KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

PREVALENCE OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) AT THE BRONG AHAFO REGIONAL HOSPITAL (BARH), SUNYANI

BY:

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APRIL, 2016

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A THESIS SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE MASTER OF SCIENCE DEGREE IN BIOTECHNOLOGY

BY

ODAI SETH ANANG

APRIL, 2016

DECLARATION

I hereby declare that this thesis is as a result of my own research work for the award of MSc. (Biotechnology) and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the university, except where due acknowledgement has been made in the text.

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ABSTRACT

Methicillin resistant *Staphylococcus aureus* (MRSA) infections are associated with higher risk of mortality and greater costs to the health-care system, however there is paucity of data on its prevalence in Ghana, and especially in the Brong Ahafo region. This research was aimed at determining the prevalence of MRSA at the Brong Ahafo Regional Hospital (BARH) and to identify sites within the hospital which may be prone to MRSA infection or transmission. In all, three hundred and twenty-seven (327) participants were recruited for the study, including outpatients, visitors, hospital personnel and some inpatients. This comprised of one hundred and twenty-four (124) males and two hundred and three (203) females. The participant population was stratified into two main groups; Group 1 included patients and visitors from the main OPD and the various clinics, and Group 2 comprised inpatients, hospital staff, and visitors attending to patients at the Wards and other departments. Nasal swab samples were obtained and inoculated on mannitol salt agar and suspected *S. aureus* colonies were confirmed through morphological and biochemical tests. Cefoxitin disc diffusion test was carried out to identify the methicillin resistant strains. The Oxoid Penicillin binding protein (PBP2′) latex agglutination test kit was used to confirm the production of the *mec*A gene product, penicillin binding protein 2a (PBP2a). The study revealed a prevalence of 8.0% at the hospital and 47.3% of the *S. aureus* isolates were resistant while 52.7% were susceptible. A comparison between the genders did not show any significant difference in prevalence ($p = 0.6273$). No significance difference was found in the prevalence between the adult and children age groups ($p = 0.3712$). It was however revealed that sample sites including the main outpatient department (OPD) and the various clinics recorded a significantly higher MRSA prevalence of 10.5% compared to the 1.1% prevalence recorded at the wards and other departments which included laboratory, pharmacy, radiology, administration, and other areas ($p = 0.0052$). It was therefore necessary to establish better equipped hand washing areas at vantage areas around the hospital as prescribed by the WHO. It is also important that appropriate hygienic practices are instilled in all hospital personnel, patients and visitors to the hospital through continuous education in order to forestall any future outbreak.

i

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TABLE OF CONTENT

DECLARATION
.....I

ABSTRACT II

ACKNOWLEDGEMENTS III

LIST OF TABLES VII

LIST OF FIGURES VIII

LIST OF SOME ABBREVITIONS USED IX

CHAPTER ONE .....
1

1.0 INTRODUCTION .....
1

1.1 OBJECTIVES 4

1.1.1 Specific Objectives 4

1.2 JUSTIFICATION .....

4 CHAPTER TWO

..... 6

2.0 LITERATURE REVIEW 6

2.1 CHARACTERISTICS OF *S. AUREUS* 6

2.2 PATHOGENESIS AND VIRULENCE OF *S. AUREUS* INFECTIONS ... 8

2.3 MECHANISMS OF INFECTION BY *S. AUREUS* 11

2.3.1 Invasive mechanisms 11
2.3.2 S. aureus toxins 13
2.3.2.1 Pyrogenic Toxin Superantigens (PTSAgs) 13
2.3.2.2 Exfoliatin or epidermolytic toxins (ETs) 14

2.4 DISEASES CAUSED BY *S. AUREUS* 14

2.5 *S. AUREUS* EPIDEMIOLOGY 19

2.5.1 *S. AUREUS* EPIDEMIOLOGY – THE AFRICAN PERSPECTIVE 22

2.5.1.1 MRSA IN GHANA 23

2.5.2 ROLE OF THE ANTERIOR NARES AND OTHER RISK FACTORS 25

2.6 METHICILLIN-RESISTANT *S. AUREUS* (MRSA) 26

2.6.1 FUNCTION OF B-LACTAM ANTIBIOTICS AND RESISTANT MECHANISMS OF MRSA 27

2.6.2 MECHANISM OF RESISTANCE TO B-LACTAM ANTIBIOTICS BY *S. AUREUS* 28

2.6.3 MECHANISM OF RESISTANCE TO SEMI-SYNTHETIC B-LACTAMS (E.G. METHICILLIN, NAFCILLIN AND OXACILLIN) 29

2.6.4 OTHER ANTIMICROBIALS MRSA IS RESISTANT TO 30

2.7 DETECTION OF MRSA COLONIZATION 30

2.8 MANAGEMENT AND TREATMENT OF MSSA AND MRSA INFECTIONS 32

2.9 INFECTION PREVENTION AND CONTROL OF *S. AUREUS* 33

2.10 PUBLIC HEALTH IMPLICATIONS AND ECONOMIC IMPACT OF MRSA INFECTIONS 35

CHAPTER THREE 37

3.0 SUBJECT AND METHODS 37

3.1 STUDY DESIGN AND SITE 37

3.2 SAMPLE COLLECTION 37

3.3 ISOLATION OF *S. AUREUS* 38

3.4 ANTIMICROBIAL SUSCEPTIBILITY TESTING 39

3.4.1 READING AND INTERPRETATION OF PLATES 40

3.5 TEST FOR THE PRESENCE OF THE *MEC*A GENE 41

3.5.1 Penicillin-Binding Protein (PBP2a) Latex Agglutination test 41

3.6 STATISTICAL ANALYSIS 43

4.0 RESULTS 44

4.1 GENERAL DEMOGRAPHICS OF *S. AUREUS* PREVALENCE 44

4.2 CEFOXITIN RESISTANCE IN *S. AUREUS* ISOLATES 46

4.3 MSSA AND MRSA PREVALENCE 46

CHAPTER FIVE 50

5.0 DISCUSSION 50

CHAPTER SIX 56

6.1 CONCLUSION 56

6.2 RECOMMENDATIONS 56

REFERENCES 57

LIST OF TABLES

Table	**Title**	**Page**
2. 1	Some clinical manifestations of *S. aureus* infection	16
4.1	General demographics of *S. aureus* prevalence	45
4.2	Cefoxitin disc diffusion zone diameter interpretive criteria.	46

4.3	MSSA prevalence at BARH	48
4.4	MRSA prevalence at the BARH	49

LIST OF FIGURES

Figure	**Title**	**Page**
2.1	Gram – positive *S. aureus* in clusters and short chains	7
2.2	*S. aureus* colonies on mannitol salt agar	7
2.3	Pathogenic and virulence factors of *S. aureus*	8
2.4	Pathogenesis of *S. aureus* by tissue invasion	12
2.5	Clinical manifestation of staphylococcal scalded skin syndrome	17
2.6	Sites of infection and diseases caused by *S. aureus*	18
2.7	Worldwide MRSA prevalence	21
2.8	Prevalence of MRSA in Africa	22
2.9	Structures of D-Alanyl-D-Alanine and a β-lactam antibiotic	28
3.1	Mannitol salt agar plate showing specimen with suspected *S. aureus* colonies	39
3.2	Effect of cefoxitin on S. aureus growth	41
3.3	PBP2a test card showing positive agglutination reactions for some isolated MRSA samples.	42
4.1	Distribution of *S. aureus* strains	47

LIST OF SOME ABBREVIATIONS

BARH – Brong Ahafo Regional Hospital

CA-MRSA – Community Acquired Methicillin Resistant *Staphylococcus aureus*

HA-MRSA – Hospital Acquired Methicillin Resistant Staphylococcus aureus

KATH – Komfo Anokye Teaching Hospital

KBTH – Korle Bu Teaching Hospital

MRSA – Methicillin Resistant *Staphylococcus aureus*

MSSA – Methicillin Susceptible *Staphylococcus aureus*

OPD – Out Patient Department

PBP – Penicillin Binding Protein

TMIAC-CDPH - The MRSA Interagency Advisory Committee and Connecticut Department of Public Health.

WHO – World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Staphylococcus is the main genus of the *Staphylococcacceae* family in the order of *Bacillales*. *Staphylococcus aureus (S. aureus)* is a non-spore-forming, nonmotile, facultative anaerobic Gram-positive coccal-shaped bacterium, which is

0.5 to 1μm in diameter. It is both catalase and coagulase positive and appears as bunch of grapes when viewed under a microscope. When grown on mannitol salt agar and blood agar, it produces round, golden-yellow coloured colonies (Ryan and Ray, 2004). When *S. aureus* reproduces asexually by binary fission, the two daughter cells may not fully separate and may remain attached to one another, which explain why the cells are often observed in clusters. The bacterium is the most pathogenic of the staphylococci species; it has a generation time of 20-30 minutes and can grow in conditions of high salt concentration and at temperatures between 10 and 46^{o}C.

A study by Sotto *et al*. (2008) highlighted the virulence potential of the bacterium, and is considered one of the main cause of community acquired and nosocomial infections, leading to high morbidity and mortality (Bhateja *et al.*, 2005). The organism forms part of the normal human microbial flora; it can be found on skin surfaces, intestines, upper respiratory tract and vagina. It can become pathogenic when temperature and pH conditions become favourable and nutrient is available to support overgrowth (Makgotlho, 2009). The pathogen is usually carried in the nasopharynx, on the skin, clothing and sometimes in the vagina, rectum or perineal areas. They can easily contaminate

any other parts of the human body from these sites through direct contact with the hands or by aerosol transfer (The Centre for Food Security & Public Health, 2011).

Methicillin-resistant *S. aureus* (MRSA) are the strains of the bacterium which have acquired the *mec*A gene that confers on them the ability to resist the effects of methicillin and several other beta-lactam based antibiotics. This resistant strain was first reported in 1961, after methicillin was approved for the treatment of penicillin-resistant staphylococci in humans (Chambers and DeLeo, 2009). MRSA was not that prevalent even in hospitals until the 1990s, when there was a surge in MRSA prevalence in hospitals and has since remained endemic (Johnson *et al*., 2001). Although MRSA causes similar infections as the methicillin-susceptible *S. aureus* (MSSA), its resistance to most common antibiotics makes its treatment very challenging, and its management can result in considerable cost to a health facility (Guleri *et al*., 2011). Many studies have shown that MRSA-related infections are associated with higher risk of mortality and greater costs to the health-care system than infections caused by MSSA (Clements *et al.*, 2008). Since MRSA was first discovered, its infection was only found in the hospitals through invasive procedures like urinary catheters, central venous lines, recent antibiotic use, and through contact with health care workers. It therefore became known as hospital-acquired MRSA (HA-MRSA). Recently, however MRSA infections have spread into the community and has become widespread (Bustamante, 2011). This strain, also referred to as community-acquired MRSA (CAMRSA), has its unique risk factors which include: crowding or low-hygiene living conditions, close contact with athletic equipment, and participation in contact

sports, and immunosuppression. In spite of their unique different genetic traits from the HA-MRSA, CA-MRSA has become more prevalent in the hospitals as well, thus blurring the line between these two distinct causes of MRSA infections. Thus the monitoring and eradication of MRSA from patients, health care workers and their family members is essential to help prevent the continuous spread between hospitals and the community (Abu-Rabie, 2010).

MRSA prevalence, ranging from 23.3% to 73%, has been reported across the globe, and an extensive research showed that MRSA is even more prevalent in most developing countries (Bustamante, 2011). In 1996, a study showed that Malaysia and South Africa had some of the highest MRSA prevalence. Odonkor and Addo (2010) reported MRSA prevalence range of 21.6% to 31.6% from routine specimen collected for microbiology analysis in some hospitals in Accra. Recently the bacteria were implicated in an outbreak at the children's ward of the Korle Bu Teaching Hospital (KBTH) which led to the death of three children and subsequent closure of the ward for decontamination (Sackey, 2012). Bustamante (2011) noted how inadequate resources and education has led to the increasing spread of MRSA around the world, and also stated that the factors that have led to the spread of MRSA throughout the world are distinct for different regions of the world. Due to its recent outbreaks and reported deaths, MRSA has become an increasing public health concern, particularly among individuals without known risk factors (Herold *et al.*, 1998; Hunt *et al.*, 1999). It has become a major cause of systemic infection in the community and hospitals, causing deaths among individuals with no known risk factors and

presents a therapeutic challenge for doctors because of the bacteria's complex mechanisms of antibiotic resistance and epidemiology (Kil *et al.,* 2008).

1.1 OBJECTIVES

The research aimed to evaluate the prevalence of MRSA at the BARH from swabs of the anterior nares obtained from some patients, personnel and other individuals at the BARH, selected at random to evaluate transient carriage of MRSA.

1.1.1 Specific Objectives

- ✓ to ascertain the prevalence of MRSA at the BARH and
- ✓ to identify areas at the hospital which may be more prone to MRSA infection.

1.2 Justification

The Brong – Ahafo Regional Hospital (BARH) was established in 1927 by the then colonial masters to cater for the health needs of the people of Western Ashanti. The hospital was further expanded and finally moved its services to the present location in 2003 (Regional Hospital-Sunyani, 2013). As earlier noted the MRSA menace has become a matter of concern for health facilities across the globe. The BARH is often faced with periods of overcrowding as seen in most hospitals around the country. Overcrowding has also been determined to be a key factor in the transmission of MRSA (Clements *et al.*, 2008). To prevent the acquisition of such nosocomial infections by its clientele and the transmission of such bacteria, it is necessary to screen to evaluate the prevalence of MRSA at the BARH to help forestall any future outbreaks as was

witnessed at KBTH in Accra. Detecting methicillin resistance in *S. aureus* has important implications for therapy and management of infected patients (Reischl *et al.,* 2000), health care workers (Rongpharpi *et al.,* 2013) and the community at large. According to the MRSA interagency advisory committee in conjunction with the Connecticut department of public health (TMIACCDPH, 1993), heath care facilities should monitor the incidence of hospital transmitted MRSA in their facility.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 CHARACTERISTICS OF *S. AUREUS*

Sir Alexander Ogston was first to identify Staphylococcus in pus from a surgical abscess in 1880 at Aberdeen, Scotland. Staphylococcus was then appended to *Staphylococcus aureus* by Rosenbach in 1884 (Ogston, 1984). *S. aureus* is a Gram-positive coccal-shaped bacterium, which are often arranged in clusters like a bunch of grapes (Figure 2.1). They are non-motile (nonflagellated), non-spore forming and non-capsulated (some rare strains are capsulated). They are aerobes and facultative anaerobes which require 37^{o}C as optimum temperature for growth and an optimum pH of 7.5. They can grow well on nutrient agar to form golden yellow pigment or sometimes white (nonpigmented) colonies. On blood agar, they grow surrounded by a zone of βhaemolysis (complete), while on mannitol salt agar, *S. aureus* ferments mannitol giving rise to colonies surrounded by yellow zones (Figure 2.2). The bacterium has long been identified as an important human pathogen and causes infections frequently in hospitalized patients with severe consequences, even when they are being given antibiotics (Rongpharpi *et al.,* 2013). Other phenotypic characteristics used to identify *S. aureus* includes their ability to ferment glucose to produce lactic acid, fermentation of mannitol (which differentiates it from S. *epidermidis*) and the production of catalase and coagulase.

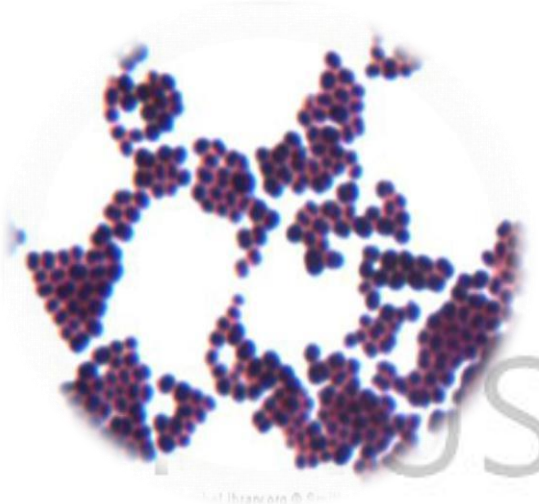

Figure 2.1: Gram – positive *S. aureus* in clusters and short chains (Makgotlho, 2009)

Figure 2.2: *S. aureus* colonies surrounded by yellow zones on mannitol salt agar

2.2 PATHOGENESIS AND VIRULENCE OF *S. AUREUS* INFECTIONS

S. aureus have an extensive armamentarium of virulence factors which includes both secreted and structural products that play various roles in the pathogenesis of infection (Figure 2.3). It has been observed that a virulence factor can play several roles in pathogenesis and also multiple virulence factors may perform similar functions (Gordon and Lowy, 2008).

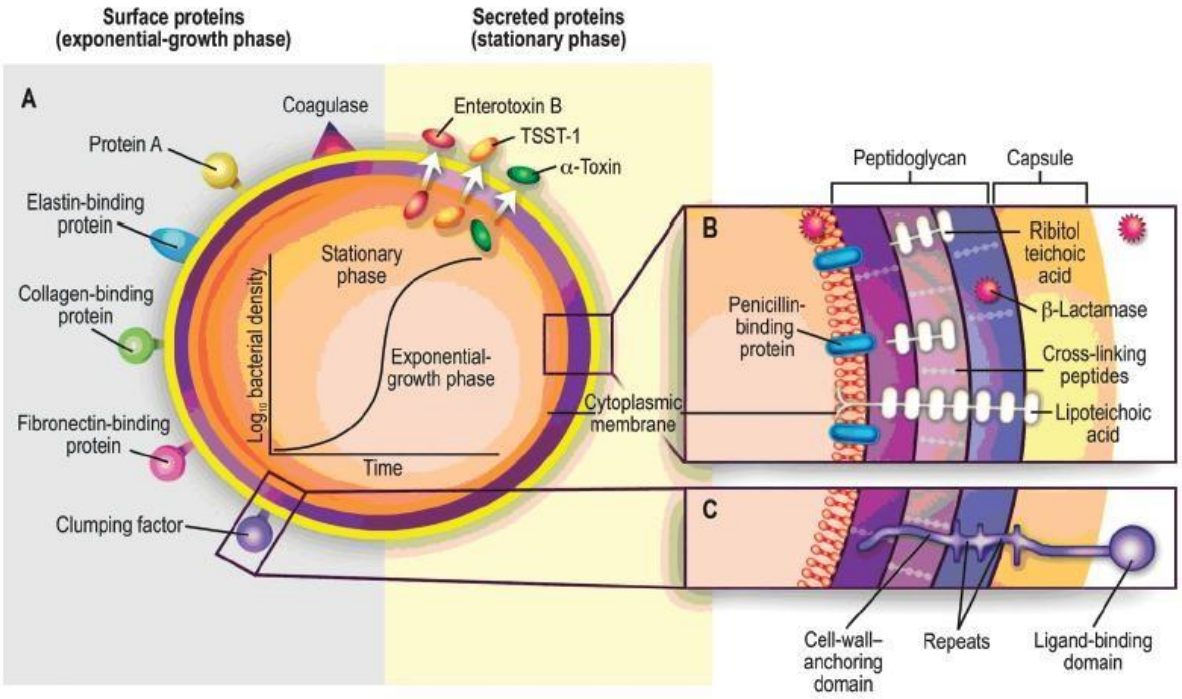

Figure 2.3: Pathogenic and virulence factors of *S. aureus*; Surface and secreted proteins (A). Cross-sections of the cell envelope (B and C) (Gordon and Lowy, 2008).

The virulence factors include capsules which inhibit phagocytosis, coagulase which may impede movement of leukocytes into an infected area by producing clots in the surrounding capillaries. Exfoliatin also separates layers of epidermis to cause scalded skin syndrome. There is also hyaluronidase which breaks down the hyaluronic acid component of tissue to promote the extension of an

infection, leukocidin which kills white blood cells by producing holes in their cytoplasmic membrane, lipase which breaks down fats by hydrolysing the bond between glycerol and fatty acids. Other factors are proteases which degrade collagen and other tissue proteins, protein A which binds to Fc portion of antibody and coats the bacteria with host's immunoglobulin to inhibit phagocytosis and toxic shock syndrome toxin which causes rash, diarrhoea, and shock (Nester *et al.*, 2009).

To establish an infection, the bacterium makes use of a number of surface proteins, called "microbial surface components recognizing adhesive matrix molecules" (MSCRAMMs), which helps the bacterium to adhere to host tissues. MSCRAMMs bind to molecules such as fibronectin, collagen and fibrinogen. Different MSCRAMMs may bind to the same host-tissue component. The proteins appear to function in initiating bone and joint infections, endovascular infections, and prosthetic-device infections (Gordon and Lowy, 2008). Different strains of the bacterium may have different collection of MSCRAMMs which can make them cause different kinds of infections. After adhering to the prosthetic material or host tissue, *S. aureus* can grow and persist in various ways. It can form biofilms on its host's or prosthetic surface, which enables it to persist by evading the host defences and antimicrobials. Biofilm formation particularly makes prosthetic device infections quite difficult to eradicate if the device is not removed. The pathogen can also form small-colony variants (SCVs) which *in vitro* can "hide" in the host's cells without causing significant host-cell damage and are thus relatively protected from antibiotics and host's defences. This may contribute to its persistence which results in recurrent infection when it later reverts to the more

virulent phenotype. In summary, the virulence factors are grouped according to their role in virulence (Gordon and Lowy, 2008). These include;

- Those involved in attachment; which includes the MSCRAMMS (e.g. fibronectin-binding proteins, collagen, clumping factors, and bone sialoprotein-binding proteins) which are associated with osteomyelitis, endocarditis, prosthetic-device and catheter infections, and septic arthritis (Gordon and Lowy, 2008).
- Those involved in persistence which includes accumulation of biofilm which are also associated with relapsing infections, endocarditis, osteomyelitis, septic arthritis, etc (Gordon and Lowy, 2008).
- Those involved in destroying the host's defences; which includes leukocidins (Panton –Valentine leukocidin [PVL]), protein A, capsular polysaccharides, etc. These are associated with necrotizing pneumonia and invasive skin infections (CA-MRSA strains that cause these diseases are often associated with PVL) and abscesses (which are associated with capsular polysaccharides) (Gordon and Lowy, 2008).
- Those involved in invading or penetrating tissues which also include virulence factors such as hyaluronatelyase, proteases, lipases, nucleases, and metalloproteases which causes tissue destruction and metastatic infections (Gordon and Lowy, 2008).
- Those involved in sepsis or toxin-mediated diseases which includes toxic shock syndrome toxin-1(TSST-1), peptidoglycan, enterotoxins, exfoliative toxins, α-toxin, and lipoteichoic acid which are associated with toxic shock syndrome, food poisoning, scalded skin syndrome, sepsis and bullous impetigo (Gordon and Lowy, 2008).

- There are also those which do not have clearly defined role in virulence and these include bacteriocin, coagulase, etc. (Gordon and Lowy, 2008).

2.3 MECHANISMS OF INFECTION BY *S. AUREUS*

2.3.1 Invasive mechanisms

An infection starts when there is a breach of the skin or mucosal barrier allowing the bacteria access to near-by tissues or the bloodstream. The infection may be contained or can continue to spread depending on a complex interaction between the bacterium's virulence determinants and the host's defence mechanisms. How the bacteria colonizes the nares (nostrils) which is the main reservoir for staphylococci, is not completely understood, although the host's mucin appear to be the key host surface that is colonized in a process involving interactions between mucin carbohydrate and staphylococcal protein (Lowy, 1998). The risk of getting infected by *S. aureus* is increased by the presence of foreign material. The host's immune response with regards to phagocytic function is seriously impaired in the presence of a foreign material. Devices like intravenous catheters gets rapidly coated with serum constituents, (eg. fibrinogen or fibronectin), which enables the bacteria to adhere to them through MSCRAMM-mediated mechanisms and also produces glycocalices which further aids colonization. Intravenous catheters have been identified as risk factors very frequently in the pathogenesis of nosocomial endocarditis (Lowy, 1998).

In nosocomial endocarditis often caused by intravenous catheters, the catheter inflicts a trauma on the surface of the cardiac valves creating a non-bacterial

blood clot on the cardiac valve which helps bacteria to adhere to subsequently. Circulating staphylococci (Figure 2.4) then bind to the sites where endovascular damage has occurred and platelet – fibrin thrombi (PFT) have formed. The bacteria may also attach itself to endothelial cells directly by means of adhesin–receptor interactions or through bridging ligands that include serum components like fibrinogen (Lowy, 1998).

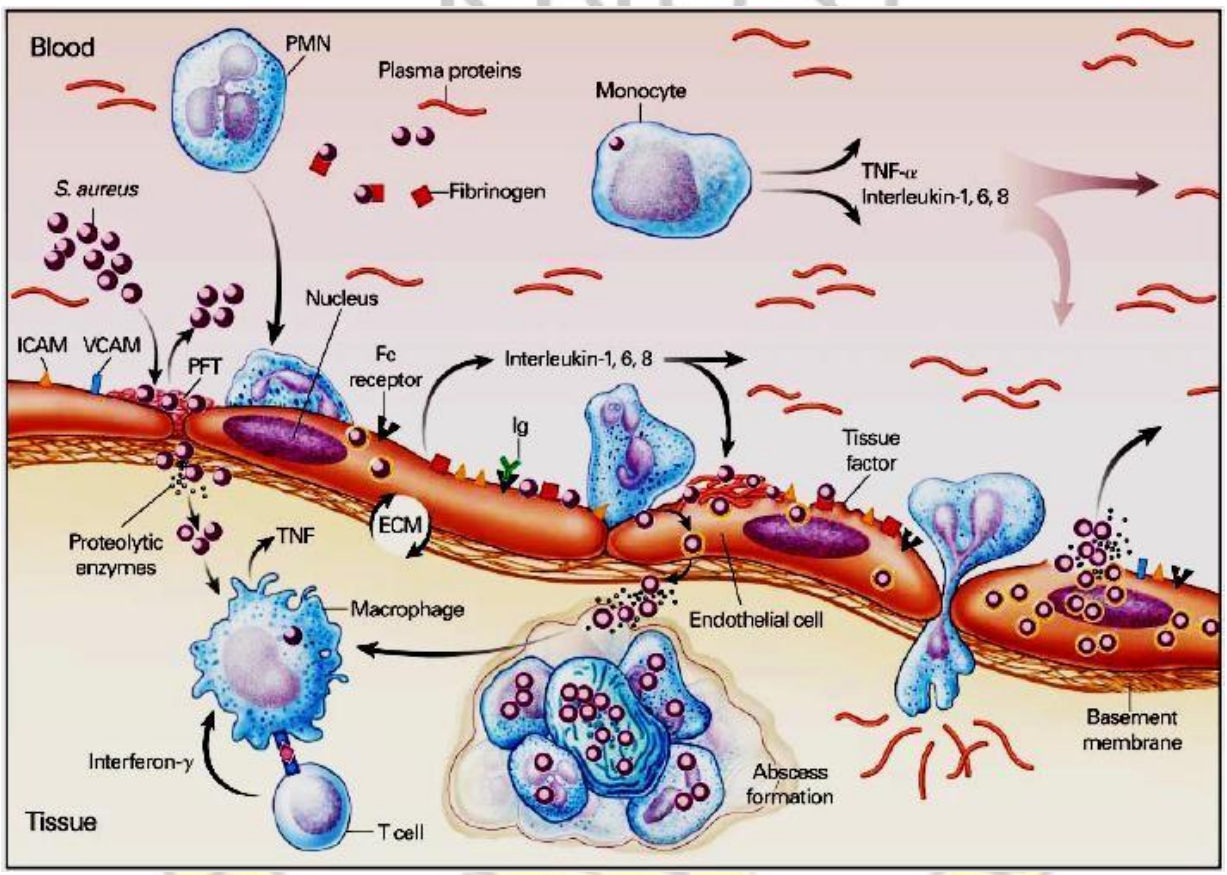

Figure 2.4: Pathogenesis of *S. aureus* by tissue invasion. TNF-Tumour necrosis factor, PFT-Platelet–fibrin thrombi, PMN-Polymorphonuclear leukocyte, ICAM-Intercellular adhesion molecule, VCAM-Vascular cell adhesion molecule, ECM-Extracellular matrix (Lowy, 1998).

Change of the endothelium due to alterations in the micro-environment can signal changes in cell's susceptibility to the infection. *S. aureus* produces proteolytic enzymes that facilitate its spread to adjoining tissues and its

subsequent release into the bloodstream after their phagocytosis by endothelial cells. Infected endothelial cells expresses a tissue factor which facilitates the deposition of fibrin and lead to the formation of vegetation. Once in the nearby sub-epithelial tissues, the bacterium elicits an inflammatory response resulting in the formation of abscess (Figure 2.4) (Lowy, 1998). The steps outlined are key to establishing the spread of the infection, and the pathogenesis of endocarditis when cardiac endothelium is involved (Lowy, 1998).

2.3.2 *S. aureus* toxins

Among their multiple virulence factors, *S. aureus* secretes several toxins and other biologically active extracellular enzymes which include α-toxin, exfoliatin, and pyrogenic toxin superantigens (PTSAgs) (Ryan and Ray, 2004).

2.3.2.1 Pyrogenic Toxin Superantigens (PTSAgs)

Superantigens can bind directly to class II major histocompatibility complexes (MHC II) of antigen-presenting cells outside the normal antigen-binding groove which can activate up to one in five T cells (Lowy, 1998). Superantigens can thus lead to extensive proliferation of T cells and release of cytokines in large amounts, which causes the symptoms associated with toxic shock; a severe disease characterized by the rapid onset of high fever, shock, capillary leak, and multi-organ dysfunction. PTSAgs also cause enhanced susceptibility to the dangerous effects of endotoxin (Ryan and Ray, 2004; Lowy, 1998).

When formed, staphylococcal enterotoxins (SE) are quite stable, and they retain activity even after being boiled or getting exposed to jejunal and gastric enzymes. There are different types including SE A, B, C, D, E, F, G etc. In the upper gastrointestinal tract, they may act directly on neural receptors there to

stimulate vomiting centre within the brain. This can result in diarrhoea and vomiting when ingested as associated with staphylococcal food poisoning. Enterotoxins B and C cause 50% of non-menstrual cases of toxic shock syndrome (TSS) (Lowy, 1998; Ryan and Ray, 2004).

Toxic shock syndrome toxin - 1 (TSST - 1) is expressed systemically and is the cause of toxic shock syndrome (TSS). TSST-1 is somehow related to enterotoxins, but does not induce vomiting (Ryan and Ray, 2004).

*2.3.2.*2 Exfoliatin or epidermolytic toxins (ETs)

ETs are directly responsible for the symptoms and clinical manifestation of staphylococcal scalded skin syndrome (SSSS) (also called Ritter's disease). ETs are highly specific serine proteases which recognize and split desmosomal cadherins only in the superficial layers of the skin (Bukowski *et al.*, 2010). This leads to the separation between the living layers and the superficial dead layers within the epidermis (Ryan and Ray, 2004).

2.4 DISEASES CAUSED BY *S. AUREUS*

The bacterium is been identified as the most common cause of wound infections after a surgery. Some strains can also produce toxins that can cause a variety of specific symptoms, which include TSS and food poisoning (WHO, 2014). An intact skin and the mucous membrane are excellent barriers against any form local tissue invasion, however if either of these is breached through trauma or surgery, *S. aureus* can enter the underlying tissue to create its characteristic local abscess lesion or cause septicaemia if it can reach the lymphatic channels

or bloodstream. Ingesting enterotoxins produced by the bacteria in contaminated food can result in food poisoning (Harris *et al.,* 2002). The bacteria have been implicated in several diseases including acute food poisoning, impetigo, folliculitis, Staphylococcal scalded skin syndrome (SSSS), cellulitis, etc. as the causative agent. It has also been implicated in systemic infections like infective endocarditis, epiglottitis, osteomyelitis, sinus infections, etc. In England it was estimated that over 4% of patients admitted into one of 96 hospitals between 1997 and 1999 for surgery acquired a nosocomial infection. A nosocomial infection is an infection acquired from a health facility in which there was no evidence the infection was present or incubating prior to a patient being hospitalized (Harris *et al.,* 2002). A study by Harris *et al* (2002) revealed that the hospital environment may also support the spread of MRSA. They stated that 81% of nosocomial infections were caused by *S. aureus*, and 61% of these isolates were methicillin resistant. Sepsis occurs when the bacteria infects the blood or other tissues of the body. Sepsis can occur in the bones, bloodstream, bowels, the liver or gall bladder, the kidneys, the lining of the brain (meningitis), the lungs (as in bacterial pneumonia), and the skin (cellulitis). Hospital patients usually get infected through surgical wounds and drains, intravenous lines, and through bedsores (Vyas, 2014). Table 2.1 gives a brief summary of some *S. aureus* infection.

Table 2. 1: Summary of some *S. aureus* infections (Zurita *et al.*, 2010)

Source of infection	Disease
Skin and soft tissue	Impetigo, boils, carbuncles, abscesses, cellulitis, fasciitis,
Foreign body-associated	pyomyositis, surgical and Intravascular catheter, urinary cathetertraumatic wound infections
Intravascular	Bacteraemia, sepsis, septic thrombophlebitis, infective carditis
Bone and joints	Septic osteomyelitis, septic arthritis
Respiratory	Pneumonia, empyema, sinusitis, otitis media
Other invasive infections	Meningitis, surgical space infection
Toxin-mediated disease	Staphylococcal toxic shock, food poisoning, staphylococcal scalded skin syndrome, bullous impetigo, necrotizing pneumonia, necrotizing osteomyelitis

Bacteraemia or septicaemia is the invasion of the bloodstream by *S. aureus* and its toxins circulating in the bloodstream (Elixhauser *et al.*, 2011). Compared with some other pathogens *S. aureus* bacteraemia is associated with higher morbidity and mortality and is also associated with some significant economic burden on healthcare facilities due to prolonged stay at the hospital (Naber, 2009).

Impetigo is a contagious, superficial infection of the skin which is spread by direct contact with lesions or with nasal carriers of *S. aureus*. It is commonly found among pre-school children and some adults.

SSSS is found most commonly in infants and children under the age of five years. In SSSS the skin becomes damaged and sheds. The bacteria produce

exfoliatin which causes the skin to damage by forming blisters as if the skin were scalded (Figure 2.5). SSSS symptoms include redness of the skin (erythema), large areas of skin peel or fall away (exfoliation), blisters, fever, and pain in the skin (Vyas, 2013).

Folliculitis occurs when hair follicles get damaged via friction with clothing, blocking of the follicles, or through shaving resulting in inflammation of one or more hair follicles. The damaged follicles often become infected with the bacteria. Symptoms of folliculitis include rashes, itching, and pimples near a hair follicle in the neck or genital area (Vorvick, 2012)

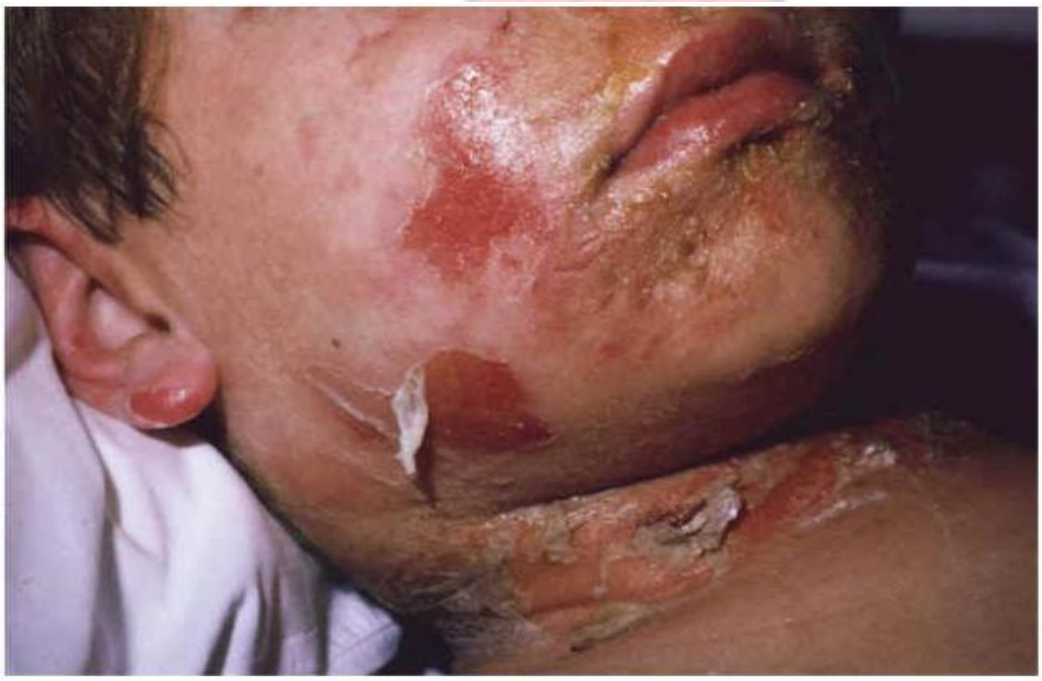

Figure 2.5: clinical manifestation of SSSS (looks like the skin is scalded by hot water (Schenfeld, 2000))

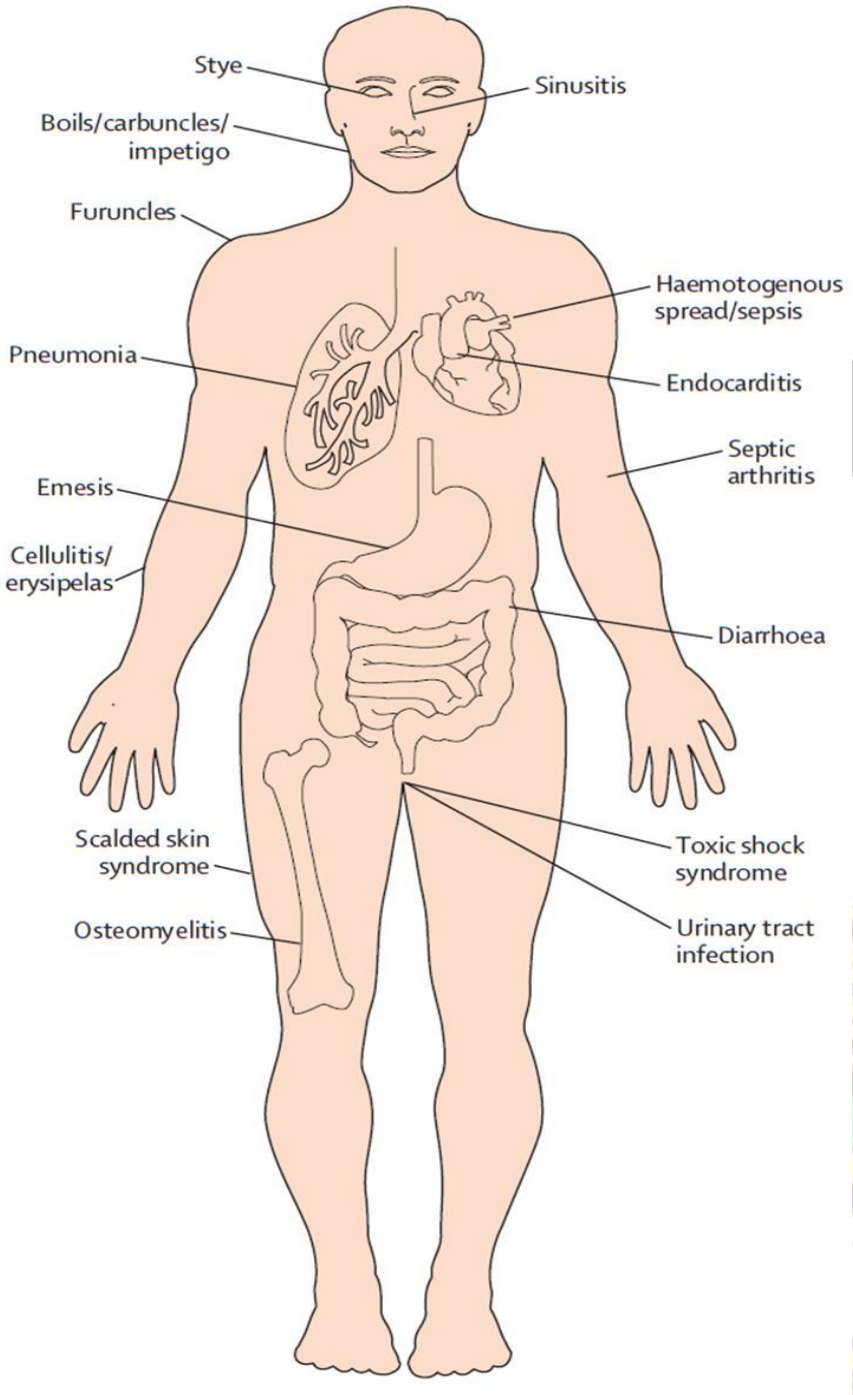

Figure 2.6: Sites of diseases caused by *S. aureus* (Wertheim *et al.*, 2005)

Staphylococcal food poisoning (SFP) is an intoxication which occurs as a result of consuming of foods which contain sufficient amounts of preformed enterotoxin(s) of *S. aureus* (Oliveira *et al.*, 2011). Staphylococcal enterotoxins are a major cause of food poisoning, which typically occurs after eating foods

contaminated with *S. aureus* through improper handling and subsequent storage at warm temperatures. The symptoms occurs rapidly (within 2 to 8 hours), and includes nausea and vomiting, abdominal cramping with or without diarrhoea. The illness usually resolves within 24 – 48 hours after onset but can also become more severe enough to warrant hospitalization in infants, the elderly or debilitated people (Centre for Health Protection, 2011).

Other diseases which are caused by *S. aureus* include styes, chalazion furuncles, carbuncles, osteomyelitis (Boucher *et al.,* 2010), endocarditis, septic arthritis, septic thrombosis, sinusitis (Wald, 2012), urinary tract infections (Ajantha *et al.,* 2011), pneumonia (McGrath *et al.,* 2008; Rubinstein *et al.,* 2008), pyomyositis (skeletal muscle infection) (Lanternier *et al.,* 2004) and several other diseases and conditions.

2.5 *S. AUREUS* EPIDEMIOLOGY

S. aureus has been identified to be the bacterial pathogen which is most frequently isolated from inpatients in United States hospitals and is the second most prevalent bacterial pathogen from clinical isolates among outpatients (Naber, 2009). According to TMIAC-CDPH (1993), colonization occurs when a patient carries the bacterium on any part of the body but does not results in any immune response or there are no clinical symptoms of a disease. Persons colonized with MRSA this way are referred to as carriers. It has been estimated that about 20.0% of individuals almost always carry one type of *S. aureus* strain and such people are called persistent carriers (TMIAC-CDPH, 1993). About 60.0% of the population harbours *S. aureus* intermittently, and the strains change with varying frequencies; such persons are referred to as intermittent

carriers. There are some 20.0% of the population who almost never carry *S. aureus* and they are referred non-carriers (Rongpharpi *et al.,* 2013). The organism is both a commensal and a pathogenic bacterium, its main ecological niche on humans are the anterior nares. Other sites which may be colonized include the groin, armpits and gastrointestinal tract. These colonized sites serve as reservoir from where the bacteria can infect its host when host defences are breached through surgery, shaving, or insertion of an indwelling catheter. Colonization enables the transmission of *S. aureus* among individuals in both the hospitals and communities (Gordon and Lowy, 2008). Infection takes place when the bacteria enters a site on the body and multiplies in number in the tissues to cause an immune response and some clinical manifestations of a disease. This is characterized by a rise in the white blood cell count, fever, or purulent drainage from a wound or body cavity (Gordon and Lowy, 2008).

Bustamante (2011) gave a worldwide prevalence which ranged between 23.3% - 73%. Europe has 26% MRSA prevalence with Greece, Italy, Portugal, and Turkey recording some of the highest rates (Bustamante, 2011). Countries such as Taiwan, Singapore, Japan, and Hong Kong in the Asia-pacific region showed very high MRSA prevalence of above 60%. A prevalence of 5%, 27.8%, and 23.8% from the Philippines, China and Australia respectively, were reported based on articles that varied in number of isolates used for the analysis, accuracy of results, and year of data collection (Bustamante, 2011).

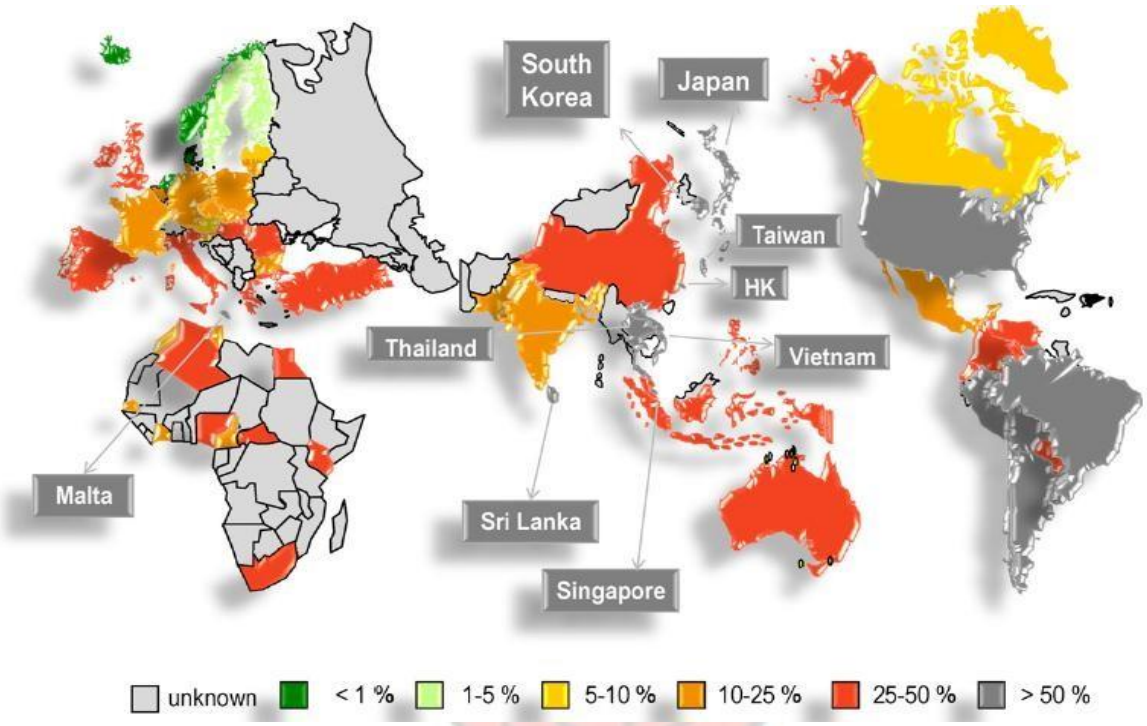

Figure 2.7: Worldwide MRSA prevalence [HK-Hong Kong (Stefania *et al.*, 2011)]

Bustamante (2011) recounted how the lack of resources and inadequate education has led to the continuous spread of MRSA and the fact that the above observed discrepancy in prevalence were due to the paucity of data from less developed countries. Scarce resources in such countries makes it difficult for adequate funds to be raised for such research.

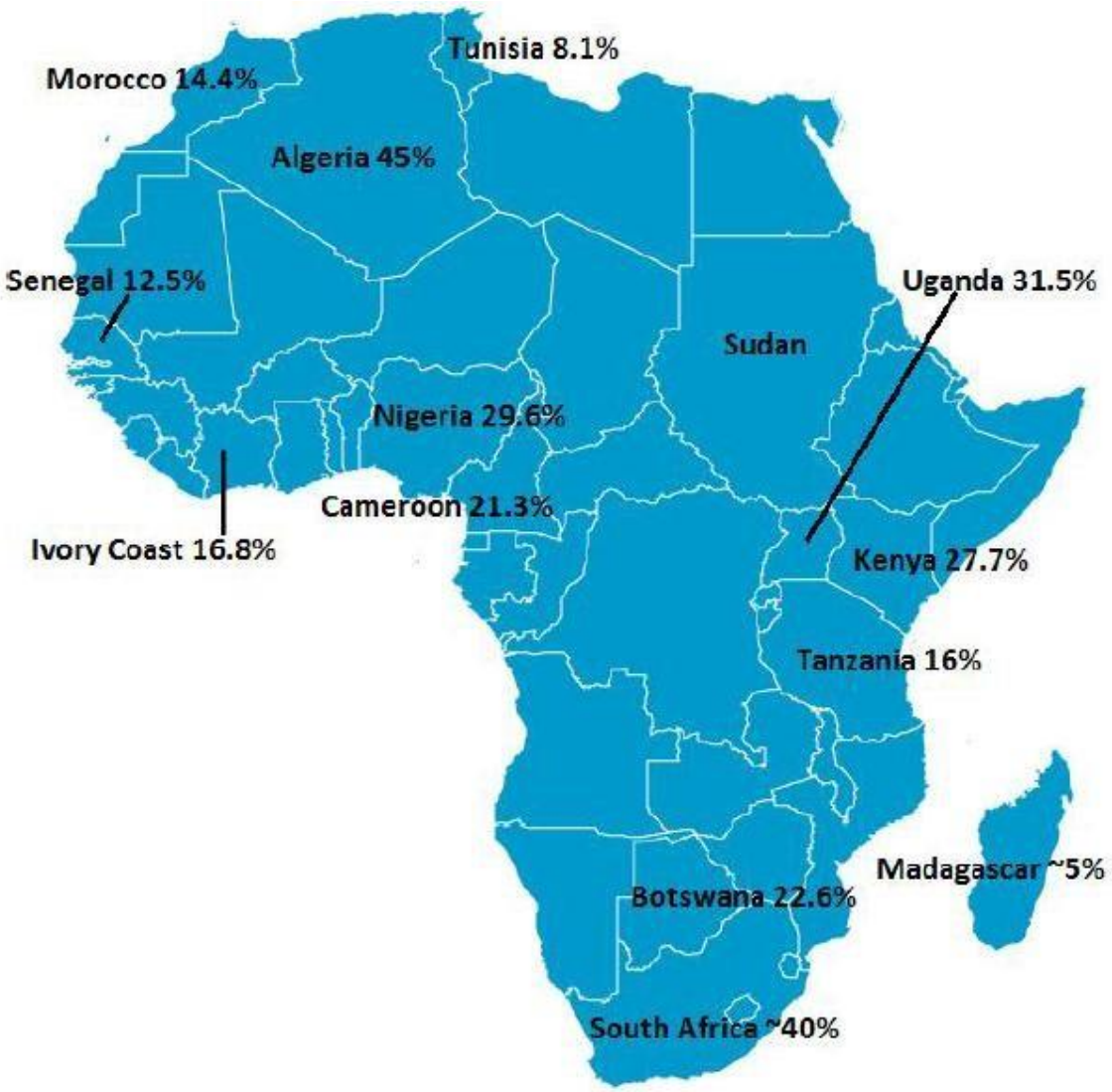

Figure 2.8: Prevalence of MRSA in Africa (Bustamante, 2011)

2.5.1 *S. aureus* epidemiology – the African perspective

Breurec *et al.,* (2011) cited the poorly documented state of MRSA-related infections in Africa as a leading cause of the spread of MRSA. This assertion was corroborated by Bustamante (2011) through a study which gave a prevalence of 5% - 45% across Africa and also cited inadequate coverage, increase in the use of antibiotic and inaccurate sensitivities as factors that aggravate the challenge of growing MRSA prevalence. Data on MRSA

prevalence in Africa is scanty, however, one of the earliest reports in the continent was made in South Africa and studies from the 1980s have been described (Obasuyi, 2013). Falagas *et al.,* (2013) sought to assess the prevalence of MRSA in Africa. It was reported that Tunisia recorded an increase in MRSA prevalence from 16.0% to 41.0% between 2002 and 2007, while in Libya a prevalence of 31.0% was recorded in 2007. In South Africa however, the prevalence decreased from 36.0% in 2006 to 24.0% during 2007– 2011. In Botswana, the reported prevalence ranged from 23.0% to 44.0% during the period between the years 2000 and 2007. In Algeria, a prevalence of 45.0% was reported during the period spanning the years 2003 and 2005. Within that same period, a prevalence of 52.0% was reported in Egypt. Generally MRSA prevalence in most African countries was lower than 50% although it appears to have risen since 2000 in many African countries, except for South Africa (Falagas *et al.,* 2013).

2.5.1.1 MRSA in Ghana

Odonkor and Addo (2010) reported MRSA prevalence range of 21.6% to 33.6% from *S. aureus* isolates obtained from routine specimen collected from hospitals in Accra for microbiological analyses, in a study to evaluate some methods for detection of MRSA. In another similar study to determine MRSA prevalence in Accra and to ascertain their antibiotic susceptibility profile, a prevalence of 33.6% was reported, indicating an increase in the prevalence (Odonkor *et al.*, 2012). In a study which determined the antibiogram of identified agents of bacterial infection in Ghana, multi-drug resistance in *S. aureus* was found to be 42.3% and it was recommended that resistance studies of such

epidemiologically significant clinical isolates be carried out (Newman *et al*., 2011). A prevalence of 34.8% was reported by Karikari (2009) in a study conducted at the Komfo Anokye Teaching Hospital (KATH).

Laryea *et al*., (2014) reiterated the assertion that MRSA is not much regarded as an issue of public health importance and as such, it's not among the priority diseases under surveillance in Ghana. Due to this, active efforts at identifying cases and instituting appropriate preventive measures are largely non-existent, due to the unavailability of laboratories with requisite human resource and logistics to run tests for MRSA. They reported MRSA case fatality rate of 12.6% and further stated that MRSA causes significant morbidity and mortality. They recommended that a surveillance system and guidelines for managing MRSA is established. In another study to determine the prevalence of *S. aureus* nasal carriage and its genetic diversity among hospital workers and inpatients at the KBTH, 13.9% prevalence was evaluated among inpatients and 23.3% among hospital staff (Egyir *et al.,* 2013). It was further revealed that the chance of being carriers was higher among hospital staff and inpatients staying ≤7 days in hospital. However, a higher chance of multidrug-resistant *S. aureus* carriage was observed among inpatients compared with hospital staff. MRSA prevalence among inpatients was evaluated at 1.3%, but none among hospital staff. The study indicated a high diversity of *S. aureus*, low levels of MRSA carriage, and a higher chance of nasal carriage of multidrug-resistant *S. aureus* among inpatients when compared with the hospital staff (Egyir *et al.,* 2013).

The bacteria has also been implicated in an outbreak at the children's ward of the Korle Bu teaching hospital (KBTH), where it was reported that five children were infected (Sackey, 2012).

2.5.2 Role of the anterior nares and other risk factors

S. aureus are primarily found in the nostrils (anterior nares) and the hands are the main vector for transferring the bacteria from other surfaces to the nostrils or vice versa. This explains the strong correlation between nasal carriage and hand carriage (Wertheim *et al.,* 2005). The association between *S. aureus* nasal carriage and staphylococcal disease was first established in 1931, and subsequently confirmed through several other studies (Wertheim *et al.,* 2005). It was confirmed that the nasal *S. aureus* strain and the disease causing strain share similar phage type or genotype. More so, when an anti-staphylococcal drug was applied in the nose, it temporarily decolonised *S. aureus* in the nose and other body parts, thereby preventing infections (Wertheim *et al.,* 2005).

S. aureus may reach or be transferred from the nose directly through airborne transmission (albeit less frequently). Such mode of transmission is important for the dispersal of staphylococci to many different sites of the body, from where, they can reach the nose or be transferred from the nose through the hands. Individuals harbouring the bacteria in the nostrils and suffering from rhinitis can disperse high loads of *S. aureus* into the environment and can be the source of an outbreak of infections (Wertheim *et al.,* 2005). Hospitalization has been found to be an important environmental risk factor in *S. aureus* transmission. Carriers from a health facility can transfer the bacteria to other household members. The transmission of the bacteria within households involving healthcare workers has also been shown to be a key risk factor for the re-introduction of the bacteria into hospitals, and patients may also get infected from their family members (Wertheim *et al.,* 2005). The bacteria can also be

spread through skin-to-skin contact with an infected person or contact with pus from an infected wound, and via contact with objects like towels, clothing, or sports equipment used by somebody infected (Illinois Department of Public Health, 2007). Hospital workers suffering from dermatitis or paronychia (infections in the tissues near the toe or finger nails) are more likely to transmit MRSA to patients (TMIAC-CDPH, 1993).

2.6 METHICILLIN-RESISTANT *S. AUREUS* (MRSA)

The first strain of MRSA was identified in 1959; within two years after the introduction of methicillin (Sakoulas and Moellering, 2008). MRSA is defined by the presence of staphylococcal cassette chromosome *mec* (SCC*mec*); which is a large mobile genetic element that carries the *mec*A gene which codes for an alternative form of penicillin binding protein (PBP2a). PBP2a has a low binding affinity to β-lactams. Since MRSA was first identified in clinical specimen in the early 1960s, the strains have spread throughout the world. By the mid - 1980s MRSA emerged as the most important hospital acquired pathogens worldwide (Pinho *et al.,* 2001). Although methicillin sensitive *S. aureus* (MSSA) can also cause outbreaks of diseases in a hospital as has been earlier discussed, MRSA infections are particularly easily spread in a hospital and there is high risk of it becoming epidemic if special surveillance program with control procedures are not implemented. The *mec* operon which is a distinctive characteristic of SCC*mec*, comprises of the *mec*A and its regulatory genes (*mecI* and *mecR1*). The operon occurs in several variants under two main categories: those with the two regulatory genes intact (also called class A *mec* operon) and

those with portions of either one or both of these regulatory genes removed (classes B, C, D, E) (Plata *et al.,* 2009).

2.6.1 Function of β-lactam antibiotics and resistant mechanisms of MRSA

β-lactams are bactericidal agents which act against the susceptible bacteria's cell wall. They target the transpeptidation step of the peptidoglycan synthesis. β-lactams acts to inactivate the transpeptidase domain of PBPs in the cell wall by binding and inactivating the transpeptidase. β-lactam are structural analogues of the natural substrate of PBPs, D-alanyl-D-alanine of the peptidoglycan stem peptide (Figure 2.9). Penicillin binding proteins (PBPs) are involved in the assembly of the bacterial cell-wall peptidoglycan. β-lactam antibiotics include penicillins, cephalosporins, and penicillinase-insensitive βlactams like oxacillin and methicillin. The reaction between a β-lactam antibiotic and PBP starts with a non-covalent association between these two molecules. The intermediate may either dissociate or undergo an irreversible reaction of acylation, and then the PBP covalently binds the antibiotic at its active site to cut the cyclic amide bond in the β-lactam ring. The D-alanyl-Dalanine undergoes quick deacylation by hydrolysis which liberates the PBP for a next round of transpeptidation. However, with a β-lactam antibiotic as substrate, the deacetylation process is very slow and the PBP is effectively inactivated which tends to inhibit the cell wall synthesis resulting in cell death (Plata *et al.,* 2009).

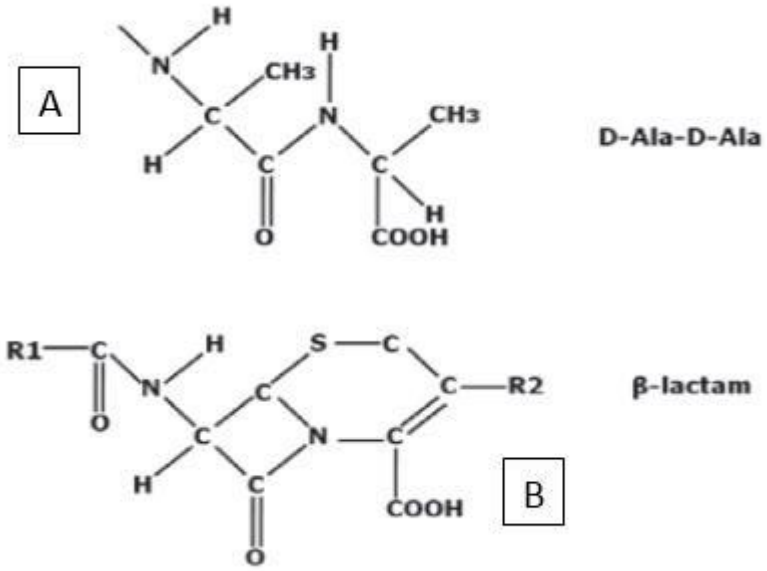

Figure 2.9: A is the structure of D-Alanyl-D-Alanine and B is the structure of β-lactam antibiotic: They both have similar structures that allow them to be bound by PBPs. R1 and R2 indicate groups that differ among various β-lactam antibiotics (Plata *et al.*, 2009).

2.6.2 Mechanism of resistance to β-lactam antibiotics by *S. aureus* β-lactamases are proteins which have enzymatic activities that aids β-lactam resistance by inactivating many of the β-lactam antibiotics in a reaction similar to the one β-lactams use to inhibit PBPs. β-lactamases attach to β-lactams leading to the formation of an acylated intermediate. This acylated intermediate leads to the cleavage of the amide bond of the β-lactam ring then the inactivated β-lactam antibiotic and active β-lactamase are then released (Plata *et al*., 2009).

2.6.3 Mechanism of resistance to semi-synthetic β-lactams (e.g. methicillin,

nafcillin and oxacillin)

S. aureus developed resistance to this type of β-lactam antibiotics by acquiring the *mec*A gene which is carried on the SCC*mec* element described earlier. Strains which carry this *mec*A gene are known as methicillin resistant *S. aureus* (MRSA), even though they are actually resistant to all β-lactam based antibiotics. β-lactam resistance in MRSA is achieved by the addition of the newly acquired PBP2a to the complement of the four native staphylococcal PBPs (Pinho *et al.,* 2001). PBP2a is encoded by the *mec*A gene and it has low affinity for β-lactam antibiotics which enables these strains of *S. aureus* to grow in antibiotic concentrations that hitherto, inactivates all native PBPs. PBP2a is a member of a group of PBPs with high molecular mass (78 kDa), comprising of a transpeptidase domain and a non-penicillin binding domain whose function is unknown. PBP2a does not appear to be an active enzyme, compared to other indigenous PBPs which synthesize very well cross-linked peptidoglycan. Even when transpeptidase activity of all the indigenous PBPs is inhibited by the presence of methicillin, PBP2a was found to rely on transglycosylase, which is the β-lactam-insensitive domain of the indigenous PBP2, thereby conferring resistance to the bacterium. Another model of methicillin resistance suggested by Pinho *et al*., (2001), assumes that the PBP2a takes over the cell wall's biosynthetic functions of normal PBPs in the presence of β-lactam antibiotics; this rapidly acylate (and inactivate) the four indigenous PBPs at concentrations far below the minimum required to inhibit the growth of most MRSA strains.

2.6.4 Other antimicrobials MRSA is resistant to

Historically, *S. aureus* has been known to develop antimicrobial resistance to most antimicrobials rapidly. The bacteria developed resistance to penicillin only

a year after the introduction of penicillin into clinical use. It is now estimated that 90%–95% of *S. aureus* strains worldwide are resistant to penicillin. Linezolid-resistant MRSA strain was also described only a year after the introduction of linezolid into clinical use in the year 2000. MRSA developed resistance to daptomycin within 2 years after it was introduced in 2003. Vancomycin which has proven quite effective against MRSA after some decades of its introduction also seems to be losing out to the MRSA threat. It took about 40 years for the first resistance strain to this antimicrobial to be identified in Japan in 1996. The bacteria acquired this resistance using the *vanA* gene complex acquired from Vancomycin-resistant enterococci (VRE) (Sakoulas and Moellering, 2008). Resistance to fluoroquinolone drugs which include ciprofloxacin, ofloxacin and norfloxacin also emerged in US hospital in 1988 after the introduction of ciprofloxacin and a 38% resistance in 616 *S. aureus* was later evaluated in 2000 (Makgotlho, 2009).

2.7 DETECTION OF MRSA COLONIZATION

MRSA identification is based on phenotypic and genotypic investigations. The methods used include culture methods and molecular testing (PCR). Phenotypic investigations include Gram staining, catalase, coagulase, DNAse, and morphological characteristics on mannitol salt agar. After identifying *S. aureus* by Gram staining (Gram-positive cocci), catalase (positive), fermentation tests (oxidase positive) and tube coagulase (positive) or DNAse (positive), the sample is grown on mannitol salt agar for 24 hours at 37^{o}C. *S. aureus* colonies appear yellow and are then subjected to Cefoxitin sensitivity test by the Kirby

Bauer disk diffusion method. Other commercially available methods include latex agglutination test kits (Brown *et al*., 2005).

The various molecular techniques for rapid identification and characterization of MRSA strains are based on the identification of the *mec*A gene which is unique to MRSA (Makgotlho, 2009). Such techniques are however more costly than the formal and rapid media culture methods (Minnesota Department of Health, 2013). For a successful MRSA confirmation, the gold standard is to detect the *mec*A gene or its product PBP2a.

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The PBP2a test kit (Oxoid Limited, UK) provides a much simpler and more rapid test to detect PBP2' by latex agglutination. This does not involve labour intensive process and no costly or complex equipment or process. The PBP2a test is also fast and much easier to perform than genotypic tests and yields very clear and visible results within minutes (Hart, 2004). The PBP2’ test kit comprises latex particles coated with a monoclonal antibody against PBP2a which will react specifically with MRSA to cause clearly visible agglutination. The Oxoid PBP2a Latex test has the advantage of being used to directly detect the PBP2a protein rapidly with less labour. It also has an advantage of avoiding false – positive results, which might occur with strains which might possess the *mec*A gene but cannot produce or express the PBP2a phenotypically. Thus the PBP2’ kit has the potential to even be more accurate than those methods that detect of the *mec*A gene only. Additionally, the PBP2’ kit does not detect strains which are hyper-producers of either PBPs or beta-lactamase, which often

interferes with sensitivity tests. The Oxoid PBP2a test kit has previously been assessed world-wide and shown to have a high sensitivity and specificity. (Hart, 2004; Marlowe *et al.,* 2002).

2.8 MANAGEMENT AND TREATMENT OF MSSA AND MRSA INFECTIONS

The drug of choice for treating MSSA infections which are susceptible to penicillin is the Penicillin G (4 million units every 4 hours). Other antibiotics which can alternatively be administered include; nafcillin (2g every 4 hours), oxacillin (2g every 4 hours), cefazolin (2g every 8 hours), and vancomycin (1 g every 12 hours). The drug of choice for treating *S. aureus* which are sensitive to methicillin include nafcillin or oxacillin (2g every 4 hours). Infections involving MRSA are treated with vancomycin (1g every 12 hours) (Lowy, 1998).

Alternatively, trimethoprim–sulfamethoxazole (TMP, 5 mg/kg of body weight every 12 hours), ciprofloxacin (400 mg every 12 hours), minocycline (100 mg every 12 hours taken orally), trovafloxacin (300 mg every 24 hours), and levofloxacin (500 mg every 24 hours). Investigational drugs which can also be used include oxzolidinones, quinupristin–dalfopristin and new carbapenem. It is advised that sensitivity tests are carried out before an alternative drug is administered. Cephalosporin can be administered to patients who are allergic to penicillin if the allergy does not involve an anaphylactic or accelerated reaction (Lowy, 1998).

Attempts at developing a vaccine against MRSA has proven quite difficult and many more companies are doing well to come out with a viable vaccine (Reuters, 2013).

2.9 INFECTION PREVENTION AND CONTROL OF *S. AUREUS*

The bacteria can survive for weeks, even months in the hospital environment, especially in dust and on dirty equipment. It can be found on staff ties, on pens and even patients' case note. The main route of transmission is through personto-person contact via patients' or healthcare workers' hands. Basic infection control practices are important to prevent and control the spread of MRSA. The required precautions are grouped under two main categories including; transmission-based precautions and standard precautions. Standard precautions apply to all patients in healthcare facilities, assuming that all body fluids, blood, non-intact skin, secretions, mucous membranes and excretions contain infectious agents that can be transmitted. Standard precautions include hand hygiene, safe injection practices, use of personal protective equipment, respiratory hygiene/cough etiquette, and safe handling of potentially contaminated equipment or surfaces in the patient environment.

Transmission-based precautions are implemented when the routes of transmission are not completely blocked when only standard precautions are employed. Transmission-based precautions covers airborne precautions contact precautions, and droplets precautions (Minnesota Department of Health, 2013)

Diseases which involve more than one route of transmission may require multiple types of transmission-based precautions. Contact precautions will be carried out to prevent transmission of pathogens which can be transferred through direct or indirect contact with the patient or his environment. For MRSA infected patients, contact precautions are recommended as well as patients with *Clostridium difficile*-associated diarrhoea (Minnesota Department of Health, 2013; TMIAC-CDPH, 1993).

In hospitals the administration are required to provide the needed resources to empower front-line teams comprising of different disciplines to provide the necessary resources, supplies and personnel (including microbiologist, infection prevention experts and environmental service experts). They are to train staff, and educate patients and families or visitors on issues related to infection prevention and control. Administration is required to put programmes in place to promote antimicrobial stewardship to optimize therapeutic outcomes and minimize accidental effects of therapeutic drug use. The universal body substance precautions should be adhered to strictly. This include proper hand washing before attending to each patient or anything relating to the patient and washing the hands again before leaving the patient's room. Appropriate personal protective equipment or clothing should be worn at all times. It is very important that patient rooms, table and floor surfaces are thoroughly cleaned and regularly disinfected, since MRSA can survive in such areas for long periods up to 56 days.

In the community, the maintenance of regular personal hygiene is critical to curb the transmission of MRSA. Bathing regularly with soap and water limits personal contamination and transmission. Individuals with lesions must cover

it up appropriately and discard dressings safely and promptly. Sharing of creams, soaps, lotions should be limited and one must avoid sharing unwashed towels. Sports equipment and children's toys should be washed regularly ideally after each use (Minnesota Department of Health, 2013).

2.10 PUBLIC HEALTH IMPLICATIONS AND ECONOMIC IMPACT OF MRSA INFECTIONS

The resistance exhibited by MRSA to most antibiotics imply that treatment for suspected or verified severe *S. aureus* infections, including common skin and wound infections, must rely on second line drugs. Thus standard prophylaxis with first-line drugs for orthopaedic and other surgical procedures will not have much effect in many settings. Second-line drugs for *S. aureus* are more expensive and also have severe side-effects for which monitoring during treatment is required which further increases costs. Through systematic reviews of scientific literature, it was established that patients with infections caused by bacteria resistant to a specific antibacterial drug generally have an increased risk of worse clinical outcomes and death, and they may consume more healthcare resources, than patients infected with the same bacteria not demonstrating that resistance pattern. However, data available are insufficient to estimate the wider societal impact and economic implications when effective treatment for an infection is completely lost as a result of resistance to all available drugs (World Health Organization, 2014).

Cummings *et al.* (2010), noted that not complying with hand hygiene has resulted in most hospital acquired infections. Based on this premise, they

embarked on a study to evaluate the cost of an incident of noncompliance with hand hygiene by a hospital worker during patient care. It was finally deduced that in a 200-bed capacity hospital, an amount of $1,779,283 was incurred in annual MRSA infection–related expenses attributable to hand hygiene noncompliance. They further stated that a 1.0% increase complying with hand hygiene compliance can result in yearly saving of $39,650 to a 200-bed hospital To conclude, they stated that not complying with hand hygiene has resulted in significant costs to the hospital and that a little adherence to hand hygiene can save the hospital substantial costs (Cummings *et al.,* 2010).

An overview of findings to address whether there is an excess cost due to infections caused by *S. aureus* resistant to methicillin revealed that there is an excess cost in hospitalization, antibacterial therapy, medical care and an excess cost in additional cost variable which include costs specifically related to the MRSA infection , daily hospital or patient costs; costs before or after infection; costs for specific allied health care; costs broken down into very specific categories; costs related to inpatient or outpatient treatment; costs reported by a specific time period (vs. entire stay), or adjusted or modelled cost variables were produced in the study (WHO, 2014).

CHAPTER THREE

3.0 SUBJECT AND METHODS

3.1 STUDY DESIGN AND SITE

A cross-sectional study was conducted between 4thJuly - 20th August, 2014 at the Brong Ahafo regional hospital (BARH), Sunyani, Ghana. The BARH is a Ghana health service secondary level facility located in the regional capital of the Brong Ahafo region. The hospital receives referrals from all districts in the Brong Ahafo region and also from the Upper East, Upper West and Northern region. The hospital has a bed capacity of 300 and an annual out-patient department (OPD) attendance of 210,963 in 2010. The most frequent cause of admissions includes deliveries, malaria, anaemia, accidents, diarrhoea, hypertension, abortions and HIV/AIDS (BARH, 2011).

3.2 SAMPLE COLLECTION

Three hundred and twenty seven (327) participants were used in the study based on *S. aureus* prevalence of 23.3% obtained by Egyir *et al.,* (2013) at 95% confidence level and 5% precision (Naing *et al.,* 2006). Nasal screening of patients, hospital personnel and visitors to the hospital was conducted at the main out-patient department (OPD), clinics, accident and emergency unit, surgical emergency ward, male ward, female ward, and other departments within the hospital which are all located in different buildings.

Approval for the research was granted by the KATH Research and Development Office (REG.NO RD/CR13/131) and from the BARH (Ref: GHS/BA/RH/RES/90/10).

Participation was voluntary and nasal screening was done after receiving informed consent from participants. Additional information regarding the age, sex, and whether the participant was using any form of antibiotic were obtained

from the participants and records. Those on any form of antibiotic treatment were not included.

All the sites from where samples were obtained were grouped under two categories based on proximity and to aid efficient sampling and analyses. Group one comprised samples obtained from participants who were outpatients or visitors at the main OPD , clinic 1, clinic 2, clinic 3, clinic 4 and clinic 5. Group two included samples of participants who were inpatients at the various wards, hospital staff, and visitors attending to patients at the wards and other departments which included laboratory, pharmacy, radiology, administration, etc.

3.3 ISOLATION OF *S. AUREUS*

Sterile swabs were partially moistened in a 2.5% saline solution and were used to take the samples by rotating it five times in both anterior nares. The swabs were then pre-enriched in test tubes containing 3ml of Mueller–Hinton broth (MHB) (Techno Pharmchem, Haryana, India) supplemented with 2.5% sodium chloride (NaCl) (UK Standards for Microbiology Investigations, 2014). The pre-enriched test tubes were then incubated at 37ºC for 24 hours and used to inoculate the mannitol salt agar (MSA) which was incubated at 37ºC for 24 hours. Suspected yellow *S. aureus* colonies surrounded by yellow halo zones (Figure 3.1) were picked and further tests such as Gram staining, microscopy, catalase production, haemolysis on blood agar, and tube coagulase, were performed to confirm the bacteria (Benson, 2001).

Figure 3.1: Colonies observed on mannitol salt agar plates (colonies surrounded by yellow halo are suspected to be *S. aureus* colonies; red colourd backgrounds have no *S. aureus* growth.

3.4 ANTIMICROBIAL SUSCEPTIBILITY TESTING

The Kirby Bauer disc diffusion method was employed to test for antimicrobial susceptibility as recommended by Clinical Laboratory Standards Institute (CLSI, 2013) which indicated Cefoxitin disk diffusion or Cefoxitin MIC tests were preferred methods to determine resistance or susceptibility to cephalosporin and a wide array of other β-lactam antimicrobial agents. The test can thus be used to predict the presence of *mec*A-mediated methicillin resistance in *S. aureus* (CLSI, 2013).

Identified colony of *S. aureus* on the MSA was sub-cultured into peptone water broth (Lab M Limited, Lancashire, UK) and incubated at 30^{o}C for 24 hours. The peptone water broth culture was used to inoculate the Mueller Hinton Agar

(MHA) using a sterile swab to streak its surface. Each streaked MHA plate was then left ajar for 5minutes to dry out any excess surface moisture and a cefoxitin (FOX 30mcg, CT0119B) antimicrobial susceptibility test disc (Oxoid Limited, UK) was aseptically placed onto the agar surface with the aid of sterile forceps. The plate was inverted and incubated at 35^{o}C for 24 hours (CLSI, 2012).

3.4.1 Reading and interpretation of plates

Circular zones of inhibition were obtained on the plates (Figure 3.2). The diameter of the zones of inhibition was measured using a ruler which was placed on the back of the inverted petri dish and held a little above a black, nonreflecting background illuminated with reflected light. The diameter of the zones of inhibition was measured to the nearest whole millimetre. The diameter of the zone of inhibition was used to determine whether the bacteria was resistant or susceptible to cefoxitin with reference to the CLSI (2013) guideline.

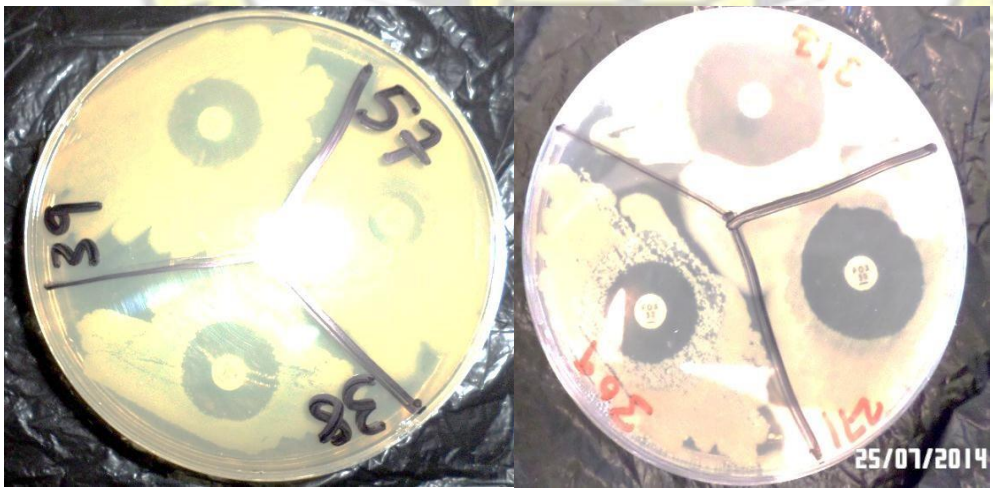


Figure 3.2: Effect of Cefoxitin on *S. aureus* growth on MHA: showing various diameters of zones of inhibition.

3.5 TEST FOR THE PRESENCE OF THE *mec*A GENE

Many methods to establish the presence of the *mec*A gene in an *S. aureus* had been reviewed (Makgotlho, 2009; Anand *et al.,* 2009). According to the CLSI (2012), identifying the *mec*A gene or its protein product, PBP2a (or PBP2'), are more accurate and the most preferred methods to predict if an isolated *S. aureus* is resistant to oxacillin (or methicillin).

3.5.1 Penicillin-Binding Protein (PBP2a) Latex Agglutination test

The penicillin binding protein (PBP2a) latex agglutination test kit (Oxoid Limited, UK), is a rapid latex agglutination test kit used to detect PBP2a in *Staphylococcus* isolates to help confirm the successful isolation of MRSA. The presence of PBP2a was determined according to the guideline outlined by the manufacturer.

Cefoxitin resistant colonies of *S. aureus* on the MHA obtained from the antimicrobial susceptibility test (section 3.4) were selected and tested for the production of the PBP2a protein. Specimen which showed resistance to cefoxitin were identified. The protein from the resistant bacteria was extracted using two extraction reagents and centrifuged at 3000 rpm at 15cm rotation radius for five minutes. The supernatant was used for the test.

The latex agglutination test was conducted on test cards provided with the kit. The specimen number was written in one circle and the other circle labeled

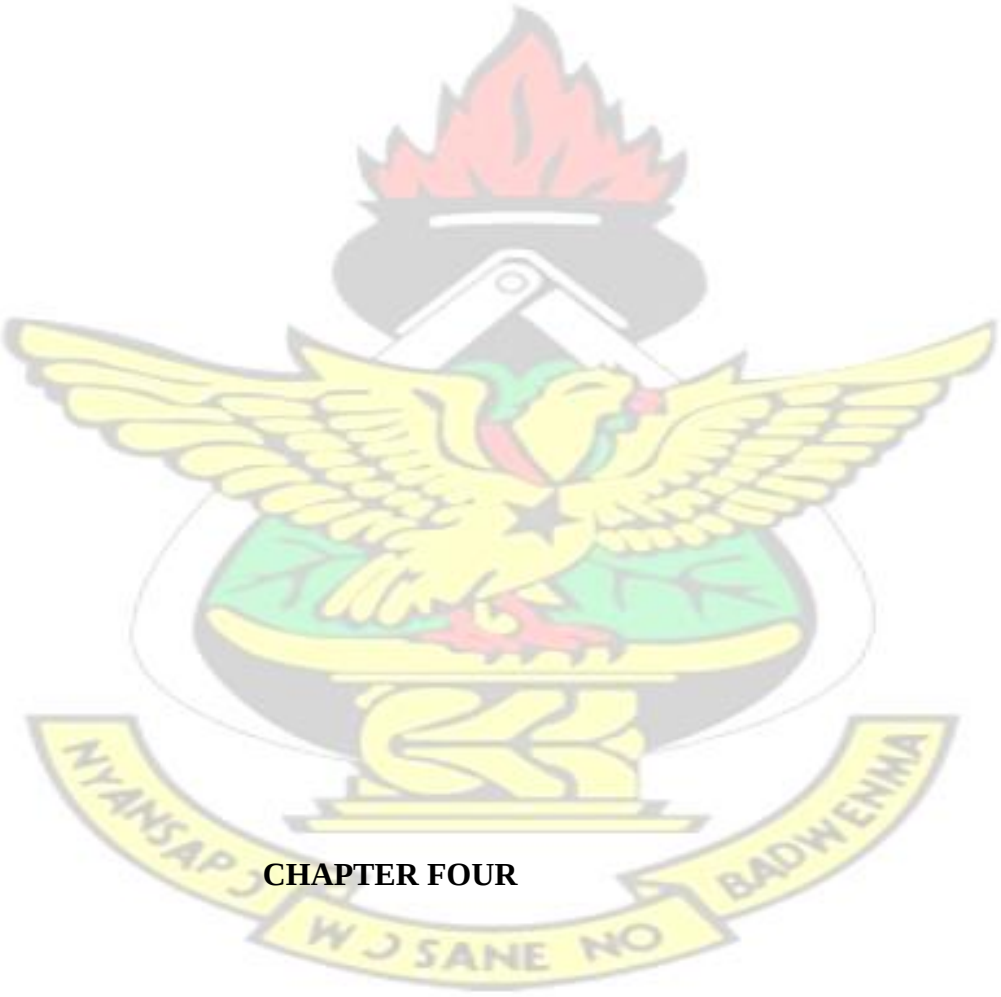
"control". The latex reagents were well mixed and a drop of the Test Latex or Control Latex was added to their respective labeled circles on the test card. 50 µL of the supernatant from the specimen and the control organism were placed on the Test circle and the Control circle respectively, and mixed with the latex thoroughly with the mixing stick provided. The card was picked up and rocked for about three minutes while looking for agglutination under normal ambient light (Figure 3.3). The outcome of the test and the control reactions were noted.

Figure 3.3: PBP2a test card showing positive agglutination reactions for some isolated MRSA samples along with a negative control test. The samples labelled 24 and 33 shows a positive agglutination reaction compared with the negative controls

3.6 STATISTICAL ANALYSIS

Analysis was carried out using SPSS 17 (SPSS Inc., U.S.A) and StatPac Statistics calculator (StatPac Inc., U.S.A). *S. aureus* and MRSA prevalence were evaluated and stratified by demographic characteristics. The ages of the study participants were grouped into two; children (1 – 17years) and adults (18years and above) (The Children's Act, 1998). The demographic attributes of

the various sampling groups were evaluated and the differences in prevalence between the individual demographic levels were evaluated using the twosample two-tailed t-test, to compute the p – value at 95% confidence level.



CHAPTER FOUR

4.0 RESULTS

4.1 GENERAL DEMOGRAPHICS OF *S. AUREUS* PREVALENCE

A total of 327 samples were obtained from the study participants and processed. Of these, 124 (37.9%) were males and 203 (62.1%) were females. Nine (2.8%)

of the participants were children aged 17 years and below whereas 318 (97.2%) were adults aged above 17years (The Children's Act, 1998). The participants' ages ranged from 2 – 105years with a mean age of 33.4 (±12.9) years. Group 1 (mainly outpatients and visitors) sampling sites provided 237 samples (72.5%) whereas 90 samples (27.5%) were obtained from Group 2 sampling sites (mainly in patients and staff). There were 55 *S. aureus* isolates from the 327 nasal swabs, representing 16.8% prevalence. Thirty-four of the *S. aureus* isolates were obtained from the female participants, which represents 16.7%, and a general prevalence of 10.4% of the total study population. Twenty-one *S. aureus* isolates were obtained from the male participants, which also represents 16.9% prevalence, and a general prevalence of 6.4%. One hundred and three of the male study population was found to be non-carriers (83.1%), whereas 169 of the female study population were also found to be non-carriers (83.3%). Forty-nine of these isolates were obtained from Group 1 sampling sites, which represented an *S. aureus* prevalence of 20.7% (49/237). Six of the *S. aureus* isolates were obtained from the Group 2 sampling sites representing a prevalence of 6.7% (6/90).

Table 4.1: General Demographics of *S. aureus* Prevalence

Parameter	**Total (*n=327*)**	***S. aureus* Prevalence (*n=55*) N (%)** *(Overall = 16.8%)*	***P – value*** [c]
Gender			
Male	124	21 (16.9)	0.9626
Female	203	34 (16.7)	
Age Group			

Children (≤17years)	9	2 (22.2)	0.6641
Adults (≥18years)	318	53 (16.7)	
Sample Group			
Group 1[a]	237	49 (20.7)	0.0027
Group 2[b]	90	6 (6.7)	

a: comprised participants from the main OPD and Clinics 1 to Clinic 5
b: comprised participants from inpatients, staff, and other departments c:
p – value of the two-sample t-test at 95% confidence level

A comparison of the *S. aureus* prevalence among the two genders revealed that there was no statistical significant difference between the 16.9% prevalence among the males and the 16.7% among the female participants (p=0.9626). Similarly, the 22.2% and 16.7% prevalence recorded for the children and adult age groups respectively was not significantly different (p=0.6641). However the difference in the prevalence between group 1 (20.7%) and group 2 (6.7%) sampling areas was significant (p = 0.0027).

4.2 Cefoxitin Resistance in *S. aureus* Isolates

The cefoxitin disc diffusion test was carried out for primary identification MRSA strains. The classification of an isolate as Methicillin-Susceptible *S. aureus* (MSSA) or Methicillin-Resistant *S. aureus* (MRSA) was based on the measured diameter to the nearest whole millimeter as indicated in Table 4.2. The diameters indicative of resistance ranged from 0 mm to 21 mm and susceptible strains produced a zone of inhibition which was greater than or equal to 22mm (CLSI, 2013). The diameter of clearance by the various

identified isolates of *S. aureus* ranged from 0mm to 40mm diameter with a mean of 20.44 mm ±8.11.

Table 4.2 Cefoxitin Disc diffusion Zone Diameter interpretive criteria.

Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm)		
	Susceptible	Intermediate	Resistant
Cefoxitin (30µg)	≥22 (MSSA)	-	≤21(MRSA)

(CLSI, 2013)

4.3 MSSA AND MRSA PREVALENCE

The 55 *S. aureus* isolates identified were further tested and classified as methicillin susceptible *S. aureus* (MSSA) or methicillin-resistant *S. aureus* (MRSA) based on their growth characteristics on MHA impregnated with cefoxitin disc and their ability to produce PBP2a. The zone of inhibition diameter was used to first classify the *S. aureus* as MSSA or MRSA (Table 4.2,). Those found to be MRSA by the Kirby Bauer cefoxitin sensitivity test, were further tested for their ability to produce the PBP2a before finally being classified as MRSA, because they carry the *mec*A gene and are expressing the gene product in the phenotype.

The results showed that 26 of the *S. aureus* isolates were MRSA strains which represent a prevalence of 47.3% and the remaining 29 were MSSA, representing 52.7% of the *S. aureus* isolates (Table 4.3, 4.4). The MSSA prevalence among

the male (8.1%) and female (9.4%) participants was not significantly different (p = 0.6891). A similar observation was made comparing the MSSA prevalence of 10.1% and 5.6% at Group 1 and Group 2 sample sites respectively (p = 0.2019). Children were found to be significant carriers of MSSA with 33.3% prevalence compared with 8.2% recorded among the adult participants (p = 0.0095).

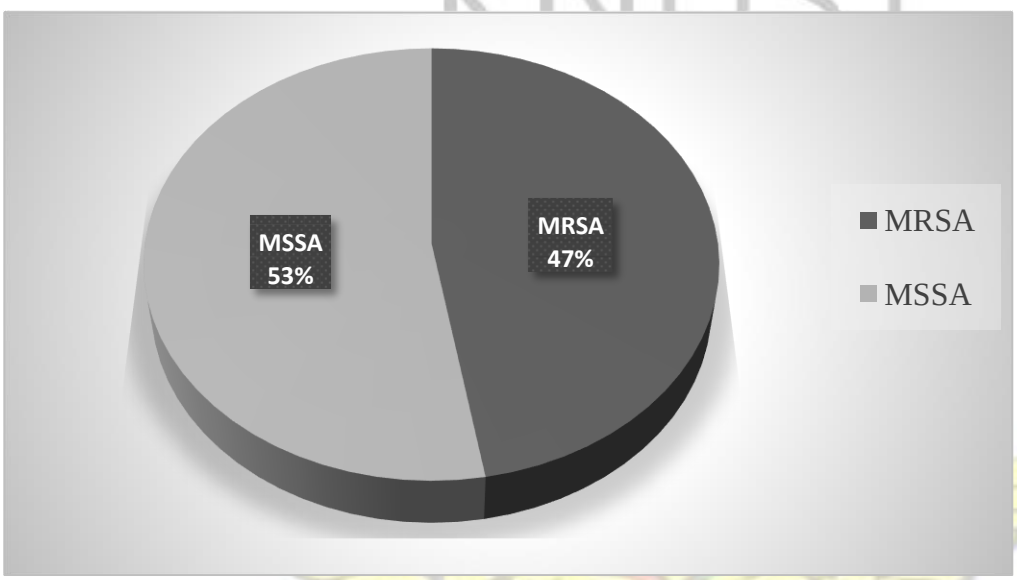


Figure 4.1 Distribution of *S. aureus* strains

Table 4.3 MSSA Prevalence at Brong Ahafo Regional Hospital (BARH)

Parameter	**Total (*n=327*)**	**MSSA Prevalence (*n=29*) N (%)** *(Overall = 8.9%)*	***P – value*** [c]
Gender			
Male	124	10 (8.1)	0.6891
Female	203	19 (9.4)	
Age Group			
Children (≤17years)	9	3 (33.3)	0.0095
Adults (≥18years)	318	26 (8.2)	

Sample Group			
Group 1[a]	237	24 (10.1)	0.2019
Group 2[b]	90	5 (5.6)	

a: comprised participants from the main OPD and Clinics 1 to Clinic5 b: comprised participants including inpatients, staff, and other departments c: p – value of the two-sample t-test computed with statpac version 4.0, 2013

A total of 26 MRSA were confirmed from the 327 study participants (approximately 8.0%). Eleven MRSA were obtained from the male participants and 15 from females. This represented a prevalence of 8.9% (11/124) and 7.4% (15/203) among the male and females respectively and the difference was not significant (p=0.6273). Similarly the difference in the prevalence of 0.0% and 8.2% recorded among the children and adult participants respectively, was not significant (p= 0.3712) (Table 4.4).

Table 4.4 MRSA Prevalence at the BARH

Parameter	**Total (*n=327*)**	**MRSA Prevalence (*n=26)* N (%)** *(Overall = 8.0%)*	***P – value*** [c]
Gender			
Male	124	11(8.9)	0.6273
Female	203	15 (7.4)	
Age Group			
Children (≤17years)	9	0 (0.0)	0.3712
Adults (≥18years)	318	26 (8.2)	

Sample Group			
Group 1[a]	237	25 (10.5)	0.0052
Group 2[b]	90	1 (1.1)	

a: comprised participants from the main OPD and Clinics 1 to Clinic5 b: comprised participants including inpatients, staff, and other departments c: p – value of the two-sample t-test computed with Statpac version 4.0, 2013

However, between the two sample groups, the prevalence recorded was found to be significantly different. Group 1 sample sites recorded a significantly higher MRSA prevalence of 10.5% compared with Group 2 prevalence of 1.1% ($p = 0.0052$).

CHAPTER FIVE

5.0 DISCUSSION

An overall *S. aureus* nasal carriage prevalence of 16.8% was obtained. In a similar study to evaluate presence of *S. aureus* in the nostrils and their diversity among the inpatients and health care workers at the Korle Bu Teaching Hospital in Ghana, a prevalence of 16.6% was reported by Egyir *et al* (2013). Conceicao *et al.*, (2014) also reported a prevalence of 15.7% in another similar study at Sao Tome´ and Prıncipe. The similarity in the above prevalence with this study's overall *S. aureus* prevalence of 16.8% could be as a result of the similar methodology used and sampling technique. Higher *S. aureus* prevalence had

been reported in other studies where samples from other parts of the body were used besides the nostrils. For example, 36% *S. aureus* isolates were obtained from urine samples collected from healthy women in Zaria, Nigeria (Onanuga *et al.*, 2005). Cavalcanti *et al* (2005), also reported a 37.7% *S. aureus* prevalence from samples obtained from the nostrils, axillary and perineal regions and from dermatosis skin rupture at the Oswaldo Cruz university hospital in Brazil.

A significantly higher overall *S. aureus* prevalence was recorded from group 1 sample areas (20.7%) which include the main OPD and the five clinics compared to the group 2 sample sites (6.7%) (p=0.0027) (Table 4.1). The reason that could be adduced for this observation could be the fact that the group 1 sample sites usually experience more overcrowding than the group 2 areas (wards, laboratory, and other departments). As already mentioned overcrowding is a key factor for *S. aureus* and MRSA transmission which could be the reason for this observation (Clements *et al*., 2008).

The children age group was also found to be significant carriers of the MSSA strain of the bacteria when their prevalence (33.3%) was compared with the adults (8.2%) (p = 0.0095). Although the threats posed by such strains of the bacteria are similar to the MRSA strains, MSSA can be easily managed because of their inability to produce PBP2a (section 1.1 and 2.6). In a similar study in Brazil, 48% of 500 children were found to be infected with *S. aureus*. 6.2% MRSA strains were identified among these children which indicates that children are also prone to *S. aureus* infection, especially in day care centres (Braga *et al.*, 2014). Children are often more infected because they come into close proximity with several other people in classrooms and other school settings, day care centres, locker rooms, playgrounds, gymnasiums and other

workout facilities. In such places, transmission and infection are more likely, because children often have skin-to-skin contact and may share toys or other equipment that have not been cleaned. They are also more likely to have frequent bug bites or scrapes which are potential entryways for infection (Cassoobhoy, 2015).

The study revealed an MRSA prevalence of 8.0% (Table 4.5) among the participants. In a similar study, Egyir *et al.* (2013) reported an overall MRSA prevalence of 0.9% from nasal swabs obtained from the Korle Bu Teaching Hospital (KBTH). The reason could be due to the fact that the majority of samples were obtained from outpatients for this study, which is in contrast to the inpatients and hospital staff from whom Egyir *et al.* (2013) obtained their samples from. Overcrowding has been determined to be a key factor in the transmission of the bacteria (Clements *et al.*, 2008), and this occurs more frequently at the main OPD and clinics from where the majority of the samples were obtained from for this study. Across Africa, prevalence less than 10% have been recorded in many countries. In Madagascar, *S. aureus* prevalence of 4.4% in hospital and 6.5% in the community was reported from pus, urinary and genital specimen collected between January 2001 and December 2005 (Randrianirina *et al.*, 2007). A prevalence of 9% was reported in *S. aureus* isolates from ear discharge and pus in Eritrea (Naik and Teclu, 2009). In Gabon, 5.8% prevalence was reported from isolates obtained from different specimen collected from 2009 and 2012 (Alabi *et al.*, 2013). Omuse *et al.* (2012) also reported an overall prevalence of 3.7% in Kenya. Across the globe, similar prevalence of less than 10% have also been reported in several studies including one conducted by an international team which obtained 32,206 nasal swabs

from Australia, Belgium, Croatia, France, Hungary, Spain, Sweden, the Netherlands, and the United Kingdom with an MRSA prevalence of 1.3% of the 6,956 *S. aureus* isolates obtained (Roos, 2013).

It has been estimated that about 40.0% of *S. aureus* infections acquired in large hospitals in the US are methicillin - resistant. The MRSA prevalence in most European and American hospitals is estimated to range from 29.0% to 35.0% of all clinical isolates (Tiwari *et al.*, 2008). The highest prevalence of more than 50.0% have been reported in North and South Americas, Asia and Malta. Intermediate prevalence ranging between 25.0 – 50.0% have been reported in China, Australia, Africa and some European countries. It is quite difficult to explain these varying prevalence observed in the various locations (Tiwari *et al.*, 2008).

The 8.0% prevalence recorded in this study is comparatively low when compared with WHO figures which generally reported MRSA prevalence of over 20.0% in all WHO regions (WHO, 2014). The reason that could be adduced for this comparatively low prevalence is that most of the participants who availed themselves for the study were outpatients or visitors to the hospital; they formed 72.5% of the total sample size. In most studies where consistent high prevalence has been recorded, a sizeable number of inpatients' samples were used. A study by Coello *et al.* (1997) revealed that inpatients or patients in intensive care stand a greater chance of acquiring MRSA infection due to the frequent opportunities for MRSA invasion from contact with nurses which makes it possible for MRSA to be transferred through staff hands from infected sites to an entry portal on the patient, such as wounds (Coello *et al.*, 1997). This

was further buttressed in another study by Egyir *et al.* (2013), who also explained that inpatients that stayed ≤ 7days at hospital facility had a higher risk of being infected. However in this study, majority of the participants were mainly from the Main OPD and Clinics which treats and discharges patients within hours. Also the low consumption of some antimicrobial agents such as fluoroquinolones and the third-generation cephalosporins in Ghana due to their high costs may also explain the lower prevalence recorded (Egyir *et al.,* 2013). Muller *et al.* (2003) established that the use of certain antimicrobials is an independent risk factor for MRSA acquisition, and that there is a causal relationship between antimicrobial use and MRSA acquisition. They also mentioned that the use of these antimicrobials contribute to MRSA spread, citing that in some Nordic countries in Europe which use the least amount of antimicrobials; 14 hospitals in such countries had very low incidences of MRSA. According to Monnet *et al.* (2004) the use of some antimicrobial drugs such as third-generation cephalosporins, macrolides, and fluoroquinolones, promoted outbreak of MRSA and also highlighted the fact that current antimicrobial drug use can also have an effect on drug resistance in future patients. According to Egyir *et al.* (2014) these drugs are usually not used in Ghana because of their high cost and are often only prescribed for acute infections. This may explain the lower MRSA prevalence (8.0%) recorded at the BARH in this study.

The 47.3% of the *S. aureus* isolates which were found to be MRSA (Figure 4.1), is indicative of an almost half a chance that *S. aureus* isolates obtained from the hospital will be MRSA. This figure albeit lower, is comparable to 52.7% MRSA

prevalence found among *S. aureus* isolates isolated in a study by Moses *et al.* (2013) in Nigeria. Such a high proportion of *S. aureus* isolates being MRSA can however prove problematic to hospital staff, care-givers and patients particularly. This observation implies that although not many of the participants may be carriers of the bacteria, there is a chance that almost half of those *S. aureus* carriers, could be harbouring MRSA. This can pose serious attendant health risks to care-givers, staff or patients when appropriate hygienic practices are not observed.

The prevalence recorded between the two genders with respect to MRSA, MSSA and *S. aureus* prevalence in general, showed no significant difference in the prevalence. Thus gender was not found to be a significant factor in MRSA prevalence among the sampled population at BARH. Likewise the prevalence between the two age groups with respect to *S. aureus* and MRSA prevalence were found not to be significantly different. This result agrees with Egyir *et al.* (2013) who found no association of age and gender with *S. aureus* carriage.

The prevalence recorded at the Group 1 sample areas compared with Group 2 sample areas was significantly different ($p = 0.0052$). Thus areas in and around the main OPD and the various clinics recorded a significantly higher MRSA prevalence of 10.5%. The observation is of significant concern because many different groups of people including nurses, doctors, patients and visitors usually converge at the OPD for one activity or the other. This often leads to occasional overcrowding which has been identified as one of the means of

transmission. Healthcare workers may become transient carriers this way and may infect those they attend to. According to Rutala *et al.* (2008), healthcare worker's hands are the most common route for transmitting microorganisms from one patient to another patient, from a patient to equipment and the environment, and from the environment and equipment to the patient. Alcoholbased hand rubs (ABHRs) should be provided in health institutions in addition to existing facilities and especially in areas where it will be difficult to have hand washing facilities provided (WHO, 2012).

CHAPTER SIX

6.1 CONCLUSION

This research revealed that MRSA was prevalent at the BARH, with a prevalence of 8.0% of the total samples taken. However, there is a 47.3% chance that an isolated *S. aureus* may be a MRSA, and it is important that adequate provisions are made to install fully equipped hand washing sinks at vantage points all around the hospital and especially at the main OPD and the clinics. The study also revealed that both males and females stand an equal chance of being infected with MRSA and also becoming agents of its transmission to others. Children and adults also stand an equal chance of being infected or becoming agents of transmitting the bacteria to others. However, prevalence of MSSA was found to be significantly high among the children age group. This study provides first baseline epidemiological information on the prevalence of nasal carriage of *S. aureus* and MRSA among patients, visitors and personnel at the BARH.

6.2 RECOMMENDATION

It is strongly recommended that further genetic analysis are carried out on the identified MRSA isolates obtained to distinguish between Hospital acquired MRSA and community acquired MRSA strains for appropriate intervention protocols to be effected as already discussed.

REFERENCES

Abu-Rabie M.M., (2010). Prevalence of Methicillin – resistant *Staphylococcus aureus* nasal carriage among patients and healthcare workers in hemodialysis centers in North West Bank- Palestine. Dissertation, An-Najah National University

Ajantha G.S., Kulkarni R.D., Upadhya A.K., Kalabhavi A.S., Patil S.S., Shetty P.C., Shubhada R.M. and Jain P.A. (2011). Urinary tract infection in a tertiary care hospital with special reference to Methicillin Resistant *S. aureus* (MRSA). IJRRMS, **1**(1): 22-6. Retrieved from http://www.ijrrms.com/pdf/2011/oct-dec-11-pdf/05.pdf

Alabi S.A., Frielinghaus L., Kaba H., Kösters K., Huson M.A., Kahl B.C., Peters G., Grobusch M.P., Issifou S., Kremsner P.G. and Schaumburg F., (2013). Retrospective analysis of antimicrobial resistance and bacterial spectrum of infection in Gabon, Central Africa. BMC Infectious Diseases **13(**455):455: Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3849904/pdf/1471-2334-13-455.pdf

Anand K.B., Agrawal P., Kumar S. and Kapila K., (2009). Comparison of cefoxitin disc diffusion test, oxacillin screen agar, and PCR for *mec*A gene for detection of MRSA. Indian Journal of Medical Microbiology, **27**(1): 27-29

Benson H.J. (2001). Microbiological applications-laboratory manual in general microbiology, eighth edition, McGraw hill companies 1000–1018

Bhateja, P., Mathur, T., Pandya, M., Fatma, T. and Rattan A. (2005). Detection of vancomycin resistant *Staphylococcus aureus*: A comparative study of three different phenotypic screening methods. Indian Journal of Medical Microbiology; **23(**1) 52-55.

Boucher, H., Miller, L.G. and Razonable, R.R., (2010). Serious Infections Caused by Methicillin-Resistant *Staphylococcus aureus*. Clinical Infectious Diseases; **51**(S2):S183–S197.

Braga, E.D.V., Aguiar-Alves, F., Freitas, M.F., Silva, M.O., Correa, T.V., Snyder, R.E., Araujo, V. A, Marlow, M.A, Riley L.W., Setúbal, S., Silva, L.E. and Cardoso, C.A.A, (2014). High prevalence of *Staphylococcus aureus* and methicillin resistant *S. aureus* colonization among healthy children attending public daycare centers in informal settlements in a large urban center in Brazil. BMC Infectious Diseases **14**:538

Breurec, S., Fall, C., Pouillot, R., Boisier, P., Brisse, S., Diene-Sarr, F., Djibo, S., Etienne, J., Fonkoua, M. C.,. Perrier-Gros-Claude, J. D, Ramarokoto, C. E., Randrianirina F., Thiberge, J. M., Zriouil, S. B., the Working Group on *Staphylococcus aureus* infections, Garin, B. and Laurent F., (2011).

Epidemiology of methicillin-susceptible *Staphylococcus aureus* lineages in five major African towns: high prevalence of Panton–Valentine leukocidin genes. Clin Microbiol Infect **17**: 633–639

Brong Ahafo Regional Hospital, Sunyani (BARH), (2011). 2010 Annual Report, BARH Library: 1

Brown, D.F., Edwards, D.I., Hawkey, P.M., Morrison, D., Ridgway, G.L., Towner, K.J. and Wren, M.W. (2005). Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). Journal of Antimicrobial Chemotherapy; **56**(6):1000-18

Bukowski, M., Wladyka, B. and Dubin, G., (2010). Exfoliative Toxins of *Staphylococcus aureus*, Toxins **2:**1148-1165

Bustamante, N.D. (2011). MRSA: A global threat, MD thesis, UT Southwestern Medical School. Retrieved on 8th December, 2014 from http://www.utsouthwestern.edu/edumedia/edufiles/about_us/admin_offices/global_health/mrsa-bustamante.pdf

Cassoobhoy, A., (2015). Staph Infections and MRSA in Children: Prevention, Symptoms, and Treatment, Retrieved on 19th August, 2015, From http://www.webmd.com/children/mrsa-and-staph-infections-in-children

Cavalcanti, S.M.M., França E.R., Cabral C., Vilela M.A., Montenegro F., Menezes D. and Medeiros Â.C.R., (2005). Prevalence of *Staphylococcus aureus* Introduced into intensive care cnits of a university hospital, The Brazilian Journal of Infectious Diseases; **9**(1):56-63

Centre for Health Protection, (2011). Review of Staphylococcal Food

Poisoning in Hong Kong; Scientific Committee on Enteric Infections and Foodborne Diseases. Department of Health, Hong Kong.

Chambers H.F. and DeLeo F.R. (2009). Resistance: *Staphylococcus aureus* in the antibiotic era. Nat. Rev. Microbiol**.; 7**(9): 629–641.

Clements, A., Halton, K., Graves, N., Pettitt, A., Morton, A., Looke, D. and Whitby, M. (2008). Overcrowding and understaffing in modern health-care systems: key determinants in meticillin-resistant *Staphylococcus aureus* transmission, Lancet Infect Dis; **8**: 427–34

CLSI, (2012). Clinical and Laboratory Standards Institute, M02-A11, Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition, **32**:1.

CLSI, (2013). Clinical and Laboratory Standards Institute, M100-S23, Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement. **33:**1

Coello, R., Glynn, J.R., Gaspar, C., Picazo, J.J. and Fereres, J., (1997). Risk factors for developing clinical infection with methicillin-resistant *Staphylococcus aureus* (MRSA) amongst hospital patients initially only colonized with MRSA; J. Hosp. Infect.; **37**(1): 39-46.

Conceicao, T., Silva, I.S., Lencastre, H. and Aires-de-Sousa, M. (2014). *Staphylococcus aureus* nasal carriage among patients and health care workers in Sao Tome´ and Prıncipe. Microbial Drug Resistance. **20**(1):57-66.

Cummings, K.L., Anderson, D.J. and Kaye, K.S. (2010). Hand hygiene noncompliance and the cost of hospital-acquired methicillin-resistant *Staphylococcus aureus.* Infection Control and Hospital Epidemiology; **31**(4): 357-364

Egyir, B., Guardabassi, L., Nielsen, S.S., Larsen, J., Addo, K.K., Newman, M.J. and Larsen, A.R. (2013). Prevalence of nasal carriage and diversity of *Staphylococcus aureus* among inpatients and hospital staff at Korle Bu Teaching Hospital, Ghana, Journal of Global Antimicrobial Resistance; **1:**189–193

Egyir B., Guardabassi L., Sørum M., Nielsen S.S., Kolekang A., Frimpong, E., Addo, K.K., Newman M.J. and Larsen A.R.. (2014) molecular epidemiology and antimicrobial susceptibility of clinical *Staphylococcus aureus* from healthcare institutions in Ghana. PLoS ONE; **9**(2): e89716

Elixhauser, A., Friedman, B. and Stranges, E. (2011). Septicemia in U.S. hospitals, 2009. Healthcare cost and utilization project. Retrieved on 8th December, 2014 from http://www.hcupus.ahrq.gov/reports/statbriefs/sb122.pdf

Falagas, M.E., Karageorgopoulos, D.E., Leptidis, J. and Korbila, I.P. (2013). MRSA in Africa: Filling the Global Map of Antimicrobial Resistance, PLoS ONE; **8**(7):e68024

Gordon, R.J. and Lowy, F.D. (2008). Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clinical Infectious Diseases; **46**:S350–S359

Guleri, A., Kehoe, A., Hartley, J., Lunt, B., Harper, N., Palmer, R., Lickiss, J.,

Mawdsley, S. and Jones A. (2011). The costs and benefits of hospital MRSA screening. British Journal of Healthcare Management; **17**(2):64-71

Harris, L.G., Foster, S.J. and Richards, R.G. (2002). An introduction to *Staphylococcus aureus*, and techniques for identifying and quantifying *S. aureus* adhesins in relation to adhesion to biomaterials: review. European Cells and Materials; **4**:39-60.

Hart, I. (2004). A test for the early detection of MRSA: clinical benefits and financial savings, Oxoid Ltd, Basingstoke, Hants, UK. Retrieved from http://www.cli-online.com/fileadmin/pdf/pdf_general/a-test-for-the-earlydetection-of-mrsa-clinical-benefits-and-financial-savings.pdf

Herold, B.C., Immergluck, L.C., Maranan, M.C., Lauderdale, D.S., Gaskin, R.E., Boyle-Vavra, S., Leitch, C.D. and Daum R.S. (1998). Communityacquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA; **279**:593-598.

Hunt, C., Dionne, M., Delorme, M., Murdock, D., Erdrich, A., Wolsey, D., Groom, A., Cheek, J., Jacobson, J., Cunningham, B., Shireley, L., Belani, K., Kurachek, S., Ackerman, P., Cameron, S., Schlievert, P., Pfeiffer J., Johnson, S., Boxrud D.,, Bartkus, J., Besser, J.,Smith, K., LeDell, K., O'Boyle, C., Lynfield, R., White, K., Osterholm, M., Moore, K. and Danila, R. (1999). Four pediatric deaths from community-acquired methicillinresistant *Staphylococcus aureus*—Minnesota and North Dakota, 19971999. Journal of American Medical Association. **282**: 1123-1125.

Illinois Department of Public Health, (2007). Health beat; MRSA – MethicillinResistant *Staphylococcus aureus*, Retrieved 31st January, 2014, from http://www.idph.state.il.us/public/hb/hbmrsa.htm

Johnson, A.P., Aucken, H.M., Cavendish, S., Ganner, M., Wale, M.C.J., Warner, M., Livermore, D.M., Cookson, B.D. and the UK EARSS participants (2001). Dominance of EMRSA-15 and -16 among MRSA causing nosocomial bacteremia in the UK: analysis of isolates from the European Antimicrobial Resistance Surveillance System (EARSS). Journal of Antimicrobial Chemotherapy **48**:141-156

Karikari A.B. (2009). The prevalence and antibiotic susceptibility patterns of methicillin resistant *Staphylococcus aureus* in Kumasi, Thesis submitted to the department of clinical microbiology, School of medical sciences, Kwame Nkrumah University of science and Technology, Kumasi.

Kil E.H., Heymann W.R. and Weinberg J.M. (2007). Methicillin-Resistant *Staphylococcus aureus*: An Update for the Dermatologist, Part 1: Epidemiology, Continuing Medical Education, Cutis. **81**:247-254

Lanternier, F., Memain, N. and Lortholary, O. (2004). Pyomyositis, Orphanet encyclopedia. Retrieved on 8th December, 2014 from http://www.orpha.net/data/patho/GB/uk-pyomyositis.pdf

Laryea, D.O., Amoako Y.A. and Asamoah J. (2014). Antibiotic Sensitivity and Clinical Outcome for Methicillin Resistant *Staphylococcus aureus*. Online Journal of Public Health**;** **6**(1):e11. Retrieved from

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4050885/pdf/ojphi-06e11.pdf

Lowy, F.D. (1998). *Staphylococcus aureus* infections, The New England Journal of Medicine; **339**(8):520-532

Makgotlho P.E. (2009). Molecular characterisation of methicillin-resistant *Staphylococcus aureus* strains, University of Pretoria, Faculty of Health, department of Medical Microbiology. DOI: 10.1111/j.1574-695X.2009.00585.x

Marlowe, E.M., Linscott, A.J., Kanatani M. and Bruckner D.A. (2002). Practical Therapeutic Application of the Oxoid PBP2' Latex Agglutination Test for the Rapid Identification of Methicillin - Resistant *Staphylococcus aureus* in Blood Cultures, American Journal of Clinical Pathology; **118**: 287-291

McGrath, B., Rutledge, F. and Broadfield, E. (2008). Necrotising pneumonia, *Staphylococcus aureus* and Panton-Valentine leukocidin. Intensive Care Society **9**:170-172

Minnesota Department of Health, (2013). Recommendations for Prevention and Control of Methicillin- Resistant *Staphylococcus aureus* (MRSA) in Acute Care Facilities. Retrieved from http://www.health.state.mn.us/divs/idepc/diseases/mrsa/rec/rec.pdf

Monnet, D.L., Mackenzie, F.M., López-lozano, J.M., Beyaert, A., Camacho, M., Wilson R., Stuart, D. and Gould, I.M. (2004). Antimicrobial drug use and methicillin-resistant *Staphylococcus aureus,* Aberdeen, 1996–2000 Emerg Infect Dis; **10(**8): 1432-1441. Retrieved from

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3320421/pdf/02-0694.pdf

Moses, A., Uchenna, U. and Nworie O. (2013). Epidemiology of Vancomycin Resistant *Staphyloccus aureus* among clinical isolates in a tertiary hospital in Abakaliki, Nigeria, American Journal of Epidemiology and Infectious Disease, **1**(3):24-26

Muller, A.A., Mauny, F., Bertin, M., Cornette, C., Lopez-Lozano, J-M, Viel, J.F., Talon, D.R. and Bertrand X. (2003). Relationship between spread of methicillin-resistant *Staphylococcus aureus* and antimicrobial use in a French university hospital. Clin Infect Dis; **36:** 971–978.

Naber, C.K. (2009). *Staphylococcus aureus* bacteremia: Epidemiology, Pathophysiology, and Management Strategies, Infectious Diseases Society of America, CID 2009:**48(**4):S231-S237

Naik, D. and Teclu, A. (2009). A study on antimicrobial susceptibility pattern in clinical isolates of *Staphylococcus aureus* in Eritrea. The Pan African Medical Journal, **3(**1):1-5. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2984300/pdf/pamj-03-01.pdf

Naing, L., Winn, T. and Rusli, B.N. (2006). Practical issues in calculating the sample size for prevalence studies, Archives of Orofacial Sciences, **1**:9-14

Nester, E.W. anderson, D.G., Roberts, C.E. and Nester, M.T. (2009). Microbiology: a human perspective, Sixth edition, The McGraw-Hill Companies. Pp 537

Newman, M.J., Frimpong, E., Donkor, E.S., Opintan, J.A. and Asamoah-Adu, A. (2011). Resistance to antimicrobial drugs in Ghana, Infection and Drug Resistance, Dove Press **4**:215–220.

Obasuyi, O. (2013). Molecular identification of methicillin-resistant *Staphylococcus aureus* in Benin City Nigeria. Afr. J. Cln. Exper. Microbiol; **14**(1): 1-4

Odonkor, S.T. and Addo, K.K. (2010). Evaluation of three methods for detection of methicillin resistant *Staphylococcus aureus* (MRSA). International Journal of Biological & Medical Research; **2**(4):1031 – 1034

Odonkor, S.T., Newman, M.J. and Addo, K.K. (2012). Prevalence and antibiotic susceptibility profile of methicillin resistant *Staphylococcus aureus* in Accra, Ghana, Microbiology Research **3**(e20): 84-87

Ogston, A. (1984). "On Abscesses". Classics in Infectious Diseases". Rev Infect Dis; **6** (1): 122–28.

Oliveira, L., Rodrigues, A.C., Hulland, C. and Ruegg, P.L. (2011). Enterotoxin production, enterotoxin gene distribution, and genetic diversity of *Staphylococcus aureus* recovered from milk of cows with subclinical mastitis. J Vet Res; **72**:1361–1368.

Omuse, G., Kariuki, S. and Revathi, G. (2012). Unexpected absence of meticillin-resistant *Staphylococcus aureus* nasal carriage by healthcare workers in a tertiary hospital in Kenya. Journal of Hospital Infection **80**: 71-73

Onanuga, A., Oyi, A.R. and Onaolapo, J.A. (2005). Prevalence and

susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolates among healthy women in Zaria, Nigeria. African Journal of Biotechnology; **4**(11):1321-1324.

Pinho, M.G., Lencastre, H. and Tomasz, A. (2011). An acquired and a native penicillin-binding protein cooperate in building the cell wall of drugresistant staphylococci. Proceedings of the National Academy of Sciences of the United States. PNAS; **98**(19):10886–10891.

Plata, K., Rosato, A.E and Węgrzyn, G. (2009). *Staphylococcus aureus* as an infectious agent: overview of biochemistry and molecular genetics of its pathogenicity, Acta biochimica polonica; **56**(4):597–612

Randrianirina, F., Vaillant, L., Ramarokoto, C.E., Rakotoarijaona A., Andriamanarivo M.L., Razafimahandry, H.C., Randrianomenjanahary, J., Raveloson Jr., Ratsima H.E., Carod J.F., Talarmin, A. and Richard V. (2007). Antimicrobial resistance in pathogens causing nosocomial infections in surgery and intensive care wards in Antananarivo, Madagascar. JIDC 4(2): 74–82

Regional Hospital-Sunyani (2013) Retrieved 13th May, 2015, from http://archive-org.com/page/3139945/2013-11-06/http://www.ghanahealthservice.org/SHP/overview.html

Reischl, U., Linde, H., Metz, M., Leppmeier, B. and Lehn, N. (2000). Rapid identification of methicillin-resistant *Staphylococcus aureus* and simultaneous species confirmation using real-time fluorescence PCR, Journal of clinical microbiology; **38**(6):2429–2433

Reuters, (2013) Pfizer takes its shot at a vaccine for evasive MRSA superbug.

Retrieved 5th August, 2013, From: http://www.foxnews.com/health/2013/05/23/pfizer-takes-its-shot-atvaccine-for-evasive-mrsa-superbug/

Rongpharpi S.R., Hazarika N.K. and Kalita H.,(2013). The prevalence of nasal carriage of *Staphylococcus aureus* among healthcare workers at a tertiary care hospital in assam with special reference to MRSA, Journal of Clinical and Diagnostic Research. **7**(2):257-260.

Roos, R. (2013). MRSA dropping in hospitals; elsewhere, not so much, Center for Infectious Disease Research and Policy (CIDRAP). Retrieved from http://www.cidrap.umn.edu/news-perspective/2013/04/mrsa-droppinghospitals-elsewhere-not-so-much. on 12th April, 2013

Rubinstein E., Kollef M.H. and Nathwani D. (2008). Pneumonia caused by methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis; **46**(5): S378S385.

Rutala, W.A., Weber, D.J. and the Healthcare Infection Control Practices Advisory Committee (HICPAC). (2008). Guideline for Disinfection and Sterilization in Healthcare Facilities, Centers for Disease Control and Prevention, Retrieved on 1st August, 2014 from www.cdc.gov/hicpac/pdf/guidelines/Disinfection_Nov_2008.pdf

Ryan, K.J. and Ray, C.G. (2004). Sherris medical microbiology: An introduction to infectious diseases. 4th edn. McGraw Hill Publishers. Pp 276-282

Sackey J. (2012). Korle Bu battles outbreak of deadly bacteria - Local News. Retrieved on 8th December, 2012, from

http://edition.myjoyonline.com/pages/news/201201/80725.php

Sakoulas, G. and Moellering, R.C. (2008). Increasing Antibiotic Resistance among Methicillin- Resistant *Staphylococcus aureus* Strains. Clinical Infectious Diseases **46:**S360–367.

Schenfeld, L. A. (2000). Staphylococcal Scalded Skin Syndrome. Conemaugh Memorial Medical Center, U.S. Retrieved on 24th June, 2015, from http://www.uv.es/~vicalegr/CLindex/Clpiodermitis/ssss.htm

Sotto A., Lina G., Jean-Louis R., Combescure C., Bourg G., Vidal L., Jourdan, N., Etienne, J. and Lavigne., J.P. (2008). Virulence potential of *Staphylococcus aureus* strains isolated from diabetic foot ulcers: a new paradigm. Diabetes Care; **31**:2318–2324.

SPSS Inc. (2008). SPSS Statistics for Windows, Version 17.0. Chicago, U.S.A

StatPac Inc. (2013). Statistics calculator version 4.0, Evaluation version, Bloomington, U.S.A

Stefania, S., Chung, D.R., Lindsay, J.A., Friedrich, A.W., Kearns, A.M., Westh, H. and MacKenzie, F.M. (2011). Meticillin-resistant *Staphylococcus aureus* (MRSA): Global epidemiology and harmonisation of typing methods. International Journal of Antimicrobial Agents; **39**:273– 282

The Center for Food Security & Public Health, (2011). Methicillin Resistant *Staphylococcus aureus*: MRSA, Iowa State University, College of Veterinary Medicine. Retrieved on 8th December, 2014 from

http://www.cfsph.iastate.edu/Factsheets/pdfs/mrsa.pdf.

The Children's Act, (1998). Act 560-Act of the Parliament of the Republic of Ghana, Retrieved on 20th August, 2015; From www.unesco.org/.../f7a7a002205e07fbf119bc00c8bd3208a438b37f.pdf

TMIAC-CDPH, (1993). The MRSA Interagency Advisory Committee in conjunction with the Connecticut Department of Public Health, Guidelines for Management of Patients with Methicillin-Resistant *Staphylococcus aureus* in acute care hospitals and long term care facilities. Retrieved from http://www.ct.gov/dph/lib/dph/infectious_diseases/pdf_forms_/mrsa_guidelines%5B1%5D.pdf

Tiwari, H.K., Sapkota, D. and Sen, M.R. (2008). High prevalence of multidrugresistant MRSA in a tertiary care hospital of northern India. Infection and Drug Resistance; **1**:57–61

UK Standards for Microbiology Investigations, (2014). Investigation of Specimens for Screening for MRSA, Public Health England (PHE). Bacteriology **29**(6):1-25. Retrieved from https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/343948/B_29i6.pdf

Vorvick, L.J. (2012). Folliculitis, A.D.A.M. Medical Encyclopedia, Retrieved on 24th June, 2015, from http://www.nlm.nih.gov/medlineplus/ency/article/000823.htm

Vyas, J.M. (2014) Sepsis: A.D.A.M. Medical Encyclopedia Retrieved 3rd February, 2014, from http://www.nlm.nih.gov/medlineplus/ency/article/000666.htm

Wald, E.R. (2012). *Staphylococcus aureus*: Is It a Pathogen of Acute Bacterial Sinusitis in Children and Adults? Clinical Infectious Diseases; **54**(6):826–831.

Wertheim, H.F.L, Melles, D.C, Vos, M.C, Leeuwen, W.V, Belkum, A.V, Verbrugh, H. A, Nouwen, J.L (2005). The role of nasal carriage in *Staphylococcus aureus* infections, Lancet Infect Dis; **5:**751–762

WHO, (2012). World Health Organization, Hand hygiene in outpatient and home-based care and long-term care facilities: Guide to the application of the who multimodal hand hygiene improvement strategy and the "my five moments for hand hygiene" approach, WHO Document Production Services, Geneva, Switzerland.

WHO, (2014). World Health Organization, Antimicrobial resistance global report on surveillance, WHO Press, Geneva, Switzerland.

Zurita, J., Mejía, C. and Guzmán- Blanco, M. (2010). Diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* in Latin America. Braz J Infect Dis 2010; **14**:S97-S106.