SCREENING FOR VANCOMYCIN RESISTANT STAPHYLOCOCCUS AUREUS AMONG PATIENTS ATTENDING HOSPITALS IN SAMARU, ZARIA- NIGERIA.

BY

ABUBAKAR OMEIZA SALAU

DEPARTMENT OF MICROBIOLOGY,

FACULTY OF SCIENCE,

AHMADU BELLO UNIVERSITY, ZARIA

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BY

Abubakar OmeizaSALAU, B.TECH (ATBU, 2008)
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DEPARTMENT OF MICROBIOLOGY,
FACULTY OF SCIENCE,
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

OCTOBER, 2014

DECLARATION

I hereby declare that this thesis entitled "**Screening for Vancomycin Resistant *Staphylococcus aureus* Infection among patients attending Hospitals in Samaru**" has been performed by me in the Department of Microbiology under the supervision of Prof. S.E. Yakubu and Prof. S.A. Ado.

The Information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Abubakar Omeiza SALAU ____________________ __________

Name of Student Signature Date

CERTIFICATION

This thesis entitled "SCREENING FOR VANCOMYCIN RESISTANT *STAPHYLOCOCCUS AUREUS* INFECTION AMONG PATIENTS ATTENDING HOSPITALS IN SAMARU" by **Abubakar Omeiza, SALAU** meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University,Zaria,and is approved for its contribution to knowledge and literary presentation.

Prof. S.E. Yakubu

Chairman, Supervisory Committee	Signature	Date

Prof. S.A. Ado

Member, Supervisory Committee	Signature	Date

Prof. S.A. Ado

Head of Department	Signature	Date

Prof. A.Z. Hassan

Dean, School of Postgraduate studies	Signature	Date

DEDICATION

This thesis is dedicated to Allah (SWT) who through His infinite mercy made me complete this programme successfully.

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ABSTRACT

This study investigated the susceptibility of *S.aureus* to Vancomycin and other antibiotics used for the treatment of patients attending Hospitals in Samaru. A total of 400 samples comprising urine (250), wound (75) and pus swab (75) were analysed. The clinical samples yielded a prevalence of 71 (17.80%) of *S.aureus* by conventional method and confirmed with microgen analytical test kit.Antimicrobial susceptibility of the isolates was tested by disc diffusion method. There was a higher prevalence of *S.aureus* from in-patient than out-patient. The highest frequency of *S.aureus* was found in age group (0-9 years). The *S. aureus* strains showed a 100.00% susceptibility to vancomycin and gentamicin but showed resistance to ampicillin (90.10%), chloramphenicol (12.70%), erythromycin (28.20%), cefoxitin (33.80%), tetracycline(14.10%), and linezolid (9.90%). In this study, the MIC of Ampicillin against*S.aureus* ranged from 3.91 µg/ml to 62.50µg/ml, Tetracycline ranged 15.63-31.25µg/ml, Cefoxitin ranged from 7.81µg/ml to 62.50µg/ml, Chloramphenicol ranged from 3.91-31.25µg/ml and Erythromycin ranged from 3.91-15.63µg/ml. While the MBC of Ampicillin against *S.aureus* ranged from 7.81-125.00µg/ml, Tetracycline ranged from 31.25-62.50µg/ml, Cefoxitin ranged from 15.63-125.00µg/ml, Chloramphenicol ranged from 7.81-62.50µg/ml and Erythromycin ranged from 7.81-31.25µg/ml. The study showed that a significant percentage of the isolates were resistant to different antibiotics used suggesting the need for control strategies to avoid dissemination of resistant strains.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Staphylococcus aureus (*S.aureus*)continues to be a major cause of community-acquired and healthcare related infections around the world (Lowy, 1998). *Staphylococcus aureus* has the ability to cause diverse array of diseases ranging from minor infection to life threatening septicaemia and its ability to adapt to adverse environmental conditions (Franklin,2003). The emergence of high levels of penicillin resistance followed by the development and spread of strains resistant to the semi-synthetic penicillins (methicillin, oxacillin and nafcillin), macrolides, tetracyclines and aminoglycosides has made the therapy of staphylococcal disease a global challenge (Lowy, 1998; Saderi *et al.*, 2005).

In the 1980s, due to the widespread occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA), empiric therapy of the infections (particularly nosocomial sepsis) was changed to vancomycin in many countries which also increased during this period because of the growing number of infections with *Clostridium difficile* and coagulase-negative Staphylococci in healthcare facilities (Saderi *et al.,* 2005). Thus, the early 1990s saw a discernible increase in vancomycin use. As a consequence, selective pressure was established that eventually led to the emergence of strains of *S. aureus* and other species of staphylococci with decreased susceptibility to vancomycin reported from Japan (Hiramatsu *et al.*, 1997; Saderi *et al.*, 2005). The vancomycin minimum inhibitory concentration (MIC) result reports for these isolates was in the intermediate range (8-16 µg/mL) using interpretive criteria defined by the Clinical and Laboratory Standards Institute (CLSI, 2006). This report was quickly followed by similar ones from other countries, including the United States (Smith *et al.,* 1999; Saderi *et al.*, 2005), Belgium (Denis *et al.,* 2002) and Germany (Bierbaum *et al.,* 1999). These strains were called vancomycin-intermediate *S. aureus* (VISA). The first clinical infection with vancomycin-resistant *S. aureus* (VRSA) (MIC $\geq$ 32 μg/mL) was reported in July 2002 from Michigan with a second case in Pennsylvania reported shortly thereafter (Anonymous, 2002). *S. aureus* with intermediate resistance to vancomycin and teicoplanin has been reported in South Africa (Ferraz *et al.,*2000 ; Amod *et al*., 2005). Vancmycin Resistant *Staphylocccus aureus* has also been reported in Zaria, Nigeria (Olayinka *et al., 2005).*

1.1. Statement of research problem

Staphylococci have a record of developing resistance quickly and successfully to antibiotics, the defence response is a consequence of the acquisition and transfer of antibiotic resistance plasmids and possession of intrinsic resistance mechanism (Kloos, 1998).

Methicillin-resistant strains of Staphylococci emerged by the late 1970s and have become increasingly more prevalent as nosocomial pathogens (Edmond *et al.,* 1996). The medical community was comfortable by the fact that vancomycin provided effective therapy for all strains of MRSA. Nevertheless, the emergence of vancomycin-resistant strains of coagulase-negative Staphylococci caused concern that such observations might presage similar developments in *S. aureus*. Adding to these concerns were observations that vancomycin-resistant enterococci caused isolated infections or epidemics in some USA and Japanese hospitals and were becoming increasingly prevalent in critical care units (Edmond *et al.,* 1996) and high-level vancomycin resistance was experimentally transferred from *Enterococcus faecalis* to *S.*

aureus in both *in vitro* and*in vivo* models (Noble*et al.,* 1992).Though, there have been only a few reports of VRSA, the high prevalence and vancomycin use both thought to be risk factors for VRSA, make the widespread dissemination of these organisms an alarmingly realistic possibility (Perl,1999). MRSA is present in our environment (Olonitola *et al.,* 2008) and this serves the need to screen for VRSA in our hospitals.

1.2. Justification

The emergence of VRSA in countries such as USA, Japan, Belgium, Germany and VISA in South Africa underscores the need to carry out a research to screen for the presence of VRSA/VISA in Nigeria and Zaria in particular. This research will provide information for policy makers and healthcare providers and will also help in monitoring and preventing the spread of antimicrobial-resistant microorganisms especially VRSA as there is no known single antibiotic that is efficacious in treating infections caused by VRSA (NCHR, 2010).

1.3. Aim

To determine the prevalence of vancomycin resistant *Staphylococcusaureus* among patients attending Hospitals in Samaru.

1.4. Objectives are to:

1. isolate and identify *Staphylococcus aureus* from patients attending Hospitals in Samaru.
2. determine the sensitivity of *S. aureus* isolates for possible presence of VRSA strains among the isolates

3. determine the minimum inhibitory concentration (M.I.C.) for the*S.aureus* isolates
4. compare the presence of vancomycin resistant *Staphylococcus aureus* among outpatients and inpatients in order to determine community versus nosocomial infection.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Microbiology of Staphylococci

2.1.1 Morphology

The name *Staphylococcus* is derived from the Greek term staphyle, meaning "a bunch of grapes". This name refers to the fact that the cells of these gram-positive cocci grow in a pattern resembling a cluster of grapes. However, Staphylococci are spherical cells about 1 mm in diameter arranged in irregular clusters. Single cocci, pairs, tetrads, and chains are also seen in liquid cultures and clinical material. Young cocci stain strongly gram-positive. On ageing, many cells become gram-negative. Staphylococci are non-motile and do not form spores (Brooks *et al*., 2001; Murray *et al*., 2002).

Staphylococcal species occur worldwide as commensals on the skin of animals and man. They are also found on mucous membranes of the upper respiratory tract and lower urogenital tract and as transients in the digestive tract. They are comparatively stable in the environment. Staphylococcal strains display a selective affinity for particular animal species. Transfer of *S. aureus* strains between animal species and between animals and man is limited (Quinn *et al*., 2002).

2.1.2 Taxonomy and biology

Kingdom: Bacteria

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: Staphylococcaceae

Genus: *Staphylococcus*

Species: *Staphylococcus aureus*

The figure 1 shows *S.aureus* in clusters as scanned by electron microscope.

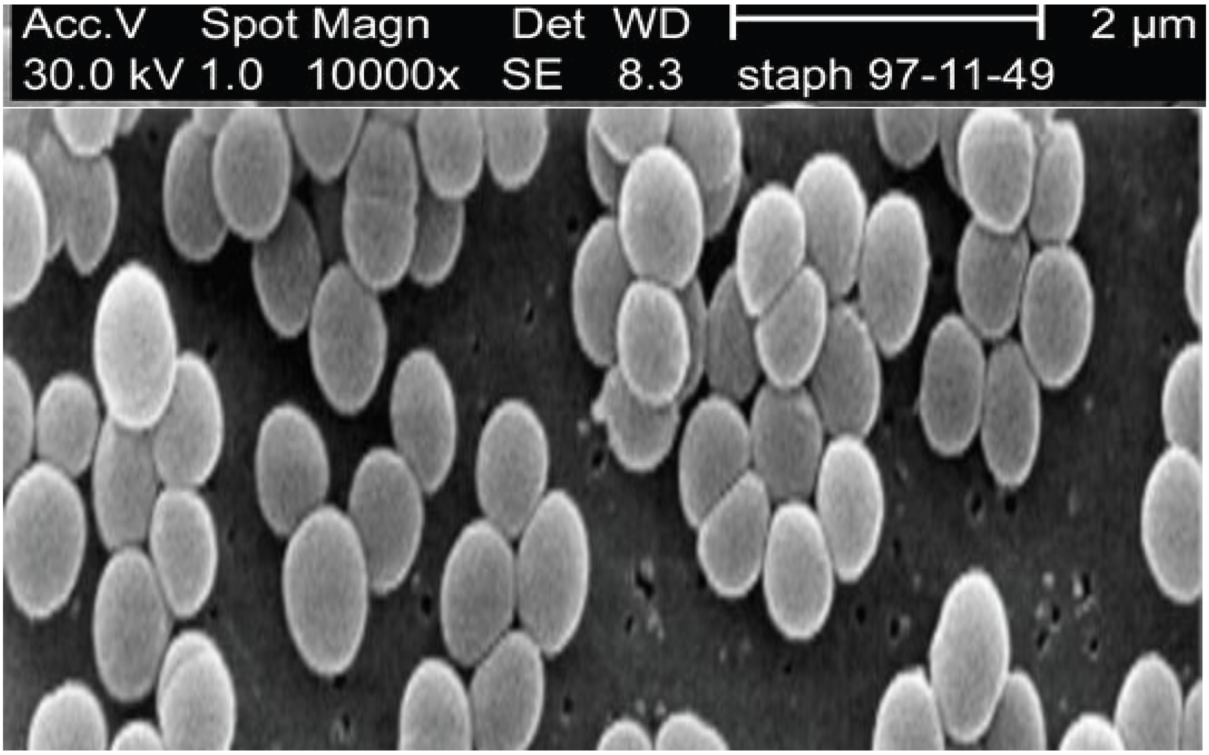

FIGURE 1. Scanning electron microscopy of *Staphylococcus aureus*

[Center for Disease Control (CDC) Public Health Image Library]

S. aureus is a potentially pathogenic bacterium found in the nasal membranes, skin, hair follicles and perineum of warm-blooded animals. Today, it is the leading cause of human infections worldwide, not only in the hospital environment but also in the community. *S. aureus* has about 2,600 genes and 2.8 millions base pairs (bp) of DNA in its chromosome.Mobile genetic elements, such as pathogenicity islands, plasmids and phages can also constitute part of the species genome. Genetic variation in *S. aureus* is very extensive, with approximately 22% of the genome comprising of dispensable genetic material. The genus *Staphylococcus* contains 37 species, 16 of which are found in humans (Table 1). The most virulent species for man in the genus include *S. aureus* (Holt *et al*., 1994*).*Staphylococciwere formerly classified in a common genus with *Micrococcus*spp. (Baird-Parker, 1971) until recently when it was grouped with *Bacillus* spp. on the basis of ribosomal RNA sequences (Ahmad *et al*., 2000).

Subsequently, about 50% of the *S. aureus* genome shares homology with non pathogenic sporulating *Bacillus subtilis*, indicating that the two organisms are quite close and have evolved from a common ancestor (Kuroda *et al*., 2001). As opposed to Micrococci, staphylococci have low guanine/cytosine content and peptidoglycan-bound teichoic-acids in their cell wall structure (Moreillon *et al*., 2005).

Table 1: *Staphylococcus* species and their host range

***Staphylococcus* species**	**Host range**
Staphylococcus aureus	Humans and animals
Staphylococcus arlattae	Chicken and goats
Staphylococcus aricularis	Humans, deer and dogs
Staphylococcus capitis	Humans
Staphylococcus caprae	Goats and humans
Staphylococcus cohnii	Humans and chicken
Staphylococcus delphini	Dolphins
Staphylococcus devriesei	Cattle
Staphylococcus epidermidis	Humans
Staphylococcus equorum	Horses
Staphylococcus felis	Cats
Staphylococcus fleuretti	Goats
Staphylococcus gallinarum	Goats and chicken
Staphylococcus haemolyticus	Humans
Staphylococcus hyicus	Pigs
Staphylococcus leei	Humans
Staphylococcus lentus	Goats, sheep and rabbit
Staphylococcus lugdunensis	Humans and goats
Staphylococcus lutrae	Otters
Staphylococcus pasteuri	Humans and goats
Staphylococcus pettenkoferi	Humans
Staphylococcus pseudintermedius	Dogs

Staphylococcus rostri	Pigs
Staphylococcus schleiferi	Humans
Staphylococcus sciuri	Humans, dogs and goats
Staphylococcus warneri	Humans
Staphylococcus xylosus	Humans
Staphylococcus simiae	Monkeys
Staphylococcus simulans	Humans
Staphylococcus chromogenes	Animals
Staphylococcus hominis	Animals
Staphylococcus intermedius	Animals
Staphylococcus microfti	Animals
Staphylococcus saprophyticus	Animals
Staphylococcus kloosii	Animals
Staphylococcus cohnii	Animals
Staphylococcus cornosus	Animals

Source: www.en.wikipedia.org/wiki/*Staphylococcus*

2.1.3. Biochemical properties

S. aureus is distinguished from other species on the basis of positive results of catalase, coagulase, mannitol-fermentation, and deoxyribonuclease tests. The bacteria can use galactose and lactose as energy sources, and as such is a notorious cause of bovine and ovine mastitis (Almeida *et al*., 1996; Mork *et al*., 2005); and food poisoning via contaminated food, dairy related especially.

Staphylococci together with Streptococci are members of a group of bacteria known as the invasive pyogenic cocci, since they can cause various suppurative or pus-forming diseasesin humans and other animals (Moreillon *et al*., 2005). On microscopic examination, *S. aureus* appear as gram-positive spherical cocci cells, between 0.5 to 1.7 μm in diameter, non-motile and non-spore forming, arranged in grape-like clusters, hence their name, from the Greek term 'staphyle' and 'aureus' for its distinctive "golden" colour. The bacteria form characteristic smooth and translucent yellow-gold colonies, owing to the production of triterpenoid carotenoids located in their cell membrane (Hammond and White, 1970). These pigments not only give *S. aureus* its characteristic colour but also shield the bacteria from the action of toxic oxidants from polymorphonuclear cells (PMN) and phagocytes (Clauditz *et al*., 2006).

More than 90% of clinical isolates of *S. aureus* elaborate polysaccharidic capsules, among which 11 serotypes have been reported (Sompolinsky *et al*, 1985). Capsules type 5 and type 8 *S. aureus* have been found in up to 75% of clinical infections. However, about 20% of human isolates and up to 86% of bovine strains of *S. aureus* have been so far non-typable (Cocchiaro *et al*., 2006). In addition, the capsule protects the bacteria from phagocytosis.

Lysozyme, a muramidase enzyme present in various host tissues (mucous membranes, respiratory and intestinal tracts) and fluids (serum, saliva, and tears) do not kill *S. aureus*. This resistance may contribute to its ability to colonize skin and mucosal tissues such as the anterior nares (Bera *et al*., 2006). Outside a host, the bacteria can withstand numerous environmental stresses, as desiccation, variable pH, osmotic stress, starvation, heat shocks or high temperatures (≤ 50°C) and are considerably salt tolerant, thus able to grow in the presence of concentrations of salt that normally inhibit most other bacteria (< 15% NaCl). Being so resistant, this bacterium can be found throughout the natural world from dust to door knobs and is common even in the cleanest health care facilities.

The remarkable ability of *S. aureus* to acquire useful genes from various organisms has been revealed through homology alignment and phylogenetic trees. The evidence of repeated lateral and horizontal gene transfers (including plasmids) to and from distantly related organisms includes homologues in vertebrates, other bacterial species and even plants (Kuroda *et al*., 2001).

In addition, a large number of mobilizable exogenous DNA stretches, including insertion sequences, transposons, bacteriophages and pathogenicity islands (also referred to as genomic islands) that contain specific determinants responsible for disease and antibiotic resistance have been identified. Overall, the staphylococcal cell wall plays an important role for the bacteria's strength and success (Moreillon *et al*., 2005). As in many gram-positive bacteria, the peptidoglycan (PDG) in *S. aureus* is a cross-linked polymer forming a layer or wall outside the plasma membrane. It consists of repeating and alternating layer units of two aminosugars, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), linked together to glycine aminoacids in a pentapeptide bridge.

In this crosslinking, a peptide bond is formed between a D-alanine on one chain and the free amino end of an L-lysine residue on the other chain. The individual PDG units are produced inside the cell, but the final crosslinking is catalysed outside the cytoplasmic membrane by a group of membrane-anchored bacterial enzymes known as the cell-wall transpeptidases or penicillin binding proteins (Scheffers and Pinho, 2005).

2.1.4 Laboratory identification

Staphylococci grow readily on most bacteriological media under aerobic or microaerophilic conditions. They grow most rapidly at 37°C but form pigment best at room temperature (20-25°C). The most important staphylococcal species is *S. aureus*, which is named for its yellow-pigmented colonies (*aureus* = golden) as the result of the carotenoid pigments that form during their growth. Colonies on solid media are round, smooth, raised, and glistening. *S. aureus* usually forms grey to deep golden yellow colonies. *S. epidermidis* colonies usually are grey to white on primary isolation; many colonies develop pigment only upon prolonged incubation. No pigment is produced anaerobically or in broth. Various degrees of haemolysis are produced by *S. aureus* and occasionally by other species. Peptostreptococcus species, which are anaerobic cocci, often resemble staphylococci in morphology (Brooks *etal.,* 2001; Tortora *et al*., 2001;Murray *et al*., 2002).

2.2 Epidemiology

Staphylococci are ubiquitous. All persons have coagulase-negative staphylococci on their skin, and transient colonization of moist skin folds with *S. aureus* is common. Colonisation of umbilical stump, skin, and perineal area of neonates with *S. aureus* is common. *S. aureus* and coagulase-negative staphylococci are also found in the oropharynx, gastrointestinal tract, and urogenital tract. Short-term or persistent *S.*

aureus carriage in older children and adults is more common in the anterior nasopharynx than in the oropharynx. Approximately 15% of normal healthy adults are persistent nasopharyngeal carriers of *S. aureus*, with a lighter incidence reported for hospitalised patients, medical personnel, persons with eczematous skin diseases, and those who regularly use needles either illicitly or for medical reasons. Although *S.aureus* rarely causes problems in otherwise healthy people, it is commonly associated with infections of prosthetic devices and surgical wounds, bacteraemia, endocarditis and pneumonia in hospitalised patients. Many of these infections start locally (e. g., skin or catheter infections) then spread to the bloodstream, thus putting patients at risk of developing endocarditis and other metastatic complications (Lee, 1998; Lowy,1998; Murray *et al.*,2002).

Because staphylococci are found on the skin and in the nasopharynx, shedding of the bacteria is common and is responsible for many hospital-acquired infections. Some characteristics of the staphylococci account for their pathogenicity, which takes many forms. They grow comparatively well under conditions of high osmoticpressure and low moisture, which partially explains why they can grow and survive in nasal secretions (many people carry the bacteria in their noses) and on the skin. This also explains how *S. aureus* can grow in some foods with high osmotic pressure (such as ham and other cured meats) or in low-moisture foods that tend to inhibit the growth of other organisms. The yellow pigment probably confers some protection from the antimicrobial effects of sunlight. Staphylococci are susceptible to high temperatures, as well as to disinfectants and antiseptic solutions. The organisms can survive on dry surfaces for long periods. The organisms can be transferred to a susceptible person either through direct contact or through contact with fomites (e. g., contaminated clothing, and bed linens)(Murray *et al.*, 2002; Tortora *et al*., 2001).

Staphylococci are among the most common causes of hospital-acquired infections, including bacteraemia, surgical site infections, pneumonia, and urinary tract infections (Fischetti *et al*., 2000; Foster, 1991; Nawas *et al*., 1998). In hospitals, the areas at highest risks for severe staphylococcal infections are the newborn nursery, intensive care units, operating rooms, and cancer chemotherapy wards. Massive introduction of "epidemic" pathogenic *S. aureus* into these areas may lead to serious clinical disease. Personnel with active *S. aureus* lesions and carriers may have to be excluded from these areas (Brooks *et al*., 2001), or at least, medical personnel must use proper hand-washing techniques to prevent the transfer of staphylococci from themselves to patients or among patients (Murray *et al*., 2002).

2.3. Pathogenicity

S. aureus is a major human pathogen (Fournier and Philpott, 2005), able to colonize and infect humans of all genetic backgrounds (Kuroda *et al*., 2001). In fact, humans are its natural reservoir. However, it can also colonize a variety of members of the animal kingdom, including diverse mammals, reptiles, birds and fish. Infection models in lower organisms, such as insects (*Drosophila melanogaster*) and worms (*Caenorhabditis elegans*), have been described as well (Needham *et al*., 2004; Sifri *et al*., 2003). Both wild-type *S. aureus* and some mutated strains are persistent colonizers (Goerke and Wolz, 2004).

The heterogeneity of the infections caused by *S. aureus* is linked to the extreme complexity of its genome and to its unique plasticity to adapt to a great variety of environments and living creatures, including antibiotics and the human immune system (Goerke and Wolz, 2004; Takeuchi *et al*., 2005). Overall, the bacteria is the leading overall cause of nosocomial infections and as more patients are treated outside the hospital setting, it is an increasing concern in the community (Furuya and Lowy, 2006; Lowy, 2003).

2.3.1. Host colonization

In order to colonize a host, and depending on their growth phase, the bacteria are able to produce and regulate a number of pathogenic proteins, such as surface proteins to colonize host tissues, factors that probably inhibit or affect normal phagocytosis (capsule, immunoglobulin, binding protein A), invasins and toxins that damage host tissue.

2.3.1.1 Physiology and Structure

2.3.1.1.1 Capsule and Slime Layer

The outermost layer of the cell wall of many staphylococci is covered with a polysaccharide capsule. Eleven capsular serotypes have been identified in *S. aureus*. Serotypes 1 and 2 are associated with very thick capsules and mucoid-appearing colonies, but are rarely associated with human disease. In contrast, serotypes 5 and 8 are associated with the majority of infections in humans (Murray *et al*., 2013). The capsule protects the bacteria by inhibiting phagocytosis of the organisms by polymorphonuclear leukocytes (PMNs). A loose-bound, water-soluble film **(**slime layer or biofilm**)** consisting of monosaccharides, proteins, and small peptides is produced by most staphylococci in varying amounts. This extracellular substance binds the bacteria to tissues and foreign bodies, such as catheters, grafts, prosthetic valves and joints, and shunts, and it is particularly important for the survival of relatively avirulent coagulase-negative staphylococci (Murray *et al*., 2013).

2.3.1.1.2 Peptidoglycan and Associated Enzymes

Half of the cell wall by weight is peptidoglycan**,** a feature common to gram-positive bacteria. The peptidoglycan consists of layers of glycan chains built with 10 to 12 alternating subunits of *N*-acetylmuramic acid and *N*-acetylglucosamine. Oligopeptide side chains are attached to the *N*-acetylmuramic acid subunits and are then cross-linked with peptide bridges (Murray *et al*., 2013). For example, the glycan chains in *S. aureus* are cross-linked with pentaglycine bridges that are attached to l-lysine in one oligopeptide chain and to d-alanine in an adjacent chain. Unlike gram-negative bacteria, the peptidoglycan layer in gram-positive organisms consists of many cross-linked layers, which makes the cell wall more rigid (Murray *et al*., 2013). The enzymes that catalyze construction of the peptidoglycan layer are called penicillin-binding proteins because these are the targets of penicillins and other β-lactam antibiotics (Murray *et al*., 2013). Bacterial resistance to methicillin and related penicillins is mediated by acquisition of a gene (*mecA*) that codes for a novel penicillin-binding protein, PBP2a, that has a low affinity for methicillin and related penicillins and cephalosporins (refer to Treatment, Prevention, and Control for additional details). The *mecA* gene is located on the staphylococcal cassette chromosome mec (SCC*mec*), and six gene sequences of this cassette (types I-VI) are described. This information is relevant because MRSA strains, previously restricted to hospital-acquired infections, are now present in the community and are responsible for the majority of staphylococcal infections. These strains most commonly have the SCC*mec* type IV, which at the time these strain initially appeared were generally not present in hospital strains of MRSA. More recently, these strains have moved from the community into the hospital (Murray *et al*., 2013).

The peptidoglycan has endotoxin-like activity, stimulating the production of endogenous pyrogens, activation of complement, production of interleukin-1 (IL-1) from monocytes, and aggregation of PMNs (a process responsible for abscess formation) (Murray *et al*., 2013).

2.3.1.1.3 Teichoic Acids and Lipoteichoic Acids

Teichoic acids are the other major component of the cell wall. Teichoic acids are species-specific**,** phosphate-containing polymers that are bound covalently to N-acetylmuramic acid residues of the peptidoglycan layer or to the lipids in the cytoplasmic membrane (lipoteichoic acids). Although the teichoic acids are poor immunogens, a specific antibody response is stimulated when they are bound to peptidoglycan (Murray *et al*., 2013).

2.3.1.2. Surface proteins

The synthesis of many of these proteins is controlled by regulatory genes (Moreillon *et al*., 2005). Surface proteins such as protein A and a number of adhesins serve the bacteria to colonize the host and cause disease.

2.3.1.2.1. Protein A

Staphylococcal Protein A (Spa) is a major surface protein of *S. aureus* strains (Fournier and Klier, 2004). It initially inhibits phagocytic engulfment but then it also allows the bacteria to interact with several host components, such as immunoglobulins (G, A and E), platelets adhesion factor in sites of endothelial damage (von Willebrand factor), etc. Spa provides the bacteria with a protective coating (immunological disguise) allowing it to bind to the Fc portion of host IgG and thus be opsonised and enter phagocytes. *S. aureus* is also able to utilize other host cell receptors, including serum factors (the subunit of the third complement system C3b), and possibly other complement receptors and coreceptors to be opsonised and internalized by phagocytes cells (Verhoef *et al.*, 1977).

2.3.1.2.2. Adhesins

The bacteria can produce around 20 different potential cell wall associated proteins called adhesins or cell adhesion molecules (CAMs), with the ability to adhere to a wide range of host proteins and interact with host extracellular matrix (ECM) components. Adhesins in *S. aureus* are present in all isolates and encoded on the chromosome. They are reassembled under the acronym MSCRAMM, for microbial surface components recognizing adhesive matrix molecules. Many MSCRAMMs have not yet been identified or characterized.

However, most of the microbial adherence factors found in the bacteria is mainly of proteinic (polypeptides) or polysaccharidic (carbohydrates or sugars) nature (Patti *etal.*, 1994). They basically confer adherence to a variety of host proteins (Dziewanowska *et al.*, 2000; Dziewanowska *et al.*,1999). Among them, cellassociated protein clumping factor (Clf) A and B and fibrinonectin-binding protein A (FnBPA), play an important role in experimental endocarditis, and collagen binding protein, in osteoarthritis (Patti *etal.*, 1994; Que *et al.*, 2001; Que *et al.*, 2005). Coagulase, extracellular adherence protein (Eap), extracellular matrix and plasma proteins (Emp) are also important (Harraghy *et al.*, 2003).

Bacterial adherence is supported by cell surface components (cell adhesion molecules:CAMs) and extracellular matrix (ECM) like several plasma proteins (Blumberg *et al.*,1988; Hauck and Ohlsen, 2006), fibrinogen, fibronectin (FN), prothrombin, collagen, fibrinogen/fibrin, elastin, vitronectin, laminin, von Willebrand factor, as well as plasma proteins; vitronectin, thrombospondin, bone sialoprotein, hemin, decorin and heparan sulphate-containing proteoglycans, immunoglobulins, integrins (á5â1) cadherins (Agerer *et al*., 2005; Hauck *et al*., 2006; Sinha *et al*.,1999), etc.

2.3.1.2.3 Secreted proteins

Once bacteria have adhered to the target host cell, they can modulate the secretion of proteins like cytotoxins or invasins. These are exoenzymes (leukocidin, hemolysins, nucleases, proteases, lipases, kinases, hyaluronidase and collagenase), able to destroy host tissues while at the same time permitting spread and conveying nutrients to the invading bacteria.

S. aureus has a minimum of four unrelated hemolysins referred to as alpha (á), beta (â), gamma (ã) and delta (ä)-hemolysins, in the order of their discovery (Dinges *et al*., 2000). Alpha-hemolysin (alpha-toxin) is the most potent membrane-damaging toxin of the bacteria; platelets and monocytes are the most sensitive to it. Alpha-hemolysin and delta-hemolysin are pore forming toxins, able to cleave the phospholipids content of the host cell membranes. Betahemolysin is a shingomyelinase that damages membranes rich in this lipid, however most human isolates do not express this toxin. Gamma-hemolysin is a major cytolysin, with activity against erythrocytes from many species. A few homologues of ã-hemolysin have been described, such as the two-component toxin, reported in 1932 by Panton and Valentine (PV leukocidin) (Kaneko and Kamio, 2004). Gamma-hemolysin is produced by virtually all strains of *S. aureus* while PV leukocidin is found in only a few strains. It is encoded by mobile phages and can be transferred among other strains (Kaneko *et al*., 1997).

PVL causes important leukocyte destruction and tissue necrosis. It is produced by less than 5% of S. aureus strains isolates, but epidemic clones are more and more frequently reported (Ho *et al*., 2007; Ma *et al*., 2006). They are frequently associated with abscesses, furunculosis, arthritis, or severe hemorrhagic pneumonia, including in young adults and children, and with high mortality rate (Gillet *et al*., 2002; Melles *et al*., 2006).

2.3.1.2.4. Staphylococcal toxins

Other secreted proteins of the bacteria are the staphylococcal toxins. They include the exfoliative toxins that produce staphylococcal scalded skin syndrome (SSSS) and the staphylococcal enterotoxins (SE), small peptides (six serotypes so far, SEA, B, C, D E, H) involved in food poisoning and toxic shock syndrome (TSST) (Moreillon *et al.*, 2005). TSST-1 and SEs are the paradigm of a large family of pyrogenic exotoxins called superantigens (SAgs) (McCormick *et al*., 2001). The bacteria can cause TSS by the release of superantigens into the blood stream. All these proteins are known to have potent effect on cells of the immune system, and other biological effects still not well understood. TSST-1 and SE are also known as pyrogenic toxin superantigens (PTSAgs), involved in the induction of the proliferation of T-lymphocytes. Staphylococcal food poisoning is due to the absorption of staphylococcal enterotoxins preformed in the food.

TABLE 2. Factors Involved in the Bacteria Pathogenesis

ACTIVITY/FUNCTION	**PRODUCT**	**GENE**
Anti-immune, antiphagocytosis	Protein A	*Spa*
	Polysaccharide capsule type 5	*Cap5*
	Polysaccharide capsule type 8	*Cap8*
Collagen binding	Collagen BP	*cna*
Fibronectin binding	Fibronectin BPA	*FnbA*
	Fibronectin BPB	*fnbB*
	Clumping factor A	clfA
Fibrinogen binding	Clumping factor B	*clfB*
Lactoferrin binding	Lactoferrin BP	*LBP*
Hemolysins, cytotoxin	α-Hemolysin	*hla*
	â-Hemolysin	hld
	δ-Hemolysin	hld
	γ-Hemolysin	*hlg*
Leukolysin	PVL leukocidin	lukS/F
Food poisoning, TSS	Enterotoxin A	sea
	Enterotoxin B	seb
	Enterotoxin C	*sec*
	Enterotoxin D	sed
	Enterotoxin H	*seh*

Scalded skin syndrome	Exfoliatin A	*Eta*
	Exfoliatin B	*etb*
Toxic shock syndrome	Toxic shock toxin-1	*Tst*
Putative protease	Serine protease-like	*splA-F*
Spreading factor	V8 protease	*ssp*
Spreading factor	Hyaluronidase	*Hys*
Spreading, nutrition	Staphopain(protease II)	Scp
	Glycerol ester hydrolase	*geh*
	Lipase (butyryl esterase)	*Lip*
Nutrition	Nuclease	*Nuc*
Fatty acid esterification	FAME	*Fme*
	PI-phospholipase C	*Plc*
Clotting, clot digestion	Coagulase	*Coa*
Plasminogen activator	Staphylokinase	Sak
Processing enzyme	Metalloprotease (aureolysin)	Aur
	Cysteine protease	*sspB*

Source: Alex and Jodi, 2010

2.3.2. Intracellular character

S. aureus has long been considered exclusively an extracellular pathogen (Lowy, 2000). However, many studies have pointed to its remarkable ability to survive and replicate intracellularly (Brouillette *et al*., 2003). The ability of *S. aureus* to escape host immunity and then establish a cell-niche, are actually crucial steps in its pathogenesis (Lowy, 1998; Lowy, 2000).

Figure 2 shows the steps involved in phagocytosis: (1.) This is adherence which is the attachment of phagocyte's membrane to the surface of microorganism or other foreign material. In some instances, adherence occurs easily and the microorganisms are easily phagocytized.(2.) Following adherence, Ingestion (Phagosome) occurs. During this process, the plasma membrane of the phagocyte extends projections called pseudopods that engulfe the microorganism. Once the microorganism is surrounded, the pseudopods meet and fuse, surrounding the microorganisms with a sac called phagosome, or phagocytic vesicle. The membrane of a phagosome has enzymes that pumps protons into the phagosome, reducing the $P^{H}$to about four.(3).Upon contact, the phagosome and lysosome membrane fuses to form a single layer called phagolysosomes. (4.) The content of the phagolysosome takes only 10 to 30 minutes to kill most types of bacteria. Lysosomal enzymes that attack microbial cells directly include lysozyme, which hydrolyses peptidoglycan in bacterial cell walls. A variety of other enzymes such as lipases, proteases, ribonuclease, and deoxyribonuclease hydrolyse other macromolecular components of microorganisms. Lysosomes also contain enzymes that can produce toxic products such as superoxide radical, hydrogen peroxide, singlet oxygen and hydroxyl radical. (5). After the enzymes have digested the contents of the phagolysosomes brought into the cell by ingestion, the phagolysosome contains indigestible material and it is called residual body. This residual body then moves towards the cell boundary and discharge wastes outside the cell (Figure 2).

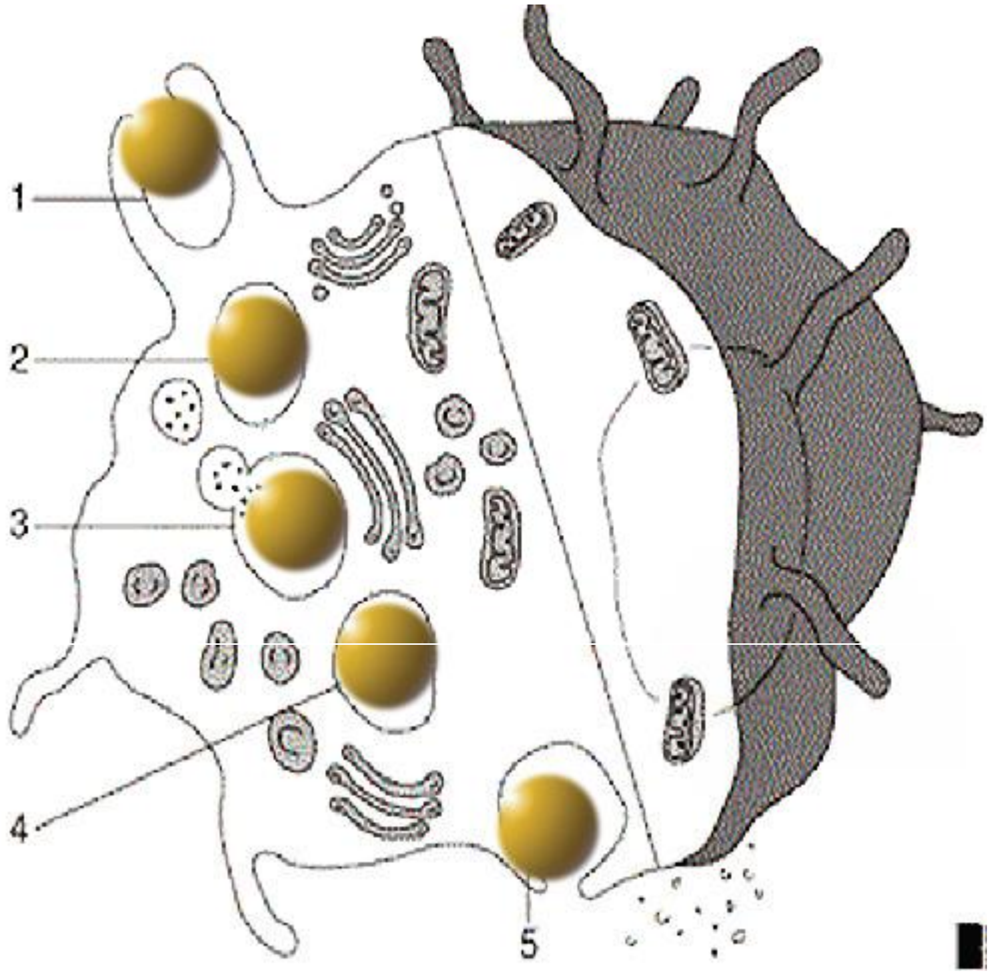

FIGURE 2. The normal process of phagocytosis: *S. aureus* is able to miss out steps 3 and 4 and reside intracellularly (Carryn *et al.*,2003). (Adapted from www.whfreeman.com)

2.3.2.1. Internalization

Normally macrophages and other leukocytes cells in the host eliminate invading bacteria by a process called phagocytosis. Phagocytes pull the bacteria into phagosomes, which eventually fuse with lysosomes forming a phagolysosome where the organisms are destined for destruction. There, the bacteria are exposed to a number of toxic chemicals and enzymatic agents, among which reactive oxidative intermediates such as superoxide anions and hydrogen peroxide, a low pH, and degradative proteases. Bacteria bind to cell surface integrins, cadherins and immunoglobulin-related host cell adhesion molecules, which triggers their internalization (Fowler *et al*., 2003; Fowler *etal.*, 2000; Hauck, 2002).

2.3.2.2. Intracellular survival

At the present time, the mechanisms allowing for the intracellular survival of *S. aureus* have not yet been fully established (Foster, 2005; Lowy, 2000). In non-phagocytic cells, with low defence mechanisms, *S. aureus* is able to escape from phagolysosomes and multiply in the cytosol. It also causes the death of these cells by apoptosis (Bayles *et al*., 1998; Menzies and Kourteva, 1998; Nuzzo *et al*., 2000). In contrast, in phagocytic cells, *S. aureus* seems to remain confined within vacuoles of the phagocytic apparatus (Gresham *et al.,* 2000; Raisanen *et al*., 2001). In this compartment, however, its multiplication is slowed down but not impaired by the acidic pH (Cotter and Hill, 2003). *S. aureus* is also known to produce enzymes degradating oxidant species (catalase, hydroperoxide reductase, superoxide dismutase (Clement and Foster, 1999; Clement *et al*., 1999), which may contribute to protect it intracellularly.

2.3.2.3. Cell types affected

S. aureus has been shown to be internalized “in vitro” and “in vivo” by different mammalian cells including professional phagocytes and non-phagocyte cells, where it is able to survive and multiply (Table 3).

TABLE 3: Studies pointing to the ability of *S. aureus* to survive intracellularly in different host cell types

INFECTED CELL-TYPE	REFERENCES
Epithelial cells	Almeida *et al*.,1996, Bayles *et al*,1998
Mammary epithelial cells	Bayles *et al*.,1998, Brouillette *et al*.,2003
Enterocytes	Hess *et al*.,2003
Keratinocytes	Kintarak *et al*.,2004,Mempel *et al*.,2002
Osteoblasts	Ellington *et al*.,2003, Ellington *et al*.,1999
Fibroblasts	Krut *etal.*,2003
Endothelial cells	Lowy *et al*.,1988,Que *et al*.,2005
Neutrophils	Brouillette *et al*.,2003, Ellington *et al*.,2005
Macrophages	Seral *et al.*,2003

2.3.2.4. Small-colony variants (SCVs)

In the intracellular milieu, *S. aureus* can switch to a phenotype known as small-colony variants (SCV's) that provides marked advantages for survival, among which are anaerobiosis and reduced growth rate (Acar *et al.*,1978; Chuard *et al.*,1997; Kohler *etal*.,2003; Lowy,2000). In fact, SCV's demonstrate a number of characteristics that are atypical to *S. aureus* and linked to interruption of electron transport (Von Eiff *et al*., 2006); they also present an altered antibiotic sensitivity (Chuard *et al.*,1997), being most notably resistant to aminoglycosides (Baumert *et al*.,2002). SCV's are auxotroph and can revert to normal growth and morphology in the presence of menadione and hemin, thiamine, or Carbondioxide(CO_2) rich culture medium (Proctor *et al*., 1998; Proctor and Peter, 1998).

Based on these characteristics, SCVs should always be suspected in indolent and recurrent infections and are increasingly implicated as a cause of persistence, recurrence, and antibiotic resistance in many clinical cases, including lung, bone, skin, wound, cystic fibrosis, and foreign body infections (Abele-Horn *et al*., 2000; Brouillette *etal*., 2004; Kahl *et al*., 1998; Looney, 2000; Von Eiff *et al*., 2000). Importantly in this context, SCV's apparently allows the bacteria to remain intracellular in a dormant metabolic state for months or even years, which could actually explain the persistent character of *S. aureus* infections (Vesga *et al.*, 1996; Von Eiff *et al*., 2001; Von Eiff *et al*., 1997; Von Eiff *et al*., 2006).

2.4 Staphylococcal diseases in human

It is important to keep in mind that the staphylococci that cause disease in humans and animals are not inherently pathogenic organisms (unlike the influenza virus, for example, which must cause disease to propagate). For every illness caused by staphylococci, many more individuals are benignly colonised with no sign of disease.It is important to realise that no single virulence factor produced by a *Staphylococcus* has been shown to date to be either necessary or sufficient for the establishment of an infection, although the infective or lethal dose does vary. No strain has yet been identified that produces all the known toxins, or even members from all toxin families. The narrowest definition of a virulence factor would be a substance that, when purified to homogeneity and introduced into a test animal, produces a pathogenic effect. Using that definition, most of the virulence factors especially those involved in attachment and probably most of the degradative enzymes (e. g., lipases, proteases, and hyaluronidase) would not be considered true virulence factors. In the broadest sense, any substance that is exported out of the cytoplasm either to the cell surface or into the extracellular environment of the *Staphylococcus* can be thought of as a potential virulence factor. Clearly, those substances that are capable of interacting with the host may affect that host in some deleterious way. This second definition is probably too broad and in many cases it is possible that the production of at least some of these factors may actually assist the host in combating an infection (Crossley and Archer, 1997).

2.4.1 Bacteraemia and infective endocarditis

S. aureus and coagulase-negative staphylococci (CoNS) are ideal infective endocarditis (IE) pathogens in that they possess a number of virulence factors, which enable them to establish 1E. Acute endocarditis caused by *S. aureus* is a serious disease, with a mortality rate approaching 50%. Although patients with *S. aureus* endocarditis may initially have non-specific influenza-like symptoms, their condition can deteriorate rapidly and include disruption of cardiac output and peripheral evidence of septic embolization. Unless appropriate medical and surgical intervention is instituted immediately, the patient's prognosis is poor. In addition, *S. aureus* continues to be an important cause of both community-acquired (CA) and hospital acquired (HA) bacteraemia. Commonly associated predisposing medical conditions include diabetes mellitus and renal insufficiency. Most patients with *S. aureus* bacteraemia have an obvious primary focus of infection, usually an intravascular catheter. Overall mortality in patients with *S. aureus* bacteraemia remains high, particularly in the absence of a removable primary focus, older age patients, rapidly fatal comorbid conditions, underlying respiratory or cardiac disease, associated infective endocarditis (IE), and septic shock. Methicillin-resistant *S. aureus* (MRSA) bacteraemia is a growing problem globally (Crossley and Archer, 1997; Murray *et al*., 2002).

2.4.2 Wound infections

Staphylococcal infections of the skin can be minor or life threatening, depending on the integrity of the skin surface and the invasiveness of the bacterial strain. Staphylococci are the most common species recovered from patients who develop a wound infection after surgery, with *S. aureus* and the CoNS rank first and third among aerobic bacteria associated with wound infections, respectively. In general, staphylococci that cause wound infections come from the flora of the patient or the personnel performing the procedure. For *S. aureus*, studies have shown that the patient's own flora serves as the source of the infecting bacteria in about one-half of cases of surgical infections (Crossley and Archer, 1997; Fischetti *et al*., 2000).

2.4.3 Infections of intravascular catheters and vascular devices

Although intravascular catheters and vascular grafts have revolutionised the practice of medicine for the betterment of patients, unfortunately they are associated with a significant incidence of infection. Staphylococci, both *S. aureus* and CoNS, play a preeminent role in these infections. Because of their unique environmental niche on the skin and mucous membranes of humans and their ability to adhere to biomaterials and cause infection, they will undoubtedly continue to cause significant morbidity and mortality as the use of prosthetic medical devices increases. From 20% to 65% of all infections of catheters and shunts are caused by CoNS. The CoNS are particularly well adapted for causing these infections because they can produce a polysaccharide slime that bonds them to catheters and shunts and protects them from antibiotics and inflammatory cells. A persistent bacteraemia is generally observed in patients with infections of shunts and catheters because the organisms have continual access to the blood stream (Crossley and Archer, 1997; Murray *et al*., 2002).

2.4.4 Skin and soft tissue infections

Staphylococci are inhabitants of the skin and mucous membranes of mammals and birds, where they exist saprophytically. However, factors ranging from tight clothing or scratching to immunosuppression or insertion of a prosthesis alter the host-parasite relationship and cause disease. Colonisation, particularly of the nares by *S. aureus*, leads to hand carriage, and from the hands, the organisms are frequently spread to other areas of the body. Thus, staphylococci often follow a nose to hands to wounds route of infection (Zimakoff *etal*., 1996). The variety of enzymes and toxins produced, especially by *S. aureus*, results in a spectrum of disease, part of which may be a direct expression of the toxin damage, but also a result of a variety of pathogenicity factors acting in concert. Purulent skeletal muscle infection (pyomyositis), folliculitis and furuncles, recurrent furunculosis, impetigo, botryomycosis and secondary cutaneous infection in patients with eczema are some staphylococcal infections due to *S. aureus* (Crossley and Archer, 1997).

2.4.5 Central nervous system

The staphylococci are important causative organisms of cerebrospinal fluid (CSF) shunt infections, acute purulent meningitis, brain abscess associated with trauma, spinal and cranial subdural empyema and epidural abscess. CoNS are the etiologic organisms in most CSF shunt infections. S. aureus is the second most common pathogen. The staphylococci typically enter the shunt system from a contaminated wound or from the patient's skin surface at operation.

S. aureus is the causative organism in most cases of staphylococcal meningitis that develop as a complication of a neurosurgical procedure, from a parameningeal focus of infection or a congenital anomaly or during the course of staphylococcal bacteraemia or endocarditis. *S. aureus* is a common causative organism of brain abscesses that develop as a result of cranial trauma, either penetrating wounds of the brain or craniotomy. *S. aureus* is the causative organism in most cases of spinal subdural empyema and in 15 to 25 percent of cases of cranial subdural empyema. CoNS are isolated on rare occasion from cranial subdural empyemas. *S. aureus* is the causative organism in 50 to 60 percent of spinal epidural abscess and in most cranial epidural abscesses that occur as a complication of craniotomy or compound skull fracture (Crossley and Archer, 1997).

2.4.6 Diseases of the eye

Staphylococcal diseases of the eye include both infections and expression of cell mediated immunity (CMI) to staphylococcal antigens. The pathogenesis of diseases due to both *S. aureus* and CoNS has been increasingly well-characterised in recent years (Crossley and Archer, 1997). A model of staphylococcal keratitis in guinea pig has been described (Davis *et al*., 1978), and a similar model in rats was used to determine the effects of antibiotics and corticosteroid treatments on the progression of *S.*

*aureus*infection of the cornea (Badenoch *et al*., 1985). Radial incision of the rabbit conjunctiva followed by inoculation of *S. aureus* resulted in purulent conjunctivitis and a model for testing antimicrobial treatments in this disease (Behrens-Baumann and Begall, 1993).

2.4.7 Osteomyelitis and other infections of bones and joints

Colonization of the bones and joints by *S. aureus* may occur as a result of either hematogenous infection or it can be a secondary infection resulting from trauma or the extension of disease from an adjacent area (Fischetti *et al*., 2000; Murray *et al*., 2002). During the 1970s, most cases of osteomyelitis were caused by strains of *S. aureus*. During the 1990s, although the incidence of osteomyelitis caused by gram-negative pathogens has increased dramatically, staphylococci are still the most commonly isolated pathogens in most types of osteomyelitis. *S. aureus* is the most frequent single pathogen in adults, accounting for 50 to 70 percent of cases of osteomyelitis. The estimated frequency of *S. aureus* in pyogenic osteomyelitis in all age groups may reach 80 to 90 percent (Crossley and Archer, 1997). *S. aureus* is the primary cause of septic arthritis in young children and in adults who are receiving intra-articular injections or who have mechanically abnormal joints.

Secondary involvement of multiple joints is indicative of hematogenous spread from a localised focus. Staphylococcal arthritis is characterised by a painful, erythematous joint, with purulent material obtained on aspiration. Infection is usually demonstrated in the large joints (e. g., shoulder, knee, hip, and elbow). The prognosis in children is excellent, but in adults, it depends on the nature of the underlying disease as well as the occurrence of any secondary infectious complications (Murray *et al*., 2002).

2.4.8 Respiratory tract infections

S. aureus, in distinct contrast to the CoNS, is a significant cause of respiratory tract disease. *S. aureus* respiratory disease can develop after the aspiration of oral secretions or from the hematogenous spread of the organism from a distant site. Aspiration pneumonia is seen primarily in the very young, the aged, and patients with cystic fibrosis, influenza, chronic obstructive pulmonary disease, and bronchioectasis.

Hematogenous pneumonia is common for patients with bacteraemia or endocarditis. While *S. aureus* is not among the most frequent of isolates from patients with sinusitis, bronchitis, or pneumonia, it is isolated from patients with these illnesses. Although *S. aureus* is an uncommon cause of severe community-acquired pneumonia, it is responsible for perhaps 20 to 30 percent of cases of nosocomial pneumonia (Crossley and Archer, 1997; Fischetti *et al*., 2000; Murray *et al*., 2002).

2.4.9 Urinary tract infections

Although staphylococci are not commonly thought of as causes of urinary tract infection (UTI), *S. aureus* may cause UTI. The infections range from asymptomatic bacteriuria to infections that are severe and associated with bacteraemia. Given the relative frequency with which the perineal area is colonised with *S. aureus*, it is perhaps surprising that this organism is relatively uncommonly recovered as a cause of UTI (Crossley and Archer, 1997). *S. saprophyticus* has a predilection for causing UTI in young, sexually active women and is rarely responsible for infections in other patients. It is also infrequently found as an asymptomatic colonizer of the urinary tract (Murray *et al*., 2002).

2.4.10 Toxin-mediated syndromes

Three diseases are associated with specific toxins: (i) toxic shock syndrome (TSS), caused by toxic shock syndrome toxin type 1 (TSST-1), (ii) food poisoning, by staphylococcal enterotoxins; and (iii) staphylococcal scalded skin syndrome (SSSS) by exfoliative toxins. Two other diseases are probably related to staphylococcal toxins: staphylococcal enterocolitis, caused by staphylococcal enterotoxins or delta-toxin; and severe wound infections associated with a-toxin (Crossley and Archer, 1997).

Staphylococcal toxic shock syndrome (TSS) is an illness that is the result of the protean effects of one or more toxins of *S. aureus* (Crossley snd Archer, 1997). Staphylococcal TSS may manifest in either of two general forms, menstrual or nonmenstrual. Menstrual TSS occurs in women whose vaginal/cervical mucosa are colonised by TSST-1-producing *S. aureus*. Tampon use is a risk factor in menstrual TSS, and a correlation between tampon absorbency and risk of developing TSS has been established. TSS acquired in this way is difficult to reproduce in animals, although some success has been achieved with a simulation of tampon use in rabbits. Attempts have also been made to induce TSS or a TSS-like syndrome by other means in laboratory animals (Quimby and Nguyen, 1985), but a definitive model for this disease has not been forthcoming. Nonmenstrual TSS may result from *S. aureus* infection elsewhere in the body. Either staphylococcal enterotoxins (SEs) or TSST-l may mediate the nonmenstrual form (Fischetti *et al*., 2000). TSST-1 in the bloodstream can trigger a massive release of cytokines that cause shock and death.

In animals, TSST-1 makes the animal hypersusceptible to LPS, which may enter the bloodstream regularly in small amounts because of lysis of gram-negative bacteria in the intestine. However, LPS might not even be the most important molecule whose action is potentiated by TSST (Salyers and Whitt, 2002). Staphylococcal food poisoning, one of the most common foodborne illnesses, is intoxication rather than an infection. Disease is caused by bacterial toxin present in food rather than from a direct effect of the organisms on the patient. Certain strains of *S. aureus* can also cause enterocolitis, which is manifested clinically by watery diarrhoea, abdominal cramps, and fever. Enterocolitis occurs primarily in patients who have received broad-spectrum antibiotics, which suppress the normal colonic flora and permit the growth of *S. aureus* (Murray *et al*., 2002).

Staphylococcal scalded skin syndrome (SSSS) is characterised by the abrupt onset of a localised perioral erythema (redness and inflammation around the mouth) and formation of the bullae or skin blisters and a potential for widespread peeling. The lesions cover the entire body within 2 days. The disease is predominant in children. Bullous impetigo is a localised form of SSSS. Specific strains of toxin-producing *S. aureus* are associated with the formation of superficial skin blisters. Unlike patients with the disseminated manifestations of SSSS, patients with bullous impetigo have localised blisters that are culture-positive (Fischetti *et al*., 2000; Melish and Glasgow, 1970; Murray *et al*., 2002).

2.5 Veterinary aspects of staphylococci

The first systematic study of pathogenic staphylococci was done by Rosenbach in 1884 (Quinn *et al*., 2002). The association of staphylococci with veterinary medicine began with the isolation of the organisms from mastitis in sheep by Nocard in 1887 (Quinn *et al*., 2002). In 1890 Guillebeau reported that staphylococci were responsible for mastitis in cattle (Quinn *et al*., 2002). Thus within six years of the first systematic study of pathogenic staphylococci in 1884, the importance of staphylococci in veterinary medicine was established (Easmon and Adlam, 1983). *S. aureus,S.intermedius*, and the coagulase-variable *S. hyicus* are important pathogens of domestic animals. Although coagulase-negative staphylococci are usually of low virulence, some occasionally cause disease in animals (Quinn *et al*., 2002).

2.5.1 Bovine mastitis

Mastitis is an inflammatory reaction of the mammary gland. The most important mastitis pathogen, worldwide, is *S. aureus*(Quinn *et al*., 2002). Staphylococcal mastitis may be subclinical, or clinical. The majority of infections are subclinical. In the clinical form, the disease may be peracute, acute, or chronic. Peracute and gangrenous forms are associated with severe systemic reactions and can be life threatening. The peracute form, which is rare, is caused by *S. aureus*, often shows a gangrenous udder, and is frequently fatal. In gangrenous mastitis the affected quarter, which becomes cold and blue-black, eventually sloughs. Tissue necrosis is attributed to the alpha-toxin which causes contraction and necrosis of smooth muscle in blood vessel walls, impeding blood flow in the affected quarter (Quinn *et al*., 2002). In addition, this toxin causes release of lysosomal enzymes from leukocytes. Usually, staphylococcal mastitis begins with an acute episode, including some or all of the cardinal signs of inflammation in the udder: tumour, rubor, calor, and dolour and functio laesa (Quinn *et al*., 2002). Systemic signs of disease are not always seen. If not successfully treated, the acute form becomes chronic, with few clots in the milk, or with no clinical signs; the disease subsequently passes into a subclinical, chronic form. An elevated somatic cell count and isolation of bacteria from the milk can only detect this form of mastitis (Gyles and Thoen, 1993; Quinn *et al*., 2002).

The route of infection in staphylococcal mastitis is via the teat, and the milking machine is considered to be the most important predisposing factor (Gyles and Thoen, 1993). However, staphylococcal mastitis does occur in hand-milked cows, in suckled cows, and during the dry period. The bacteria pass the teat duct into the teat and udder cisterns, and subsequently into the milk ducts (Gyles and Thoen, 1993). They may subsequently establish in an area of secretory tissue. *S. aureus* and other mastitis pathogens, at 37°C, are able to rise with the milk fat. The phenomenon seems to involve IgA antibodies associated with membranes of fat globules in previously infected animals. This phenomenon may aid to invade upper parts of the milk ducts and udder. The mechanism of penetration via the teat duct is not fully understood, but it is suggested that the organisms gain entrance to the teat cistern by propulsion and/or by bacterial growth. The staphylococci colonise the tip of the teat, especially in areas of teat lesions or erosions. Colonisation often precedes an intramammary infection but does not always result in mastitis (Gyles and Thoen, 1993). Staphylococci can colonize the teat duct for a prolonged period of time. In the intact teat, the organisms probably exist as distinct microcolonies in the stratum corneum, as they do in other areas of skin. When contact is made between the staphylococci and milk, then a medium is provided in which the staphylococci can grow into the gland. The nearer the organisms are to the milk in the teat cistern, the greater is the risk for mammary infection to occur. Once the organisms pass the barriers of the teat duct, adherence to epithelial cells is thought (but still not proven) to be the next critical step in pathogenesis (Gyles and Thoen, 1993). Adherence is believed to involve a combination of cation-bridging, hydrophobic interactions, and bacterial adhesin-specific epithelial surface receptors. It has been shown that the ability of *S. aureus* to bind to epithelial cells of the ductules and alveoli in the bovine mammary gland is an important virulence factor, and that antibodies against whole cells inhibit the adherence. Once established on the surface, nucleases and phosphatases degrade pus into substances that can be utilised as nutrients for the bacteria. Although several potential virulence determinants have been purified, or partly purified, and tested for toxicity in different experimental systems, their relative importance as virulence determinants is not known (Gyles and Thoen, 1993).

The mouse model of intramammary inoculation has been used extensively in studying this disease and represents a reproducible and cheap simulation of the disease in dairy cattle (Chandler, 1970). Various attempts have been made in the past to design vaccines directed against the bacterial surface polysaccharides and proteins (adhesins) and against the toxins of *S. aureus* and to test their efficacy in bovine, mouse, and rabbit models (Foster, 1991; Sutra and Poutrel, 1994). More recently, a live attenuated *S. aureus* strain was used to provide protection in mice when inoculated locally during late pregnancy or early lactation (Gomez *et al*., 1998).

2.5.2 Mastitis in other species

Staphylococci are capable of causing mastitis in domestic animals other than the cow though these infections are far less well documented than staphylococcal mastitis in the cow. In broad terms the mode of infection and the host defence mechanisms are similar to those that operate in the cow. However, staphylococcal mastitis has been seen in other species such as sheep, goats, pigs, rabbits, dogs, cats and horses (Easmon and Adlam, 1983). In sheep and goats, the mastitis is predominantly of the gangrenous type (Gyles and Thoen, 1993).

2.5.3 Exudative epidermitis

Exudative epidermitis (greasy pig disease) is an acute generalised dermatitis, caused by *S. hyicus*, which involves the entire body surface of pigs, 5-35 days of age. It is highly contagious and is characterised by hyperhidrosis, excess sebaceous secretion, exfoliation and exudation but no pruritis. These changes result in a loss of skin function, extreme dehydration and rapid exhaustion, which may terminate in death. Piglets under 3 weeks of age may die within 24 to 48 hours. Morbidity rates range from 20% to 100%, and mortality rates can reach 90% in severely affected litters. *S. hyicus* can be isolated from the vaginal mucosa and skin of healthy sows. The organisms probably enter the skin of young pigs through minor abrasions such as bite wounds (Easmon and Adlam, 1983; Quinn *et al*., 2002).

Prevention of the disease is by scrubbing the sow with a suitable disinfectant prior to entering the farrowing crate. The farrowing crate and pen should be clean and all sharp edges and ridges should be removed (Easmon and Adlam, 1983).

2.5.4 Tick pyaemia of lambs

Tick pyaemia is a prenatal infection of lambs with *S. aureus*, characterised by septicaemia and death or by bacteraemia and the localisation of staphylococci in various tissues. In the bacteraemic form of the disease arthritis or meningitis may first appear during the first 2-4 weeks of life; there may also be suppurative lesions in the skin, muscles, tendon sheaths and viscera especially the heart and kidneys. Tick pyaemia was originally found to co-exist withlouping ill, tick-borne fever, lamb dysentery and pulpy kidney disease (Easmon and Adlam, 1983; Quinn *et al*., 2002).

The association between the disease and hill-grazing regions of Britain and Ireland, where there are suitable habitats for the tick, Rodes ricinus, is strong (Quinn *et al*., 2002). The first line in control of tick pyaemia must be to ensure that hygienic conditions exist for lambing and carrying out surgical procedures so that staphylococcal contamination and infection is reduced to a minimum (Easmon and Adlam, 1983).

2.5.5 Staphylococcosis of poultry

As in other animals the staphylococci that cause disease in poultry are inhabitants of the skin of the animals and do not differ biochemically from other skin staphylococci. The lesion traditionally associated with staphylococci in poultry is bumblefoot. This is a localised bulbous lesion of the ball of the foot, which arises from the penetration of a foreign body followed by secondary invasion by *S. aureus*. Lameness is often the first symptom of the condition and the swelling may not be obvious until the planter aspect of the foot is examined. However, if *S. aureus* gains entrance to the circulation of birds, then a septicaemia is produced which if not fatal, leads to arthritis and synovitis (Easmon and Adlam, 1983). *S. aureus* can also cause arthritis and septicaemia in turkeys, and omphalitis in chicks (Quinn *et al*., 2002).

2.5.6 Pyoderma

Pyoderma is one of the most common skin diseases of dogs, but it is relatively rare in cats. Coagulase-positive *Staphylococcus* is the most commonly isolated organism from pyoderma in dogs. Prior to 1982, the organism was called *S. aureus*, but subsequently it hasbeen identified as *S. intermedius*. It is suggested that changes in sebum production provide a milieu in which *S. intermedius* may multiply (Gyles and Thoen, 1993).

2.5.7 Endometritis

The clinical features of endometritis vary from species to species. Frequently no more is observed in the cow than a slight vaginal discharge and repeated return to service. On the other hand the bitch usually presents as an endometritis which has advanced to pyometritis. The uterus is palpably enlarged, there is a vaginal discharge, anorexia and depression. There are often signs of concomitant renal involvement such as polyuria, nocturia and polydipsia (Easmon and Adlam, 1983).

2.6 ANTIBIOTIC RESISTANCE

2.6.1. Major phenotypes of antibiotic resistance

S. aureus possesses a remarkable ability to develop resistance to antibiotics. It started shortly after the introduction of penicillin, a beta-lactam antibiotic, into clinical use.

2.6.1.1. Beta-lactam resistance

All beta-lactam antibiotics have a common element in their molecule structure: a four-atom ring (cyclic amide) known as a beta-lactam. Beta-lactams antibiotics are analogous of D-Alanyl- D-Alanine, the terminal aminoacid residues of the precursor N-acetyl muramic acid (NAM)/N-acetyl glucosamine (NAG)-peptide subunits of the nascent Peptidoglycan(PDG) layer. This structural similarity facilitates their binding to the active site of the penicillin binding proteins (PBPs) and prevents the final crosslinking of the nascent PDG layer, disrupting cell wall synthesis, causing cell lysis and death. The inhibition of PBPs also leads to the activation of autolytic enzymes in the bacteria cell wall.

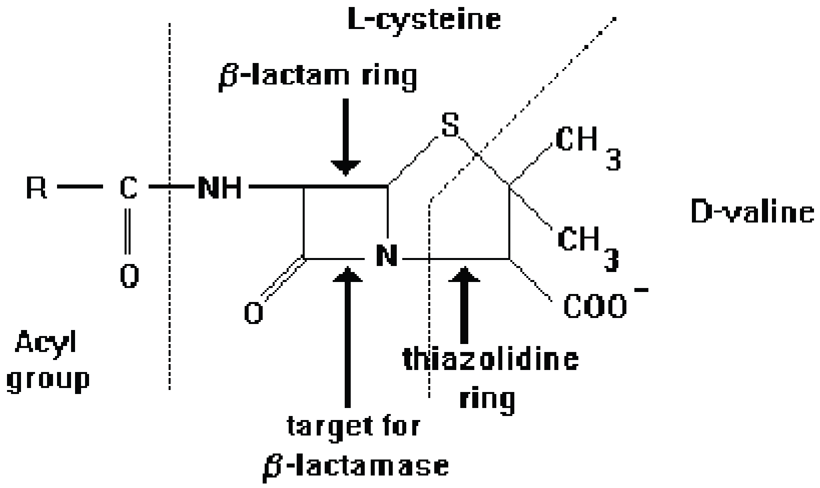

FIGURE 3. Common core of beta-lactam antibiotics (beta: to denote four-membered ring size), (lactam: grammatical blend from lactone plus amide).

Staphylococcihave at least two major mechanisms for resistance to beta-lactam antibiotics. The first one is the production of beta-lactamasesalso known as penicillinases, enzymes that hydrolytically destroy beta-lactams rendering the antibiotic ineffective, and the other is the intrinsic expression of a new PBP, called penicillin binding-protein 2A or PBP2A, which has low affinity for present beta-lactam compounds and make the bacteria resistant to inhibition by all currently available beta-lactam antibiotics (Livermore, 2006).

Methicillin-Sensitive-*S.aureus* (MSSA)is the term used to denote the bacteria susceptibility to all beta-lactams including beta-lactamase inhibitors combinations, cephems and carbapenems. A less known and understood form of penicillin resistance mechanism in *S. aureus* is toleranceto the bactericidal effects of beta-lactam antibiotics (Lyon and Skurray, 1987; Sabath, 1982; Sabath *et al*., 1977). It is manifested in strains deficient in autolytic enzymes due to an excess of autolysin inhibitors and causes cross-tolerance to the killing effects of other cell wall inhibitors such as vancomycin (Lyon and Skurray, 1987). Bacteriophages have also been implicated on the conversion of non-tolerant organisms to tolerance (Sabath *et al*., 1977).

2.6.1.1.1. BlaZ- Penicillin resistance: beta-lactamase production

The introduction of penicillin in the early 1940s dramatically improved the prognosis of patients with staphylococcalinfections (Lyon and Skurray, 1987). In fact, penicillin G still remains one of the best choices for the fully susceptible strains (MIC ≈ 0.01 μg/ml).

However, as early as 1942, penicillin-resistant staphylococciwere recognized, first in hospitals and subsequently in the community. These penicillin-resistant (MIC ≥ 0.25 μg/ml), oxacillin-susceptible strains (MIC ≤ 2 μg/ml) are resistant to beta-lactamaselabile penicillins but susceptible to other beta-lactamase stable penicillins, beta-lactamase inhibitor combinations, cephems and carbapenems.

The staphylococcalresistance to penicillin can be found inherently present on the bacterial chromosome or may be acquired via plasmid transfer. It is mediated by the *blaZ* gene that encodes a beta-lactamase enzyme when the bacteria are exposed to beta-lactam antibiotics, opening the beta-lactam ring and forming inactive penicilloic acid and thus, rendering thebeta-lactam antibiotic inactive. By the late 1960s, more than 80% of both community- and hospital-acquired staphylococcalisolates were resistant to penicillin. At the present time, beta-lactamase-dependent resistance is found in > 95% isolates (Lowy, 1998; Lowy, 2003).

a

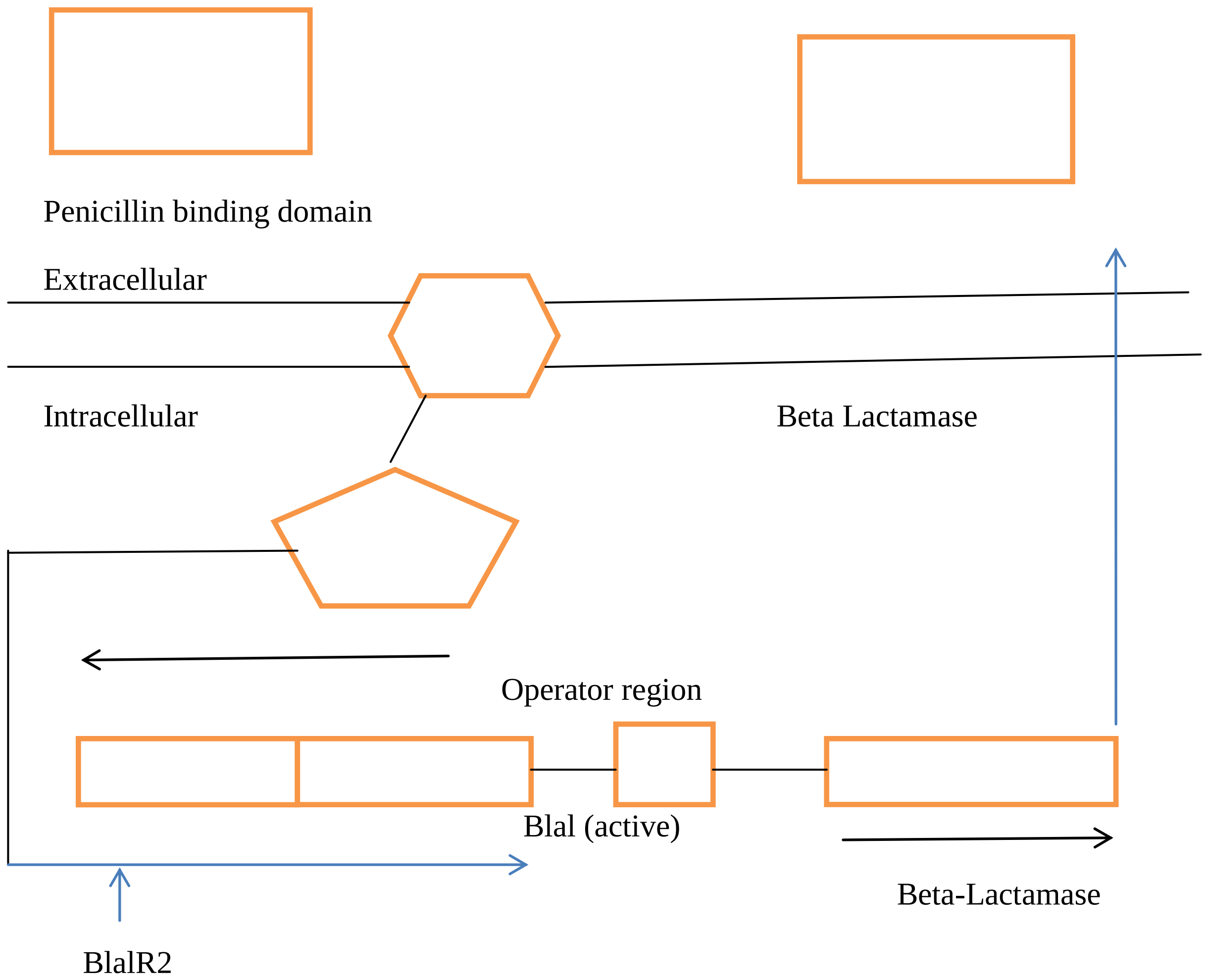

FIGURE 4. Induction of beta-lactamase synthesis in the presence of the beta-lactampenicillin: The presence of penicillin in the extracellular medium stimulates the transmembrane sensor-transducer BlaR1 to autocatalytic activation. Active BlaR1 cleaves Bla into inactive fragments, allowing transcription of both blaZ and blaR1 to commence. Beta-lactamase, the extracellular enzyme encoded by blaZ is produced and hydrolyzes the beta-lactam ring of the penicillin antibiotic rendering it inactive. (Adapted from Lowy, 2003).

2.6.1.1.2. PBP2A mediated-resistance

In the late 1950's new beta-lactam semi-synthetic compounds were developed and became available. They were stable to the action of BlaZ mediated penicillinase and hence categorized as penicillinase-stable penicillins. The group includes methicillin, oxacillin, nafcillin, flucloxacillin, and dicloxacillin, methicillin being the prototype (Fisher *et al*., 2005, Guignard *et al*., 2005).

Methicillin use in the 1960's was rapidly followed by reports of mutants being resistant to the whole new group of beta-lactams and from the 1970s to today, MRSA has become the main cause of nosocomial infection worldwide and created great clinical difficulties for treatment on a global scale (Fuda *et al.,* 2005). *S. aureus* strains resistant to this group of antibiotics are known as methicillin-resistant isolates or MRSA (Mastouri *et al*., 2006), even though methicillin is no longer the agent of choice for testing or treatment.

MRSA are by definition not inhibited by less than 25 ug/ml methicillin, whereas ordinary strains are usually inhibited by 1.6 to 3.2 ug/ml (Sabath *et al*., 1972). They are also oxacillin resistant (MIC 4 ug/ml), hence also named ORSA. Multiresistant MRSA (MDR-MRSA) have been defined as *S. aureus* isolates with a MIC for oxacillin at .4 ug/ml and with four or more additional resistances (Diekema *et al*., 2000).

MRSA resistance is mediated by the bacterial acquisition of a novel mobile genetic element, designed SCC (Staphylococcal-Chromosomal.Cassette) or mec chromosomal mecA genethat codes the alternative supplementary target protein PBP2A, with low affinity for betalactams, including cephalosporins, carbapenems and beta-lactamase inhibitor combinations. MecA is a large 20- to 65-Kb mobile element in *S. aureus*. Moreover, the cassettes have also been found to act as a trap for additional unrelated drug resistance genetic determinants, thereby leading to multiple resistances. SCCmec consists of the mec gene complex, containing mecA and the mecA regulatory genes, and the cassette chromosome recombinase (ccr) complex, which encodes a recombinase(s) responsible for SCCmec movement. The SCC elements are classified according to the type of recombinase they carry and their general genetic composition. SCC types I, II, III, IV and V have been described and SCC lacking mecA have also being reported (Hanssen and Ericson Sollid, 2006). In addition, this mechanism of resistance seems to be shared with induction of a gene(s) in the penicillinase plasmid (Ubukata *et al*., 1989).

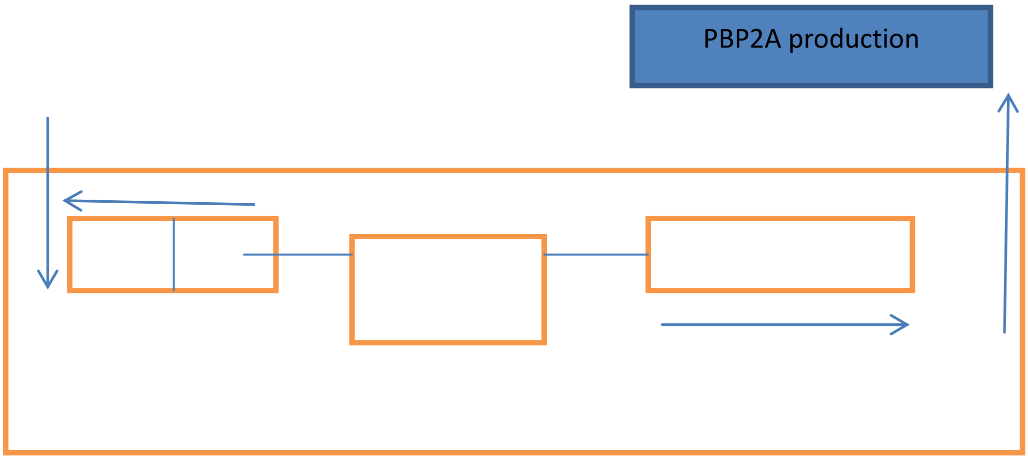

FIGURE 5. Mechanism of *S. aureus* resistance to methicillin: Synthesis of PBP2A proceeds in a fashion similar to that described for beta-lactamase. mecI and Blal have coregulatory effects on the expression of PBP2A and beta-lactamase. Exposure of mecR1 to a betalactam antibiotic induces mecA activation. MecR1 inactivates mecI, allowing expression of PBP2A.

In MRSA, the PBP2A acts as a surrogate enzyme able to perform both transpeptidase and transglycosylase activities of the normal four PBPs of the bacteria (Pinho *et al*., 2001). The lateral and then horizontal gene transfer of mecA, has played a fundamental role in the evolution of *S. aureus* (Fitzgerald *et al*., 2001). Several clones with different types of SCCmec have been reported, such as type IV found mainly in Community-MRSA (CA-MRSA) in Australia and the United States and types I to III being prominent in the Hospital-MRSA setting (HA-MRSA) (Lu *et al*., 2005).

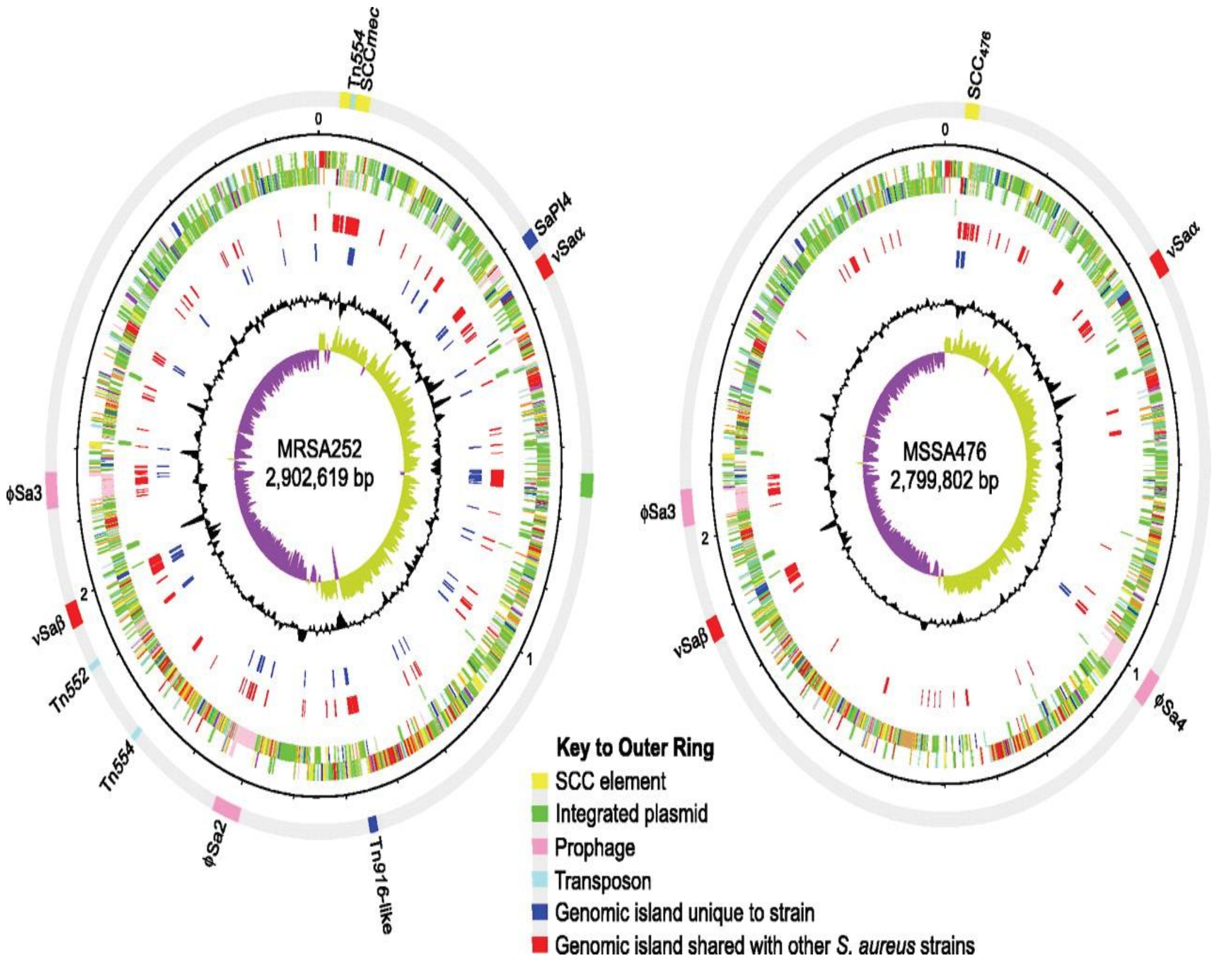

Figure 6: Schematic circular diagrams of the MRSA252 and MSSA476 chromosomes.This comparative genome approach between an epidemic MRSA clone (MRSA252) and aninvasive MSSA (MSSA476) illustrates the role of accessory elements in the rapid evolution of the bacteria (Holden *et al*., 2004).

Hospital-acquired MRSA constitutes 25-50% of clinical isolates all over the world (Diekema *et al*., 2000). It has reached more than 40% in Europe (Lowy, 1998; Lowy, 2003). In Belgium MRSA accounts for 25-30% (EARSS data, figure 7). Between 1997 and 1999 MRSA was found to be the most prevalent cause of bloodstream infection, skin and soft-tissue infection and pneumonia in hospital cases in USA, Canada, Latin America, Europe and the Western regions (Diekema *et al*., 2001), see figure 7. Today it is thought that MRSA kills more Americans than HIV, mainly because of hospital practices of no screening for MRSA in blood donors or MRSA asynthomatic carriers entering their presmises.

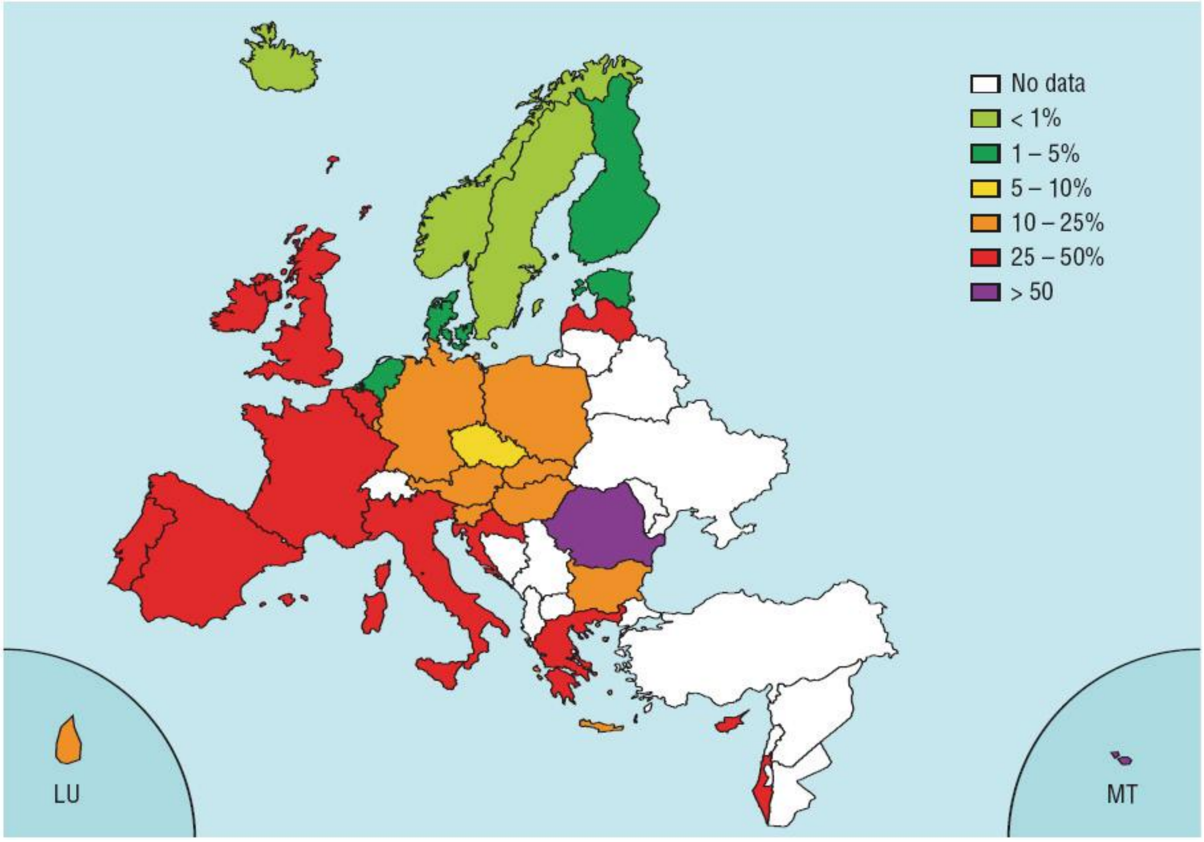

FIGURE 7. Percentage of MRSA resistance in Europe in 2004: *S. aureus* proportion of invasive isolates MRSA in 2004 (Data from the European antimicrobial resistant surveillance system, EARSS).

Strains of *S. aureus* exhibiting either beta-lactamases or PBP2A directed resistance, or both, have unfortunately already established a considerable ecological niche among humans. So far today, the frequencies of both community-acquired and hospital-acquired *staphylococcal*infections have increased steadily, with little change in overall mortality from the pre-antibiotic era. Multitude of factors that include the widespread and sometimes inappropriate use of antimicrobials, not only in humans but also extensive use of these agents as growth enhancers in animal feed and husbandry, together with the increase in regional and international travel, facilitates the relative ease with which antimicrobial-resistant bacteria cross geographic barriers (Lowy, 1998; lowy, 2003).

A typical MRSA possesses several insertion sequences with antibiotic resistance genes. Thus, MRSA resistance may encompass cell wall inhibitors such as β-lactams, ribosomal inhibitors including macrolide-lincosamide-streptogramin-B- (MLSB), aminoglycosides, tetracyclines, fusidic acid, and the new oxazolidinones, the RNA polymerase inhibitor rifampin, the DNA gyrase blocking quinolones, and also the antimetabolite trimethoprim-sulfamethoxazole (Barrett, 2005).

2.6.1.1.2.1. MRSA-Hospital acquired: HA-MRSA

Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for the largest outbreak ofhospital-acquired infection that the world has ever seen (Gould, 2005). MRSA hospital isolates aremultiresistant and clonal, they are associated with risk factors including recent hospitalizationor surgery, living in a nursing home, and having an indwelling catheter or device. Theprevalence is highly variable among geographical regions, ranging from 2 to 40 % betweenNorth and South European countries (Stefani and Varaldo, 2003), with about 30 % in Belgium. Early diagnosticand strict hygiene measures appear therefore as the best strategies to control theirspreading (Loveday *et al*., 2006, Struelens and Denis, 2006).

2.6.1.1.2.2. MRSA-Community acquired: CA-MRSA

Although originally confined to the hospital environment, MRSA have emerged in communityacquired infections (Lu *et al*., 2005). As compared to hospital acquired MRSA, CA-MRSA carry a distinct molecular makeup; they are less resistant but more virulent and invasive (Chapman *et al*., 2005; Drews *et al*., 2006)

They are frequently found in young, healthy patients without significant health care contact, especially in populations with close physical contacts (Sport Teams, Prisons (Kazakova *et al*., 2005; Pan *et al*., 2003).

Although both HA-MRSA and CA-MRSA harbour a SCC*mec* carrying the *mecA* gene, their SCC*mec*s are of quite different sizes and most likely of different origin. Thus, the two types of organisms are not alike. Furthermore, clinical and molecular epidemiology indicates that they represent two separate evolutions of the strain. CA-MRSA may represent a hybrid between HA-MRSA, which escaped from the hospital environment, and the more easily treatable community organisms. These hybrids strains have in turn acquired new virulence factors, which make their infections after only minor cuts or scrapes, more aggressive and invasive (Barrett, 2005). The prevalence rates of CA-MRSA vary widely among studies, in part because of the use of different definitions to distinguish between CA-MRSA and HA-MRSA, but also because of the different settings in which studies have been performed (It should be noted that relatively few studies have been conducted among randomly selected healthy members of the community, most studies being based on hospitalised patients, or patients upon admission to the hospital, which has probably resulted in an overestimation of the 'true' prevalence of CA-MRSA (Kluytmans-Vandenbergh and Kluytmans, 2006). Thus, prevalence of CA-MRSA was about 30 % among MRSA isolates from hospitalised patients, but only 0.2% among community members (Salgado *et al*., 2003).

The main features that distinguish CA-MRSA from HA-MRSA is their susceptibility to other classes of antimicrobial agents, their genetic diversion from HA-MRSA (including toxins), and the inclusion of new markers including Panton-Valentine Leukocidin (PLV) and SCC MEC type IV.

2.6.1.1.2.3. Beta-lactams anti- MRSA in development

The consensus that beta-lactam antibiotics are inherently more efficacious than vancomycin against MSSA has prompted more research on the field. MRSA resistance to beta-lactams depends upon the supplementary peptidoglycan transpeptidase PBP2A that has low affinity for these compounds. PBP2A does not lack affinity for beta-lactams but the beta-lactam compounds developed so far have low affinity. So, it is though that in principle beta-lactams can be re-engineered to bind beta-lactams strongly. On this basis, new anti-MRSA compounds with improved affinity towards PBP2A have been designed (Livermore, 2006). Studies of MRSA strains have shown that PBP2A can be effectively blocked by beta-lactams with improved affinities for PBP2A and stability against degradation by s*taphylococcal* penicillinase (Entenza *et al*., 2002) and hence restore beta-lactam affinity. These new compounds include carbapenems and cephalosporins (Livermore, 2006), in particular LB11058, ceftobiprole (formerly BAL9141, Ro 63-9141), RWJ-54428 (MC-02,479) (Cho *et al.*, 2001; Vouillamoz *et al.,* 2004). New developmental anti-MRSA carbapenems include SM-197436, SM-232721 and SM-232724 (Ueda and Sunagawa, 2003). Faropenem and doripenem carbapenems also include MRSA in their spectrum (Bosso, 2005; Guignard *et al.*, 2005; Hanaki, 1997; Schmidt-Ioanas *et al*., 2005).

2.6.2 Vancomycin-resistance

Vancomycin and teicoplanin are still the only glycopeptide antibiotics approved to treat multiresistant bacterial strains in human use (Van Bambeke, 2004; Van Bambeke *et al*., 2004). In sensitive *S. aureus* strains, these large hydrophilic molecules are able to form a five-point hydrogen bond interactions with the terminal D-alanyl-D-alanine moieties of the N-acetylmuramic acid (NAM)/ N-acetylglucosamine (NAG)-peptide subunits preventing the incorporation of these subunits into the peptidoglycan matrix (PDG) (Arthur *et al*., 1996; Reynolds, 1989) (Figure 8). Teicoplanin is not used in the USA, but vancomycin has been widely used orally to treat *Clostridium spp*. and *Enterococcus spp.* infections (Gerding, 1997; Mylonakis *et al*., 2001).

S. aureus has already developed different ways to overcome the action of these antibiotics. One of them is the tolerance of the bacteria to continue to survive in presence of glycopeptides at clinical relevant doses (Appelbaum, 2006; Arthur *et al*., 1996; Courvalin, 2006); the other depends on the acquisition of genetic material from a transposon found in the vancomycin resistant *Enterococcus spp*. (Gonzalez-Zorn and Courvalin, 2003). We therefore also get an insight into glycopeptide resistance in *Enterococcus spp*.

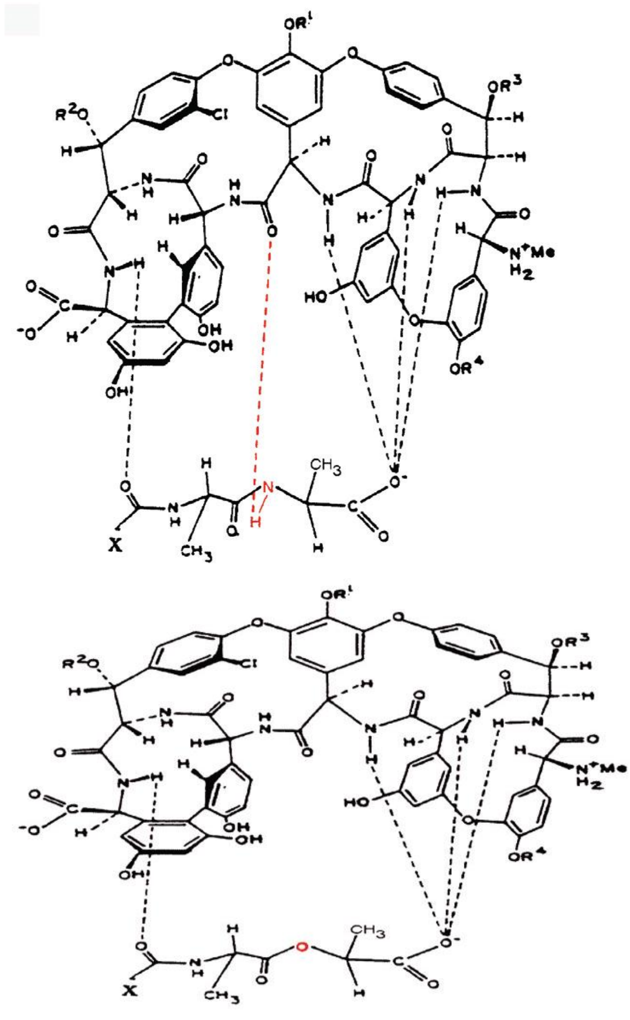

FIGURE 8: Hydrogen bonding interaction in vancomycin susceptible (first) and vancomycin resistant (second) bacteria (Arthur *et al*., 1996).

2.6.2.1. Vancomycin resistance in Enterococcus spp: (VRE)

Among enterococci, six types of acquired glycopeptide resistance have been described (VanA, VanB, VanC, VanD, VanE, and VanG). They are named based on their specific ligase genes (e.g., vanA, vanB,) (Courvalin, 2006). A related gene cluster, vanF, has been found in *Paenibacillus popilliae* strains (formerly *Bacillus* spp.), a vancomycin-resistant biopesticide used in the United States which could be the progenitor of the resistance genes acquired by enterococci (Courvalin, 2006).

In resistant strains a novel cell wall precursor with reduced glycopeptide affinity is synthesized, a hydrogen bond is lost in the five-hydrogen interaction pocket and the antibiotic looses 1000X times' affinity. The new dipeptides of the PDG wall are not then D-ala-D-ala but D-Ala-D-Lac or D-Ala-D-Ser, depending of the genetic material (Arthur *et al*., 1996).

2.6.2.1.1. The vanA operon

The most common genotype of vancomycin and teicoplanin resistance (vanA) in enterococcus arises as the result of the horizontal acquisition of operons (transposon Tn1546) carrying genes coding for enzymes for the synthesis of PDG with low affinity precursors (the C-terminal D-Ala residue is replaced by D-lactate), resulting in 4-point hydrogen bonding interaction and 1000 times-fold decrease in vancomycin affinity (Arthur *et al*., 1996; Courvalin, 2006).

This vanA phenotype requires a five-gene-resistance-cassette to be assembled in a transposon (embedded in a plasmid) for a re-programming of the enzymes that make the D-ALa-D-Ala terminus of the peptidoglycan precursor. What is more, one of the enzymes in the genetic package (VanX) also eliminates the high-affinity precursors (D-

Ala) that are normally produced by the bacteria and thus removes the vancomycin-binding target.

2.6.2.2 Resistance of *S.aureus*to vancomycin

There are two forms of *Staphylococcus aureus* becoming resistant to vancomycin. One form has been identified in the VISA strains, which have MICs to vancomycin of 8-16ug/ml. Pre-VISA stage of resistance (heterogeneously resistance) have been identified. Only subpopulation remains susceptible to vancomycin for heteroresistance (Franklin, 2003) .The VISA isolated was selected from the vancomycin resistance subpopulation. Result shows that the reduction in the susceptible to the vancomycin appeared due to the changes in the peptidoglycan synthesized. The VISA strains were detected for the additional quantities of synthesized peptidoglycan that results in irregularly shaped, thickened cell wall. This also decreases cross-linkage of peptidoglycan strand, which leads to the exposure of more D-ala-D-ala residue. The change causes reducing of L-glutamine that was available for amidation of D-glutamate in the peptide bridge (Franklin, 2003). Vancomycin binds to two classes of targets in *Staphylococcus aureus* cells, they are D-alanyl-D-alanine residues in the completed peptidoglycan chain and the murein monomers found in the cytoplasmic membrane that serves as the precursor for peptidoglycan synthesis (Hiramatsu, 2001). Binding of vancomycin to the D-alanyl-D-alanine residues in the completed peptidoglycan layers does not inhibit the peptidoglycan synthesis but binding to the murein monomers in the cytoplasmic membrane completely stops the peptidoglycan synthesis and the cells die (Hiramatsu, 2001).

However, cell-wall thickness is a major contributor to vancomycin resistance. It is suggested that the first clinical VRSA strain, Mu50 (VRSA) produces increased amount of peptidoglycan, meaning more peptidoglycan layers and murein monomers are found in the cell wall (Hiramatsu, 2001). Hence, more vancomycin molecules are trapped in the peptidoglycan layers before reaching the site of peptidoglycan synthesis on the cytoplasmic membrane (Hiramatsu, 2001) .In addition, a higher concentration of vancomycin was needed to saturate all the murein monomers that are being produced at an increased rate in Mu50 (Hiramatsu, 2001). According to the experiments done by researchers, it was suggested that the mesh structure of the outer layers of thickened peptidoglycan was destroyed by the trapped vancomycin molecules themselves (Hiramatsu, 2001). Since the mesh structure of the outer layers of thickened peptidoglycan was destroyed, thus vancomycin molecules can no longer recognize the structure and bind to it, hence preventing the trapped vancomycin molecules of having the chance to penetrate further into the inner part of cell-wall layers (Hiramatsu, 2001)

The second form of the vancomycin resistant S. aureus is mediated by vanA operon (encoded on a conjugative plasmid) (Fey, 2011). The MICs for complete vancomycin resistance is more than or equal to 128ug/ml. Resistance of these isolates caused by alteration of the terminal peptide to D-Ala-D-Lac instead of D-Ala-D-Ala. D-glutamic acid replaces the D-alanine in the pentapeptide chain (Patrick, 2009). Thus, terminal ester link was formed instead of amide bond which is needed for hydrogen bonding with vancomycin (Patrick, 2009). As a result, the binding affinity of vancomycin to the pentapeptide chain decreases, thus making the antibiotic ineffective. Furthermore, lactate acts as leaving group compared to D-alanine (Patrick, 2009). Synthesis of D-Ala-D-Lac occurs only with exposure to low concentrations of vancomycin. As a result, the additional biosynthetic demands are limited and the VRSA strain is ecologically fit (Franklin, 2003).

2.7 TREATMENT, PREVENTION AND CONTROL

2.7.1 Treatment

Staphylococci quickly developed drug resistance after penicillin was introduced and today less than 10% of the strains are susceptible to this antibiotic. This resistance is mediated by penicillinase which hydrolyses the ß-lactam ring of penicillin. The genetic information encoding the production of this enzyme is carried on transmissible plasmids, which facilitated the rapid dissemination of resistance among staphylococci.

Because of the problems with penicillin-resistant staphylococci, semisynthetic penicillins resistant to ß-lactamase hydrolysis (e. g., methicillin, nafcillin, oxacillin, dicloxacillin) were developed. Unfortunately, the staphylococci developed resistance to these antibiotics, as well. *S. aureus* which is resistant to an antibiotic called methicillin is referred to as methicillin-resistant *Staphylococcus aureus* or MRSA.

Currently, 30% to 50% of the strains of *S. aureus* and more than 50% of the coagulase-negative staphylococci are resistant to these semisynthetic penicillins. Resistance occurs as the result of acquisition of a gene, mecA, that codes for a novel penicillin-binding protein, PBP2'. The penicillins and other ß-lactam antibiotics kill bacteria by their ability to bind to penicillin-binding proteins, which are enzymes responsible for construction of the cell wall peptidoglycan. PBP2' is not bound by penicillins but retains its enzymatic activity.

Staphylococci have demonstrated the remarkable ability to develop resistance to most antibiotics. Until recently, the one antibiotic that has remained uniformly active against staphylococci has been vancomycin, the current antibiotic of choice for treating staphylococci resistant to oxacillin. Unfortunately, isolates of *S. aureus* have been found with decreased susceptibility to vancomycin, and frank resistance to vancomycin has been observed in coagulase-negative staphylococci. The mechanism of this resistance is unknown. However, there is evidence that changes in cell wall synthesis have led to reversible vancomycin binding that effectively prevents vancomycin from disrupting peptidoglycan synthesis (Murray *et al.,* 2002).

New agents are being developed against MRSA. Some of them are expected to have considerable activity against hetero-VRSA and VRSA strains as well. A new quinolone antibiotic, DU-6859a, has MICs of 0.5 and 1 mg/L against Mu3 (Hetero-VRSA) and Mu50 (VRSA) which are resistant to other quinolones such as levofloxacin, ciprofloxacin, sparfloxacin, and tosufloxacin [Hiramatsu, 2001]. Normally, antibiotic combination therapies were used against VISA and VRSA.

Quinupristin-dalfopristin (trade name Synercid) which is a combination of two antibiotics has potent activity against hetero-VRSA and VRSA strains (Hiramatsu, 2001). ß-lactam agents work synergistically with vancomycin against VISA. The isolates of VISA can be inhibited at lower vancomycin concentration when they are exposed to nafcillin or cefazolin [Fridkin, 2001] .Moreover, ampicillin or sulbactam would be a good partner for vancomycin; even the agent alone has good anti-microbial activity against VRSA in an experimental infection model [Hiramatsu, 2001].

Linezolid has either a synergistic or additive effect on hetero-VRSA and VRSA strains when combined with ampicillin or sulbactam. When vancomycin was used, it can be seen from its mechanism of action and mechanism of resistance that efforts have to be put to reduce the bacterial burden from the patient’s body with procedures such as surgical drainage, debridement, and removal of contaminated lines, foreign bodies, or prosthetic materials. When vancomycin therapy is still unsuccessful with these procedures, triple therapy is used which includes vancomycin, rifampicin (oral) and co-trimaxole (TMP-SMZ)[Hiramatsu, 2001] .A report from Nevada found that a patient treated with surgery together with linezolid, TMP-SMZ, and doxycycline therapy was successful in treating VISA[Fridkin, 2001].

2.7.2 VACCINATION

Until the 1970s a variety of whole staphylococcal preparations was used in clinical and veterinary trials as a vaccine. These included cultured, attenuated, fixed or lysed organisms. None showed convincing benefit in patients, animal models or farm animals. Staphylococci were known to produce haemolysins and leukocidins. Toxoids prepared from these were largely unsuccessful as vaccines and were accompanied by many adverse reactions. Some toxoids of veterinary value, such as the alpha toxoid, protect ewes against gangrenous mastitis, did not reduce the overall infection rate.

Polyvalent polysaccharide components of the bacterial wall have given some success in animal models. However, clinical trials of staphylococcal vaccines, including those utilising polysaccharides, were beset with problems with loss of potency of vaccine material and difficulties in interpretation of trial data (Easmon and Adlam, 1983; Michie, 2002).

Aerosol vaccination with *S. aureus* endotoxins have shown good results in animal models and now await clinical trials. In last few years, scientists have tried DNA vaccination against the clumping factor A (C1fA) and passive immunisation with monoclonal antibodies as new strategies in staphylococcal vaccination (Michie, 2002). The results suggested that DNA immunization against adhesins represents a new and valuable approach to combat *S. aureus* infections (Brouillette *et al*., 2002).

2.7.3 Prevention and control

Even though there are some antibiotics that can be given to treat infections caused by VISA and VRSA, prevention is the primary infection control issue. These precautions need to be taken in order to prevent the transmission of the infections among the patients in the hospitals. The VRSA infection can be prevented by hygienic precaution such as:

• Prevent contact with other patient's wounds. Cover the wound infections with hygienic, dry bandages until they are healed. Put the used bandages in a sealed plastic bag before disposing.

• Avoid sharing personal items with other people such as plate, cup, toothbrush and others.

• Limit the use of antibiotics.

• Place contaminated laundry in a plastic bag and wash with hot water and detergents. Dry them in clothes dryer on the hot situation.

• Use alcohol or chlorine-based disinfectants and wear gloves, mask and gown when cleaning.

• Wash hands frequently with soap and warm water. If there is no water available, germ-killing hand lotion can be used to clean hands. Although recommended measures to control the spread of VRSA have been promoted for several years, it is still not appreciably slow the increasing rate of infection or colonization of the *Staphylococcus aureus* especially at the country like United States. Preventing the emergence of multidrug resistant organism require a systematic and comprehensive approach that build up the health care and public health system. The encouragement to the public health care system are very important to recommend prevention and control guidelines, conduct active surveillance and ensure vigorous antibiotic stewardship by health care provider [Chang *et al*., 2003].

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Zaria is located between latitude 11°07'N and longitude 7° 44'E within the Sudan savannah zone. Grass is the dominant vegetation. The major trees in this vegetation are dumb palm, fan palms and Shea butter. The average rainfall ranges from 1000 to 1250 mm and the average daily temperature ranges from 19°C – 33°C (Sa'idu *et al.*, 2001).

3.2 Ethical consideration

Ethical approval for the study was obtained from the authorities of the Hospitals (Appendix VIII)

3.3 Questionnaire

Four hundred questionnaire to obtain socio-demographic and risk factors of the study were distributed to the patients (Appendix I).

3.4 Study population

The study was carried out among inpatients and outpatients attending some Hospitals in Samaru, Zaria: University Health Services Ahmadu Bello University Samaru (Sickbay), Savannah Hospital Samaru, Rangwame Hospitaland Maternity Hayin Dogo Samaru (Rangwame Hospital) and Jama'ah Hospital Samaru, Zaria. The study population were comprised of children and adults of both sexes (males and females).

3.5Sample size determination

Sample size was obtained using the following equation described by Sarmukaddam and Garad (2006).

$$n = \frac{z^2 \mathrm{pq}}{L^2}$$

Where n = number of sample to be collected

z = standard normal distribution at 95% confidence limit = 1.96 from z table.

p = prevalence of previous study 57.7% (0.577) (Olayinka *et al*., 2005).

q = (1 – p) = 1 – 0.5 = 0.5

L = allowable error, which is taken as 5% = 0.05

Therefore

$$n = \frac{(1.96)^2 \times 0.577 \times 0.423}{(0.05)^2}$$

$$= 375.05$$

≈ 400 samples

A total of 400 samples were collected for analysis.

3.5.1Inclusion criteria

Samples were collected from patients who gave there consent.

3.5.2 Exclusion criteria

Patients who did not give there consent were not recruited for sample collection

3.6 Collection of specimens

A total of 400 specimens (Urine,Pus and wound swab) of patients were collected from the Microbiology laboratory of University Health Services (Sickbay) Ahmadu Bello University samaru (100), Jamaa Hospital samaru (100), Savannah Hospital (100) and Rangwame Hospital and maternity Hayin dogo samaru (Rangwame Hospital) (100) using appropriate containers and swabs. The clinical specimens included urine (250), pus (75) and wound swabs (75). Mid-stream urine was collected in sterile universal bottles from the patients. Each bottle was used once for an individual. The wound swab was collected from the deep part of the wound after cleansing of the wound surface with sterile saline.

3.7 Processing of specimens

The Specimens were processed for the isolation of *Staphylococcus aureus* within an hour after collection. The media used were: Mannitol salt agar, Blood agar and MacConkey agar.

The spcimens were plated on Mannitol salt agar medium and incubated at 37°C *for* 48 hours. Pure colonies of the isolates were identified based on colony morphology and kept on nutrient agar slants at 4°C until used.

3.8. Laboratory methods

3.8.1 Gram stain: The Gram staining reaction is used to help identify pathogens in specimens and cultures by their Gram reaction (Gram positive or Gram negative) and morphology. Gram staining was carried out as outlined by Cheesbrough(2005).Smears of the isolated colonies were prepared on clean grease free slides containing a drop of distilled water using wire loops. The smears were air-dried and heat-fixed. Crystal violet stain was applied on the smears for 30 seconds followed by rinsing with water and mordanting with lugol’s iodine for 30 seconds.This was washed off with water and brief decolorization with acetone after which the smears were again rinsed with water and counterstained with Neutral red for 1-2 minutes before being washed off finally with water.The smear was allowed to air-dry and examined microscopically at times 100 magnification using oil immersion.

3.8.2. Catalase test: Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. Few fresh colonies of the isolates were immersed in test tubes containing 2-3 ml of hydrogen peroxide (H_2O_2) solution using wooden applicator and observed for gas bubbling. *Staphylococcus aureus* is catalase positive (Cheesbrough, 2004).

3.8.3. Coagulase test

Coagulase causes plasma to clot by converting fibrinogen to fibrin.

3.8.3.1. Slide Test Method (Detects bound coagulase)

A drop of distilled water was placed on two separate slides. The colony of the test organism was emulsified in each of the drops to make two thick suspensions. A loopful of plasma was added to the suspension and mixed gently. The mixture was observed for clumping of the organisms within 10 seconds.

No plasma was added to the second suspension. This was used to differentiate any granular appearance of organism from true coagulase clumping (Cheesbrough, 2004).

3.8.3.2. Tube Test Method (Detects Free coagulase)

Three small test tubes were labelled:

T= Test organism (18-24 broth culture)

Pos= Positive control (18-24 hours. *S. aureus* broth culture)

Neg= Negative control (sterile broth)

A 0.2ml of plasma was pipetted in each tube and 0.8 ml of the test broth culture was added to tube T.

A 0.8 ml of the *S. aureus* culture was added to the tube labelled Positive. And also 0.8ml of sterile broth was added to the tube labelled Negetive.

After mixing, the three tubes were incubated at 35-37°C. After 1 hour the clot was examined. If no clotting had occurred, it was examined after 3 hours. If the test was still negative, the tubes were left at room temperature over night and it was examined again. Interpretation of result: clotting of the tube content indicates the presence of *S. aureus* while no clotting or fibrin clot indicates a negative test (Cheesbrough, 2004).

3.8.4. Characterization of Staphylococcal Isolates by Microgen Staph ID kit

Staphylococcal isolates were subjected to further characterization using a commercial identification kit called microgene staph ID according to manufacturer's instructions.The Microgen Staph-ID system comprises of a single microwell test strip containing 12 standardized biochemical substrates which have been selected on the basis of extensive computer analysis i.e. each well contains dehydrated substrates. A colour change occurs if the individual substrates are metabolized by the organism during incubation, or after addition of specific reagents.

3.8.4.1 Procedure-inoculation and incubation

Gram stain, catalase test and slide agglutination test were carried out on the isolates to confirm it belongs to the genus *Staphylococcus*. Colony pigment production was noted. A single colony from an 18-24 hours culture was emulsified in the suspending medium supplied in the kit and mixed thoroughly. The adhesive tape sealing the micro well test strip(s) was carefully peeled and was not discarded as it was required for later use. Three to four drops of the bacterial suspension was added to each well of the strip(s) using Pasteur pipette. After inoculation, wells 10 and 11 were overlayedwith 3-4 drops of mineral oil. These wells (10, 11) were highlighted with a black circle around the well to assist in adding the mineral oil to the correct well. The top of the microwell test strips was sealed with the adhesive tapes removed earlier and was incubated at 35-37°C. The microwell test strips was read after 18-24 hours of incubation.

3.8.4.2. Procedure-Reading and adding of reagents

The adhesive tape was removed and all positive reactions were recorded with the aid of a colour chart. The result was recorded on the form provided (1-11). A drop of pyr reagent was added to well 12 and was read after 10 minutes. A positive result was indicated by a very deep pink/red colour.

A nitrate reduction test was performed on well 9 after reading and recording the beta glucuronidase reaction. One drop of nitrate A and one drop of nitrate B reagent were added to the well and was read after 60 seconds. The development of a red colour indicated that nitrate had been been reduced to nitrite. These additional results were recorded on the forms provided 9a and 12.

3.8.4.3. Identification

On the microgen staph ID report form, the substrate has been organised into triplets (sets of 3 reactions). With each substrate assign a numerical value (1, 2, or 4). The sum of the positive reactions for each triplet forms a single digit octal code that was used to determine the identity of the isolate. The octal code was entered into the micogen identification system software (MID-60), which generates a report of the most likely organisms in the selected data base.

3.8.5 Screening Test to Detect Vancomycin Resistant *Staphylococcus aureus*

Mueller-Hinton Agar (MHA) was used for antimicrobial disc diffusion susceptibility testing of the isolates by the Bauer-Kirby method as standardized by the Clinical and Laboratory Standards Institute (CLSI, 2006).

3.8.5.1 Media preparation

Thirty-eight grams (38 g) of the MHA powder was suspended in 1 L of purified water, thoroughly mixed and heated with frequent agitation and boiled for 1 minute to completely dissolve the powder. The medium was autoclaved at 121°C for 15 minutes, cooled to 45°C and aseptically poured into sterile Petri dishes and cooled to room temperature (CLSI, 2006).

3.8.5.2. Antibiotic Susceptibility Test

This was done according to Clinical Laboratory Standards Institute (CLSI, 2006) guidelines. At least three to five well-isolated similar colonies of the test organism and standard strain(*Staphylococcus aureus*, ATCC 25923 obtained from National Institute of Pharmaceutical Research and Development (NIPRD), Abuja) were selected and transferred with a loop into 4-5 ml sterile normal saline. These were incubated at 35°C until it achieved the turbidity of the 0.5 McFarland barium sulphate standards (usually 2-6 hours). The turbidity was adjusted to be equivalent to the barium sulphate standard using sterile broth. The turbidity of the standard and the test inoculum were compared by holding both tubes in front of a white background. Within 15 minutes after adjusting the turbidity of the inoculum, sterile cotton swab was immersed into the properly diluted inoculum volume and rotated firmly several times against the upper inside wall of the tube to expel excess fluid. The entire agar surface of the plate was inoculated, rotating the plate 60° between streakings to obtain even inoculation before applying the antimicrobial disks. The single disks used included vancomycin (30ug), erythromycin (15ug), tetracycline (30ug), gentamicin (10ug), ampicillin (10ug), chloramphenicol (30ug), cefoxitin (10ug), and linozolid (10ug). Within 15 minutes after the discs were applied, the plates were incubated at 35°C-37°C for 18-24 hours. Diameters of zones of inhibition were measured based on CLSI (2006) interpretative chart (Appendix V).

3.8.6 Minimum Inhibitory Concentration

This was determined by broth microdilution method. Standard antibiotics powders were obtained from Glaxosmithkline and Evans pharmaceutical companies. Two-fold dilutions of the antibiotics were prepared in Muller Hinton broth (Wayne, 2002). Ten (10) dilutions were made with concentration gradient covering from 0.244-125µg/ml of each of the antibiotic using a stock concentration of 250µg/ml. For each series of the dilution, all the first tubes of the Mueller Hinton broth were double- strengthened (10ml) , the remaining tubes were single strength (10ml). The concentration of each of the suspension of the test organisms were prepared by picking a 24 hours colony of the organism using sterile wire loop into sterile test tube containing sterile normal saline to form turbidity that match with 0.5 scale of MacFarland's standard (1.5×10^8 cells/ml) (Coyle, 2005). The tubes containing 10ml of the sterile Mueller-Hinton broth were used to carry out two-fold dilution using 10ml of the antibiotic (125µg/ml as the highest concentration). Three controls were set up: positive control (containing Mueller Hinton broth and standard strain), negative control (containing Mueller Hinton broth and antibiotic), and sterility control (containing only Mueller Hinton broth) (Terry-Alli *et al*., 2011). All the tubes were inoculated with 0.1ml of the isolates using micro pipette except negative control and sterility control and were incubated at 35°C for 24 hours. The lowest concentration that inhibited the growth or showed no growth of the bacterial isolate in the tube was taken as the MIC. Readings were taken and recorded according the guidelines of CLSI, 2008.

3.8.7 Minimum Bactericidal Concentrations (MBC)

The MBC was determined by subculturing the last tube that showed visible growth and all the tubes in which there was no growth of the bacterial isolates on freshly prepared plates of Mueller Hinton agar containing 5% of tween 80(which inactivate the antibiotic). The plates of the inoculated medium were incubated at 37°C for 24 hours. The lowest concentration on the plate that showed no growth of the bacterial isolate was the MBC (Cheesbrough, 2005).

3.9 Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR index was determined for each isolate by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics tested (Krumperman, 1983; Olayinka *et al*., 2004; Olonitola *et al*., 2009). Multiple antibiotic resistances was calculated as

$$\text{MARI} = \frac{\text{Number of antibiotics to which the isolate is resistant}}{\text{Number of antibiotics tested}}$$

(Olayinka *et al*., 2004).

3.10 Statistical Analysis

The data were analysed usingChisquare ($\chi 2$) test. Results were considered significant when $P \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS

A total of 400 samples were collected from four Hospitals in Samaru and were analysed for *Staphylococcus aureus*. In all, 71 (17.80%) were positive for *Staphylococcus aureus (S. aureus).* However, in each Hospitals 100 samples were collected. From Sickbay, 30 (30.00%) were positive for *Staphylococcus aureus,* while from Jamaa Hospital, 17 (17.00%) were positive. Rangwame Hospital had 14 (14.00%) positive for *S. aureus*; while in Savannah Hospital only 10 (10.00%) were positive for *S. aureus* (Table 4.1).

However, on the in-patients and out-patient 200 samples were collected from in-patient with 40 (20.00%) positive for *S.aureus* while in Out-patient 31 (15.50%) were positive for *S. aureus* (Table 4.2).

Table 4.3 shows the prevalence of *S.aureus* in relation to specimen type among patients attending Hospitals in Samaru, Zaria. The result shows that 46 (18.40%) of the 250 urine samples collected were positive for *S. aureus* and 14 (18.70%) out of the 75 samples collected from wound swabs were positive for *S. aueus* while 11 (14.70%) of the pus swabs collected were positive for *S.aureus*.

Table 4.4 shows the distribution of *S. aureus* in relation to age and sex among patients attending Hospitals in Samaru, Zaria. The result shows that 45 (11.25%) out of 400 specimens collected were positive for *S. aureus* isolates which were from male patients while the remaining 26 (6.50%) were from female patients. The result shows that age group 0-9 (28.36%) had the highest *S. aureus* isolates, followed by age 30-39 (27.27%), followed by age range 40-49 (19.64%), then age range 20-29 (14.04%) and

Table 4.1. Distribution of *S. aureus*isolates in relation to Health facility among patients attending Hospitals in Samaru, Zaria

Hospitals	No of Samples collected	Samples Positive	
		(n)	(%)
Sickbay	100	30	30.00
Jamaa Hospital	100	17	17.00
Rangwame	100	14	14.00
Savannah	100	10	10.00
Total	400	71	17.80

Key: n=number of positive samples, %=percentage.

Table 4.2. Distribution of *S. aureus*isolates in relation to patient category attending Hospitals in Samaru, Zaria

Patients	No of samples collected	No positive	(%)
IN-Patient	200	40	20.00
OUT-Patient	200	31	15.50
Total	400	71	17.80

Table 4.3. The Prevalence of *S. aureus* in relation to specimen type among patients attending Hospitals in Samaru, Zaria

Sample type	No of sample collected	No of positive	(%)
Urine	250	46	18.40
Wound	75	14	18.70
Pus	75	11	14.70
Total	400	71	17.80

Table 4.4 Distribution of *S. aureus* in relation to Age and Sex among Patients attending Hospitals in Samaru, Zaria

Age group (years)	No of specimens	No examined	No (%) positive	No examined	No (%) positive	Total (%) positive
		Males		Females		
0−9	67	34	10 (14.93)	33	9 (13.43)	19 (28.36)
10−19	60	30	4 (6.67)	30	1 (1.67)	5 (8.33)
20−29	57	28	6 (10.53)	29	2 (3.51)	8 (14.04)
30−39	66	30	11 (16.67)	36	7 (10.61)	18 (27.27)
40−49	56	28	9 (16.07)	28	2 (3.57)	11 (19.64)
50−59	53	33	5 (9.43)	20	3 (5.66)	8 (15.09)
60−69	41	17	0 (0.00)	24	2 (4.88)	2 (4.88)
≥70	0	0	0 (0.00)	0	0 (0.000	0 (0.00)
Total	400	200	45 (11.25)	200	26 (6.50)	71 (17.80)

50-59 (15.09%). Age range 10-19 had 8.33% while the least came from age range 60-69 which had 2 (4.88 %). No samples were collected in age group greater than seventy (70) years as in table 4.4.

Table 4.5 shows the antibiotic susceptibility profile of *S.aureus* isolates from patients attending Hospitals in Samaru, Zaria. All the 71 (100.00%) isolates were susceptible to vancomycin and gentamicin. For ampicillin, *S. aureus* had 64 (90.10%) resistant and 7 (9.90%) sensitive strains, while for tetracycline, *S. aureus* had 10 (14.10%) resistant, 1 (1.40%) intermediate strains and 60 (84.50%) susceptible strains, while for cefoxitin, *S. aureus* had 24 (33.80%) resistant and 47 (66.20%) susceptible strains. For linezolid, *S. aureus* had 7 (9.90%) resistant and 64 (90.10%) susceptible strains while for erythromycin, *S. aureus*had 20 (28.20%), 4 (5.60%) intermediate and 47 (66.20%) susceptible strains. For chloramphenicol, *S. aureus* had 9 (12.70%) resistant and 62 (87.30%) susceptible strains.

Table 4.6 shows the Susceptibility of*S. aureus* isolates in relation to patient category attending Hospitals in Samaru, Zaria. The percentage of resistance / intermediate sensitivity and full sensitivity of the isolates to the antimicrobials used among the patients is shown in Table 4.6. Out of 71 isolates, out-patients had 28 (39.40%) resistant/intermediate sensitivity and 3 (4.20%) fully susceptible to ampicillin. However, 5 (7.00%) of the isolates showed resistance/intermediate sensitivity and 26 (36.60%) were fully susceptible to tetracycline, but for cefoxitin 15 (21.10%) resistance/intermediate sensitivity were observed while 16 (22.50%) were fully susceptible. On Linezolid, 3 (4.20%) showed resistant/intermediate sensitivity while 28 (39.40%) were fully susceptible. On erythromycin 14 (19.70%) showed resistant/intermediate sensitivity while 17 (23.90%) were fully susceptible. On chloramphenicol, 7 (9.90%) showed resistant/intermediate, while 24 (33.80%) were

Table 4.5. The antibiotic susceptibility profile of *S. aureus* isolates from patients attending Hospitals in Samaru, Zaria

ANTIBIOTIC (Conc)	**R**	**I**	**S**
Ampicillin (10ug)	64(90.10%)	0(0.00%)	7 (9.90%)
Gentamicin (10ug)	0(0.00%)	0(0.00%)	71 (100.00%)
Tetracycline (30ug)	10 (14.10%)	1 (1.41%)	60 (84.50%)
Cefoxitin (30ug)	24 (33.80%)	0(0.00%)	47 (66.20%)
Vancomycin (30ug)	0(0.00%)	0(0.00%)	71 (100.00%)
Linezolid (10ug)	7 (9.90%)	0(0.00%)	64 (90.10%)
Erythromycin (15ug)	20 (28.20%)	4 (5.60%)	47 (66.20%)
Chloramphenicol (30ug)	9 (12.70%)	0(0.00%)	62 (87.30%)

Key

R=resistant, I= intermediate sensitivity, S= sensitive

Table 4.6 The Susceptibility of *S.aureus*isolates in relation to patient category attending Hospitals in Samaru, Zaria

Antibiotics (Conc)	Out-patients		In-patients		χ^2	*P*
	R/I (%)	SEN (%)	R/I (%)	SEN (%)		
Ampicillin (10ug)	28 (39.40)	3(4.20)	36(50.70)	4 (5.60)	0.002	0.964
Gentamicin (10ug)	0(0.00%)	31(43.70)	0(0.00%)	40(56.30)	NA	NA
Tetracycline (30ug)	5 (7.00)	26(36.60)	6 (8.50)	34(47.90)	0.017	0.896
Cefoxitin (30ug)	15 (21.10)	16(22.50)	9(12.70)	31(43.70)	5.230	0.022*
Vancomycin (30ug)	0(0.00%)	31(43.70)	0(0.00%)	40(56.30)	NA	NA
Linezolid (10ug)	3(4.20)	28(39.40)	4(5.60)	36(50.70)	0.002	0.964
Erythromycin (15ug)	14(19.70)	17(23.90)	10(14.1)	30(42.30)	3.173	0.075
Chloramphenicol(30ug)	7(9.90)	24(33.80)	2(2.80)	38(53.50)	4.877	0.027*

R/I = Resistant/Intermediate Sensitivity
SEN = Sensitivity

NA= Not Available

*=significant association exists at $p \leq 0.05$

X^2= Chisquare

P= Confidence level

fully susceptible. Gentamicin and vancomycin all had 31(43.70%) fully susceptible.While in in-patients, 36 (50.70%) of the isolates showed resistance/intermediate sensitivity and 4 (5.60%) werefully susceptible to ampicillin.However, 6 (8.50%) of the isolates showed resistance/intermediate sensitivity and 34 (47.90%) were fully susceptible to tetracycline, but for cefoxitin 9 (12.70%) resistant/intermediate sensitivity were observed while 31(43.70%) were fully susceptible. On Linezolid, 4 (5.60%) showed resistant/intermediate, while 36 (50.70%) were fully susceptible. On erythromycin 10 (14.10%) showed resistant/intermediate sensitivity while 30 (42.30%) were fully susceptible. On chloramphenicol, 2 (2.80%) showed resistant/ intermediate sensitivity while 38 (53.50%) were fully susceptible. Gentamicin and vancomycin all had 40 (56.30%) fully susceptible. Statistically, there is no significant difference between the susceptibilities of isolates to ampicillin, tetracycline, linezolid and erythromycin from the patients as all have a p-value of greater than 0.05. While there was significant difference between the susceptibility of the isolates to Cefoxitin and chloramphenicol from the patients as the p-value was less than 0.05. Statistical association could not be established for vancomycin and gentamicin because the isolates were all susceptible.

Table 4.7 shows the multiple antibiotic resistant pattern of *S.aureus* from clinical specimens. Fourteen (14) resistance patterns were identified, all from single (1) and multiple resistance type with varying combinations of 2, 3 and 4 antibiotics. The highest frequency 31(44.00%) was found in single antibiotics. Multidrug resistance is taken as resistance to 3 or more antibiotics (Tillotson *et al*, 2008). Also 27(38.00%) of the isolates were multidrug resistant.

Multiple Antibiotic Resistance Index (MARI) of the isolates is shown in Figure 4.1. Multiple Antibiotic Resistance Index (MARI) gives an indirect suggestion of the **Table**

4.7. Multiple antibiotic resistance pattern of *S. aureus* from clinical specimens; n=71

No of antibiotics	No of isolates with pattern (%)	Resistance pattern/phenotype
1	31 (44.00)	AMP,
	6 (9.00)	LZD
2	1 (1.00)	E, AMP
	1 (1.00)	AMP, C
	1 (1.00)	AMP, TE
	3 (4.00)	AMP, E
	1 (1.00)	LZD, E
3	4 (6.00)	AMP, FOX, C
	2 (3.00)	AMP, E, C
	4 (6.00)	AMP, TE, FOX
	8 (11.00)	AMP,FOX,E
	1 (1.00)	AMP, TE, E
4	5 (7.00)	AMP, TE, FOX, E
	3 (4.00)	AMP, FOX, E, C

AMP=Ampicillin, FOX=cefoxitin, TE=Tetracycline, E=Erythromycin, C=Chloramphenicol, LZD=Linezolid.

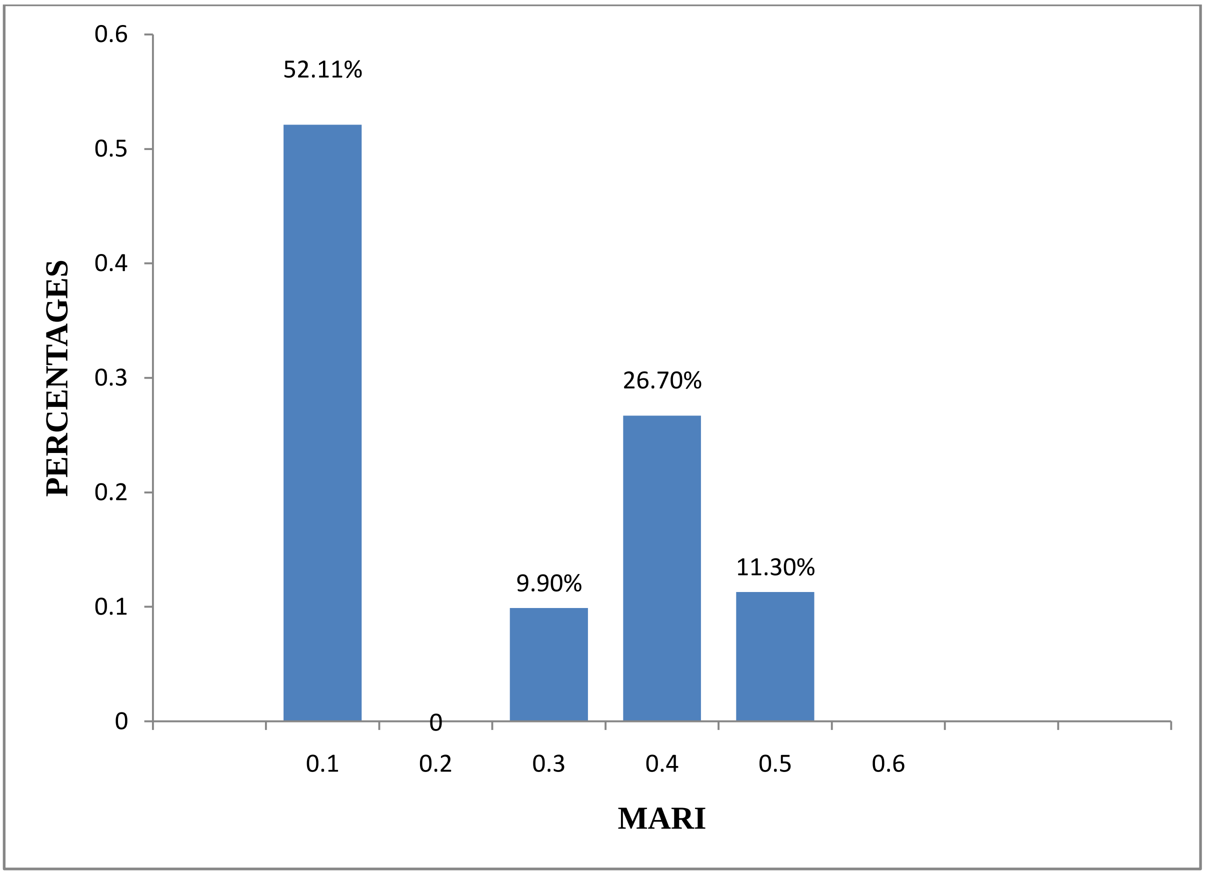

Figure 4.1. Multiple antibiotic resistance indices of *S. aureus* from clinical specimens (47.90% of isolates had an MARI of >0.2)

probable source of the organism. MAR index greater than 0.2 indicates that an organism must have originated from an environment where antibiotics are often used (Olayinka *et al*., 2004).Among the isolated *S. aureus* from clinical specimen, 34 (47.90%) of the tested bacteria had an MAR index greater than 0.2.

Table 4.8 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *S. aureus* from clinical samples. The MICs of ampicillin against the *S. aureus* ranged from 3.91– 62.50µg/ml while MBCs ranged from 7.81 – 125.00µg/ml; more were inhibited and killed at 15.63 µg/ml and 31.25µg/ml respectively. The MICs of cefoxitin were 7.81µg/ml, 31.25µg/ml, 62.50 µg/ml and killed the organism at 15.63µg/ml, 62.50µg/ml and 125.00 µg/ml. The MIC of tetracycline was at 15.63 µg/ml and 31.25 µg/ml and killedthe organism at 31.25µg/ml and 62.50 µg/ml also; The MIC of chloramphenicol was 3.91 µg/ml, 7.81 µg/ml, 15.63 µg/ml,and 31.25 µg/ml but more organism were inhibited at 15.63 µg/ml while the organisms were killed at 7.81 µg/ml, 15.63 µg/ml, 31.25 µg/ml and 62.50 µg/ml but the highest number of organisms were killed at 31.25 µg/ml. The MIC of erythromycin was at 3.91µg/ml, 7.81µg/ml and 15.63 but more organisms were inhibited at 15.63µg/ml while the organism were killed at 7.81 µg/ml,15.63 µg/ml and 62.50 µg/ml but were killed most at 31.25 µg/ml.

Table 4.8: The MIC and MBC of the antibiotic against the resistant strains of the isolates from clinical samples

Organism	Antibiotics	MIC(ug/ml)	No of isolates	MBC(ug/ml)	No of isolates
S. aureus	AMP	3.91	4	7.81	4
		7.81	7	15.63	7
		15.63	9	31.25	9
		31.25	6	62.50	6
		62.50	1	125.00	1
	TE	15.63	3	31.25	3
		31.25	6	62.50	6
	FOX	7.81	7	15.63	7
		31.25	10	62.50	10
		62.50	6	125.00	6
	C	3.91	2	7.81	2
		7.81	2	15.63	2
		15.63	4	31.25	4
		31.25	1	62.50	1
	E	3.91	7	7.81	7
		7.81	4	15.63	4
		15.63	8	31.25	8

KEY

AMP=Ampicillin, TE=Tetracycline, FOX=Cefoxitin, C=Chloramphenicol, E=Erythromycin

MIC= Minimum Inhibitory Concentration

MBC= Minimum Bactericidal Concentration

CHAPTER FIVE

5.0 DISCUSSION

Staphylococcus aureus is a facultative anaerobic gram positive coccal bacterium. It is the most common species of *Staphylococcus* to cause staphylococcal infection. *S. aureus* has emerged as one of the mainly important pathogens and has over the past numerous decades been a leading foundation of hospital and community –acquired infections (Johnssons *et al*., 2004; Loffler *et al*., 2005; Shittu and Johnsson, 2006). In the present study a prevalence of 17.80% (71 out of 400) detected in this study was similar to that detected by Opere *et al*., (2013) who detected a prevalence of 18.06% in clinical specimens.

There is a higher prevalence of *S. aureus* among in-patient (20.00%) than out-patient (15.50%), this was similar to the work of Kitara *et al*., 2011 who found a higher prevalence of *S. aureus* in in-patient than out-patient. The higher prevalence is expected due to long hospital stay, ward condition such as bed making, changing of clothes, sneezing, nose picking and other personal habits like poor hygiene which contaminate every patient in the ward (Collin *et al*., 1991).

Staphylococcus aureus was found to be a frequent cause of burns and wound sepsis (Emmerson, 1994). A study by Ndip *et al*.,(1997) at Ilorin, Nigeria reported wound infection of 38% as the highest frequency of *S. aureus* isolates. This agrees with the result in the present study where *S. aureus* had the highest prevalence in wound followed by urine and then pus.

Staphylococcus aureus were isolated more in males than females. This agrees with the work of Nwankwo and Nasiru, 2011. However it is not clearly understood why males where more infected than females in the present study. However, it could be mostly because men are more likely to experience traumatic events because of their physical activity in conjunction with their care free attitude to hygiene. The prevalence of *S. aureus* was not gender dependent.

The highest frequency of isolates of *S. aureus* in the present study was observed in the (0-9) years age group in which neonates and infants were included. It is 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 hospitalized. The older children have also been observed to be more active than adults during interaction with there play mates 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* (Nwankwo and Nasiru, 2011).

S. aureus develops resistance very quickly and successfully to different antimicrobials over a period of time. The highest frequency of *S. aureus* with the susceptibility to the antibiotic vancomycin (100.00%), gentamicin (100.00%), followed by linezolid (90.10%), the least was ampicillin (9.90%).

Vancomycin and gentamicin were the only antibiotics with 0.00% resistance even with multi drug resistant strains and thus remain the best therapeutic options in our setting. A 100.00% gentamicin susceptibility to antibiotics by *S. aureus* was reported by Assefa *et al*., 2013. Vancomycin is widely unavailable and other available therapeutic options must be considered. Many studies have reported complete sensitivity to vancomycin (Nwankwo *et al*., 2010, Fayomi *et al*., 2011, Kesah *et al*., 2003, Vidhani *et al*., 2001). Vancomycin is a glycopeptide and is currently a drug of choice for *S. aureus* infections especially MRSA infection (Stevens *et al*., 2005). Since there is recognition of vancomycin Resistant Enterococcus (VRE), the emergence of vancomycin Resistant *S. aureus* (VRSA) has been anticipated in the future. There are few reports of VRSA/VISA (Vancomycin Intermediate *S. aureus*) cases from United States (Brief report, 2004). One of the common risk factor for acquiring VISA is the long term use of vancomycin (MDPH, 2007). It is therefore important to consider alternative treatment options for MRSA infections to prevent VRSA/VISA acquisition. The emergence of *S. aureus* with reduced susceptibility to vancomycin presents the potential for infection with a virulent organism for which therapeutic options are severely limited. Vancomycin is still the primary agent used for suspected invasive CA-MRSA infections. It is generally well tolerated and has few adverse effects. However, concerns have been raised about its poor penetration into lung tissue, under dosing, reported treatment failure in the case of necrotizing pneumonia caused by PVL + CA-MRSA strains (Wang *etal*., 2008), increasing low level (intermediate) resistance (Hiramatsu,1998), and "MIC Creep" (Rutland *et al*., 2009).

Oral therapy with linezolid, a bacteriostatic oxazolidonone, is effective in treating patients with SSTI, resistance to linezolid among MRSA isolates has been reported with prolong use (Besier *et al*., 2008) but it is rare with short term use. The use of linezolid is limited by high cost , limited availability of the suspension, the occurrence of thromcytopenia with prolong use and the availability of less-expensive effective oral antimicrobial drugs. Linezolid is well suited for the therapy of MRSA respiratory tract infection because of its excellent penetration into the lungs (pleura) and it may be an appropriate choice in cases in which the MIC of vancomycin is high (Sing *et al*., 2008). In this study a 9.90% resistance of *S.aureus* to linezolid was recorded which was in contrast to the 0% resistance (100.00% susceptibility) reported by Kaleem *et al*., 2010.

In this study ampicillin recorded the highest rate of resistance of 90.10% with a sensitivity of 9.90%. The ability of *S. aureus* to resist this antibiotic is due to the ability of *S. aureus* to produce a plasmid encoded beta-lactamase that hydrolyses the beta-lactam ring of this class of antibiotic which is essential for its antimicrobial activities (Opere *et al*., 2013) and also the high resistance to ampicillin could be due to the mis-use or abuse of antibiotics which is common phenomenon in developing countries (Olasupo *et al*., 2003).

In this study, cefoxitin had a sensitivity of 66.20%. Cefoxitin is a second generation cephalosporin and resistance to cefoxitin by disc diffusion has been used for the detection of MRSA strains in routine testing because cefoxitin is a potential inducer of the system that regulates *mecA* gene (Philip and Shannon, 1993; Madhusudhan *et al*., 2011). Compared with the first generation cephalosporins, the second and third generation antibiotics are said to have an inferior in-vitro activity against *S. aureus* with the exception of cefamandole, cefuroxime and possibly cefaclor, cephalosporin of later generations generally have lower activity against Staphylococci and offers no advantage over first generation cephalosporin when they need to be used in the management of staphylococcal infection (Turnidge *et al.,* 2008). This explains the high rate of cefoxitin (33.80%) resistance. Although cephalosporin are no longer used by CDC guidelines (2007), this study's sensitivity result indicated that cefoxitin remains effective in the population studied. It should also be noted that CDC guidelines (Gorwitz *et al* and CDC, 2006) also stated that antimicrobial therapy should also be guided.

Resistance to chloramphenicol in this study was low (12.70%), however, its role in the management of *S. aureus* (MRSA) soft tissue infection is yet to be defined. There are certain reports of trial of usage of chloramphenicol in multi-drug resistant Gram positive organism like MRSA, Vancomycin-intermediate *S. aureus* (VISA), Vancomycin Resistant *S. aureus* (VRSA) and Vancomycin Resistant Enterococcus (VRE) (Anderson *et al*., 2008).

In this study, erythromycin had a resistance of 20 (28.20%) and an intermediate resistance of 4 (5.60%) given a total resistance of 24 (33.80%), this is in line with the work of opera *et al*., 2013 which recorded 32% resistance of *S. aureus* to erythromycin.

Tetracycline had a resistance of 10 (14.10%), intermediate resistance of 1 (1.41%) and a total resistance of 11 (15.51%). Findings of opera *et al*., (2013) who reported a resistance of 68% which is in contrast with the present findings that accounted for 15.51% of resistance of *S. aureus* to tetracycline.

Comparing drug resistance among the patients, there was no significant difference in the resistance pattern of *S. aureus* to ampicillin, linezolid, and tetracycline between out patient and in-patient (P>0.05) this is in line with the work of Abdallah *et al*., (2007) while erythromycin was not significant in this study and in contrast to the report of Abdallah *et al*., 2007 who recorded a significant difference. In this study there was also a significant difference in resistance pattern of chloramphenicol and this differ from the finding of Abdallah *et al*., 2007. while statistical association could not be established for vancomycin and gentamicin because they were all susceptible. In this study there was no resistance of *S.aureus* to gentamicin in both in patient and out-patient and this is in line with the work of Kitara *et al*., 2011. In this study statistical association could not be established on vancomycin and was in contrast to the study of Abdallah *et al*., 2007 that recorded a non significant association. Because of low specificity and sensitivity, cefoxitin disc diffusion was introduced by CLSI that is known as a good surrogate marker for mecA gene detection (Okon *et al*., 2013). In this study those *S.aureus* resistant to cefoxitin were considered as MRSA. In this study MRSA was higher in out patient than in the in-patient and this is in contrast to the report of Abdul Jabbar *et al*., (2013). Resistance rate could be as a result of incessant mis-use of antibiotics by people in Zaria, Kaduna State without any restriction unlike in developed world where access to antimicrobials is restricted. Economic situations when considering the cost of diagnosis and other hospital bills have made almost everybody to embark on self-medication even without knowing the cause of the illness. Even those who are hospitalized must have taken barrage of antibiotics to no avail before going to the hospital.

There was higher proportion of *S.aureus* isolates from clinical samples. This study recorded 14 resistance patterns/phenotypes (table 4.7) among the isolates as against 20 patterns of resistance recorded by Tula *et al*., (2013). This may be due to differences in source of isolates and choice of the antibiotics used. Multidrug resistance is taken as resistance to 3 or more antibiotics (Tillotson *et al.*, 2008). As shown in Table 4.7 the multidrug resistance *S.aureus* from clinical samples (38.00 %) in this study was not higher than that reported by Fagade *et al*., 2010.

In this study multiple antibiotic resistance (MAR) index was found to be 47.90% (MARI > 0.2). According to Krumperman (1983), Olayinka *et al*., (2004) and Olonitola *et al*., (2009), MAR indices greater than 0.2 indicates that an organism originates from the environment where antibiotics are habitually used without prescription.

Staphylococcus aureus isolated from clinical samples(Table 4.8) were resistant to ampicillin at minimum inhibitory concentrations (MICs) ranging from 3.91–62.50µg/ml, since the MIC break point of ampicillin against *S. aureus* is sensitive at ≤ 0.25µg/ml as recorded by Clinical and Laboratory Standard Institute (CLSI), 2008 guideline. The MIC of ampicillin against *S. aureus* in finding was not similar to MIC of ampicillin from 512-1024µg/ml as reported by Dudhagara *et al*., (2011). The minimum bactericidal concentrations (MBCs) of ampicillin were at 7.81 µg/ml, 15.63µg/ml, 31.25µg/ml,62.50µg/ml and 125.00 µg/ml but the organisms were more killed at 31.25µg/ml and 62.50µg/ml. The MICs of cefoxitin (FOX) against *S. aureus* which ranged from 7.81 to 62.50µg/ml shows that this pathogen was resistant to this antibiotic with MBC from 15.63-125.00µg/ml. Because the CLSI, 2008 recommend that the MIC break point of FOX against *S. aureus* is considered sensitive at ≤ 4µg/ml. The MIC of the antibiotic from 20µg/ml and above agreed with earlier report of Salimna and Brown, 2005 in which reported the MIC of this antibiotic to be ≥20µg/ml. The MICs of tetracycline was from 15.63-31.25µg/ml lower to ≥32µg/ml as reported by Eze *et al*., (2013). The minimum concentration of chloramphenicol (C) that inhibited *S.aureus* was 15.63µg/ml and 31.25µg/ml with MBC at 31.25µg/ml and 62.50µg/ml. This result is slightly lower to the earlier report of Kamel *et al*., (2013) that the MIC of chloramphenicol on *S.aureus* was 32µg/ml. CLSI, 2008 recommend that the MIC of chloramphenicol against *S.aureus* should be considered resistant at ≥32µg/ml. The MIC and MBC for erythromycin against *S.aureus* was 3.91, 7.81 µg/ml, 15.63 µg/ml and MBC 7.81 µg/ml, 15.63 µg/ml, 31.25 µg/ml respectively, unlike a report by Tang *et al*., (2002) who had an MIC of ≥8 µg/ml for all isolates of *S.aureus*. According to CLSI, 2008 an MIC of erythromycin of ≥8 µg/ml was considered resistant.

CHAPTER SIX

6.0 SUMMARY AND CONCLUSION

This study has been able to establish a high prevalence of *S.aureus* (17.80%) in clinical samples. It also established a higher prevalence of *S.aureus* from in-patients (20.00%) than out-patients (15.50%). The *S.aureus* was isolated more in males (63.38%) than in females (36.62%). The highest frequency (28.36%) of *S.aureus* was found in the age group (0-9 years). *S.aureus* develops resistance very quickly and successfully to different antimicrobials overtime. A total susceptibility of *S.aureus* to vancomycin (100.00%) and gentamicin (100.00%) was detected in this study while *S.aureus* was resistant to cefoxitin (33.80%), chloramphenicol (12.70%), linezolid (9.90%), erythromycin (28.20%), tetracycline (14.10%), and ampicillin (90.10%).

The society is presently characterised by inappropriate, unethical dispensing and indiscrimate use of antibiotics. Drugs are made available to the public in the country through means commonly known as over-the-counter availability of antimicrobial drugs which allow people practice self medication. This increases the rate at which antibiotics are losing the battle of treatment of infections. Antibiotics are also sometimes prescribed without determining bacterial sensitivity to antibiotics. Further more, physicians administer antimicrobial drugs to patients with cold and viral diseases and most patients frequently stop taking the medications when syndrome goes away but the infection is not completely gone. All these encourage the emergence of resistant strains. It can be concluded therefore, that there is a need to re-access policies on antimicrobials within and outside hospital environment. The control of multidrug resistance will provide a major challenge to both health care community and the public in general. The inability to isolate Vancomycin Resistant *Staphylococcus aureus* inthis studyis a plus to health care providers here since there is still hope to treat infections caused by multidrug resistant organisms with vancomycin which is regarded as the drug of last resort if others failed.

6.2 RECOMMENDATIONS

The following recommendations are essential in the containment of resistance toAntimicrobial agents:

1. There should be formulation and implementation of a national drug policy by the government to ensure rational use of antibiotics.
2. There should be an effective education and training of the public on the appropriateness and limitations of antibiotics to ensure that they are utilized wisely and also the need to adopt a high personal hygiene.
3. There should be a Proper prescription of antibiotics and the implementation of an agreed control infection policies.
4. The Laboratories should screen patients properly to give an early warning of the presence of resistant organism in order to allow the assessment of barrier and infection control techniques.
5. The use of antibiotics in livestock and animal feeds should be adequately controlled because this may aid transmission of resistant strains from animals or livestock to humans and further complicating the epidemiology of this organism.

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APPENDIX I

Questionnaire

DEPARTMENT OF MICROBIOLOGY,
FACULTY OF SCIENCE,

AHMADU BELLO UNIVERSITY, ZARIA.
QUESTIONNAIRE

Dear respondent,

This questionnaire is aimed at assessing the demographic and epidemiologic factors associated with the Screening for Vancomycin Resistant *Staphylococcus aureus* (VRSA) infection among patients attending hospital clinic samaru.

Kindly assist by ticking the appropriate answer as information provided here will be kept confidential and used solely for the advancement of knowledge.

SECTION A: BIODATA

1. Reference No: …………

2. Patient: 1. IN 2. OUT

3. Sample type: 1.URINE 2. WOUND 3.PUS

4. Age:1. 0-9( ) 2. 10-29( ) 3.30-39( ) 4.40 -49( ) 5.50-59 ( ) 6.60-69( ) 7. ≥70 ( )

5. Sex: 1. Male ( ) 2. Female ( )

6. Marital Status: 1. Single ( ) 2. Married ( )

7. Educational Background: 0. None ( ) 1. Primary ( ) 2.Secondary ( ) 3.Tertiary ( )

8. Occupation : 1.Civil servant 2. Student 3. Farmer 4. Trader 5. Housewife

SECTION B:

9. Have you been previously diagnosed of any urinary tract infection before? 1.Yes( ) 2.No ( )

10. Have you been placed on Urinary Cathether before? 1.Yes ( ) 2. No ( )

11. Have you undergone recent hospitalization? 1. Yes ( ) 2. No ( )

12. If yes,what was the duration of your stay? 1. 1 day 2. 3 days 3. 5 adys

13.Were you treated or injected with vancomycin or any other antibiotics? 1.Yes( ) 2.No ( )

14. If yes, please specify the other range of antibiotics.

15 Have you undergone surgery before?1. Yes ( ) 2. No ( )

16. If yes, for how long ?(Please specify)

17. Have you undergone dialysis before or suffered a kidney ailment? 1. Yes 2. No

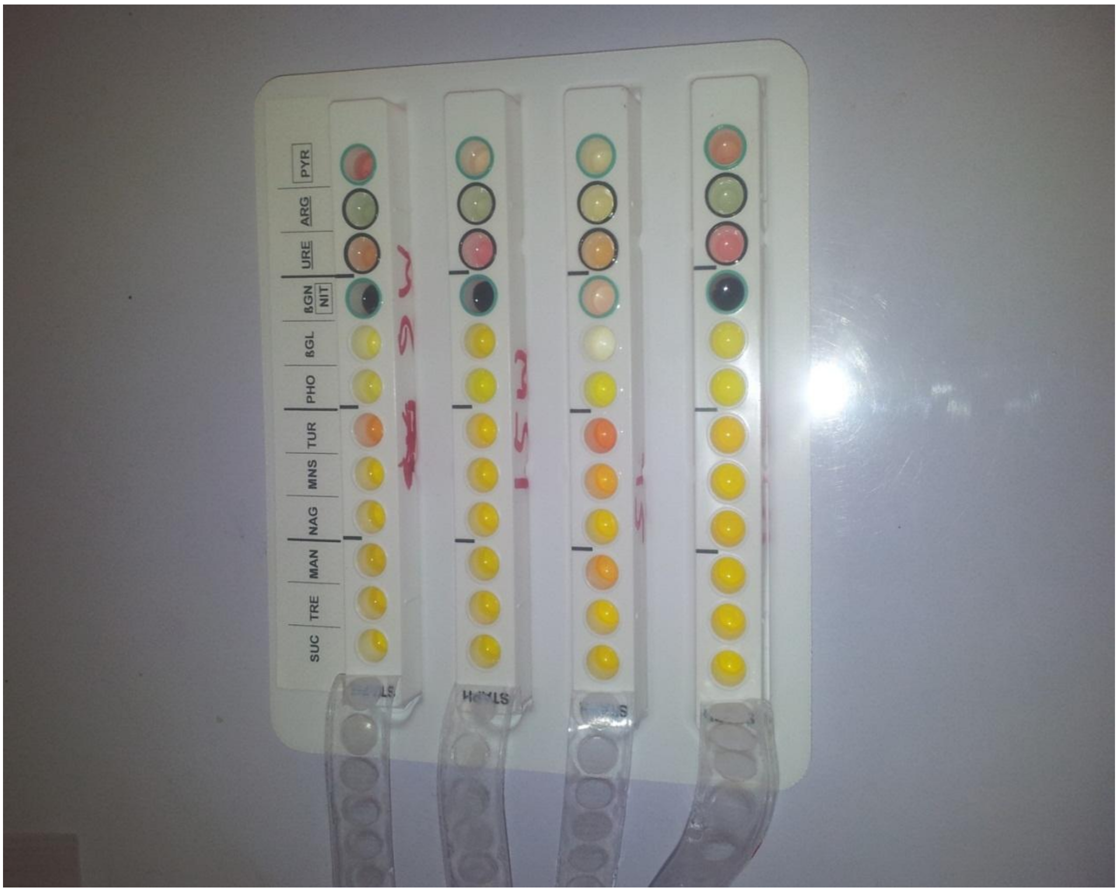
18.If yes, for how long?(Please specify) ………………..

19.Have you been diagnosed of diabetes before?1. Yes ( )2. No ( )

20.Have you had any previous Methicillin resistant *Staphylococcus aureus* infection before? 1.Yes ( ), 2.No ( )

Thank You for your co-operation.

Appendix II: Pictures of a positive test reaction to confirm *S.aureus* using microgen test kit



Suc=Sucrose, Tre=Trehalose, Man=Mannitol, NAG=N-Acetyl glucosamine, MNS=Mannose,TUR=Turanose, PHO=Alkaline phosphate, BGL=Glucosidase , BGN=Glucorunidase, NIT=Nitrate, URE=Urease, ARG= Arginine, PYR=Pyrrolidonyl arylammidase.


STAPH
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Appendix III: Identification profile of *S. aureus* using Microgen STAPH-ID kit

Substrate	LAT	CPG	Nitrate	Sucrose	Trehalose	Mannitol	N-acetyl Glucoseamine	Manose	Turanose	Alkaline Phosphatase	Glucosidase	Glucuronidase	Urease	Argenine	PYR
Results	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Reaction Index	4	2	1	4	2	1	4	2	1	4	2	1	4	2	0
Sum of positive reaction	7			7			7			7			6		
Profile No.	77776														

PYR = L-Pyrrolidonyl-α-naphthylamide CPG = Colony Pigmentation, LAT = Latex Agglutination Test(Coagulase Test).

Appendix IV: Preparation of 0.5 Macfarland's standard (turbidity standard)

1. A solution of 1% v/v of sulphuric acid was prepared by adding 1 ml of concentrated sulphuric acid to 99 ml of water and was mixed well.

2. A solution of 1% w/v barium chloride was prepared by dissolving 0.5g of dehydrated barium chloride ($BaCl_2.2H_20$) in 50ml of distilled water.

3. 0.6 ml of the barium chloride solution was added to 99.4 ml of the sulphuric acid solution and was mixed well.

4. A small volume of the turbid solution was transferred to a capped tube (Cheesbrough, 2004).

Appendix V: The Table showing the interpretation of zone sizes by CLSI, 2006

Antimicrobial agent	Concentration	Diameter of zone of inhibition (mm)		
		Susceptible	Intermediate	Resistant
Ampicillin	10 µg	≥29	–	≤28
Erythromycin	15 µg	≥23	14 – 22	≤13
Gentamicin	10 µg	≥15	13 – 14	≤12
Cefoxitin	30 µg	≥22	–	≤21
Vancomycin	30 µg	≥15	–	–
Chloramphenicol	30 µg	≥18	13–17	≤12
Linezolid	30 µg	≥21	–	–
Tetracycline	30 µg	≥19	15-18	≤14

Source: CLSI, 2006

Appendix VI :*S.aureus* Isolates and zones of inhibition in millimetre(mm)

Sample no	Patient type	AMP (mm)	CN (mm)	TE (mm)	FOX (mm)	VA (mm)	LZD (mm)	E (mm)	C (mm)
1	In	16 R	23 S	25 S	21 S	18 S	25 S	11 R	27 S
3	In	12 R	21 S	22 S	16 R	16 S	28 S	11 R	27 S
9	In	0 R	16 S	13 R	10 R	15 S	22 S	13 R	27 S
12	out	29 S	25 S	28 S	23 S	18 S	20 R	28 S	29 S
14	In	12 R	21 S	20 S	21 S	15 S	25 S	11 R	22 S
15	out	15 R	21 S	19 S	22 S	17 S	25 S	10 S	12 R
22	In	16 R	21 S	25 S	24 S	17 S	26 S	24 S	21 S
25	In	13 R	20 S	22 S	25 S	15 S	23 S	25 S	25 S
31	In	14 R	21 S	23 S	23 S	16 S	23 S	23 S	28 S
32	IN	14 R	21 S	24 S	22 S	18 S	23 S	25 S	27 S
34	In	13 R	22 S	21 S	22 S	15 S	23 S	23 S	11 R
52	In	10 R	23 S	20 S	23 S	16 S	25 S	25 S	28 S
55	IN	19 R	25 S	30 S	26 S	16 S	29 S	30 S	27 S
57	Out	29 S	19 S	21 S	20 S	16 S	9 R	25 S	22 S
59	In	6 R	21 S	26 S	22 S	17 S	27 S	25 S	25 S
66	IN	13 R	15 S	28 S	22 S	15 S	27 S	16 I	29 S
71	In	18 R	23 S	25 S	25 S	19 S	27 S	29 S	25 S
75	Out	16 R	22 S	13 R	19 R	15 S	25 S	13 R	24 S
76	Out	10 R	24 S	24 S	23 S	17 S	21 S	26 S	22 S
85	In	18 R	15 S	27 S	23 S	17 S	25 S	28 S	30 S
87	Out	17 R	25 S	30 S	21 R	19 S	30 S	12 R	12 R

92	In	11 R	19 S	20 S	22 S	15 S	21 S	12 R	12 R
97	IN	15 R	26 S	24 S	25 S	19 S	29 S	25 S	25 S
98	IN	14 R	21 S	22 S	23 S	18 S	27 S	25 S	29 S
102	In	17 R	22 S	25 S	23 S	19 S	23 S	23 S	29 S
103	Out	15 R	22 S	14 R	10 R	17 S	25 S	11 R	26 S
104	Out	15 R	22 S	20 S	24 S	17 S	24 S	24 S	21 S
111	IN	11 R	25 S	29 S	22 S	20 S	29 S	30 S	21 S
112	In	17 R	28 S	14 R	20 R	17 S	21 S	30 S	26 S
113	In	29 S	21 S	23 S	23 S	16 S	20 S	25 S	27 S
114	Out	0 R	21 S	23 S	20 R	15 S	23 S	12 R	22 S
115	In	0 R	15 S	14 R	10 R	16 S	21 S	24 S	19 S
116	Out	15 R	22 S	25 S	16 R	17 S	26 S	12 R	12 R
118	Out	19 R	23 S	27 S	21 R	18 S	25 S	26 S	12 R
212	In	13 R	21 S	22 S	25 S	15 S	22 S	25 S	24 S
278	Out	5 R	19 S	21 S	24 S	16 S	23 S	20 I	20 S
280	In	14 R	22 S	25 S	23 S	16 S	24 S	25 S	26 S
289	In	14 R	24 S	23 S	23 S	18 S	28 S	28 S	23 S
292	IN	10 R	18 S	21 S	20 R	15 S	21 S	12 R	20 S
293	Out	15 R	22 S	25 S	22 S	17 S	24 S	27 S	25 S
295	In	19 R	25 S	27 S	24 S	18 S	27 S	28 S	30 S
300	Out	17 R	20 S	21 S	22 S	15 S	21 S	25 S	25 S
312	Out	0 R	15 S	14 R	25 S	16 S	21 S	13 R	22 S
317	In	29 S	23 S	26 S	27 S	23 S	19 R	24 S	24 S
320	IN	0 R	17 S	14 R	16 R	16 S	22 S	11 R	2 S5

321	Out	18 R	25 S	25 S	21 R	16 S	24 S	29 S	12 R
327	Out	11 R	20 S	20 S	23 S	17 S	21 S	24 S	22 S
329	Out	7 R	21 S	25 S	24 S	15 S	22 S	28 S	27 S
333	In	9 R	24 S	24 S	16 R	15 S	25 S	27 S	12 R
334	In	30 S	24 S	25 S	23 S	16 S	20 R	22 I	25 S
335	In	12 R	19 S	20 S	23 S	17 S	24 S	24 S	26 S
337	Out	15 R	22 S	26 S	21 R	18 S	26 S	13 R	12 R
342	In	10 R	23 S	26 S	23 S	16 S	23 S	27 S	22 S
343	In	17 R	23 S	26 S	24 S	17 S	26 S	29 S	25 S
345	Out	23 R	22 S	21 S	21 S	20 S	29 S	30 S	27 S
346	In	16 R	23 S	24 S	24 S	16 S	22 S	26 S	28 S
348	Out	15 R	21 S	23 S	22 S	15 S	21 S	25 S	26 S
350	In	17 R	25 S	27 S	25 S	19 S	29 S	29 S	22 S
351	In	0 R	15 S	10 R	10 R	15 S	24 S	9 R	26 S
352	In	9 R	23 S	26 S	23 S	18 S	21 S	23 S	20 S
353	Out	15 R	21 S	20 S	24 S	16 S	21 S	15 I	24 S
357	Out	0 R	15 S	20 S	0 S	15 S	22 S	13 R	25 S
358	In	30 S	24 S	22 S	24 S	16 S	18 R	24 S	24 S
371	Out	7 R	20 S	14 R	6 R	15 S	22 S	24 S	24 S
373	Out	16 R	22 S	23 S	23 S	16 S	27 S	23 S	26 S
378	Out	29 S	25 S	23 S	22 S	15 S	20 R	24 S	22 S
390	Out	17 R	20 S	12 R	17 R	16 S	22 S	25 S	21 S
393	In	14 R	21 S	24 S	23 S	16 S	26 S	13 R	31 S
395	Out	13 R	20 S	22 S	20 R	15 S	22 S	11 R	22 S

396	In	6 R	19 S	15 I	22 S	16 S	22 S	25 S	22 S
400	Out	6 R	20 S	22 S	21 R	16 S	24 S	23 S	11 R

The antibacterial susceptibility profile of standard strain

Standard strain	**Amp**	**CN**	**Te**	**Fox**	**Va**	**Lzd**	**E**	**C**
S.aureus(ATCC25923)	29 S	25 S	19 S	23 S	18 S	26 S	24 S	22 S

R=Resistance S=susceptible I=Intermediate susceptible

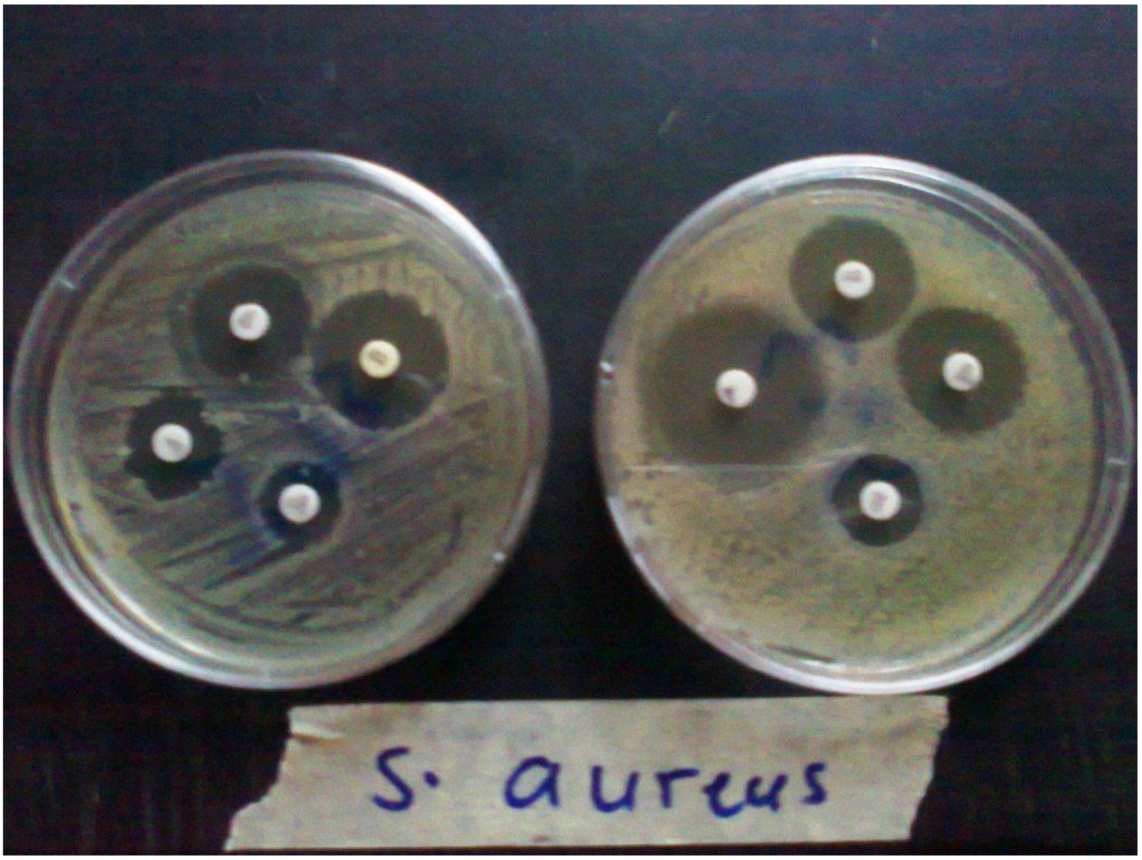
In=In patient Out=out patient Amp=Ampicillin

CN=Gentamicin Te=Tetracycline Fox=Cefoxitin

VA=Vancomycin Lzd= Linozelid E=Erythromycin

C=Chloramphenicol ATCC = American Type Culture Collection

Appendix VII: plates showing susceptibility test of *S.aureus*



APPENDIX VIII: Ethical approval

UNIVERSITY HEALTH SERVICES

MAIN CAMPUS CLINIC
AHMADU BELLO UNIVERSITY
ZARIA, NIGERIA.

Vice-Chancellor: **Professor Abdullahi Mustapha**; B.Sc. (Hons) Pharm. (A.B.U), Ph.D. (London), FPSN
Director: **Dr. M. A. Usman**; MBBS (ABU), FWACS.

Telegrams: UNIBELLO
Telephone: 069-550660
e-mail: uhs@abu.edu.ng

Our Ref: UHS/GEN/4 8th July, 2013

Your Ref:

The Head,
Department of Microbiology,
Faculty of Science,
Ahmadu Bello University,
Zaria.

Dear Sir,

RE: LETTER OF INTRODUCTION:
SALAU, ABUBAKAR OMEIZA (MSc/SCIE/00855/9-10)

After careful review of the appropriateness of the research protocols on the topic: Screening for Vancomycin Resistant Staphylococcus aureus Infection Among Patients attending Hospital Clinics in" Samaru and its justifications, approval was granted accordingly.

Thanks.

Yours faithfully,

Dr. M. A. Usman
Director.

JAMA'A HOSPITAL

ADDRESS:
No 90 Sarkin Pawa Road,
Samaru G.P.O. Box 1783
Zaria, Kaduna State

Our Ref: JHH/ISGP/VOL 1/13

Your Ref:

Date: 30/04/2013

The Head of department,

Department of Microbiology,

Faculty of science,

Ahmadu Bello University,

Zaria,

Dear Sir,

RE: LETTER OF INTRODUCTION

SALAU, ABUBAKAR OMEIZA

M.sc/SCIE/00855/9-10

We refer to yours dated 27/07/12 on the above in caption and write to confirm that the student has completed his research work in which the hospital supplied him with samples for his laboratory work.

Yours sincerely

G.O. Kolawole

For Medical Director

JAMA'A HOSPITAL

Appendix IX: Inform consent form

DEPARTMENT OF MICROBIOLOGY

SCHOOL OF POSTGRADUATE STUDIES

INFORMED CONSENT FORM (ICF)

Serial No.: …………. Hospital No.: ……………… Age: …………… Phone No.: ………..……………………….

This Informed Consent Form is basically for out -patient and in-patient, who attend University Health Services, Ahmadu Bello University Zaria, Jammaa Hospital, Rangwame Hospital and Savannah Hospital Samaru . We are inviting you to participate in this research work titled "Screening for Vancomycin resistant *Staphylococcus aureus* among patients attending Hospitals in Samaru, Zaria". It involves the collection of pus swab, wound swab and urine samples of participants. The procedure involve will be fully explained to all participants. The results obtained thereby may be used in any way to improve the understanding and management of the disease in our community. All participants may be given copies of their test results. Individual data will be treated with strict confidentiality. Participation is strictly voluntary and refusal to participate in the research study will not in any way affect your right and benefit in this clinic/hospital.

CERTIFICATE OF CONSENT

I ……………………………………………………….of …………………………………………….. do hereby consent to participate in this study. The full procedures of the test/study have been explained to me by the investigator. I understand that sample of my pus , urine, and wound will be taken for test. I therefore give this consent voluntarily without being subjected to any pressure.

Name of Participant …………………………………………………….…

Signature/right thumb print of participant ………………………………. Date …………………….

Statement by Witness:

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

Name of witness…………………………………………………………

Signature of witness……………………………………………….…. Date ……………….

Statement by the Researcher/Person Taking Consent

I confirm that sufficient information, including about risks and benefits, to make an informed decision have been fully explained to the participant. The participant was given an opportunity to ask questions about the study, and all the questions asked by participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Researcher ……………………………………………………….

Signature ………………………………………………………………...   Date. ………………