc is then placed onto the agar medium and incubated for full 24 h at 33-35°C. The plate is read using transmitted light as against reflected light for 13mm is considered susceptible, ≤ 10mm is considered methicillin resistant, while between 10-13mm is intermediate susceptibility and should be confirmed by performing testing for *mec*A or PBP 2a, the cefoxitin MIC or cefoxitin disk test, an oxacillin MIC test, or the oxacillin-salt agar screening test.

In the cefoxitin-based method, the inoculum is also prepared using direct colony suspension and inoculated onto Muellar Hinton Agar. Cefoxitin is used as a surrogate for oxacillin resistance. Thus, a 30μg cefoxitin disc is placed on the inoculated lawn, and incubated at 35 ± 2°C for 16-20h. The plate is read using reflected light, and zone diameters ≥ 22 mm are considered susceptible and zone diameters ≤ 21mm are considered methicillin (oxacillin) resistant (CSLI,2009).

2.10.2.4 Automated systems

Automated systems have been in use for the detection of MRSA since the early 1980s, Jorgensenand colleagues described the performance of two automated test systems; MS-2 and the AutoMicrobic systems.Some automated systems for blood culture incorporate methicillin susceptibility and have been reported to be reliable. These include: Vitek/Vitek2 (bioMe´rieux), Phoenix (Becton Dickinson) and Microscan (Dade Behring), though some false resistance have been reported. Another method is the Quenching fluorescence technique found to be also reliable but requires hours of incubation (Brown *et al.*, 2005).

2.10.2.5 Confirmatory Methods

a. Latex agglutination for detection of MRSA

Detection of the protein product of the mecA gene (PBP2a protein) is a rapid and easy method of predicting resistance to methicillin. A rapid (10-20 minutes for a single test) slide latex agglutination test is commercially available. The method involves extraction of PBP2a from suspensions of colonies and detection by agglutination with latex particles coated with monoclonal antibodies to PBP2a. It requires no special equipment and is suitable for rapid confirmation of resistance or equivocal tests in routine clinical laboratories. Isolates producing small amounts of PBP2a may give weak agglutination reactions or agglutinate slowly. Reactions tend to be stronger if PBP2a production is induced by growth in the presence of a penicillin or oxacillin (Brown *et al.*, 2001; Brwon *et al.*, 2005). Studies have shown the method to have high specificity (100%) and high sensitivity (97% - 100%). It also has a potential advantage of not detecting false positives from strains that possess mecA but are unable to produce the protein.

2.10.3Chromogenic agar

More recently, media employing chromogenic enzyme substrates that were used to identify *S. aureus* have been utilized for the detection of MRSA. With the addition of various selective agents they have been employed in screening for MRSA, performing better than the more traditional media (Flayhart *et al*., 2005). They were also associated with significant reduction in turnaround time, improved sensitivity and specificity. Use of enrichment broths prior to inoculation also improved performance, as in the traditional culture methods. In the chromogenic agars, α-glucosidase produced by *S. aureus* cleaves the chromogenic substrate in the medium and gives a

blue or green color to the *S. aureus* colony. Cefoxitin, oxacillin, methicillin or ciprofloxacin supplement inhibits the growth of *S. aureus* sensitive to methicillin (Perry *et al*., 2004).

2.10.4 Molecular method

The majority of molecular methods used for the detection of MRSA are in-house, relying on multiplexed polymerase chain reaction (PCR) primers detecting genes specific for *S. aureus* (nuc, fem) and mecA detecting methicillin resistance. Most are only suitable for use with pure cultures and not screening of swabs due to the presence of coagulase negative staphylococci carrying the methicillin resistant gene mecA (Brown *et al*., 2005).

Some commercially available amplification assays such as the isothermal signal amplification method (CytAMP ® —British BioCell International, Cardiff, UK) target mecA in combination with other specific markers such as coagulase have shown encouraging results. By targeting these markers together it is able to simultaneously identify *S. aureus* and methicillin resistance, without the fear of false positives from coagulase negative staphylococci(CoNS) (Warren *et al.,* 2004).

Warren *et al.,* (2004) described a real time PCR assay (IDI-MRSA) for the detection of MRSA directly from nasal swabs, identifying MRSA colonization, with a turn-around-time of about 1 h. This assay simultaneously detects the staphylococcal cassette chromosome *mec* (*SCCmec*), containing the mecA gene that confers methicillin-resistance, and a *S. aureus* specific sequence located within the *orfX* gene. These two genes permit the detection of methicillin-resistance and the differentiation of *S. aureus* from coagulase-negative staphylococci, yielding the reliable identification of the presence of MRSA.

The turnaround time has been further reduced to less than 1 hour by Huletsky and colleagues, who developed a new real-time multiplex PCR assay which utilizes five primers specific to the different staphylococcal cassette chromosome *mec* (SCC*mec*) right extremity sequences, including three new sequences, in combination with a primer and three molecular beacon probes specific to the *S. aureus* chromosomal *orfX* gene sequences located to the right of the SCC*mec* integration site (Huletsky *et al*., 2005).

2.11 Eradication of MRSA Colonization

Eradication of *S. aureus* including MRSA carriage may serve to achieve two purposes: prevention of infection and prevention of transmission. In a 2003 Cochrane review of antimicrobial drugs for treating MRSA colonization, Loeb *et al*. (2008)concluded that there was insufficient evidence to support use of topical or systemic antimicrobial therapy for eradicating nasal or extra nasal MRSA (Loeb *et al.,* 2008). On the other hand, Albrich and Harbarth concluded that as part of a multipronged approach, decolonization of healthcare workers contributed to successful termination of nosocomial MRSA outbreaks and was easier and more cost effective when applied early before MRSA became endemic in an institution (Albrich and Harbath, 2008). In a systematic review performed to determine the effectiveness of different forms of approaches to the eradication of MRSA carriage, Ammerlaan *et al*. (2009) summerised that application of short term nasal mupirocin was the most effective therapy for eradicating MRSA carriage, with an estimated success rate of 90% after a week's therapy and approximately 60% after a longer follow-up (Ammerlaan *et al.,* 2009). This has also been corroborated by Albrich and Harbarth (2008). Despite these successes with mupirocin for nasal decolonization, it has not been very useful for

extranasal MRSA carriage, and the efficacy improved when combined with chlorhexidine body wash (Albrich and Harbath, 2008).

Furthermore, mupirocin was associated with relapse within several months following therapy, and subsequent reuse was associated with rising resistance, suggesting the need for newer antibiotics with more bactericidal properties than mupirocin. Oral co-trimoxazole, plus nasal fusidic acid also in combination with chlorhexidine produced similar results with the mupirocin-based combination for extra nasal sites. Other antimicrobial agents that have been used but with less success include topical agents such as: bacitracin nasal ointment, tea tree oil, systemic agents (orally administered) like tetracyclines, ciprofloxacin, fusidic acid, Rifampin, or a combination of both topical and systemic (Ammerlaan *et al.,* 2009).

2.11.1 Mupirocin Resistance

The use of Mupirocin for MRSA prevention and control has been limited by emergence of resistance fuelled by widespread use. Three levels of Mupirocin susceptibility have been described for *S. aureus*. These are Mupirocin susceptibility with minimum inhibitory concentrations (MICs) ≤4µg/mL which is the wild type, low-level Mupirocin resistance with MICs from 8 to 64µg/mL due to mutations in native tRNA synthetase, and high-level mupirocin resistance with MICs ≥512µg/mL which is secondary to a novel tRNA synthetase coded by the *mupA* gene borne on a plasmid (Patel *et al.,* 2009).

2.11.2 Mupirocin Susceptibility Testing

Several methods have been described for the detection and differentiation of Mupirocin resistance. These include MIC methods like broth microdilution, agar dilution, and the commercial E-test (bioMe´rieux). Disk diffusion methods were proposed by Moreira de Oliveira and colleagues in 2007, and have been recently incorporated into the CLSI susceptibility testing document. Methicillin-resistant *Staphylococcus aureus* isolates can be successfully classified into three Mupirocin susceptibility groups by the disk diffusion method using 5 and 200 µg Mupirocin disks. The zone diameter observed for a 5 µg disk distinguished Mupirocin susceptible from the resistant strains (either low or high). On the other hand, a 200 µg disk distinguished the high-resistance strains from the other two (Mupirocin susceptible or low level resistance) strains. Aside from the phenotypic methods, PCR assays have also been described for detection of *mupA*-mediated high-level Mupirocin resistance (Patel *et al.*, 2009).

2.12 The Internally Displaced Persons (IDPs)

2.12.1 Definition

When the issue of internal displacement emerged onto the international agenda in the early 1990s, no definition of "internally displaced persons" existed. Yet, having a definition was essential for identifying the populations of concern and their particular needs, compiling data, and framing laws and policies designed to assist them.

The internally displaced persons (IDPs) are persons or group of persons who have been forced or obliged to flee or to leave their homes or places of habitual residence, in particular as a result of or in order to avoid the effects of armed conflict, situations of generalized violence, violations of

human-made disasters and who have not crossed an internationally recognized state border (OCHA, 2015). At the end of 2014, it was estimated that there are more than 38.2 million IDPs worldwide, the highest since 1989, the first year for which global statistics on IDPs are available (UNHCR, 2015). The countries with largest IDPs population were : Syria 7.6 million, Colombia 6 million, Democratic Republic of Congo 2.8 millon, Sudan 2.2 million, South Sudan 1.6 million, Parkistan 1.4 million, Nigeria 1.2 million, and Somalia 1.1 million (UNHCR, 2015).

The causes of displacement are multi-faceted, complex and often overlapping. The National Emergency Management Agency (NEMA) had reported that Boko Haram terrorists had forced residents of various communities to take refuge in different camps across northern Nigeria and more than one third of such camps were domiciled in the northeast, while more than half of them are in Maiduguri the Borno State capital (NEMA report, 2014)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area/Location

This study was conducted in selected IDP camps located within Maiduguri, Nigeria. Maiduguri, the capital and largest urban city of Borno State, in the north eastern part of Nigeria.It is cosmopolitan in nature, situated at an elevation of 354metere above sea level, located between latitude 11° and 14° North and longitude 10° and 14° East, within the conventional Sahel zone and has a total land mass of 50,778 square kilometers (BMLS, 2007). It has a population density of 1,738 people per square kilometers, and a total population of 521,492(NPC, 2006).It is is borderd by the Republic of Niger to the North, Cameroun Republic to the East and Chad Republic to the Northeast.Within Nigeria, its neighbouring states are Adamawa to the South, Yobe to the West and Gombe to the Southwest.The temperature ranges from 35-40°C for most parts of the year with two distinct seasons,raining season with meN annual Rainfall 647mm from July to October and a prolonged dry season for the rest of the year (LCRI, 2007).

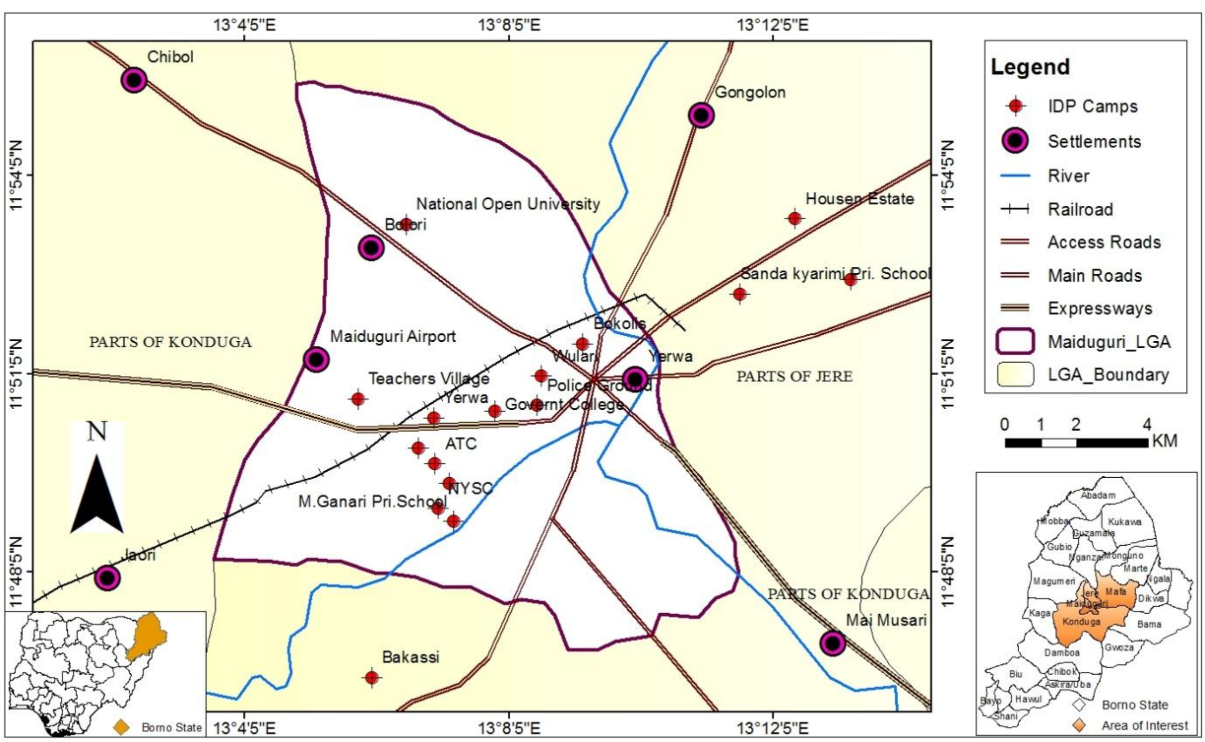


Figure3.1: Map of Maiduguri Metropolis showing some IDP camps.

3.2 Sample Size Determination

The sample size was determined using the formular for Sample size when population >10,000

$$n = \frac{z^2 pq}{d^2} \text{ (Araoye, 2003).}$$

n= the desired sample size (when population>10,000)

z= the standard normal deviation, usually set at 1.96, which corresponds to the 95% confidence level.

p= Prevalence = 47.6 % (Adelowo *et al*., 2014)

q=1.0 – p = 1-0.476 = 0.524

d= degree of accuracy desired, usually set at 0.05

$$n = \frac{(1.96)^2 (0.476)\ (0.524)}{(0.05)^2}$$

= 383 but 400 samples were collected to increase positivity of isolates.

3.3 Study Population

The study population comprised of both male and female of all age groups who are staying in IDP camps in Maiduguri, Nigeria.

3.3.1 Inclusion Criteria and Exclusion Criteria

3.3.1.1 Inclusion criteria

Male and female (IDPs) of all age groups living in the IDP camps and who have given their consent.

3.3.1.2 Exclusion criteria

The study excludes IDPs that are not staying in the IDP camps and those IDPs living in the camps but have not given their consent.

3.4Ethical Approval and Clearance

Ethical approval was obtained from the Borno State Ministry of Health and clearance letter was gotten from the State Emergency Management Agency (SEMA).Consent forms and questionnaires were issued out to the participants (appendix vii and viii).

3.5 Sample Collection

Four hundred (400) IDPs were recruited based on convenience from different IDP camps. Swab sample was collected from the anterior nares of each IDP by a Medical laboratory scientists using sterile swab stick. A sterile swab moistened with sterile physiological saline was used to swab the inside of the nostril by rotating evenly about 3-4 times. A single swab was used for both nostrils of one subject (Bocher *et al.,* 2012). Swabs collected were immediately labeled and transported on ice pack to Microbiology laboratory, University of Maiduguri Teaching Hospital for processing before transporting to Department of Microbiology, ABU Zaria for further analysis

3.6 Isolation and Characterization

The swabs were inoculated directly onto Mannitol Salt Agar and 5% Blood Agar plates. These inoculated plates were incubated at 37ºC for 48 h and 24 h respectively (Cowan and Steel, 2004). Blood Agar plates were examined after 24 h while Mannitol Salt Agar plates were examined after 48 h to allow visible growth.Out of the four hundred (400) samples, One hundred and five (105)suspected *S. aureus* colonies that were Mannitol-fermenting appeared as yellow colonies on MSA but creamy white colonies on BA. Sub culturing of the 105 isolates was carried out from the agar plates in order to obtain pure cultures. Thereafter, discrete yellow colonies and creamy white colonies on MSA and BA respectively were picked aseptically and stored on Nutrient Agar slants (Baba *et al.,* 2015*).* The slants were transported on ice packs to Department of Microbiology, Ahmadu Bello University Zaria for further characterization and analysis.

The colonies suspected to be *S. aureus* were sub cultured on nutrient agar plates to obtain pure cultures and further confirmed by Gram staining and biochemical test according to Cowan and Steel (2004) as followed.

3.6.1 Gram Staining

All the isolates suspected to be *S. aureus* on MSA were subjected to Gram staining. A well isolated discrete colony from Nutrient Agar was picked with a sterile wire loop and emulsified in a drop of distilled water on clean grease free glass slide and allowed to dry. The dried smear was fixed by passing over a Bunsen burner flame; this was covered with crystal violet for 60 seconds. The slide was then rapidly washed off with clean water from running tap. Next the smear was covered with Lugol's iodine for another 60 seconds and again washed off with clean water. The smear was

decolorized with acetone and was washed off immediately, before covering with neutral red to counter stain for 60seconds. Finally, the slide was washed and air dried before viewing with microscope under an oil immersion objective. Suspected *S. aureus*appeared as gram positive cocci, in clusters, singles, pairs and violet in colour.

3.6.2Biochemical tests

The suspected colonies from the cultured medium were confirmed by biochemical tests as described by Cowan and Steel (2004).

3.6.2.1 Catalase Test

After confirming the organisms to be Gram positive cocci, the ability to produce the enzyme catalase by the organisms was observed. A wooden applicator stick was used to transfer a colony of the test organism to the surface of a glass slide which was placed in a petri dish. A drop of 3% H_2O_2 was added on the glass slide and observed for bubble formation. A rapid and sustained bubble formation was indicative of a positive test. *Staphylococcus aureus* is catalase positive. A few tiny bubbles after 20-30 seconds were not considered as *S. aureus*.

3.6.2.2 Tube Coagulase Test

All the suspected *S. aureus* isolates were subjected to coagulase test to differentiate them from *S. epidermidis* and *S. saprophyticus*. This was carried out by emulsifying several isolated colonies of test organism in 0.5 ml of physiological saline contained in a clean test tube. Citrated human plasma (1ml) was added and incubated at 35^0C for 1-4 h and even over night for proper coagulation. Clot formation was observed, those that were negative after four hours, were incubated at room temperature overnight and checked again for clot formation. *Staphylococcus aureus* are positive for

coagulase and some are negative. Only the coagulase positivewere subjected for further biochemicals.

3.6.2.3 DNAse Test.

The suspected *S. aureus* isolates that were coagulase positive were subjected to DNAse test to differentiate them (producer of the enzyme deoxyribonuclease) from other staphylococci spp. This was carried out by heavily spot-inoculating DNAse agar (Oxoid) with several colonies of the suspected (Gram, catalase and coagulase positive)Staphylococcal colonies from the nutreint agar plate, and was incubated for 24 h at 35°C. After an overnight incubation, the colonies were tested for DNase production by flooding the plates with a weak hydrochloric acid solution (1mol) and excess acid was drained off. The acid precipitated the unhydrolyzed DNA. DNase-producing colonies were surrounded by clear zone due to DNA hydrolysis withing 5mins after floading.

3.6.2.4 Sugar fermentation test (Mannitol, Sucrose, Arabinose and Rafinose)

Sugar fermentation test was carried out for the 52 suspected *S.aureus* by preparing series of test tube containing sterile phenol red, peptone water with 1% each of the fermentable sugar and inverted Durham's tubes. Each of the test tubes was inoculated with suspected *Staphylococcus aureus* isolate. The inoculated tubes were incubated at 35^0C for 24 h. At the end of the incubation period, all tubes were examined for acid and gas production and then compared with the control for interpretation. Tubes showing negative results were incubated for further confirmation.

3.7 Antibiotic Susceptibility Testing

3.7.1 Preparation of McFarland Standard

A 0.5 McFarland standard was prepared by adding 0.05ml of 1% (g/l) solution of Barium chloride $BaCl_2$ to 9.95ml of 1% Sulphuric acid (H_2SO_4).

3.7.2 Determination of antibiotic susceptibility pattern of the isolates

The antibiotic susceptibility pattern of the isolates was determined by the Kirby Bauer disk diffusion method. Susceptibility of the isolates to the following antibiotics was tested: Oxacillin (1µg), cefoxitin (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), ofloxacin (5µg), tetracyclin (30µg), erythromycin (15µg) and penicillin (10 µg).

3.7.2.1 Inoculation of test plates

Four to five well isolated colonies of the same morphology were picked aseptically from an agar plate culture with a sterile wire loop and transferred to a tube/bottle containing normal saline, the turbidity of medium was matched with 0.5 McFarland turbidity standards. This was then kept at room temperature for three hours.

After adjusting to McFarland turbidity standard, a sterile swab was dipped inside, rotated several times, pressing firmly on the inside wall of the tube and above the fluid level to remove excess inoculums from the swab. The swab was used to inoculate dried surface of the Muller- Hinton Agar plate by swabbing over the entire surface. The plate cover was replaced and allowed to stand for 3 to 5mins, for any excess surface moisture to be absorbed. Appropriate antibiotic-impregnated disks were placed on the surface of the agar plates using a drug dispenser (Oxoid). Plates were covered,

inverted and incubated immediately in 35^0C incubator for 16-18h. Afterwards, the plates were read by measuring the diameter of zone of inhibition to the nearest millimeter. Interpretation of the zone of inhibition was done in accordance to CLSI (2015) break point for each antibiotic.

3.8 Detection of Methicillin Resistance using Cefoxitin Disc Diffusion

A 0.5 McFarland standard suspension of the isolates were made and a lawn culture was done on MHA plate. Cefoxitin 30 µg discs were placed on each plate and were incubated at 35^oC for 18 h and zone diameter was measured in reflected light (CLSI, 2015).

3.9 Determination of Multiple Antibiotic Resistance (MAR) Index

The Multiple Antibiotic Resistance (MAR) index was determined for each isolate by dividing the number of antibiotics to which the organisms is resistant to by the total number of antibiotics tested (Christopher *et al*., 2013;Obajuluwa, 2014).

3.10 Molecular Detection of *mecA* Gene by Polymerase Chain Reaction (PCR)

3.10.1 DNA extraction

The procedure was carried out according to the method adopted by Obajuluwa (2014): Lysate preparation: *Staphylococcus aureus* culture (1.5 ml) was vortexed for 15 seconds and 1 ml was aliquot into a microcentrifuge tube and centrifuged at 14,000 rpm. The supernatant was poured out and resuspended in 100 µl of digestion buffer containing lysozyme/lysostaphin mix. The tube was incubated at 37 ^{0}C for 45 minutes.

After incubation, 300 µl of lysis solution and 10 µl of reconstituted proteinase K was added to the digestion mixture and mixed well by vortexing. The lysate was then incubated at 55^{0}C for 45 minutes. After incubation, 40µl of binding solution and 180 µl of 96-100% ethanol were added to the lysis mixture, and mixed by vortexing. The sample was spinned for 10 seconds at 14,0000rpm. Using a pipette, the clear aqueous phase only was carefully transferred to a spin column that has been attached to a collection tube. The column assembly was centrifuged for 3minutes at 14,000 rpm to bind the bacterial DNA. Five hundred (500) µl of wash solution I was applied to the column and centrifuged for 2 minutes at 14,0000 rpm. The flow-through was discarded and the column reassembled and the collection tube. Five hundred (500) µl of wash solution II was added to the column and centrifuged again for 2 minutes at 14,0000 rpm. The elution was done by transferring the spin column to the provided 1.7 ml elution tube. Then 75 µl of elution buffer were applied to the column and centrifuge at 6,000 rpm for 2 minutes. This was spinned for an additional 2 minutes at 14,0000 rpm to complete the DNA elution.

3.10.2 Amplification of the mecA gene

The polymerase chain reaction (PCR) amplification of mecA gene was conducted according to the method of Strommenger *et al.* (2003) as shown below;1× Phusion GC buffer containing 1.5 mM $MgCl_2$, 200 µm each dNTP, 2 µm each primer, 0.1 µg template DNA, 3% (v/v) DMSO and 1 U Phusion DNA polymerase (Finnzymes). The amplification conditions was initial denaturation at 95°C for 30sec, for 50 cycles, annealing 55°C for 30secs, extension at 72°C for 1min and final extension at 72°C for 5min. The primer sequences specific formecA used in the study is shown in table 3.1.

Table3.1: Nucleotide sequence and amplicon size of the mecA specific primer.

Gene	Primer Sequence	Amplicon Size	Reference
mecA	F-5'-AAAATCGATGGTAAAGGTTGGC-3'	533bp	Strommenger *et al.,* 2003
	R-5'-AGTTCTGCAGTACCGGATTTGC-3'		

3.10.2.1 Visualization of the product

For the visualization of the product, 10 µl of each PCR reaction product was mixed with 5 µl 5× loading dye and loaded on 1.5% agarose gel for electrophoresis and visualization of the amplified amplicons. A 100 bp molecular weight DNA ladder was used for the validation of length of the amplified products.

3.11 Data Analysis

The data obtained were analyzed statistically using Graphpad QuickCals (www.graphpad.com). Chi square was used to determine the association between *S. aureus* colonization, gender and age group.

CHAPTER FOUR

4.0 RESULTS

4.1 Prevalence of *S. aureus* isolated from IDP's in Maiduguri.

In this study, 400 nasal swabs were collected from IDP's in some selected camps within Maiduguri metropolis. Out of the 400 samples, 51 *S. aureus* were isolated giving a prevalence of 12.75% (Figure 4.1).

4.2 Prevalence of *S. aurues* isolated from some selected IDP camps in Maiduguri.

Out of the 400 samples collected, 50(12.5%) were from MOGCOLIS camp out of which 5 were positive giving a prevalence of 10%, 86(21.5%) from Arabic Teachers College camp out of which 9 were positive with a prevalence of 10.47%, 108(27%) from Govt.Girls College camp out of which 18 were positive with a prevalence of 16.67%, 97(24.25%) from Fedral Training Center camp giving a positive of 12 with a prevalence of 12.37% and 59(14.75%) from Sandakyarimi camp out of which 7 were positive giving a prevalence of 11.86% (Figure 4.2). The difference observed is not statistically significant (χ^2 = 2.289, df = 4, P = 0.6828).

4.3 Prevalence of *S. aureus* based on gender of the IDPs

Figure 4.3 shows the prevalence of *S.aureus* based on gender .Out of the 400nasal swabs collected, 273(68.25%) were collected from female IDPs out of which 37 were positive giving a prevalence of 13.55% and 127(31.75%) was from male IDPs out of which 14 were positive with a prevalence of 11.02%.The difference observed is not statistically significant (χ^2 =0.497,df=1, P= 0.4808).

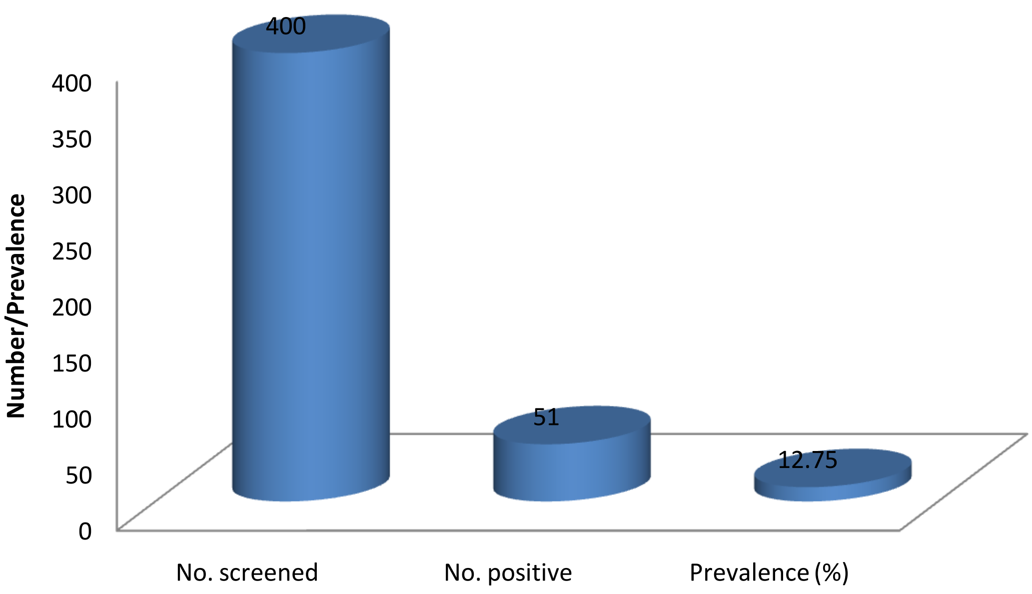
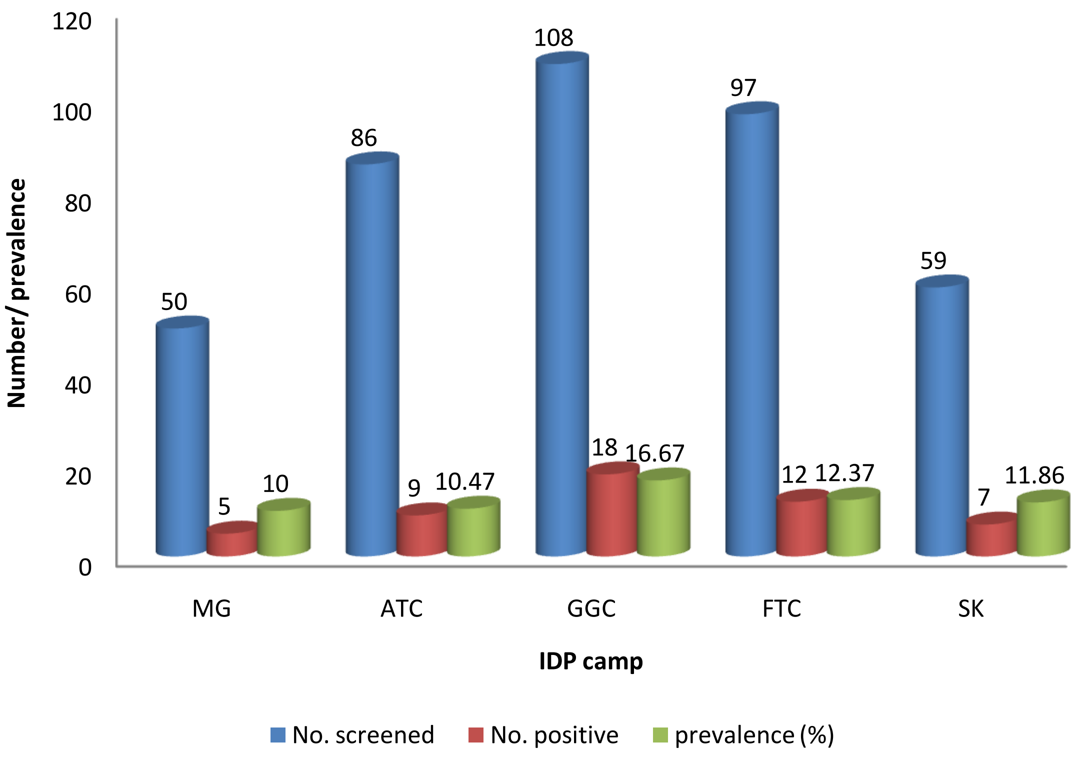


Figure4.1: Prevalence of *S. aureus* isolated from the anterior nares of IDPs in Maiduguri, Nigeria.



Key: MG=Mohamed Goni College of Legal and Islamic studies, ATC=Arabic Teachers College, GGC=Government Girls College, FTC=Federal Training Center, SK=Sanda Kyarimi.

Figure 4.2: Prevalence of *S. aureus* isolated from different IDP camps in Maiduguri, Nigeria.

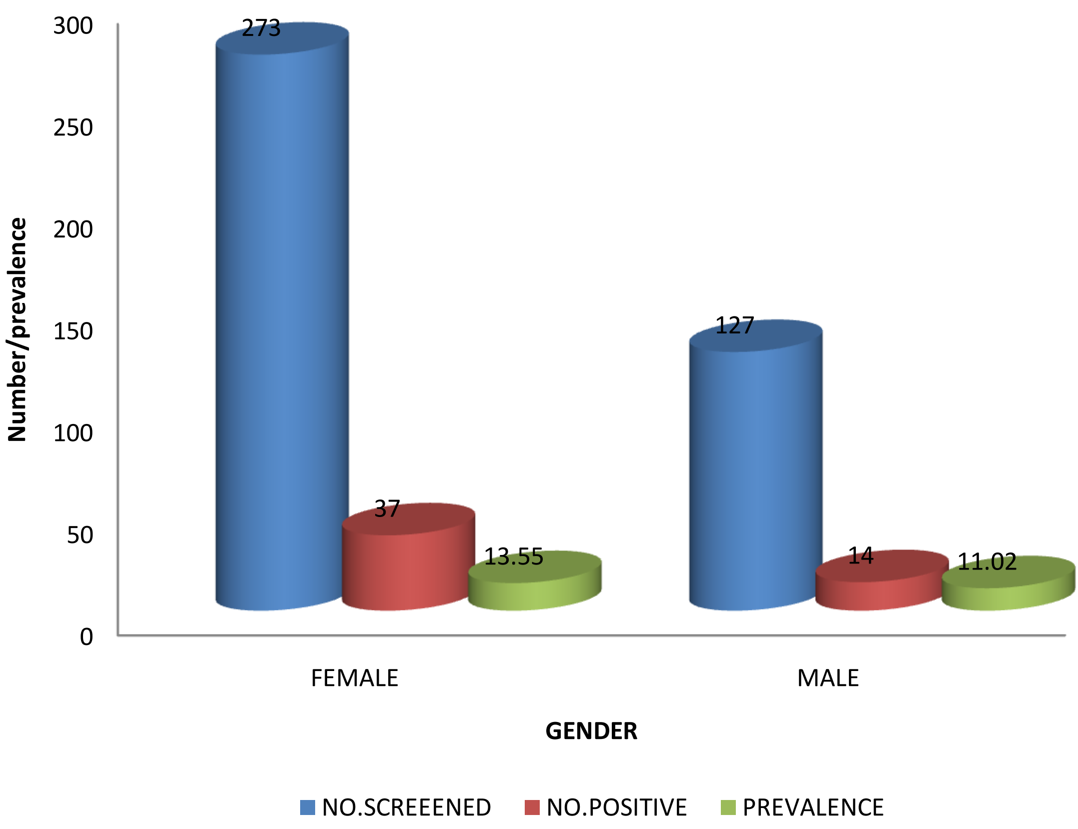


Figure 4.3: Prevalence of *S. aureus* based on gender of the IDPs in Maiduguri, Nigeria.

4.4 Prevalence of *S. aureus* by age group of the IDPs

The age group prevalence of *S. aureus* is presented in Figure 4.4.The age group with the highest prevalence was from ages <10years giving a prevalence of 18.46% followed by age group 11-19years with a prevalence of 16.94%. Zero (0%) prevalence was observed in age group >60.The difference observed is not statistically significant (χ^2 = 11.445, df= 6, P= 0.0756)

4.5 Antibiotic Susceptibilities Testing.

4.5.1 Antibiotic Susceptibilities of the *S. aureus* isolates

Out of the 51 isolates screened, 46 were found to be resistant to oxacillin and cefoxitin (90.20%) and is the highest resistance recorded in the study followed by penicillin with a percentage resisitance of 84.31%. Lowest resistance was recorded to ciprofloxacin (1.96%).The level of susceptibility was also recorded which was found higher to chloramphenicol and ofloxacin (100%) and lowest recorded to oxacillin and cefoxitin (9.80%).The result of the susceptibility pattern is presented in Table 4.1.

4.5.2 Multiple Antibiotic Resistance (MAR) Indices and resistance pattern of the *S.aureus*isolates.

Table 4.2 shows the antibiotic resistance pattern and the Multiple Antibiotic Resistance Index of the *S. aureus* isolates. All the isolates screened were resistant to at least one of the antibiotics tested. The MAR index of the isolates was between 0.13 and 0.75.Most of the isolates (17) were resistant to 4 out of the 8 antibiotics tested (MAR index = 0.50) and 11 of the isolates screened were resistant to 3 out of the 8 antibiotics tested (MAR index = 0.38). Six of the isolates were resistant to 2 out of the 8 antibiotics tested (0.25). None of the isolates was resistant to all the 8 antibiotics tested.

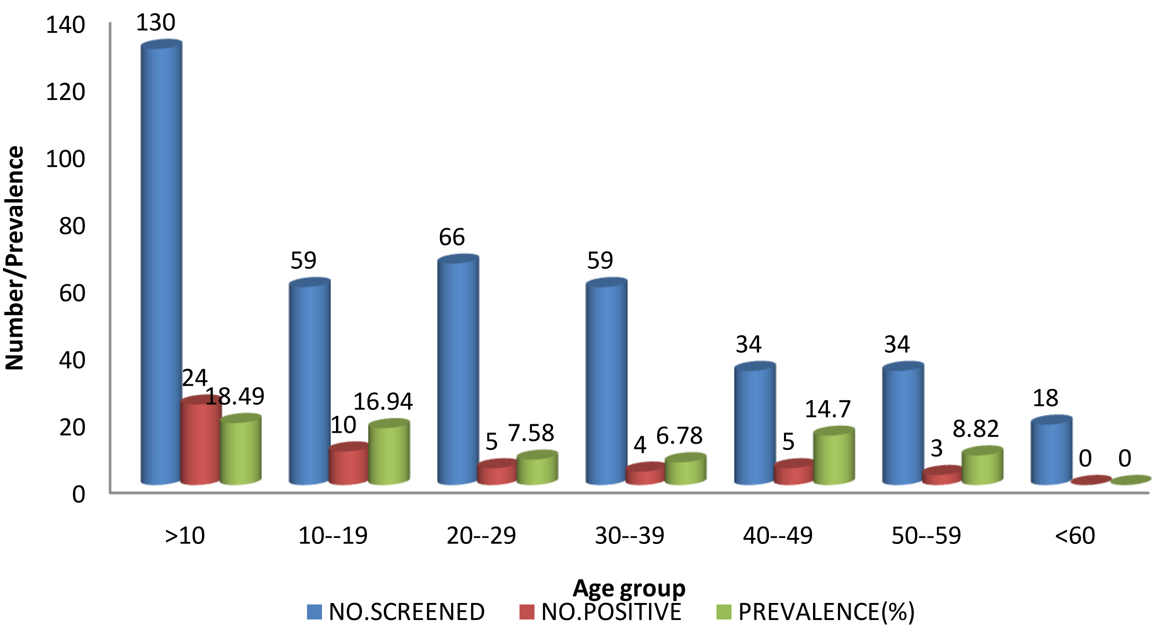


Figure 4.4: Prevalence of *S. aureus* isolated based on age group of the IDPs in Maiduguri, Nigeria.

Table 4.1: Antibiotic susceptibility pattern of *S. aureus* isolated from IDP's in Maiduguri.

Antibiotic (disk content)		Number		
	*n = 51	Susceptible (%)	Intermediate (%)	Resistant (%)
Oxacillin	(1µg)	05(9.80)	0(0.00)	46(90.20)
Tetracyclin	(30µg)	21(41.18)	4(7.84)	26(50.98)
Penicillin	(10 iu)	08(15.69)	0(0.00)	43(84.31)
Ciprofloxacilin	(5µg)	50(98.04)	0(0.00)	01(1.96)
Erythromycin	(15µg)	24(47.06)	8(15.69)	19(37.25)
Ofloxacin	(5µg)	50(98.04)	1(1.96)	0(0.00)
Cefoxatin	(30µg)	05(9.80)	0(0.00)	46(90.20)
Chloramphenicol	(30µg)	50(98.04)	1(1.96)	0(0.00)

Table 4.2: Multiple Antibiotic Resistance (MAR) Indices and resistance patterns of *S. aureus* isolated from IDP's camp in Maiduguri.

Number of isolates	Number of antibiotics Resisted	Resistance pattern	MAR index
1	1	E	0.13
4	2	TE,E	0.25
2	2	OX,FOX	0.25
10	3	P, OX, FOX	0.36
1	3	TE,OX,FOX	0.36
7	4	P,E,OX,FOX	0.50
10	4	TE,P,OX,FOX	0.50
13	5	TE,P,E,OX,FOX	0.63
2	6	C, TE, P, E, OX, FOX.	0.75
1	6	OFX,TE,P,CIP,OX,FOX	0.75

Key: E = erythromycin, TE = tetracycline, OX = oxacillin, FOX = cefoxatine, P = penicillin, CIP = ciprofloxacin, OFX = ofloxacin, C = chloramphenicol.

4.6 Prevalence of Phenotypic Methicilin Resistance among the *S. aureus* isolates.

Figure 4.5 presents the prevalence of phenotypic MRSA obtained among the *S. aureus* isolates. Out of the 51 isolates screened, 46 were found to be MRSA phenotypically with a prevalence of 90.20% and 5 were MSSA giving a prevalence of 9.08%.

4.7 Prevalence of Phenotypic MRSA obtained from each IDP camp.

Figure 4.6 shows the prevalence of phenotypic MRSA that was obtained from each camp. Highest prevalence was recorded at MG and GGC camp (100%) followed by FTC camp with a prevalence of 91.67% while low prevalence was recorded at SK camp giving a prevance of 71.42%.

4.8 Detection of MecAgene among the Phenotypic MRSA isolates by PCR

Eight MRSA isolates were selected at random from the five camps with at least 1 from each camp. Plate I shows the agarose gel electrophoresis of PCR amplicons of mecAgene. Primer and Nuclease free water were loaded into well 8 and 10 as controls respectively. All the amplicons produced bands corresponding to 533bp which is the expectedamplicon size of the mecA gene.

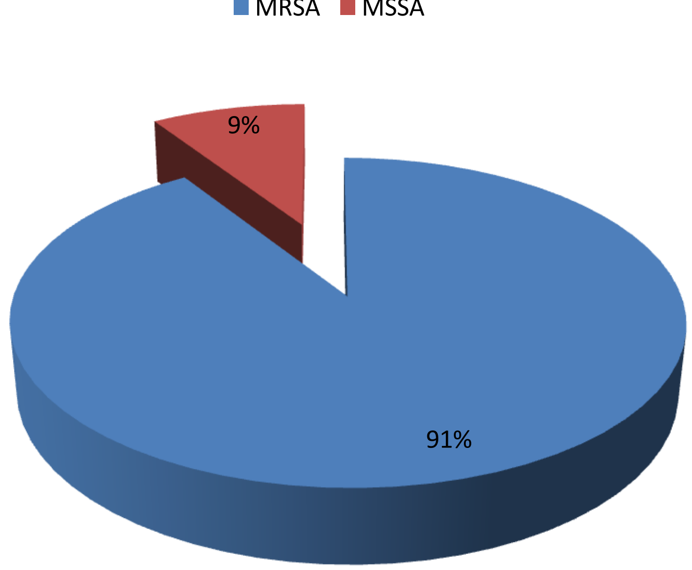
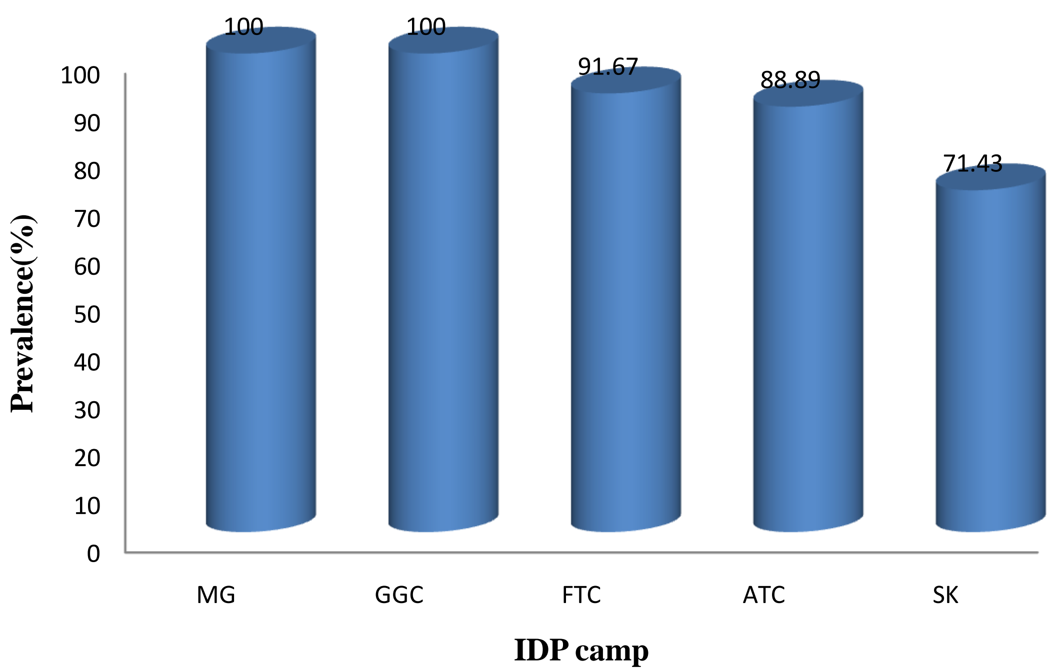


Figure 4.5: Prevalence of Phenotypic Methicilin resistance among the *S. aureus* isolate



Key: MG=Mohamed Goni College of Legal and Islamic studies, ATC=Arabic Teachers College, GGC=Government Girls College, FTC=Federal Training Center, SK=Sanda Kyarimi

Figure4.6: Prevalence of Phenotypic MRSA obtained from each IDP camp in Maiduguri, Nigeria.

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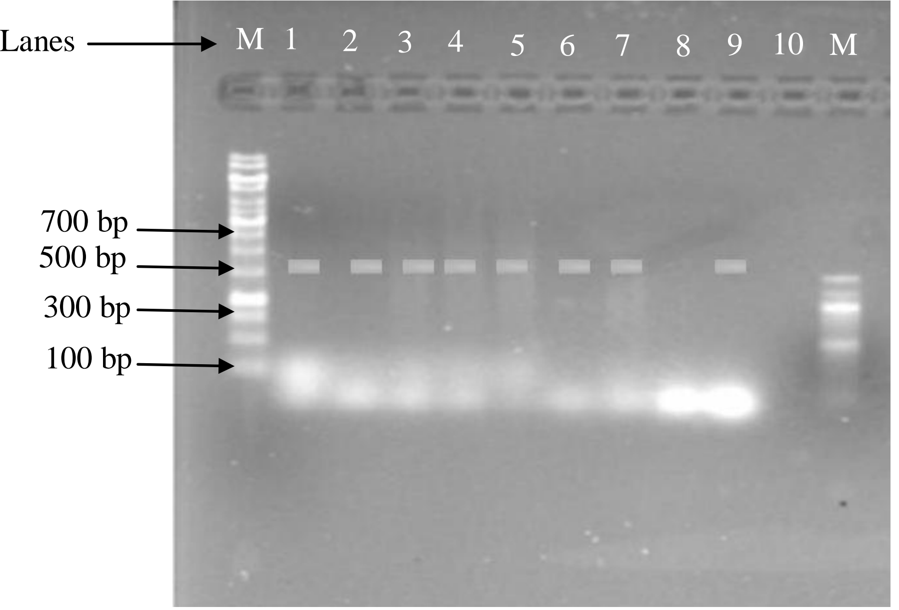

Plate I: Amplicon of *S.aureus* mecA gene size 533bp.Lanes 1-7 and 9 PCR amplicons, lane 8 and 10 negetive controls, lane M100bp DNA ladder.

CHAPTER FIVE

5.0 DISCUSSION

In this study, the overall prevalence of *S. aureus* isolated from the nasal swab of internally displaced persons in Maiduguri Nigeria was 12.75%. The reason for this prevalence rate could be attributed to the poor living condition of the IDPs. A lower prevalence of 2.1% and 4.3% of *S. aureus* from nasal swab were reported by Adelowo *et al.* (2014) and Adetayo *et al.* (2014) in Maiduguri and Ibadan respectively. However the prevalence rate of 12.75% observed in this study is considered low when compared to that of Nworie *et al.* (2013) who reported a prevalence of (23%) and the reported prevalence level that ranged between 16.8%-90% in Saudi Arabia, Delhi and Iran (Alghaithy *et al.*, 2000; Goyal *et al.*, 2002; Heininjar *et al.*, 2007; Askarian *et al.*, 2009). The *S. aureus* colonisation varies with geographical location, studied population and sampling procedures (Bell and Turndge, 2002). Therefore, the difference observed in the prevalence could be due to the difference in study subjects and geographical location.

Distribution of *S. aureus* colonization varies from one location to another even within the same setting (Adelowo *et al.,* 2014). As seen in this study, GGC camp had the highest prevalence of *S. aureus* colonization (16.67%) while MG camp had the lowest prevalence of 10%. The fact that GGC camp is overcrowded and the level of hygiene in this camp is poor argues in favour of the high prevalence observed in this camp. So also the IDPs found in GCC camp are mostly from the same location (Bama).

Staphylococcus aureus colonisation level is known to be influenced by age and gender (Huh *et al.*, 2012; Adelowo *et al.*, 2014). In this study, a higher prevalence was obtained from females (13.55%) compared to that obtained from males (11.02%). The higher prevalence observed in

females could be due to the fact that the females are engaged in more activities, always confined in the camps and their rooms or tents are congested which favours transmission of *S. aureus*. It may also be due to the fact that a higher number of females were screened (68.25%). However, it is in disagreement with the findings of Nwankwo and Nasiru (2011) who reported a higher prevalence in male (62.0%) than females (38.0%).The difference could be due to difference in study subjects, geographical location, sample site and number of samples collected.

Higher prevalence was also observed in age group <10 years (18.49%) in which both neonates and infants are included. Followed by age group 10-20 years (16.94%) and none were obtained from age group ≥60 years. The high prevalence observed in lower age groups could be attributed to the life style and low hygiene level of members of these age groups. It is also believed that their immunity is not properly developed at this stage to cope with bacterial infections hence they are vulnerable and easily infected especially when stressed and malnourished. The older children have also been observed to be more active than adults during their interaction with their playmates and while playing for hours, come in contact with various objects. In this process, they become a target to ubiquitous bacteria such as *S. aureus*. The prevalence of *S. aureus* observed in the lower age group in this study agrees with that of Nwankwo and Nasiru (2011) who also observed a higher prevalence of 47.3% in the lower age group.

Antimicrobial resistance has been noticed as one of the paramount microbial threats in the twenty first century. The development of antimicrobial resistance is a natural phenomenon. However, certain human actions accelerate it emergence and spread. The inappropriate use of antimicrobial drugs, including animal husbandry, favours the emergence and selection of resistant strain. Poor infection prevention and control practices contribute to further emergence and spread of antimicrobial resistance (WHO, 2015).

Staphylococcus aureus develops resistance very quickly and successfully to different antimicrobials over a period of time (Nwanko and Nasiru, 2011).The susceptibility test of *S. aureus* in this study showed a high level of resistance to oxacillin and cefoxatin (90.20%), followed by penicillin (84.31%). Relatively high level of resistance was observed to tetracycline (50.98%) and erythromycin (37.25%) but lower resistance was observed to ciprofloxacin (1.96%) and no resistance was observed to chloramphenicol and ofloxacin. The high level resistance could be associated with earlier exposure of these drugs to isolates which may have enhanced development of resistance and there is high level antibiotic abuse in this environment arising from self-medication. This is often associated with inadequate dosage and failure to comply with treatment and availability of antibiotics to consumers across the counters with or without prescription (Nwankwo and Nasiru, 2011).This agrees with the findings of Balami *et al.* (2016) who also reported a high level of resistance to oxacilin, penicillin and cefoxitin in Maiduguri, Borno State.

Resistance of *S. aureus* to antibiotics appeared within a few years after the onset of the antibiotic era, and this problem has reached epic proportions owing to over use and improper use of antibiotics. *Staphylococcus aureus* resistance to antibiotics currently spans all known classes of natural and synthetic compounds. Staphylococcal infections are typically associated with death of tissue, and evidence suggests intracellular bacteria are capable of inducing apoptosis. *Staphylococcus aureus*-mediated apoptosis has been reported in epithelial cells, keratinocytes, endothelial cells, and osteoblasts (Chakraborty *et al*., 2012).

The high level of resistance to oxacillin, cefoxatin and penicillin could be attributed to the emergence and spread of β-lactamases among *S. aureus* and microorganisms in general.*Staphylococcus aureus* have being reported to have ability to acquire many resistance gene

from other bacteria for example acquisition of vancomycin and methicillin resistance gene from *Enterococcus faecalis.* This is consistent with the observation that staphylococcal isolates are resistant to a large number of commonly prescribed antimicrobial agents and to β- lactams in particular (Olukoya *et al*., 2005).The low resistance to ciprofloxacin (1.96%) and non to chloramphenicol and oflaxacin (0.00%) observed in this study is an indication that these drugs could be used in the treatment of infections caused by *S. aureus*.

Antibiotic resistance has no border to cross especially in settings where proper infection control is not in practice. It doesn't discriminate between the specimen from which the organism was isolated or even the gender and age of the individual from which it was isolated as demonstrated by the isolates screened in this study.

Ciprofloxacin, a quinolone is a potent inhibitor of nucleic acid synthesis. The susceptibility level of *S. aureus* isolates to ciprofloxacin (98.04%) in this study is high.This is because ciprofloxacin is relatively expensive and less available for drug abuse in this environment. This is similar when compared with the findings of Balami *et al* (2016) and Obajuluwa (2014) who alsoreported 100% susceptibility to ciprofloxacin. It also agrees with the findings of Ehinmidu (2003) who reported a 97.06% sensitivity level in *S. aureus* isolated from urine and Onanuga *et al*., (2005) who reported a 96.7%.

The use of antibiotic and its abuse by human (e.g. tetracycline and erythromycin) could be attributed to the resistance to these antibiotics. Adelowo *et al.* (2014) reported a high level of resistance to erythromycin (90%) and tetracycline (70%). However, in this study, a lower resistance to erythromycin was observed (37.25%) as compared to the 90% reported by Adelowo *et al.* (2014) in Maiduguri the same place where this study was carried out. The reason for the

difference obtained could be due to difference in antibiotic usage, sample site as well as study subjects.

High susceptibility was recorded to chloramphenicol and ofloxacin (98.04%) in this study. This is considered high when compared with the work of Nwanko and Nasiru (2011) who observed a lower percentage 76.6%. His findings is in consistent with the reports from Abakaliki by Amadi *et al.* (2008) and in Owerri by Uwazuoke and Aririatu(2004) all in Nigeria who also reported a lower percentage as compared with the reports of this work .The high susceptibility to ofloxacin may be due to its limited use and the susceptibility to chloramphenicol could be due to the fact that it is no more available for oral use.

The high level of drug resistance and MAR indices (0.13-0.75) observed among the isolates screened in this study is alarming. The MAR index gives an insight on the number of isolates showing antibiotic resistance and the consequence risk zone in routine susceptibility testing.

Isolates with MAR index value higher than 0.2 are considered to have originated from high risk sources where antibiotics are often used (Christopher *et al.,* 2013). It then follows that 98.04% (50/51) of the isolates in this study originated from high risk environment where antibiotics are frequently used. However, the high MAR index may be due to antibiotics abuse or it may be as result of increase in the emergence of multidrug resistant strains. The major reason for the spread of antibiotic resistance observed may be due to inappropriate antibiotics prescription, incorrect dose or duration of use, adulteration of drugs and use of antibiotics without prescription. This is in agreement with the findings of Balami *et al*., 2016 who also reported isolates with MAR index higher than 0.2.

The use of antibiotics for treating people in most developing countries is unregulated such that antibiotics could be purchased in pharmacies, general stores, markets and even motor parks with the implication that there is a widespread and uncontrolled abuse. For example patients often do not take full course of treatment, furthermore, many antibiotics in the developing countries are of low quality or fake and adulterated coupled with bad storage and management processes. All these may lead to phenotypic adaptations resulting in resistant isolates. Methicillin-resistant *Staphylococus aureus* can produce a host of conditions ranging from mild to severe skin infections to fatal pneumonia, osteomyelitis, septicarthritis, endocarditis, abscesses, bacteremia and septicemia.

The increasing frequency of drug resistance has been attributed to combination of microbial characteristics, selective pressure of antimicrobial use and societal and technological changes that enhance the transmission of drug resistant organisms (Orozova *et al*., 2008).

The prevalence of phenotypic MRSA isolated from the nasal swab of IDPs in Maiduguri as observed in this study is 90.20%. The reason for this high prevalence could be due to the poor living condition of the IDPs which favours transmission of MRSA. This however disagrees with the findings Adelowo *et al* (2014) and Okon *et al.*(2013) who reported 47.6% and 12.5% prevalence respectively in Maiduguri. Obajuluwa(2014) also reported a prevalence of 64.7% and 56.3% from skin and bed swabs respectively in Zaria. However, a much lower prevalence (4.8%) of MRSA from nasal swab of patients in India was reported by Bala *et al.* (2010). In comparison with other reported MRSA isolates from different part of the country, Nigeria: Fayomi (2009) reported a prevalence rate of 31% of MRSA among in-patients at a tertiary health facility in Ido-Ekiti; Azeez- Akande *et al*. (2008) reported a MRSA prevalence rate of 37.5% from clinical specimens at University of Calabar Teaching Hospital; 43% prevalence was reported in Jos by

Ikeh (2003) and from Ilorin, 34.7% was reported by Taiwo *et al*., (2004). Olonitola *et al*. (2007b) reported a rate of 33.3% from a Federal Medical Centre, Onanuga *et al*. (2006) reported a rate of 71.7% from urine of healthy women in Abuja while it is in agreement with the findings of Ikeh and Yakeu (2006) who reported an alarming 92.6% MRSA out of the *S. aureus* isolated from bacterial flora on the hands of nursing service workers in Jos University Teaching Hospital. The reason for difference and higher prevalence observed in this study could be attributed to the fact that the IDPs are more exposed to predisposing factor of MRSA colonization. Moreover the IDPs are malnourished and stressed which could lead to a supressed immunity making them more prone to MRSA colonization and other diseases as well. All these could account for the higher prevalence observed.

Prevalence of MRSA varies from one area to another (Adetayo *et al*., 2014). As seen in this study the prevalence of phenotypic MRSA was found higher in MG and GGC camp (100%) followed by FTC camp with a prevalence of 91.67%.The reason for the higher prevalence in these camps could be due to the fact that these camps are overcrowded and the level of hygiene among the inhabitants of these camps is poor.

The gold standard for detection of MRSA is the detection of mecA gene by polymerase chain reaction (PCR) (Obajuluwa, 2014). Expression of mecA gene yields an altered penicillin binding protein(PBP2a) with reduced affinity for β- lactam antibiotic binding. In this study, the PCR result for the detection of mecAgene shows the presence of mecAgene in all the isolates screened (100%). This suggests that the screening technique used (cefoxitin as surrogate) is sensitive and specific. In North-eastern Nigeria where this work was carried out, there has not been any reported case on the detection of mecA gene from nasal swabs of the internally displaced persons to the best of our knowledge. This agrees with the findings of Obajuluwa, 2014 where two MRSA isolates

with mecAgene were detected in Ahmadu Bello University Teaching Hospital Zaria. Shittu *et al*. (2011) also reported two MRSA isolates with mecAgene which were detected in Ile-Ife, one from Lagos and two from Ibadan (all in South western Nigeria) respectively. In the same study, five MRSA isolates with mecA gene were detected in Maiduguri (North eastern Nigeria) (Shittu *et al*., 2011). Okon *et al*. (2013) reported the presence of mecA gene in twelve MRSA isolates from clinical specimens from six tertiary hospitals in North-eastern Nigeria.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1CONCLUSION

In conclusion, the prevalence of *S. aureus* and MRSA isolated from nasal swab of IDPs were found to be 12.75% and 90.20% respectively. The susceptibility test result shows that most of the isolates were resistant to the beta lactam antibiotics.

The PCR result confirms the presence of mecA gene among the MRSA isolates. This shows that the resistance was mediated by possession of mecA gene by the MRSA isolates.

Antibiotic resistance is on the increase in Nigeria. Based on the findings of this study we can state that the prevalence of MRSA among the IDPs is relatively high (90.20%) and the resistance pattern is of public health concern. The reason has been that, these resistance strains and genes could be transmitted and disseminated within the IDPs, the community they live in and subsequently to the next environment.

6.2 RECOMMENDATION

1. The prevalence of MRSA observed in this study is extremely high, therefore it is very important to put in measures to contain their spread and continuous surveillance both in the IDP camps and the community.
2. Further studies are needed to define optimum use of ciprofloxacin, chloramphenicol and ofloxacin as agents for treatment of MRSA colonization and infection.
3. Since mecA gene mediated MRSA has been detected in different parts of the country, there is need for the various institutions and the government to formulate policy tocombat its spread

either in the camps, hospitals or within the community before it becomes a major health problem in Nigeria.

4. It is critical to find out new alternative therapies to antibiotics and the need to produce new antibiotics to combat drug resistance.
5. Robust antimicrobial stewardship and strengthened infection control measures are required to prevent spread and reduce emergence of resistance.

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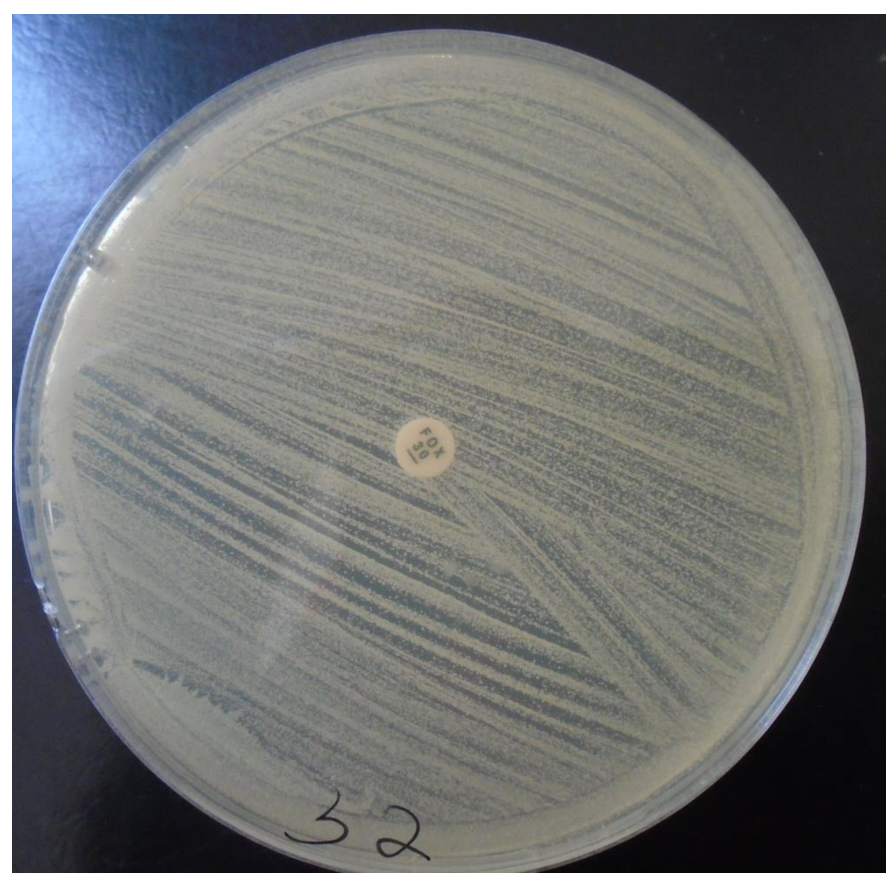
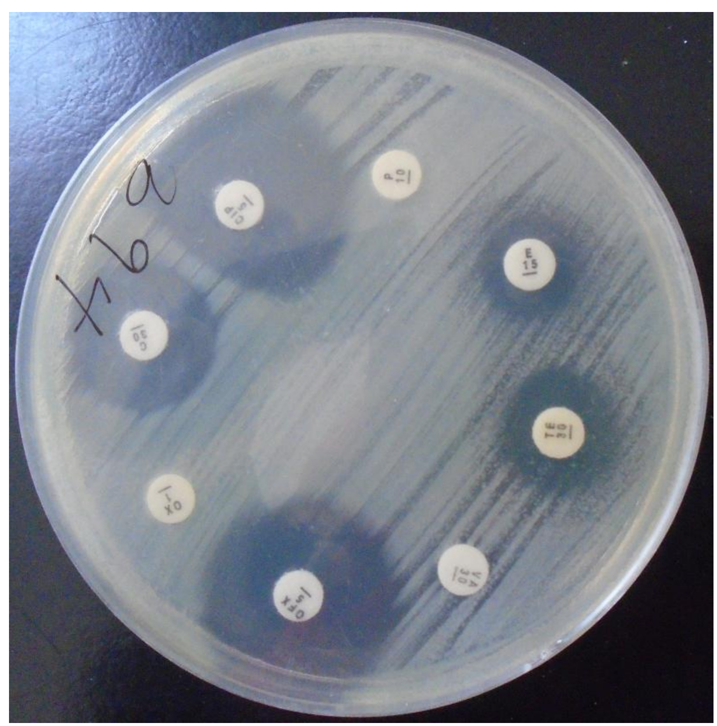
APPENDICES

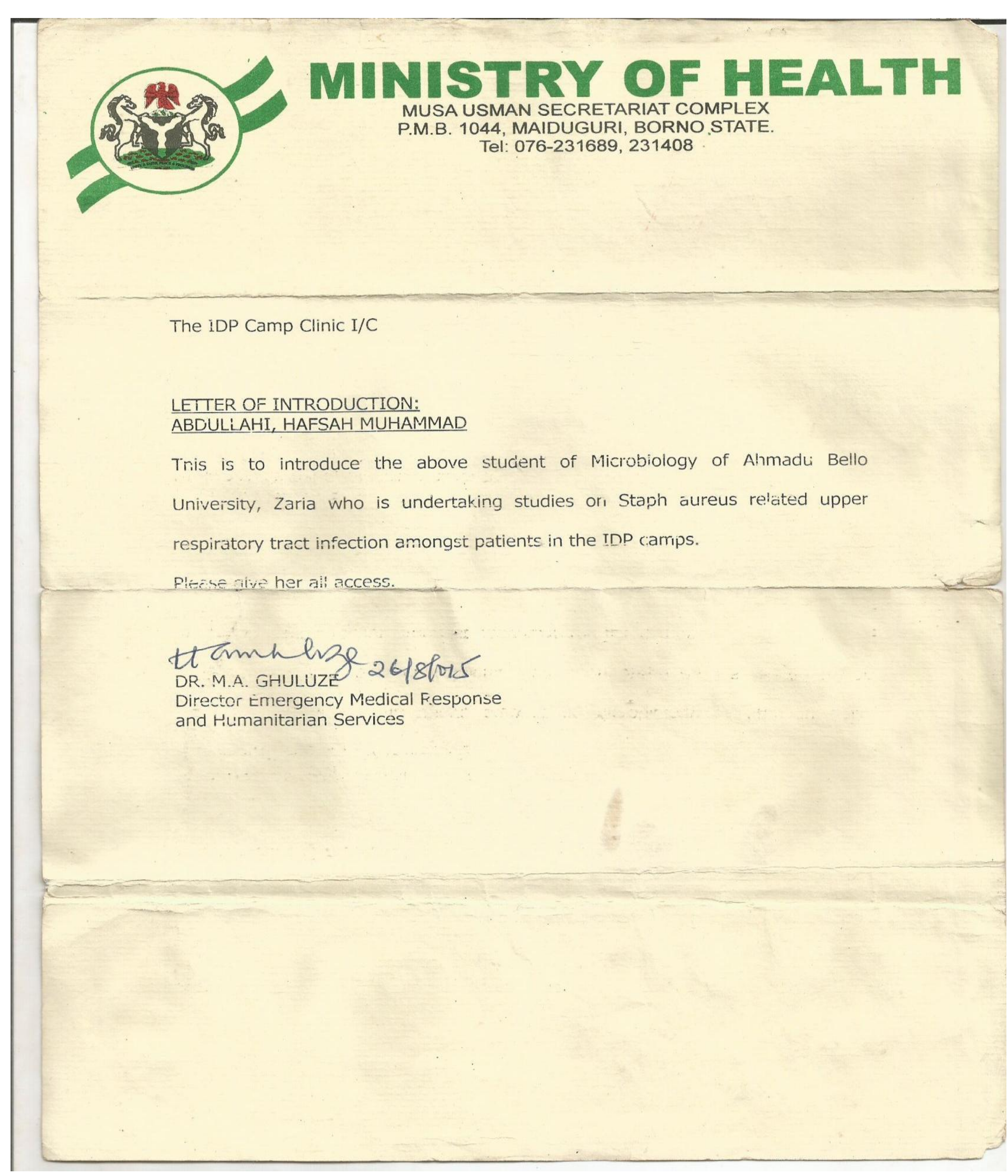
Appendix I: Phenotypic detection of methicillin resistance in *S.aureus* on MHA (Cefoxitin used as a surrogate for oxacillin to detect for MRSA , mecA mediated)



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



Appendix III: Ethical approval from Ministry of Health Maiduguri, Borno State.



MINISTRY OF HEALTH
MUSA USMAN SECRETARIAT COMPLEX
P.M.B. 1044, MAIDUGURI, BORNO STATE.
Tel: 076-231689, 231408

The IDP Camp Clinic I/C

LETTER OF INTRODUCTION:
ABDULLAHI, HAFSAH MUHAMMAD

This is to introduce the above student of Microbiology of Ahmadu Bello University, Zaria who is undertaking studies on Staph aureus related upper respiratory tract infection amongst patients in the IDP camps.

Please give her all access.

26/8/2015
DR. M.A. GHULUZE
Director Emergency Medical Response
and Humanitarian Services

Appendix IV: Clearance letter from State Emergency Management Agency.

SEMA

STATE EMERGENCY MANAGEMENT AGENCY

No. 47 Toro Road, Off Shehu Laminu Road, behind former Dujima Hotel, Maiduguri, Borno State.
website: www.bosema.gov.ng

Our Ref: ____________ Your Ref: ____________ Date ____________

BO/SEMA/1/17

08/04/2015

The Camp Commander

....

....

INTRODUCTORY LETTER

I am directed to inform you that the bearer Hafsah M. Abdullahi is a post graduate student of Ahmadu Bello University, Zaria; she is working on a research topic as per the attached letter.

2. Please allow her access into the camp to carry out her assignment.

Thank you.

STATE EMERGENCY MANAGEMENT AGENCY SEMA 10 APR 2015 BORNO STATE

Alhaji Bulam Dawud Kyari

SECRETARY

For: EXCUTIVE CHAIRMAN