KWAME NKRUMAH UNIVERSITY OF SCIENCE AND

TECHNOLOGY KUMASI

SCHOOLOF GRADUATE STUDIES

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF CLINICAL MICROBIOLOGY

THE PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF METHICILIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN KUMASI

AKOSUA BONSU KARIKARI

APRIL, 2009

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY KUMASI

SCHOOLOF GRADUATE STUDIES

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF CLINICAL MICROBIOLOGY

THE PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF METHICILIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN KUMASI

A THESIS SUBMITTED TO THE DEPARTMENT OF CLINICAL MICROBIOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE MASTER OF SCIENCE DEGREE (MSC.) IN CLINICAL MICROBIOLOGY

BY

AKOSUA BONSU KARIKARI

APRIL, 2009

DECLARATION

I hereby declare that this submission is my own work towards the M.Sc (Clinical Microbiology) 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.

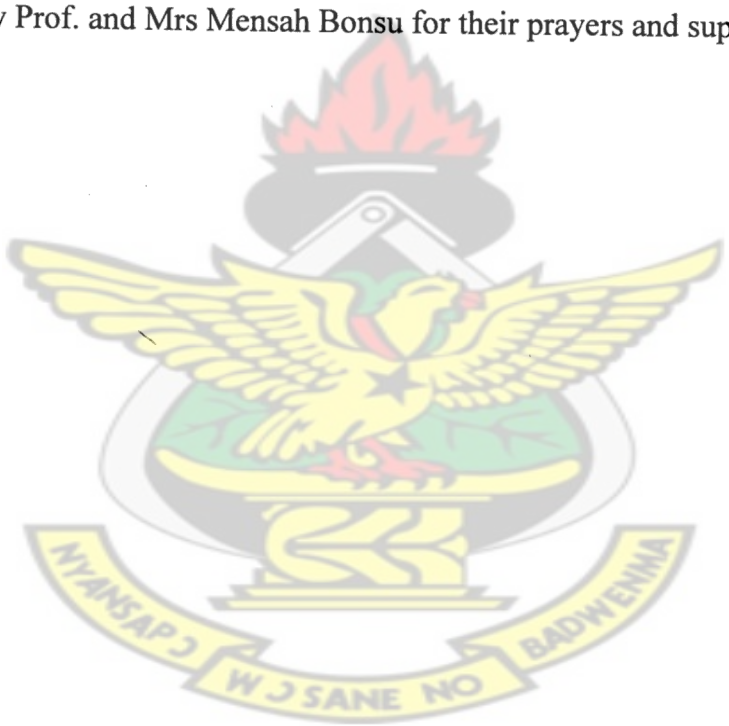
AKOSUA BONSU KARIKARI Signature..... Date: 12/12/08
(STUDENT)

PROFESSOR E.H. FRIMPONG Signature..... Date: 25/04/09
(SUPERVISOR)

PROF. YAW ADU SARKODIE Signature..... Date..... 24.04.09
(HEAD OF DEPARTMENT)

AKNOWLEDGEMENTS

I am grateful to Prof. E.H Frimpong whose excellent supervisory role and support has led to the successful completion of this work. I thank all the staff at the microbiology department of KATH, especially Mr R.A Lartey for his immense support and contributions to this work. The kind assistance of Dr. Alex Owusu-Ofori, Head of Diagnostics; KATH is appreciated. My thanks again go to all the doctors, nurses and health aids in the various departments, who assisted in the filling and collection of questionnaire forms. I also wish to express my profound gratitude to Rev Prof. and Mrs Mensah Bonsu for their prayers and support.



DEDICATION

I dedicate the work to the Almighty God for giving me the strength and grace to complete the work. This work is also dedicated to my parents, Mr. and Mrs. Karikari Danquah for their support and prayers.

TABLE OF CONTENT

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Methicillin resistant *Staphylococcus aureus* (MRSA) poses a serious therapeutic problem worldwide, however data on prevalence and antibiotic susceptibility patterns is lacking in Africa and for that matter Ghana. This study was aimed at determining the prevalence and antibiotic susceptibility patterns of MRSA in Kumasi.

MRSA was diagnosed using 1µg oxacillin discs and 10 unit penicillin, on a total of 250 clinical isolates of *S. aureus*. Susceptibility of MRSA isolates to penicillin, ampicillin, cotrimoxazole, flucloxacillin, erythromycin, tetracycline, gentamicin and cefuroxime were determined using modified Kirby-Bauer disc diffusion method. The MIC of 50 MRSA isolates was determined using E-test (AB-Biodisk, Solna, Sweden).

The study determined a prevalence rate of 34.8%, (hospital acquired (HA) MRSA was 26.8% (67), and community acquired (CA) was 8%- though not statistically significant), compared with the rate of 12.1% reported in 2004. Penicillin, ampicillin, cotrimoxazole, tetracycline, gentamicin and flucloxacillin showed resistance rates ranging from 50-100% but cefuroxime and erythromycin, showed resistance of below 50%.

The MIC 0f 50 isolates tested were as follows: oxacillin; 4 - ≥256µg/ml, gentamicin; 0.125 - ≥256µg/ml, trimethoprim sulfamethoxazole; 0.064 - ≥32µg/ml and ceftriaxone; 1.5 -≥32µg/ml.

The study showed that the problem of MRSA was urgent. To reduce the prevalence of MRSA, regular surveillance of hospital and community associated infections, monitoring of antibiotic susceptibility patterns and formulation of definitive antibiotic policy may be helpful. Several measures to help reduce the spread of Staphylococci have been discussed.

CHAPTER ONE

1.0 INTRODUCTION

Staphylococcus aureus is a Gram-positive, catalase positive, coagulase positive, non-motile bacterium that causes a variety of human infections in all age groups (Boyce et al, 1981). It has emerged as one of the most important human pathogens and has over the past several decades, been a leading cause of hospital and community-acquired infections (Lowy, 1998). They live as commensals in the anterior nares of over half the population of humans (Doig, 1981). They are spread from these sites into the environment by the hands, handkerchief, clothing and dust (Burnett et al, 1996). It is associated with a variety of clinical infections including septicaemia, pneumonia, wound sepsis, septic arthritis, and osteomyelitis and post surgical toxic shock syndrome with substantial rates of morbidity (Klodkowaska-Farner et al, 1995)

Antibiotic resistance in *S. aureus* was almost unknown when penicillin was first introduced in 1943. By 1950; 40% of hospital *S. aureus* isolated were penicillin resistant and by 1960, this had risen to 80 % (Chambers, 2001). *Staphylococcus aureus* resistance to penicillin is mediated by penicillinase; a form of β-lactamase production; an enzyme, which breaks down the β-lactam ring of the penicillin molecule. Penicillinase-resistant penicillins such as methicillin, oxacillin, cloxacillin, dicloxacillin and flucloxacillin are able to resist degradation by *S. aureus* penicillinase. These antibiotics were developed to treat penicillin resistant S. *aureus* and are still used as first line treatment (Jevons, 1961).

Methicillin was the first antibiotic in this class to be used. It was introduced in 1959, but only two years later the first case of methicillin resistance *S. aureus* (MRSA) was reported in England (Jevons, 1961). Despite this, MRSA generally remained an uncommon finding even in hospital settings until the 1990s when there was an explosion in prevalence in hospitals where it is now endemic (Johnson, 2001).

MRSA infections in both the hospital and community setting are commonly treated with non β-lactam antibiotics such as clindamycin (a lincosamine) and co-trimoxazole. Resistance to these has also lead to the use of new broad- spectrum anti-Gram positive antibiotics such as linezolid. Those strains resistant to methicillin and related penicillins are particularly difficult to treat because they are resistant to most other common antibiotics such as streptomycin, tetracycline, chloramphenicol, lincomycin etc. First line treatment for serious invasive infections due to MRSA is currently glycopeptide antibiotics; vancomycin and teicoplanin (Blot et al, 2002). Recent reports describing the therapeutic failure of vancomycin for MRSA infections have aroused considerable concern regarding the emergence of MRSA strains for which there will be no effective therapy (Hiramatsu et al, 1997).

Methicillin – resistant *Staphylococcus aureus* (MRSA) was first reported in 1961 and was recognized as a nosocomial pathogen by the late 1960s (Jevons, 1961). Known MRSA risk factors include recent surgery or hospitalization, presence of indwelling catheter, or recent dialysis (Brumfit, 1989). Hospital acquired *Staphylococcal* infections (HA) MRSA are common in newborn babies, surgical patients and hospital staff (Tuo et al, 1995). In

the 1980s, MRSA infections were reported in persons who lacked traditional MRSA risk factors. The Centres for Disease Control and Prevention (CDC), Active Bacterial Core Surveillance Program defined a CA-MRSA case as a patient with an MRSA infection and no history of the following: surgery, hospitalization, within the year before infection, presence of a percutaneous device or indwelling catheter, dialysis within the previous year, hospitalization for more than 48 hrs before MRSA culture or previous MRSA infection (Jessica et al, 2003). These infections appeared to be acquired in the community and are now known as community- associated (CA) MRSA infections. These infections have been reported worldwide (Saravolatz et al, 1982). Outbreaks have occurred in many settings and among different populations (CDC, 2003). The most common clinical manifestations of CA- MRSA are skin and soft tissue infections such as abscesses and cellulitis (Naimi et al, 2003). Less commonly, CA-MRSA can cause severe disease such as necrotizing pneumonia, osteomyelitis and septicaemia (Herold et al, 1998).

MRSA is at present the most commonly identified antibiotic-resistant pathogen in many parts of the world, including Europe, the Americas, North Africa, the Middle East and East Asia in contrast with the assumptions that predicted little or no in vivo relevance of the methicillin-resistant phenotype in the 1960s (Fridkin et al, 2002). Moreover, MRSA rates have been swiftly increasing worldwide over the past decades, as data from continuing surveillance initiatives such as the National Nosocomial Infection Surveillance System and European Antimicrobial Resistance Surveillance System show (Fridkin et al, 2002).

The prevalence rates in most African countries have not been reported. However, a study of the prevalence and antibiotic susceptibility patterns of MRSA in eight large hospitals in Africa and Malta was undertaken from 1996 to 1997. It was revealed that the prevalence rate ranged from 21% to 30% in Nigeria, Kenya and Cameroon and rates of below 10% in Tunisia, Malta and Algeria (Kesah et al, 2003). Earliest reports on MRSA in South Africa on the antimicrobial susceptibility patterns and characterization of *S. aureus* in Kwa-Zulu-Natal province revealed a prevalence of 26.9%. The majorities i.e. more than 60% of the MRSA strains were multi resistant. There is therefore the need to maintain surveillance and control of MRSA infections in Africa (Kesah et al, 2003).

In Ghana no comprehensive research has been done on MRSA, as such there is no publication on it. A B.Sc. project carried out in 2004 on the prevalence rate of MRSA at the Komfo Anokye Teaching Hospital (KATH) indicated 12.1% rate out of a sample size of 132 (Kyei, 2004).

Antimicrobial sensitivity testing can be performed using the dilution technique and disc diffusion technique (Cheesbrough, 2000). Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial sensitivity (Cheesbrough, 2000). The WHO recommends the CLSI formerly National Committee for Clinical Laboratory Standards (NCCLS) modified Kirby-Bauer disc diffusion technique for clinical and surveillance purposes, and to promote reproducibility and comparability of results between laboratories. Stokes disc diffusion technique is not as highly standardized as the Kirby Bauer technique and is used in the laboratories particularly when the exact amount of

antimicrobial in a disc can not be guaranteed due to difficulties in obtaining discs and storing them correctly or when the other conditions required for the Kirby Bauer technique can not be met (Cheesbrough, 2000).

Current antimicrobial susceptibility testing methods are based either on quantitative dilution techniques or qualitative diffusion procedures. Dilution methods have the ability to detect certain resistance patterns that may not be detected by disc diffusion or automated systems (Sahm et al, 1989). They are not routinely applied to all microorganisms but rather are used in unusual situations. Results of these tests may aid in determination of optimal antimicrobial therapy, elucidation of resistant mechanism, or epidemiologic analysis of resistant isolates (Charles et al, 1982). They are used both in the clinical setting and in research. In research, they are most often used to predict antimicrobial dose responses (Charles et al, 1982).

Dilution methods are based on two-fold serial dilutions of antibiotics in broth or agar media. These methods generate the MIC value i.e. minimum inhibitory concentration of a given antibiotic in µg/ml that will inhibit the growth of a particular bacterium under defined experimental conditions. The MIC value is not an exact entity and the true MIC is between the lowest concentration that inhibits the organism's growth and the next lower concentration (Baker, 1991).

Epsilometer test (E-test) is a useful addition to the array of diagnostic procedures and consist of antimicrobial agent-impregnated strips that are placed on the surface of agar.

The antimicrobial agent content of the strips is graded and the concentration is printed linearly along the strip (Hamilton-Miller, 1995). The concentration range of the antimicrobial in the E-test strip corresponds to two-fold dilutions in a conventional MIC method (Jorgensen, 1991).

Traditionally confirmation of *S. aureus* is performed using the slide coagulase test (clumping factor) and the tube coagulase test (free coagulase). Agglutination kits are also available and can be used to confirm *S. aureus* by detecting protein A and clumping factor. Newer agglutination kits now work by also detecting surface antigen. Other latex kits detect PBP2a, which occurs within the cell membrane and requires lysis of the cells for detection. Commercial biochemical test systems such as the API Staph-Ident system and DMS Staph Trac (Analytab products), Vitek system may also be used for routine identification of *S. aureus* in certain clinical laboratories (Almeida, 1983).

Laboratory screening for MRSA is a complex balance between speed of result, sensitivity, specificity and cost. Of the penicillinase-stable penicillins, oxacillin is preferred for in-vitro testing. However the cefoxitin disc test is another preferred method for testing *S. aureus* for resistance to the penicillinase-stable penicillins (CLSI, 2006). MRSA have also been identified by using DNA probes, peptide nucleic acid probes, gel-based PCR, and real-time PCR (Chung et al, 2004). However, such molecular assays are associated with specialized equipment and expertise, increased cost, and specific laboratory organization into pre- and post amplification areas (Oliveira et al, 2002). There have also been a number of developments in bioluminescence. Currently the majority of

MRSA screening is carried out using plate based methods such as Mannitol Salt Agar containing 7% NaCl with either 4mg/L methicillin or 2mg/L oxacillin; Oxacillin Resistant Screening Agar with 5.5% NaCl and 2mg/L oxacillin; Baird Parker Medium with 8mg/L ciprofloxacin; Mueller Hinton Agar with 4% NaCl and 6mg/L oxacillin. DNase plates can also be used in addition. (CLSI, 2006)

1.1 STATEMENT OF THE PROBLEM

MRSA has assumed increasing importance internationally as a cause of both nosocomial and community acquired infections. However, in Africa, and specifically Ghana, current data are lacking on MRSA as proved by searches in the Pubmed medical and Google academia have not generated any papers.

1.2 JUSTIFICATION

Today, MRSA is the most common drug resistant bacteria in North America, Europe, North Africa, the Middle East and East Asia (Fridkin et al, 2002). MRSA is now a huge burden in addition to methicillin susceptible *S. aureus* and it is by far the most significant antibiotic resistant hospital acquired pathogen that has ever been encountered (Diekema et al, 2004). As with other multiresistant organisms, MRSA infections are associated with high mortality, prolonged illness and extended hospital stay as well as greater potential for further infections with even more resistant strains including death (Mackenzie et al, 2002).

The situation in many countries in Africa is not well established. Continuous surveillance of drug resistance in MRSA in Sub-Saharan Africa is imperative for the detection of emerging trends and the development of appropriate therapeutic schedules. There is therefore the need to maintain surveillance and control of MRSA infections in Africa. (Kesah et al, 2003).

In view of these the study hopes to come up with the prevalence of hospital and community acquired MRSA infections, the MIC of some of the antibiotics used in treating *S. aureus* infections at Komfo Anokye Teaching Hospital. This will add to the information and knowledge on MRSA situation in Kumasi.

1.3 OBJECTIVES

General Objectives;

- To provide data on MRSA in Kumasi.

Specific Objectives

- To establish the prevalence rate of MRSA in Kumasi
- To determine the antibiotic resistance patterns of the MRSA isolates using Kirby Bauer disc diffusion method and determination of MIC.
- To categorize MRSA into hospital acquired and community acquired MRSA.

CHAPTER TWO

2.0 LITERATURE REVIEW

Members of the genus *Staphylococcus* are gram-positive cocci (0.5 to 1.5μm) that occur singly and in pairs, tetrads, short chains and irregular grape-like clusters (Kloos et al, 1985). Ogston introduced the name *Staphylococcus* for the group of micrococci causing inflammation and suppuration (Ogston, 1883). *Staphylococci* are non motile, non-sporeforming, and usually catalase positive. The catalase test is useful to distinguish *Staphylococci* from Enterococci and Streptococci (Ryan et al, 2004). They are usually unencapsulated or have limited capsule formation (Kloos et al, 1985). *Staphylococcus aureus* grows in large, round, opaque colonies at an optimum temperature of 37°C, though it can grow anywhere between 10°C and 46°C. The colonies are usually large, smooth, and translucent. The colonies of most strains are pigmented, ranging from cream-yellow to orange after 18-24 hours of incubation (Talaro et al, 1993).

The species is a facultative anaerobe, and growth is enhanced in the presence of oxygen and carbon dioxide. It is non fastidious, its nutrient requirement can be satisfied by routine laboratory media. Most strains are metabolically versatile, that is, they can digest proteins and lipids and ferment a variety of sugars. This species is considered the most resistant of all non- spore forming pathogens, with well developed capacities to withstand high salt (7.5% - 10%), extremes pH, and high temperatures up to 60°C for 60 minutes (Talaro et al, 1993). The traditional marker for identifying *S. aureus* is *the* coagulase test. It differentiates *S. aureus* from most other *Staphylococci. S. aureus is* coagulase -positive, while most other *Staphylococci* species are coagulase-negative.

Nearly all strains of *S. aureus* produce the enzyme coagulase (Ryan et al, 2004).

2.1 GENETIC COMPOSITION OF *S. AUREUS*

S. aureus has about 2,600 genes and 2.8 million bp of DNA in its chromosome. Plasmids can also comprise part of the species' genome. The Staphylococcal cell wall is 50 percent peptidoglycan by weight. Peptidoglycan consists of alternating polysaccharide subunits of *N*-acetylglucosamine and *N* acetylmuramic acid with 1, 4- linkages. The peptidoglycan chains are cross-linked by tetrapeptide chains bound to *N*-acetylmuramic acid and by a pentaglycine bridge specific for *S. aureus*. Peptidoglycan may have endotoxin-like activity, stimulating the release of cytokines by macrophages, activation of complement, and aggregation of platelets. Differences in the peptidoglycan structure of *Staphylococcal* strains may contribute to variations in their capacity to cause disseminated intravascular coagulation (Kessler et al, 1991). Ribitol teichoic acids covalently bound to peptidoglycan are major constituents of the cell wall. Lipoteichoic acid is a glycerol phosphate polymer linked to a glycolipid terminus anchored in the cytoplasmic membrane (Kessler et al, 1991).

2.2 PATHOGENESIS OF *S. AUREUS*

S. aureus strains can express a wide array of potential virulence factors, including surface proteins that promote adherence to damaged tissues (Foster et al, 1998) bind proteins in blood to help evade antibody-mediated immune responses (Foster et al, 1998), and promote iron uptake (Mazmanian, 2003). The organism also expresses a number of membrane-damaging toxins and super antigen toxins that can cause tissue damage and the

symptoms of septic shock, respectively (Bohach et al, 1999). There is a growing realization that *S. aureus* has multiple mechanisms for evading both innate immunity mediated by polymorphonuclear leukocytes (Fedtke et al, 2004) and induced immunity mediated by both B and T cells (Goodyear et al, 2003). Some virulence factors are expressed by genes that are located on mobile genetic elements called pathogenicity islands e.g., toxic shock syndrome toxin–1 and some enterotoxins; or lysogenic bacteriophages e.g., Panton-Valentine leucocidin [PVL]; (Novick, 2003) and factors associated with suppressing innate immunity such as the chemotaxis inhibitory protein and staphylokinase which are integrated in the bacterial chromosome (de Haas, 2004).

2.3 EPIDEMIOLOGY OF *S. AUREUS.*

S. aureus is a common commensal of humans and its primary habitat is the moist squamous epithelium of the anterior nares (Peacock et al, 2001). About 20% of the population is always colonized with *S. aureus*, 60% are intermittent carriers, and 20% never carry the organism (von Eiff et al, 2001). It can survive on domesticated animals such as dogs, cats and horses, and can cause bumble foot in chickens. It can survive for some hours on dry environmental surfaces (Curran et al, 1980). Outbreaks may result from exposure to a single long-term carrier or environmental sources, but these modes of transmission are less common (Sheretz et al, 1996).

Humans are the natural reservoir for *S. aureus*, and asymptomatic colonization is far more common than infection. Colonization of the nasopharynx, perineum, or skin, particularly if the cutaneous barrier has been disrupted or damaged, may occur shortly

after birth and may recur anytime thereafter (Payne et al, 1966). Family members of a colonized infant may also become colonized. Transmission occurs by direct contact to a colonized carrier. Carriage rates are 25% to 50%; higher rates than in the general population are observed in injection drug users, persons with insulin- dependent diabetes, patients with dermatologic conditions, patients with long-term indwelling intravascular catheters, and health-care workers (Wadlvogel, 2000). Young children tend to have higher colonization rates, probably because of their frequent contact with respiratory secretions (Adcock et al, 1998). Colonization may be transient or persistent and can last for years (Sanford et al, 1994).

2.4 INFECTIONS OF *S. AUREUS*

S. aureus is the causative agent of a wide variety of infections in humans including diseases of the skin and soft tissues. Pustules, impetigo as well as more serious infections such as bacteraemia, osteomyelitis, renal abscess, pneumonia, endocarditis, meningitis, gastroenteritis, food poisoning, and toxic shock syndrome are among these diseases (Kloos et al, 1986). It can infect other tissues when normal barriers have been breached (e.g. skin or mucosal lining). This leads to furuncles (boils) and carbuncles. In infants *S. aureus* infection can cause a severe disease known as Staphylococcal Scalded Skin Syndrome (SSSS) (Curran et al, 1980). It is the major causative agent in surgical wound infections and epidermal skin diseases in newborn infants (Baldwin et al., 1957). *S. aureus* infection may also be superimposed on superficial dermatological diseases such as eczema, pediculosis and mycosis (Kloos et al, 1995).

S. aureus infections can be spread through contact with pus from an infected wound, skin-to-skin contact with an infected person, and contact with objects such as towels, sheets, clothing, or athletic equipment used by an infected person. Prosthetic joints put a person at particular risk for septic arthritis, Staphylococcal endocarditis and pneumonia, which may be rapidly fatal (Talaro et al, 1993).

2.5 EVOLUTION OF ANTIBIOTIC RESISTANCE IN *S. AUREUS* (MRSA)

One of the reasons for the success of this human pathogen is its great variability, occurring at different periods and places with diverse clonal types and antibiotic resistant patterns within regions and countries (Jevons, 1961). Antibiotic resistance in *S. aureus* was almost unknown when penicillin was first introduced in 1943 (Chambers, 2001). Shortly after the introduction of benzyl-penicillin into clinical use in the early 1940s, isolates of *S. aureus* were found that were resistant to penicillin, owing to the production of β-lactamase. Under the selective pressure of increasing penicillin usage, the proportion of *S. aureus* that were penicillin-resistant increased, such that by 1948, over 50% of isolates in many hospitals were resistant (Barber et al, 1948).

As part of the strategy for combating penicillin-resistant *S. aureus,* a series of semi-synthetic penicillin derivatives that were stable to *Staphylococcal* β-lactamase were developed and introduced into clinical use during the1960s (Rolinson, 1998). The first of these was methicillin, followed by the isoxazolyl penicillins; oxacillin, cloxacillin, dicloxacillin and flucloxacillin. The latter agents were not only more active against penicillin-resistant Staphylococcal than methicillin, but had the advantage of being

suitable for oral administration. The first isolate of methicillin resistant *S. aureus* (MRSA) was reported from the UK in 1961, the year after the drug was introduced (Jevons, 1961).

They were first reported in Australia in 1966 in the eastern states and in the United States in 1968 (Lee et al, 1997). Subsequent work showed that the resistance to methicillin was mediated by expression of a novel penicillin-binding protein with low binding affinity not only for methicillin, but for all licenced β-lactams (Brown et al, 1980). Although methicillin is no longer used in clinical practice, having been superseded by the isoxazolyl penicillins, particularly flucloxacillin in the UK, the acronym MRSA has continued to be used when referring to *S. aureus* resistant to these agents (Marples et al, 1992). The isolate of MRSA reported in 1961 was the only one found among about 5000 isolates examined, and MRSA remained uncommon in the UK for several years thereafter. However, their prevalence gradually increased in the late 1960s, and by 1971 they comprised of 5% of *S. aureus,* referred to the Staphylococcal Reference Laboratory (Marples et al, 1992). There was a subsequent decline in their prevalence in the mid-1970s, possibly owing to increased prescribing of aminoglycosides, to which many MRSA were susceptible to at that time. However, by the late 1970s outbreaks owing to gentamicin- resistant MRSA were seen in a number of hospitals (Shanson, 1981).

2.6 GENETIC BASIS OF METHICILLIN RESISTANCE

MRSA continues to be a major cause of serious infection to man, both in hospitals and in the community (Shanson, 1981). Until the early 1980s, MRSA reports consisted of

isolated cases, later in 1982 epidemic MRSA strains (EMRSA) were described as multi-resistant strains with special capacity to colonize patients and hospital staff and cause widespread outbreaks of infections. These epidemic MRSA strains have subsequently spread to various parts of the world (Pavillard et al, 1982). ß-lactam antibiotics such as methicillin inactivate penicillin binding proteins 1, 2 and 3 (PBPs 1, 2 and 3) by the acylation of the catalytic site of the PBP. The PBPs occur in the bacterial cell wall and have an enzymatic role in the synthesis of peptidolgycan. PBPs normally possess a high affinity for ß-lactam antibiotics; in MRSA this affinity is reduced resulting in antibiotic resistance (Matsuhashi et al, 1986).

MRSA carry the mecA gene which encodes an additional low-antibiotic affinity penicillin binding protein, known as PBP2a (Matsuhashi et al, 1986). MRSA strains have emerged by acquisition of mobile genetic elements called SCC*mec* cassettes, which carry the *mecA* gene that encodes PBP2a. There are 5 different cassettes (SCC*mec* types I–V (Ito, 2004). It is now clear that major MRSA clones were created on multiple occasions by acquisition of SCC*mec* by prevalent strains that have continued to flourish (Enright et al, 2003)).

The origins and mechanism of transfer of SCCmec are still unclear and so far no bacterial isolates of any other genera have been reported to carry this element. A mecA homologue, ubiquitous in the antibiotic-susceptible animal species *S. sciuri* was a possible evolutionary precursor of the mecA of the MRSA strains (Wu et al, 1996).

Health-care associated and community-acquired MRSA strains have been proved

genetically distinct with respect to the SCCmec type they contain. Although some epidemic nosocomial MRSA clones contain SCCmec type IV, most health-care associated MRSA strains carry one of three types of SCCmec (type I, II, or III) (Enright et al, 2003), whereas most community-acquired MRSA strains carry SCCmec typeIV (Vandenesch et al, 2003). Type V has also been identified in a community-acquired isolate (Vandenesch et al, 2003). The extreme heterogeneity of the genetic backgrounds in community-acquired MRSA strains and the small size of SCCmec types IV and V suggest that these SCCmec allotypes are more readily transmissible between *Staphylococci* than the larger SCCmec types and, once introduced, do not compromise the fitness of the pathogen (Ito, 2004).

The detection of divergent MRSA lineages by different molecular typing techniques, including multilocus sequence typing and SCCmec typing, suggests that MRSA have arisen by the introduction of SCCmec into distinct successful methicillin- susceptible *S. aureus* lineages (Robinson et al, 2003). Conversely, there is evidence that resistance has been transferred to *S. aureus* on more than twenty occasions, since some lineages have acquired different structural types of the element (Robinson et al, 2003).

2.7 TREATMENT OF MRSA INFECTIONS

Staphylococcus aureus continues to be a major cause of community-acquired and health-care related infections in the United States and around the world (Lowy, 1998). Approximately 20% of community-acquired and nosocomial bacteraemias in the United States are caused by *S. aureus* (Cockerill et al, 1997). The emergence of high levels of

penicillin resistance followed by the development and spread of strains resistant to the semi synthetic penicillins (methicillin, nafcillin, and oxacillin), macrolides, tetracyclines, and aminoglycosides has made therapy of *Staphylococcal* disease a global challenge (Lowy, 1998).

In the 1980s, because of widespread occurrence of methicillin-resistant *S. aureus* (MRSA), empiric therapy for Staphylococcal infections (particularly nosocomial sepsis) was changed to vancomycin in many health-care institutions (Ena et al, 1993). Vancomycin use in the United States also increased during this period because of the growing numbers of infections with *Clostridium difficile* and coagulase-negative *Staphylococci* in health-care facilities (Ena et al, 1993). Thus, the early 1990s saw a discernible increase in vancomycin use. As a consequence, selective pressure was established that eventually led to the emergence of strains of *S. aureus* and other species of *Staphylococci* with decreased susceptibility to vancomycin and other glycopeptides. In 1997, the first strain of *S. aureus* with reduced susceptibility to vancomycin and teicoplanin was reported from Japan (Hiramatsu, 1997). Shortly thereafter, two additional cases from the United States were reported (CDC, 1997). Reduced susceptibility was often heterogeneously expressed and associated with thickening of the bacterial cell walls (Shieradzki et al, 1997). Finally in 2002, MRSA with vancomycin resistance (VRSA) was isolated from two independent patients who were co-infected with vancomycin resistance Enterococci (CDC, 2002). In 2004, a third case was reported in New York, USA (CDC, 2004).

Reports also provided evidence of failure by frequently used automated antimicrobial-susceptibility testing to detect VRSA (CDC, 2004). The shortcomings of routine laboratory procedures to identify reduced susceptibility to vancomycin and the poor effectiveness of vancomycin in eliminating even vancomycin susceptible MRSA from deep-seated infections, hampers a thorough appraisal of the importance of vancomycin resistance in clinical practice. Although few publications have addressed this issue, heterogeneously expressed vancomycin resistance has proved to be associated with treatment failure, which is defined as persistent bacteraemia and fever for longer than seven days (Charles et al, 2004)

Staphylococcus aureus has emerged as one of the most important human pathogens, and has over the past several decades, been a leading cause of hospital and community-acquired infections (Lowy, 1998)). Although infections caused by antibiotic-resistant *S. aureus* bring about serious problems in the general population, such infections can be particularly devastating for the very young, the elderly and the immunocompromised (Update, 1997). Antimicrobial resistance among nosocomial pathogens is a significant problem in many countries with severe consequences including increased medical costs, morbidity and mortality of patients (Bouchillon et al, 2004). Since the emergence of *S. aureus* strains with resistance to penicillin and methicillin in 1948 and 1961 (Barber et al, 1948, Jevons, 1961) respectively, it has become a well- known etiologic agent of a wide variety of infections, and has assumed increasing importance internationally as a cause of both nosocomial and community-acquired infections.

Methicillin-resistant *S. aureus* (MRSA) infections are additional to the burden of methicillin susceptible *S. aureus* (MSSA) and are particularly difficult to treat especially if they are located at anatomical sites, where antibiotic penetration is reduced (Duckworth, 2003). Cohort studies of patients with MRSA bacteremia have reported increased morbidity, longer hospital length of stay, and higher costs compared with patients with MSSA bacteraemia (Cosgrove et al, 2003).

2.8 EVOLUTION OF COMMUNITY ASSOCIATED INFECTION (CA-MRSA)

Traditionally, MRSA has been considered a major nosocomial pathogen in healthcare facilities, but in the past decade, it has been observed emerging in the community as well. Most documented MRSA infections were acquired nosocomially with community acquired MRSA (CA-MRSA) restricted to patients with frequent contact with health facilities, such as residents of long-term care facilities and intravenous drug users (Levine et al, 1982). The Centers for Disease Control and Prevention (CDC), Active Bacterial Core Surveillance Program defined a CA-MRSA case, as a patient with an MRSA infection and no history of the following; surgery, hospitalization, or long residence in health-care facility within the year before infection presence of a percutaneous device or indwelling catheter, dialysis within the previous year, hospitalization for more than 48 hours before MRSA culture, or previous MRSA infection or colonization (CDC, 2003).

In 1993, novel MRSA strains were reported from Western Australia. The strains had been isolated from indigenous Australian patients who had not been previously exposed to the health-care system (Udo et al, 1993). Publication of this information heralded the

worldwide recognition of the striking evolution of genuine CA-MRSA, strains which were transmitted in the community and differed from conventional endemic nosocomially acquired MRSA strains in several ways. First, they were more susceptible to antibiotic classes other than β-lactam antibiotics (Herold et al, 1998); secondly their genotypes were not the same as isolates from local hospitals; (Vandenesch et al, 2003), thirdly they remained harboured in different methicillin-resistant cassettes; (Vandenesch et al, 2003, Ito et al, 2004), and finally, community isolates were more likely to encode a putative virulence factor called Panton-Valentine Leukocidin (Dufour et al, 2002).

Ever since this recognition, CA-MRSA has been isolated from children and adults with skin and soft tissue infections, septic arthritis, bacteraemia, toxic shock syndrome (Lina et al, 1999), necrotizing fasciitis and necrotizing pneumonia (Miller et al, 2005). CA-MRSA has been reported most often from indigenous populations, (Groom et al, 2001), homosexuals, jailed inmates, military recruits, children in day care centers (Shahin, 1999), and competitive athletes (CDC, 2003). Common to all these groups are high intensity physical contact, which might help with transmission (Kaplan et al, 2005). Not unexpectedly CA-MRSA has also found its way into hospitals where outbreaks have been reported (Bratu et al, 2005).

CA-MRSA has now emerged as an epidemic that is responsible for rapidly progressive, fatal diseases including necrotizing pneumonia, severe sepsis and necrotizing fasciitis Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most frequently identified antimicrobial drug-resistant pathogen in US hospitals (Lord, 2005). Outbreaks of

community-associated (CA)-MRSA infections have been reported in correctional facilities, among athletic teams, among military recruits, in newborn nurseries, and among active homosexual men. CA-MRSA infections now appear to be endemic in many urban regions and cause most CA- *S. aureus* infections (Lord, 2005).

2.9 MRSA PREVALENCE

In the past two decades, the prevalence of MRSA strains has steadily increased in hospitals in the United States and abroad. National Nosocomial Infections Surveillance (NNIS) data collected by the Centers for Disease Control in the early to mid-1980s indicated that MRSA were limited mainly to relatively large urban medical centers. MRSA rates ranging from 24% to 30% has been reported in some hospitals in Switzerland, Belgium and Spain, while rates of 34% and 40% have been reported from France and Italy, respectively (Voss et al, 1994). The Netherlands, Sweden and Denmark have recorded MRSA rates of below 10% (Voss et al, 1994). MRSA rates between 10% and 20% have been obtained from hospitals in Austria and Germany (Voss et al, 1994). By the 1990s, rates among these smaller (<200-bed) community hospitals had increased to 20%, and twice that rate was found in the larger urban centers. More recent surveillance data from NNIS indicate that rates have continued to rise, with the prevalence of MRSA isolates from intensive care units approaching 50% by the end of 1998. Unless this upward trend has reversed, the prevalence rate of MRSA in U.S hospitals likely has reached 50% (Layton et al, 1995). At these high rates, the emergence of correspondingly high rates of MRSA strains in the community can be anticipated. Because no systematic, population-based surveillance of community isolates of *S. aureus*

exists, the true prevalence of MRSA cannot be determined (Layton et al, 1995).

One hospital-based study found that up to 40% of MRSA infections in adults were acquired before admission to the hospital. Published reports of MRSA colonization and infection among study participants who lack traditional risk factors indicate that community prevalence rates are rising (Layton et al, 1995).

By contrast with the assumptions that predicted little or no in-vivo relevance of the methicillin-resistant phenotype in the 1960s, MRSA is at present the most commonly identified antibiotic resistant pathogen in many parts of the world, including Europe, the Americas, North Africa, the Middle East and East Asia. Moreover, MRSA rates have been swiftly increasing worldwide over the past several decades, as data from continuing surveillance initiatives such as the National Nosocomial Infection Surveillance System and European Antimicrobial Resistance System show (Fridkin et al, 2002). Even in Scandinavian countries and the Netherlands where MRSA rates have been low and fairly stable for many years, the frequency is beginning to rise; and this trend should be taken seriously since the threshold for losing control might be low and is not well defined (Tiemersma et al, 2002). Most MRSA infections are of nosocomial origin and as such manifest themselves as complications of health-care procedures or underlying disorders. There is still evidence that hospital-acquired MRSA infection increases morbidity, the risk of mortality, and costs (Cosgrove et al, 2005). These infections also cause suffering and harm patients psychologically and financially (Tarzi et al, 2001). The societal costs accrue either directly as expenses caused by extension of hospital stay, additional

diagnostic or therapeutic procedures, and additional antibiotic use or indirectly through the loss of productivity, long-term disability and excess mortality (Scott et al, 2005). Other financial repercussions include the cost for containment of outbreaks, and the deliberate or unwitting changes of empirical antibiotic prescribing habits (Oliveira et al, 2001).

2.9.1 MRSA PREVALENCE IN AFRICA

Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a serious therapeutic problem worldwide, and its frequency in most African countries has not been reported. A study aimed at determining the prevalence and antibiotic susceptibility patterns of MRSA in eight large hospitals (>500 beds) in Africa and Malta, was carried out from 1996 to 1997. Susceptibility to methicillin (oxacillin) and to other drugs was determined by E- test (AB Biodisk, Solna, Sweden) on a total of 1440 clinical isolates of *S. aureus*. Methicillin resistance was detected in 213 (15%) of the 1440 isolates tested. In the study, a 14.8% rate of MRSA was recorded from the nine hospitals. MRSA rates of 21%–30% were noted in Nigeria, Kenya, and Cameroon. In Morocco, Senegal and Cote d'Ivoire, MRSA rates were between 10% and 20%. MRSA rates were below 10% in Algeria, Tunisia and Malta. All the MRSA isolates were sensitive to vancomycin. The isolates were also highly sensitive to ciprofloxacin, except in Kenya, Morocco, and Tunisia, where relative resistance to this drug was noted. Susceptibility to rifampin and fusidic acid seems to be correlated with the clinical use of these compounds. Only 46% of 59 MRSA strains analyzed were susceptible to rifampin, fusidic acid, and ciprofloxacin. The majority (> 60%) of MRSA strains were multiresistant (Kesah et al, 2003).

Continuous surveillance of drug resistance in MRSA in Sub-Saharan Africa is imperative for the detection of emerging trends and the development of appropriate therapeutic schedules. Characterization of resistance mechanisms may aid in tracing infection sources and the spread of resistance traits. All these should culminate in the eradication or the implementation of measures to curtail the spread of MRSA (Kesah et al, 2003). Although data on the prevalence of *Staphylococcal* infections in Africa are limited, one of the earliest reports of MRSA in the continent was in South Africa (Scragg et al, 1978). A study carried out on the antibiotic susceptibility and characterization of *S. aureus* from clinical samples in Kwa Zulu Natal Province, South Africa revealed that, all the isolates were susceptible to vancomycin, teicoplanin and fusidic acid, and 26.9% of isolates studied were confirmed as MRSA (Scragg et al, 1978).

In Ghana no comprehensive research has been done on MRSA, as such there is no publication on it. A project report carried out in 2004 on the prevalence rate of MRSA at the Komfo Anokye Teaching Hospital (KATH) indicated 12.1% rate out of a sample size of 132 (Kyei, 2004).

2.10 METHODS OF ANTIMICROBIAL SUSCEPTIBILITY TEST FOR MRSA.

Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial sensitivity. For clinical and surveillance purposes and to promote reproducibility and comparability of results between laboratories, the WHO recommends the CLSI modified Kirby-Bauer disc diffusion technique. The validity of this carefully standardized technique depends on using discs of correct antimicrobial content, an inoculum which gives confluent growth, and a reliable Mueller Hinton agar (Cheesbrough, 2000).

In the Stokes disc diffusion technique both the test and control organisms are inoculated on the same plate. The zone sizes of the test organism are compared directly with that of the control. This method is not as highly standardized as the Kirby-Bauer technique and is used in laboratories particularly when the exact amount of antimicrobial in a disc cannot be guaranteed due to difficulties in obtaining discs and storing them correctly or when the other conditions required for the Kirby-Bauer technique cannot be met (Cheesbrough, 2000).

Bacterial susceptibility to antimicrobial agents may be measured in vitro by using the principles of agar diffusion. Reasonably accurate and precise results can be obtained with agar diffusion techniques as long as all procedural details are carefully standardized and controlled. Diffusion techniques can be used as quantitative tests (D'Amato et al, 1985). However, most procedures simply categorize microorganisms as being susceptible, moderately susceptible, intermediate (indeterminate), or resistant to different antimicrobial agents (D'Amato et al, 1985).

Antimicrobial agents are commonly applied to the test plates in the form of dried filter paper disc. When a disc is placed on the inoculated surface of the test medium, several events progress simultaneously. First, the dried discs absorb water from the agar medium, and thus the drug is dissolved. The antimicrobial agent is then free to diffuse through the adjacent agar medium according to the physical laws that govern the diffusion of molecules through an agar gel (Barry, 1986). The result is a gradually changing gradient of drug concentrations in the agar surrounding each disc. As the diffusion of the

antimicrobial agent progresses, microbial multiplication also proceeds. After an initial lag phase, a logarithmic growth phase is initiated. At that point, bacterial multiplication proceeds more rapidly than the drug can diffuse, and bacterial cells that are not inhibited by the antimicrobial agent will be able to continue multiplying until a lawn of growth can be visualized. No growth will appear in the area where inhibitory concentrations of the drug is present; the more susceptible the test organism, the larger the zone of inhibition will be (Barry, 1986).

Methicillin is the least active member of the penicillinase (β-lactamase)-resistant penicillins, but oxacillin discs are preferred as the class representative because of their stability and because they may detect hetero-resistant *Staphylococci* more efficiently (Barry et al, 1987).

Dilution susceptibility testing methods are used to determine the minimal concentration, usually expressed in units or micrograms per milliliter, of an antimicrobial agent required to inhibit or kill a microorganism. Procedures for determining antimicrobial inhibitory activity are carried out by either agar-or broth-based methods. Antimicrobial agents are usually tested at log2 (twofold) serial dilutions, and the lowest concentration that inhibits visible growth of an organism is recorded as the minimum inhibitory concentration (MIC). The concentration range used may vary with the drug, organism identification, or site of infection. Ranges include concentration that allows determinations of interpretive categories (i.e., susceptible, moderately susceptible, or intermediate, and resistant) and the acceptable ranges for quality control reference strains. Other dilution methods include

those that test a single or a selected few concentrations of antimicrobial agents (i.e. breakpoint susceptibility testing and single-drug concentration screens) (Barry, 1986).

The macrodilution broth method is a well- standardized and reliable reference method that is useful for research purposes, but because of the laborious nature of the procedure and the availability of more convenient dilution systems (i.e. microdilution), this procedure generally is not useful for routine susceptibility testing in most clinical microbiology laboratories (Washington et al, 1978).

Current antimicrobial susceptibility testing methods are based either on quantitative dilution techniques or qualitative diffusion procedures. Epsilometer test (E-test) is a useful addition to the array of diagnostic procedures and consist of antimicrobial agent-impregnated strips that are placed on the surface of agar. The E-test gradient technology is based on a combination of the concepts of dilution and diffusion principles for susceptibility testing. As with other dilution methods, E-test directly quantifies antimicrobial susceptibility in terms of discrete MIC values. However, in using a pre-defined, stable and continuous antibiotic concentration gradient, E-test MIC values can be more precise and reproducible than results obtained from conventional procedures based on discontinuous two-fold dilutions (Baker, 1991).

The principle is an expansion of the disc diffusion method. The antimicrobial agent content of the strips is graded and the concentration is printed linearly along the strip (Hamilton-Miller, 1995). The concentration range of the antimicrobial on the E-test strip

corresponds to twofold dilutions in a conventional MIC method (Jorgensen, 1991). Diffusion of antimicrobial agents begins immediately after placement of the strip, which can therefore not be moved once the impregnated surface has touched the agar. After incubation, whereby bacterial growth becomes visible, a symmetrical inhibition ellipse centered along the strip is seen. The MIC value is read from the scale in terms of μg/ml where the ellipse edge intersects the strip.

The E-test has proven to be effective for general use. The expense of this approach makes it difficult to justify for testing multiple antimicrobial agents against organisms that grow well in one of the dilution or disc diffusion procedures. It is invaluable, however, for testing highly selected antimicrobial agents against fastidious organisms that do not grow well in other test (Baker, 1991).

2.11 DIAGNOSIS OF *S. AUREUS* AND MRSA

Confirmation of *S. aureus* is performed using the slide coagulase test (clumping factor) and the tube coagulase test (free coagulase). Positives on the slide coagulase test should be confirmed with the tube coagulase test (Cheesbrough, 2000)

Agglutination kits are widely available and can be used to confirm *S. aureus* by detecting protein A and clumping factor, although some strains of MRSA have low levels of these proteins. Newer kits now work by also detecting surface antigen. Other latex kits detect PBP2a, which occurs within the cell membrane and requires lysis of the cells for detection (CLSI, 2006). Several manufacturers of commercial kit identification systems

or automated instruments have released products that can identify a number of the *Staphylococcus* species with an accuracy of 70% to more than 90% with relative speed and simplicity (Crouch et al, 1987). The commercially available systems with latex agglutination for the identification of *S. aureus* include: SeroSTAT, Scott Laboratories, Inc. Fiskeville, RI; Accu-staph, and Carr-Scaborough Microbiologicals, Inc. Most routine methods for the identification of MRSA in clinical specimens are based on antibiotic susceptibility disc diffusion and agar method. These methods detect phenotypic expression rather than the presence of the mecA gene (Gradelski, 2001).

MRSA have been identified using the 1µg oxacillin disc. Of the penicillinase-stable penicillins, oxacillin is preferred for in-vitro testing. Oxacillin susceptibility test result can be applied to the other penicillinase-stable penicillins. Oxacillin is more resistant to degradation and is more likely to detect heteroresistant *S. aureus* strains than methicillin or nafcillin discs. However the cefoxitin disc test is the preferred method for testing *S. aureus* for resistance to the penicillinase-stable penicillins. The cefoxitin disc test is comparable to the oxacillin disc test for prediction of mecA-mediated resistance to oxacillin; however, the cefoxitin disc test is easier to read and thus is the preferred method. In this case, cefoxitin is used as a surrogate to report oxacillin (CLSI, 2006).

MRSA have been identified by using DNA probes, peptide nucleic acid probes, gel-based PCR, and real-time PCR. Molecular typing methods have in the last few years paved the way for sophisticated techniques to track the source and transmission route of bacterial pathogens in hospital outbreaks and have also helped in establishing epidemiological

investigations comparing strains across continents (Chung et al, 2004). However, such molecular assays are associated with specialized equipment and expertise, increased cost, and specific laboratory organization into pre- and post amplification areas (Oliveira et al, 2002). The majority of molecular methods used for the detection of MRSA are in-house, relying on multiplexed PCR primers detecting genes specific for *S. aureus* (nuc, fem) and mecA detecting methicillin resistance (CLSI, 2006).

There have also been a number of developments with bioluminescence in particular, the use of adenylate kinase (AK), an enzyme found in all cells that produce ATP from ADP. AK measurement is more sensitive than ATP-based systems and allows routine detection of 50 organisms or more in a sample. Early performance data shows results equivalent to conventional plate culture methods whilst providing results within 5 hours.

Currently the majority of MRSA screening is carried out using plate based methods. Surveys suggest that this methodology group accounts for more than 90% of the screening tests performed. There are no universal standardised methods for screening and isolation of MRSA using solid agar media. Many selective media are available, and these rely on inhibitors such as NaCl and/or antibiotics to aid selection, together with a pH indicator to highlight presumptives. Examples are Mannitol Salt Agar containing 7% NaCl with either 4mg/L methicillin or 2mg/L oxacillin; Oxacillin Resistant Screening Agar with 5.5% NaCl and 2mg/L oxacillin; Baird Parker Medium with 8mg/L ciprofloxacin; Mueller Hinton Agar with 4% NaCl and 6mg/L oxacillin. DNase plates can also be used but positives require additional information (CLSI, 2006).

Bekkaoul et al recently described the development of a 2-hr assay utilizing cycling probe technology with a DNA-RNA-DNA chimeric probe designed to detect the *mecA* gene in *S. aureus*. The resulting Velogene Rapid MRSA Identification Assay (ID Biomedical Corp., Vancouver, British Columbia, Canada) is a colorimetric enzyme immunoassay (EIA) utilizing a fluorescein-labeled *mecA* probe. This subtractive assay uses a streptavidin-coated 96-well microtiter plate format, and the detection of uncut probe from *mecA* negative strains results in the development of a blue color, whereas *mecA*-positive strains result in a colorless reaction (Bekkaoul et al, 1999).

A detailed knowledge of the susceptibility to antimicrobial agents is necessary to facilitate the development of effective strategies to combat the growing problem of resistance. A nationwide knowledge base is also important for optimal patient management, control of nosocomial infection and for the conservation of antibiotics.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY SITE

The study was done at the microbiology laboratory of the Komfo Anokye Teaching Hospital (KATH). It is a tertiary care hospital with 800-bed capacity. It is a referral center for the northern sector of the country. The microbiology laboratory services the entire Ashanti region as well as the neighbouring Regions in Ghana.

3.2 STUDY POPULATION

Six thousand two hundred specimens comprising venous blood samples, wound swabs, pus and pleural effusions (miscellaneous samples) of outpatients and inpatients were screened from 9th October, 2006 to 9th March, 2007. The diagnoses were sepsis, septicaemia, other invasive infections and skin and soft tissue infections. Patients were grouped into hospital-acquired MRSA (HA-MRSA) and community acquired MRSA (CA-MRSA). HA-MRSA included all inpatients and outpatients who have had recent surgery or hospitalization in the year before they tested a positive MRSA culture. CA-MRSA were identified as outpatients who have not had recent surgery or hospitalization. Factors such as infections of the lungs, heart, liver, diabetes, were considered in both HA and CA infections.

Patients' information was obtained from their medical records, questionnaire and personal interview. The patients were interviewed and briefed on the nature and

importance of the research. When they gave their consent, they were assisted to fill in the questionnaire. Nurses and medical assistants in the wards assisted in the filling and collection of questionnaire forms of inpatients. Interview and filling of questionnaire forms of outpatients was done when they came for their medical reports in the laboratory. The interview confirmed the information in the medical records. The questionnaire provided the risk factors for grouping the isolates into HA and CA infections as well as the underlying infections of the patients. A copy of the questionnaire form is presented in appendix 3B.

3.3 SAMPLE COLLECTION

Specimens collected and received in the laboratory were processed as follows:

3.3.1 INVASIVE INFECTIONS

3.3.1.1 BLOOD

Blood was aseptically drawn at the venipuncture site of patients. In case of adults and children 5ml and 3ml of blood were collected respectively. It was dispensed into a universal bottle containing 20ml of brain-heart infusion medium. Specimens were then incubated at 37°C overnight and then cultured on blood and MacConkey agar. The specimens were incubated for up to 7 days before it was declared as negative culture.

3.3.1.2 PLEURAL AND PERITONEAL FLUID

These specimens were collected by the medical officers and then brought to the laboratory. It was aseptically dispensed into cooked meat medium and incubated overnight at 37°C. After the incubation it was cultured on MacConkey and blood agar.

3.3.2 SKIN AND SOFT TISSUE INFECTIONS

3.3.2.1 WOUND SWAB, PUS

Sterile swab stick was used to collect discharges from wounds and pus. The swab stick was then inserted into cooked meat medium, breaking off the stick to allow the bottle top to be replaced. It was incubated overnight at 37°C and then cultured on blood agar.

3.3.3 RESPIRATORY AND EAR INFECTIONS

3.3.3.1 THROAT AND EAR SWAB

Throat and ear swabs were collected by the medical officers and brought to the laboratory. The specimen was then cultured on blood or MacConkey agar and incubated aerobically at 37°C for up to72 hrs, checking for growth after overnight incubation. On chocolate agar it was incubated in a carbon dioxide enriched atmosphere at 37°C for up to 48 hrs, checking for growth after overnight incubation.

3.4 TEST PROCEDURE FOR SCREENING *S. AUREUS* ISOLATES

3.4.1 GRAM STAIN

The Gram stain was performed on all colonies resembling *S. aureus* to confirm Gram positive cocci in clusters.

3.4.2 CATALASE TEST

The catalase test was performed on colonies resembling *S. aureus.* This test was done to differentiate *S. aureus* from *Streptococci*. Catalase acts as a catalyst in the breakdown of hydrogen peroxide into water and oxygen. *S. aureus* tests positive.

3.4.3 COAGULASE TEST

The coagulase test was performed to confirm *S. aureus.* The slide test was used in this study. The tube coagulase test was performed when the result of the slide test was doubtful or negative.

3.5 STORAGE OF ISOLATES

The isolates were put in 1ml of glycerol broth and stored at 8°C in a refrigerator.

3.5.1 PREPARATION OF GLYCEROL BROTH

The broth was prepared by weighing 7.4g of Brain-heart infusion media and added to 160ml of distilled water. Glycerol 40ml was added to the mixture and stirred to obtain a uniform mixture. Using sterile pipette 1ml of the broth was put into Eppendorf tubes and autoclaved at 121 °C for 15 minutes.

3.5.2 ISOLATION OF *S. AUREUS* FROM STORAGE

The stored isolates were cultured on blood agar and incubated at 37°C overnight. The isolates were then sub- cultured on nutrient agar to obtain a pure culture for catalase test ,coagulase test and antibiotic susceptibility testing.

3.6 DETERMINATION OF MRSA USING THE DISC DIFFUSION METHOD

The Kirby-Bauer disc diffusion method was employed in this study. Penicillin 10 units discs and oxacillin 1µg discs were used in the MRSA determination.

3.6.1 MEDIA PREPARATION

Mueller-Hinton sensitivity testing agar was used. The media was prepared and sterilized as instructed by the manufacturer. The pH of the medium was 7.2. The media was then aseptically poured into 90mm diameter sterile Petri dishes to a depth of 4mm depth. To obtain a uniform media it was ensured that the plates were poured on a level surface. The plates were dried and stored in ziplock bags (Cheesbrough, 2000).

3.6.2 DISC DIFFUSION PROCEDURE

The Kirby-Bauer disc diffusion method was employed in this study. Penicillin and oxacillin disc were used in the determination of MRSA strains. Three to four isolated colonies of similar appearance were picked using a sterile wire loop. This was emulsified in, 2ml, 0.85% sterile saline. A densimat (bio merieux sa France 69280 Marcy-I'Etoile with serial number IDN004874) was used to adjust the turbidity of the suspension to match the 0.5 Mcfarland standards. A sterile swab stick was dipped in the saline suspension.Excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension.The swab stick was then used to streak evenly over the surface of the Mueller-Hinton agar in three directions, rotating the plate approximately sixty degrees to obtain even distribution. The inoculated plate was allowed to dry for three to five minutes with the Petri dish lid in place.

Penicillin 10µg disc and oxacillin 1 µg disc were placed on the inoculated plate with the aid of sterile forceps. The plates were allowed to stand in the upright position for at least 5 minutes before they were inverted and incubated at 35°C for 16-18 hours. After

incubation the plate was examined to ensure a confluent growth. The diameter of each zone of inhibition was measured in millimeters on the underside of the plate with ruler and calipers. The endpoint of inhibition was taken at the point where growth started (Cheesbrough, 2000). A control plate containing *S. aureus* ATCC 25923 was included in every batch of test done.

3.6.3 INTERPRETATION OF ZONE SIZES

The zone sizes were interpreted as resistant, intermediate and susceptible using the interpretive chart. The zone size interpretive criteria used were as follows; with 10µg penicillin disc a zone size of ≤ 28 mm was considered resistant, while zone size of ≥ 29 mm was considered susceptible, however, no intermediate zone size has been established by CLSI . With 1µg oxacillin disc a zone size of≤ 10mm was considered resistant; zone size of 11-12 mm was considered intermediate, while zone size of ≥ 13 mm was considered susceptible.

3.7 ANTIBIOTIC SUSCEPTIBILITY TESTING OF MRSA ISOLATES USING MODIFIED KIRBY-BAUER METHOD.

The CLSI Kirby-Bauer disc diffusion technique was used in this study. The susceptibility of the following commonly used antibiotics, were tested using dics obtained from Abtek Biologicals LTD: penicillin 1.5 units, gentamicin 10µg, ampicillin 10µg, flucloxacillin 5µg, erythromycin 5µg, tetracycline 10µg, cotrimoxazole 25 µg, and cefuroxime 30 µg.

3.7.1 PROCEDURE

Mueller-Hinton sensitivity agar was used in the sensitivity testing. Pure culture of *S. aureus* was obtained after culturing the stored sample on nutrient agar media. A sterile wire loop was used to touch three to five well-isolated colonies of similar appearance and emulsified in 2 ml of peptone water to form the inoculum's suspension. The sensitivity test agar plate was inoculated by dipping a sterile swab stick in the suspension. Excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension. The swab stick was then used to streak evenly over the surface of the Mueller-Hinton agar in three directions, rotating the plate approximately sixty degrees to obtain even distribution. The inoculated plate was allowed to dry for three to five minutes with the Petri dish lid in place. The antibiotics in the form of a multodisc were aseptically placed on the inoculated plates using sterile forceps.

The plates were incubated aerobically at 37°C within thirty minutes of applying the discs. The plates were examined after overnight incubation. The diameter of the zone of inhibition in millimeters of each antibiotic was measured using a ruler and calipers. The CLSI interpretive chart was used to interpret the zone size of each antimicrobial. A control plate containing *S. aureus* ATCC 25923 was set up when each batch of test was done.

3.8 DETERMINATION OF MIC USING THE E-TEST METHOD

The MIC of fifty of the MRSA isolates was determined using the E-test method. Oxacillin, trimethoprim sulfamethoxazole (co-trimoxazole), gentamicin and ceftriaxone E-test strips obtained from AB-Biodisk, Solna, Sweden were used.

3.8.1 STORAGE OF ANTIBIOTIC STRIPS

The antibiotic strips were stored following the manufacturer's instructions. Oxacillin test strips with lot number B10492 and ceftriaxone strips with lot number B10526 were stored in a refrigerator at 8°C. Gentamicin strips with lot number B10404 and trimethoprim sulfamethoxazole with lot number B10893 were stored at 22 °C.

3.8.2 INOCULUM PREPARATION

Well isolated colonies of similar morphology were picked from an overnight nutrient agar plate with a sterile straight loop and emulsified into 2ml sterile saline. The inoculum was prepared to a turbidity of 0.5 McFarland standard and compared to 0.5 calibrator standard (Phoenix Spec. BD Lot No P787006, exp date 21/12/08).

3.8.3 INOCULATION PROCEDURE

The sterile swab was dipped at the surface of the suspension medium. Excess fluid was removed by pressing the swab against the inside wall of the bijou bottle. The suspension was inoculated on to Mueller-Hinton agar plates of 90 mm diameter and 4 mm depth. The entire agar surface was carefully streaked three times, rotating the plate at 60 °C each time to evenly distribute the inoculum. The plates were left for about 10 minutes to ensure that excess moisture was absorbed leaving dried agar surface. Using sterile forceps; antimicrobial strips were applied onto the dried agar surface. One plate per antimicrobial strip was done.

3.8.4 INCUBATION

The inoculated plates were incubated in an inverted position (lid down) at 35 °C for 24 hours.

3.8.5 INTERPRETATION AND REPORTING OF RESULTS

On incubation an elliptical zone of inhibition was produced, and the MIC read directly from the graduated E- test strip at the point of intersection of the zone of inhibition of growth with the strip. MIC breakpoints for defining interpretive category as published by the CLSI were used for interpreting the E-test MIC values. The breakpoints for the antibiotics tested in this study are shown in Table15 in appendix2.

3.8.6 REPORTING GUIDE

-When growth occurred along the entire strip i.e. no inhibition ellipse was seen the MIC was reported as ≥ the highest value on the MIC scale.

-An E-test MIC value, which fell between standard two-fold dilutions, was rounded up to the next upper two-fold value before categorization.

3.8.7 TEST STRIPS QUALITY CONTROL

The four E-test strips were tested with each batch of test performed with *S. aureus* ATCC 25923. Results obtained are shown in the Table 16. The concentration ranges of the antibiotic strips were as follows: Oxacillin (0.016- 256µg/ml), gentamicin (0.016-256 µg/ml), SXT (0.002-32 µg/ml), and ceftriaxone (0.002-32 µg/ml).

3.9 QUALITY CONTROL

Staphylococcus aureus ATCC-25923 of known coagulase production was used as control strain in the screening for *S. aureus* isolates. This control was also used in the diagnosis of the *S. aureus*, disc diffusion tests, and the MIC determination by the E-test.

3.10 DETERMINATION OF MIC$_{(50)}$ AND MIC$_{(90)}$

The formulas (N+1)/2 and (90/100)*(N +1) were respectively used to calculate MIC $_{(50)}$ and MIC $_{(90)}$. The procedure is shown in appendix 2.

3.11 LIMITATIONS OF THE STUDY

The slide coagulase test was used in the confirmation of *S. aureus* instead of latex agglutination test, which is more sensitive in picking *S. aureus*, isolates.

Determination of the mecA gene by PCR was not used. Cefoxitin 30µg disc which is another preferred method for testing MRSA was not available; instead 1µg oxacillin disc was used in the MRSA screening. Vancomycin, which is the drug of choice for treating multidrug resistant MRSA infections, could not be tested due to difficulty in acquisition of discs and strips.

3.12 STATISTICAL ANALYSIS

Frequencies were obtained and percentages were calculated for study variables. Analysis of variance (ANOVA) was used to test for significant difference in the prevalence and antibiotic resistance patterns between HA and CA isolates. The excel output for the calculation of ANOVA is presented in appendix 3A. Logistic regression analysis was

carried out to find association between variables. All statistical tests were two-tailed and p-value ≤ 0.05 was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 MRSA PREVALENCE

A total of 6200 blood and miscellaneous samples were processed. There were 250 *S. aureus* isolated. The rate of isolation of *S. aureus* in reference to other clinical isolates such as *E. coli and Salmonella* was 4 % (250/6200). Out of the 250 *S. aureus*, 87 were MRSA giving a prevalence of 34.8% (87/250). Of the 87 MRSA, 67 were HA-MRSA giving a prevalence of 26.8% (67/250) and CA-MRSA was 20, giving a prevalence of 8% (20/250). The difference in HA and CA MRSA was not statistically significant given a p value equal to 0.9. As shown in Table 9, the geometric mean for MRSA of community-based individuals was 1.1 (SD=3.7) and that of hospital-based individuals was 1.0 (SD=3.6) but the difference in geometric means was not statistically significant (p=0.791).

4.2 ANTIBIOTIC RESISTANCE PATTERNS OF MRSA USING DISC DIFFUSION METHOD

Antibiotic resistance patterns of HA and CA-MRSA using disc diffusion method are shown in Figure 4:1. The number of isolates, which were sensitive, intermediate and resistant, is shown in Table 1. There was no significant difference between HA and CA isolates in the resistance patterns with a p value equal to 0.76. With the exception of cefuroxime and erythromycin, which showed resistance of below 50%, the rest showed rates ranging from 50-100%. Penicillin and ampicillin resistance was 100% in HA and CA-MRSA isolates. In the HA-MRSA 81 % (54/67) of isolates were resistant to

cotrimoxazole and 70% (14/20) of isolates in CA-MRSA. In HA-MRSA, 75% (50/67) of the isolates were resistant to tetracycline and 80% (16/20) of isolates in CA-MRSA. In HA-MRSA, 70% (47/67) of isolates were resistant to gentamicin and 25% (5/20) of isolates in CA-MRSA. For HA-MRSA 60% of isolates were resistant to flucloxacillin and 55% (37/67) of isolates (12/20) in CA-MRSA. In HA-MRSA 33 % (22/67) of isolates were resistant to cefuroxime and 30% (6/20) of isolates in CA-MRSA. For HA-MRSA 31% (21/67) of isolates were resistant to erythromycin and 45% (9/20) of isolates in CA-MRSA.

Figure 4:1 Percentage resistance of HA and CA-MRSA.

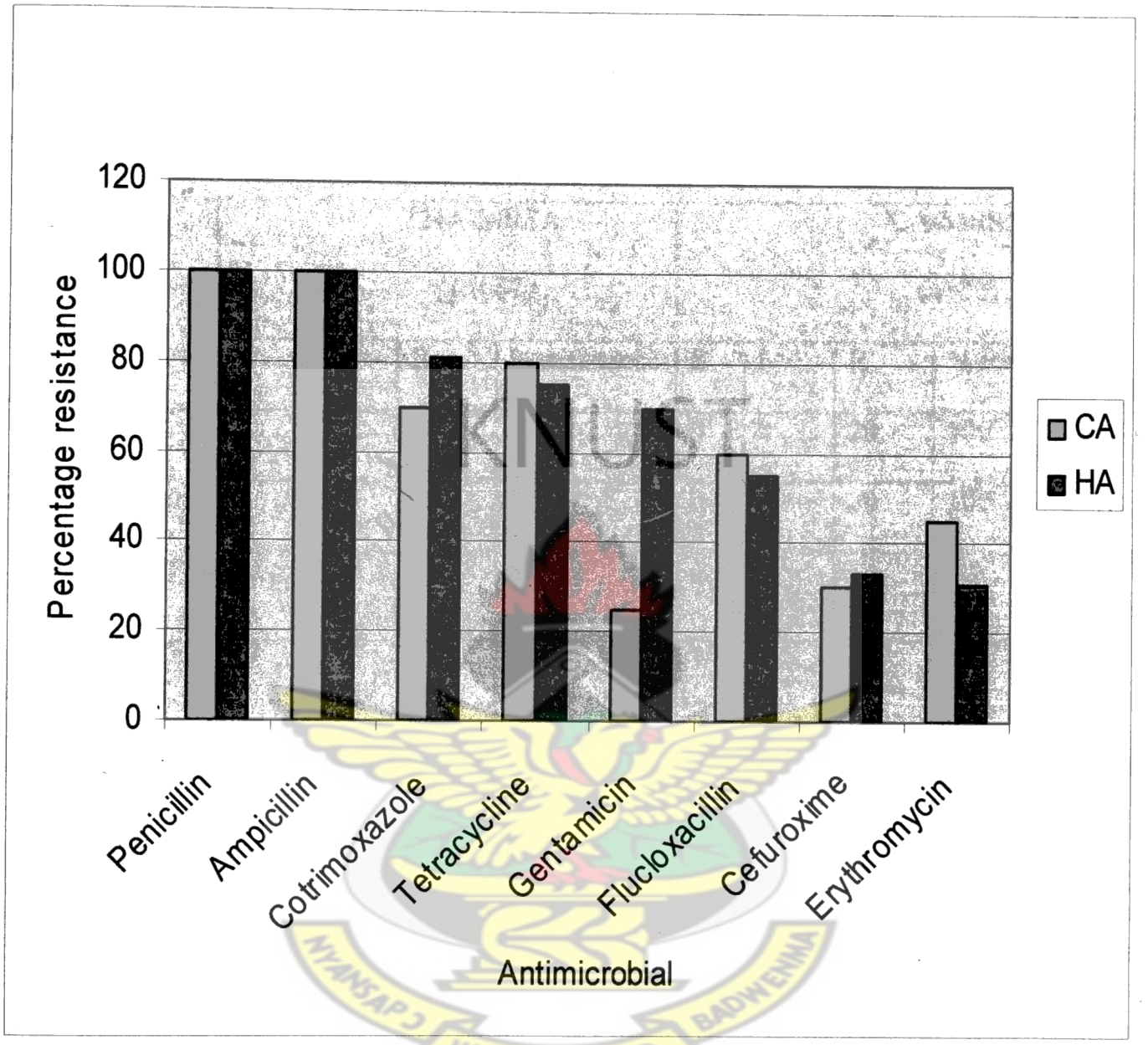


Table 1 Antibiotic resistance patterns of HA and CA-MRSA using disc diffusion method.

Antimicrobial	HA-MRSA (n=67)				CA-MRSA (n=20)			
	S	I	R	% resistance	S	I	R	% resistance
Penicillin (1.5U)	0	0	67	100	0	0	20	100
Ampicillin (10µg)	0	0	67	100	0	0	20	100
Cotrimoxazole(25 µg	10	3	54	81	6	0	14	70
Tetracycline(10 µg)	12	5	50	75	3	1	16	80
Gentamicin (10 µg)	17	3	47	70	13	2	5	25
Flucloxacillin(5 µg)	12	18	37	55	3	5	12	60
Cefuroxime (30 µg)	38	7	22	33	14	0	6	30
Erythromycin (5 µg)	37	9	21	31	11	1	9	45

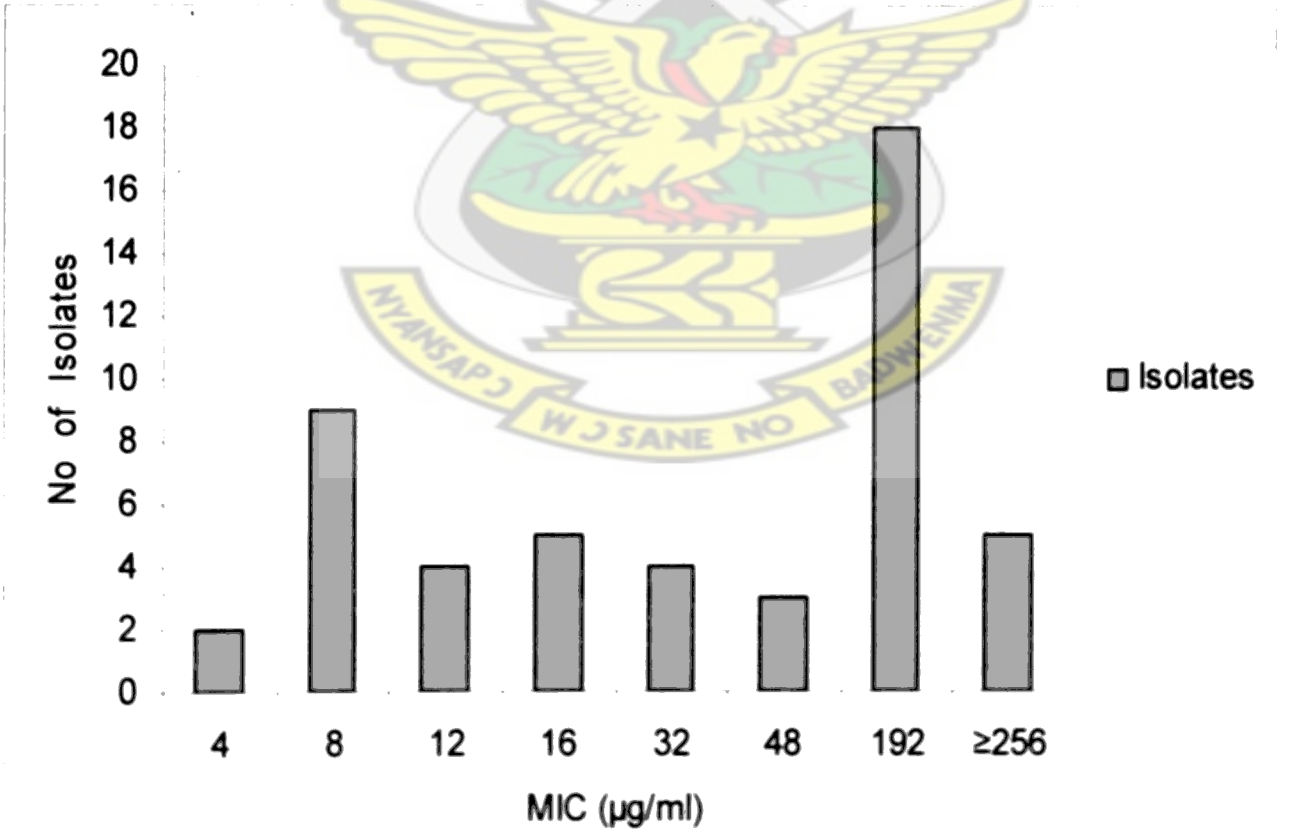
S-Sensitive, I- Intermediate, R- Resistant

4.3 THE MINIMUM INHIBITORY CONCENTRATION OF FIFTY MRSA ISOLATES USING THE E-TEST

4.3.1 MIC FOR OXACILLIN

Figure 4:2 shows the MIC of 50 isolates to oxacillin. The MIC for oxacillin ranged from 4- ≥256µg/ml. The MIC at which 90% of the isolates were inhibited (MIC_{90}) was ≥224µg/ml. The MIC at which 50% of isolates were inhibited (MIC_{50}) was 48µg/ml. According to CLSI interpretive criteria, all the 50 isolates tested were resistant.

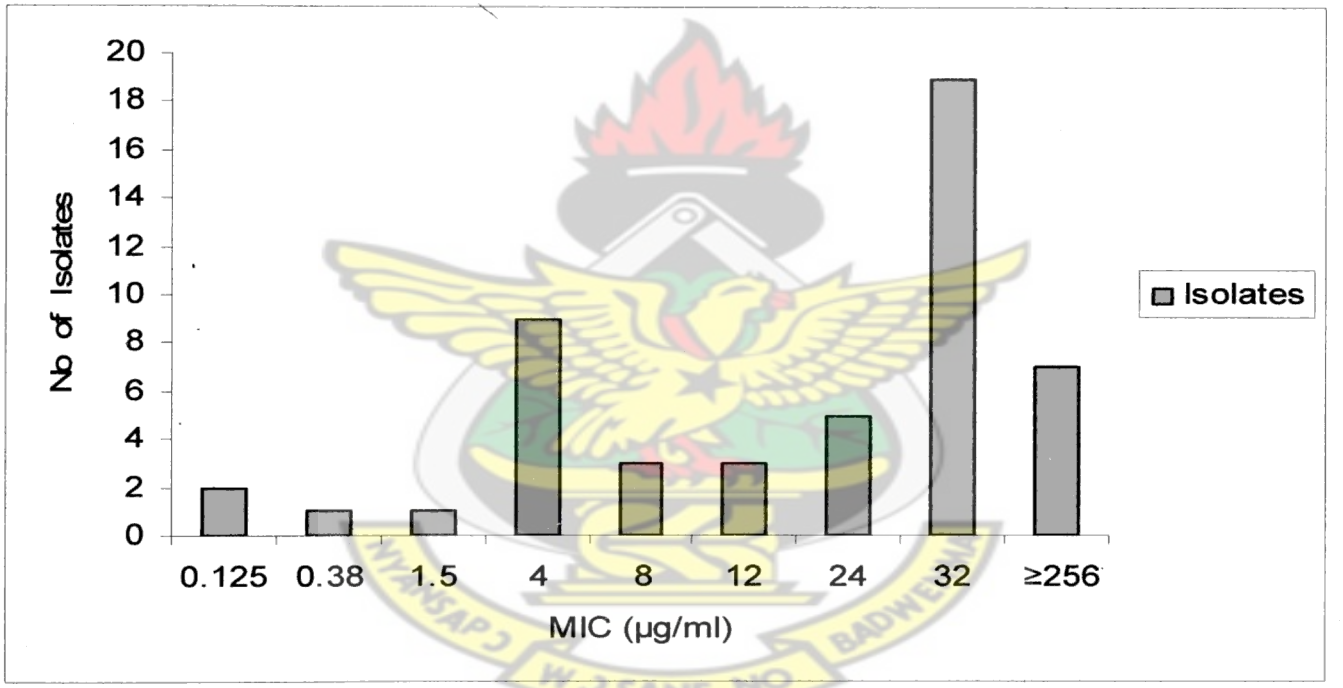
Figure 4:2 MIC of fifty MRSA isolates to Oxacillin as determined by the E- test



4.3.2 MIC FOR GENTAMICIN

Figure 4:3 shows the MIC of 50 isolates to gentamicin. The MIC for gentamicin ranged from 0.125 - ≥256µg/ml. The MIC at which 90% of the isolates were inhibited (MIC_{90}) was ≥256. The MIC at which 50% of isolates were inhibited (MIC_{50}) was ≥32µg/ml. According to the CLSI interpretive criteria used, 13 isolates were susceptible, 3 were intermediate and 34 were resistant.

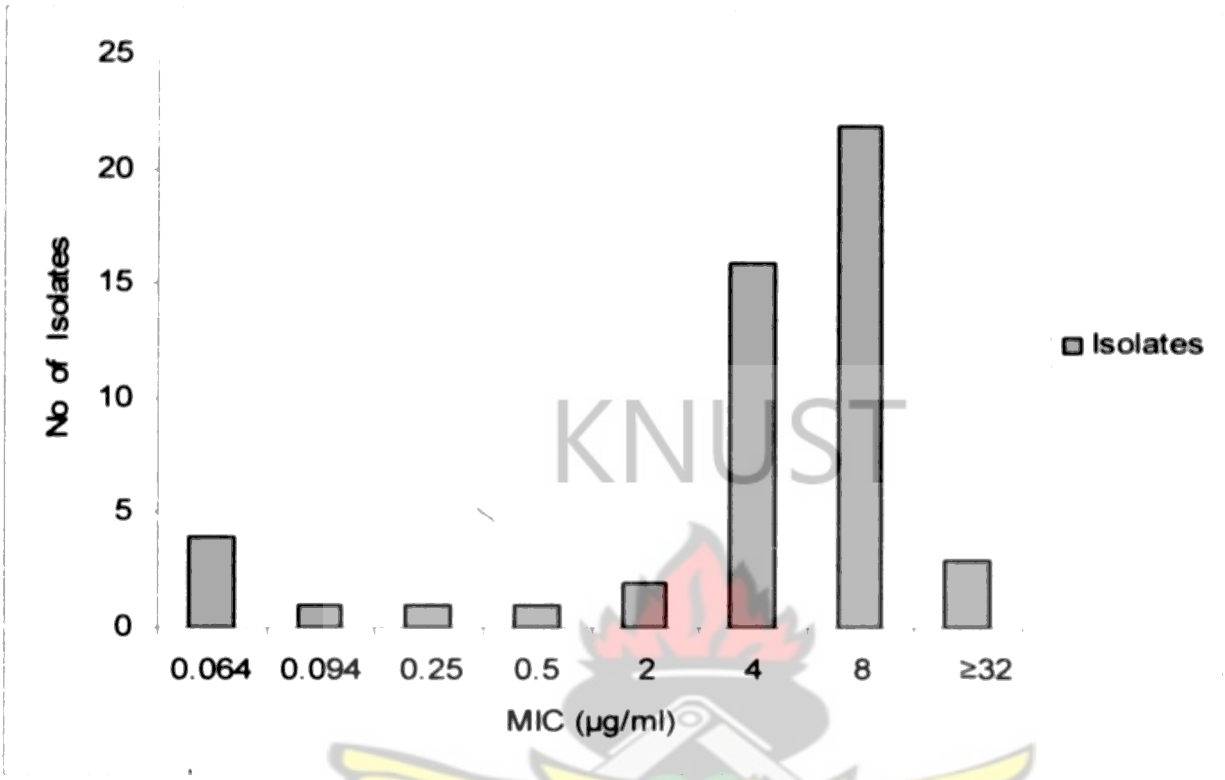
Figure 4:3 MIC of fifty MRSA isolates to gentamicin as determined by the E- test



4.3.3 MIC FOR TRIMETHOPRIM-SULFAMETHOXAZOLE (SXT)

Figure 4:4 shows the MIC of 50 isolates to trimethoprim-sulfamethoxazole. The MIC for SXT ranged from 0.064 - ≥32 µg/ml. The MIC at which 90% of the isolates were inhibited (MIC_{90}) was 8 µg/ml. The MIC at which 50% of the isolates were inhibited MIC (MIC_{50}) was 4 µg/ml. There were 9 isolates, which were sensitive according to CLSI standards. There were no intermediates, however 41 isolates were resistant.

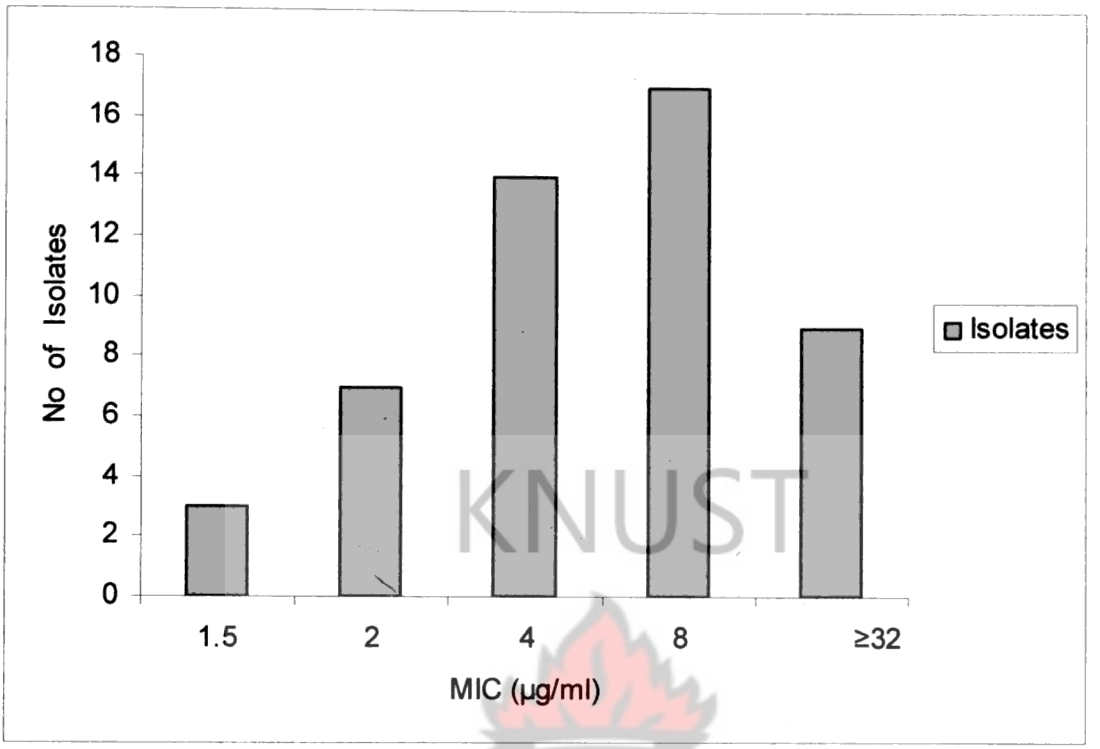
Figure 4:4 MIC of fifty MRSA isolates to Trimethoprim-sulfamethoxazole as determined by the E- test



4.3.4 MIC FOR CEFTRIAXONE

The MIC for ceftriaxone ranged from 1.5- ≥32 µg/ml. This is shown in Figure 4:5. The MIC at which 90% of the isolates were inhibited (MIC_{90}) was ≥32 µg/ml. The MIC at which 50% of the isolates were inhibited (MIC_{50}) was 8µg/ml.

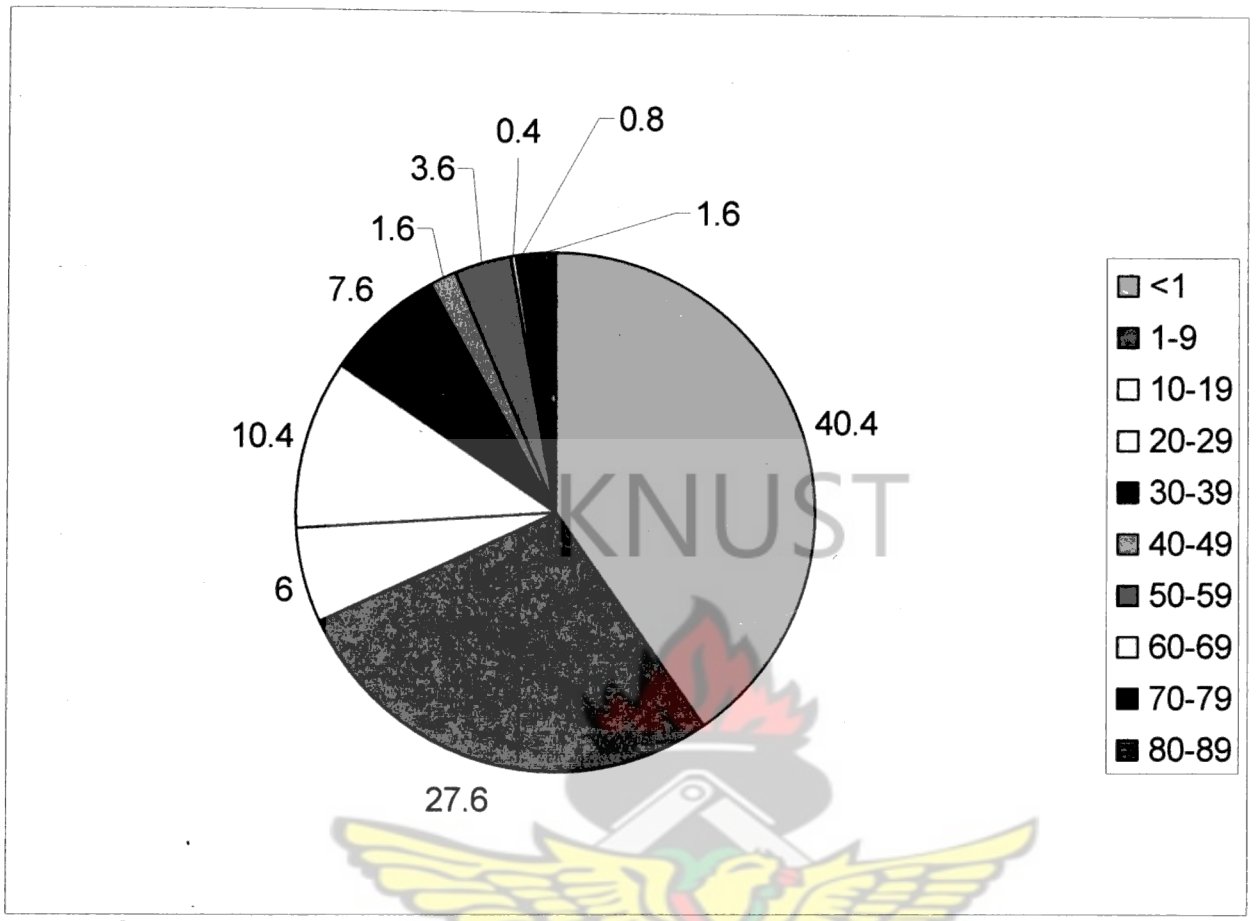
Figure 4:5 MIC of fifty MRSA isolates to ceftriaxone as determined by the E- test.



4.4 DEMOGRAPHIC DATA

The age distribution of studied population is exhibited in figure 4:6. Out of the 250 *S. aureus*, 40.4 % (101) was obtained in the age group of less than one year. Age group of 1-9 years had 27.6 % (69). Age group of 50-59 years had 3.6 % (9).

Fig 4:6 Age Distribution of study population



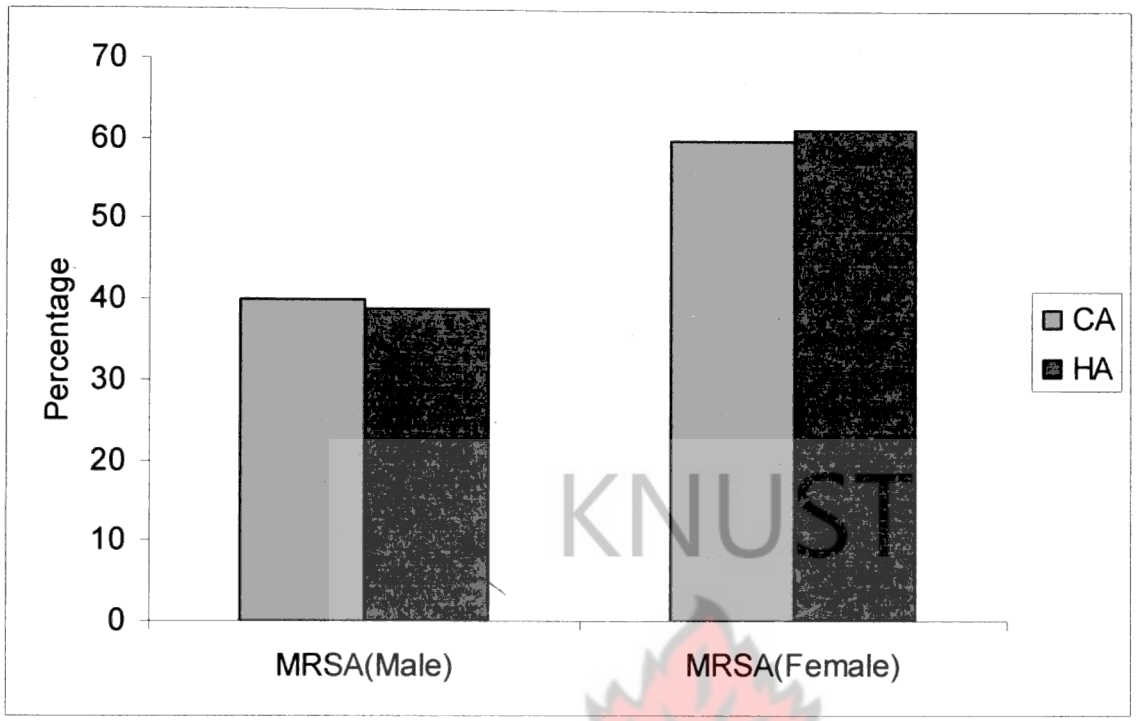
The mean age in this study is 11 years. The age distribution of MRSA patients is exhibited in Fig 4:7. Patients with HA-MRSA infection had their ages ranging from-, day old to 57 years, approximately 65.7% (44) of the isolates came from patients in the age group of less than 1year. Patients between the ages of 1-9 years formed 25.4% (17) of the isolates. The remaining 8.9 % (6) of the isolates were distributed among the other age groups. In the CA-MRSA the ages of patients ranged from 2 to 85 years. Patients in the age group of 20-29 years formed 35% (7/20) of the isolates. This was followed by age group of 1-9 years forming 20 % (4/20) of the isolates. The remaining 45 % (9/20) was distributed among the other age groups.

Figure 4:7 Distribution of MRSA isolates among the age groups of patients

4.5 SEX DISTRIBUTION OF MRSA

The 87 MRSA isolates were distributed among the sex groups as follows; 39% was recovered from male patients while 61% was from females. For the HA-MRSA, 38.8% was obtained from male patients and 61.2% from female patients. In the CA-MRSA 40% and 60% were obtained from male and female patients respectively. Forty percent (40%) of males based in the community had MRSA and 38.8% of males in the hospital had MRSA but the difference was not significant (p=0.923). Although the proportion of females in the community was 60% and that of the females in the hospital was 61.2% the difference in their proportions was not significant (p=0.923) as shown in Table 7.

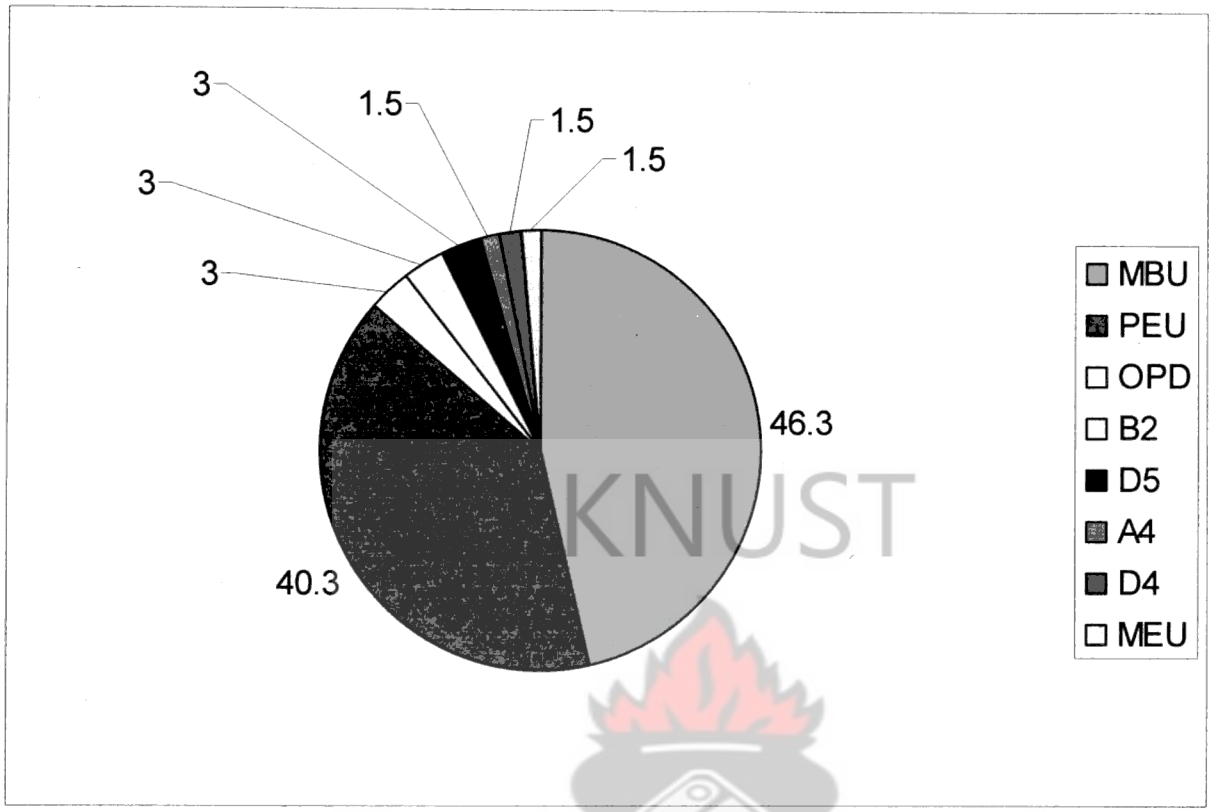
Figure 4:8 Sex distribution of MRSA isolates



4.6 DISTRIBUTION OF MRSA FROM VARIOUS DEPARTMENTS

The CA-MRSA isolates came from the OPD. The distribution of HA-MRSA according to the source of infection is shown in Figure 4:9.

Figure 4:9 Percentage distribution of HA-MRSA from various departments

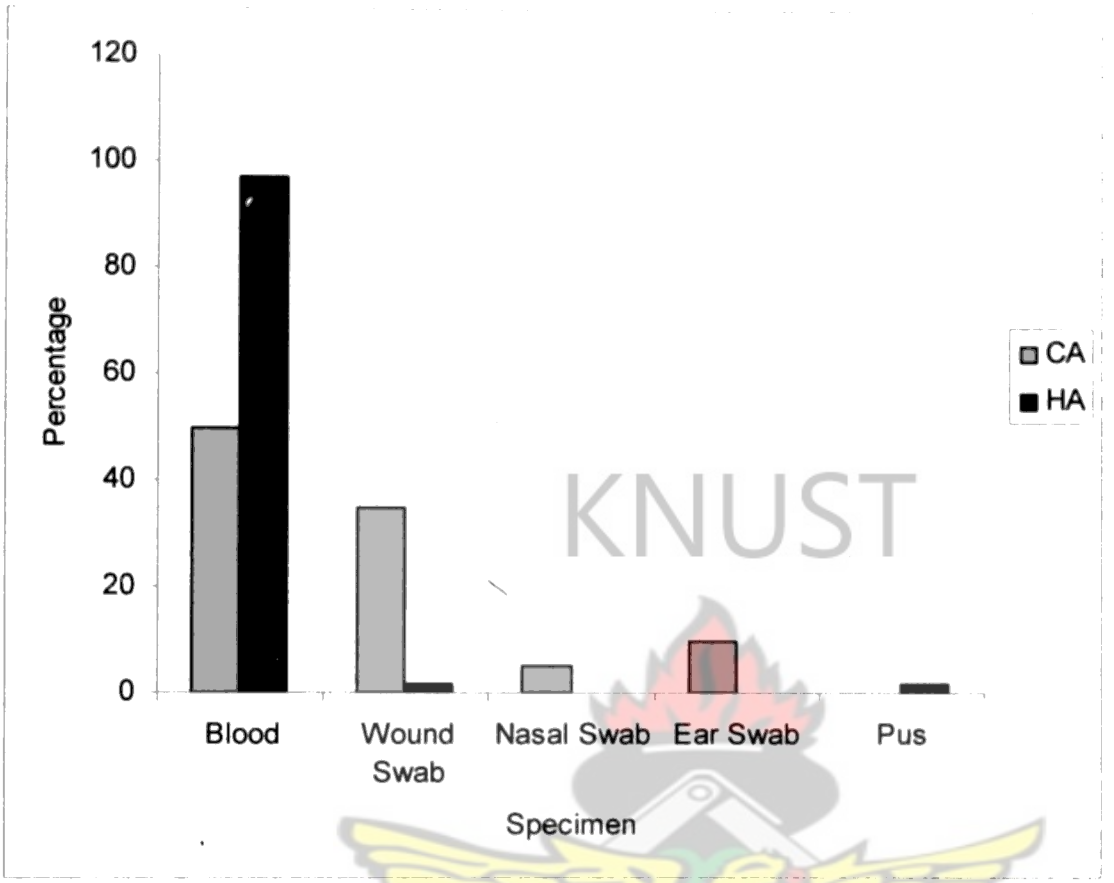


MBU= Mother and baby unit; PEU= Paediatric emergency unit; OPD= Outpatients department; MEU = Male emergency unit; B2, D5, A4, D5 = blocks A, B, C, D (wards) of the hospital.

4.7 RATE OF ISOLATION FROM CLINICAL SPECIMENS

Figure 4:10 exhibits the distribution of MRSA from clinical specimens. Of the 250 S. *aureus,* 84% (210/250) was from blood and 16% (40/250) was from miscellaneous samples. Out of the 87 MRSA, 86.2% (75/87) was obtained from blood, whiles 13.8% (12/87) was from miscellaneous samples.

Figure 4:10 Distribution of MRSA isolates from clinical specimens



4.8 DISTRIBUTION OF MRSA IN RELATION TO PRESENTING CONDITION

The distribution of MRSA in relation to presenting condition is presented in Table 5 in appendix 2. In the HA-MRSA the three main sources of infection were sepsis, 52.2%, pneumonia 8.9% and septicaemia 5.9% In the CA-MRSA, the highest infection isolation was from sepsis with 40%, followed by ear infections with 10%. Patients with underlying infections formed 9.2% (8/87) of the isolates. The breakdown was as follows: 5.7 % (5) had heart disease, 1.1% (1) had diabetes, 1.1 % (1) had HIV and 1.1% (1) had liver infection.

4.9 RELATIONSHIP OF SEX AND AGE ON MRSA PREVALENCE

4.9.1 UNIVARIATE MODEL

The MRSA status, which was treated as binary outcome, was examined with regards to the individual's sex and age in the logistic models. Table 8 summarizes the results of both the univariate and multivariate logistic model. In the univariate model, females were 1.4 times more likely to have MRSA as males (OR=1.4, 95% CI [0.84 – 2.43], p=0.185). However, this was not statistically significant. Those within the age group 1-9 were 40% less likely to have MRSA than children less than 1 year old (OR=0.6, 95% CI [0.30 – 1.08], p=0.791). This association was not statistically significant. The age groups 20-29 (OR=0.8, 95% CI [0.33 – 1.96], p=0.693), 40-49 (OR=1.3, 95% CI [0.18 – 9.56], p=0.800), 50-59 (OR = 0.6, 95% CI [0.15 – 2.74], p = 0.555) and 80-89 (OR=3.9, 95% CI [0.39 – 38.65], p = 0.247) were not associated with MRSA. In addition, those within age group 30-39 were 80% less likely to have MRSA than children less than 1 year old (OR=0.2, 95% CI [0.07 – 0.89], p=0.032) and this was significant.

4.9.2 MULTIVARIATE MODEL

In the univariate model the age group 30-39 was associated with MRSA (OR = 0.2, 95% CI [0.07 – 0.89], p = 0.032). After controlling for sex in the multivariate model, only age group 30-39 was significant (OR = 0.2, 95% CI [0.07 – 0.90], p=0.034).

Table 8 Univariate and Multivariate Model

Characteristics	Univariate (Unadjusted)		Multivariate (Adjusted)	
	Odds Ratio (95% CI)	P value	Odds Ratio (95% CI)	P value
Sex				
Male	1		1	
Female	1.4 (0.84 – 2.43)	0.185	1.1 (0.61 – 1.89)	0.796
Age				
<1	1		1	
1-9	0.6 (0.30 – 1.08)	0.791	0.6 (0.30 – 1.11)	0.097
10-19	-	-	-	-
20-29	0.8 (0.33 – 1.96)	0.639	0.8 (0.35 – 1.97)	0.648
30-39	0.2 (0.07 – 0.89)	0.032	0.2 (0.07 – 0.90)	0.034
40-49	1.3 (0.18 – 9.56)	0.800	1.3 (0.17 – 9.50)	0.805
50-59	0.6 (0.15 – 2.74)	0.555	0.7 (0.15 – 2.76)	0.561
60-69	-	-	-	-
70-79	-	-	-	-
80-89	3.9 (0.39 – 38.65)	0.247	3.9 (0.39 – 38.41)	0.249

CHAPTER FIVE

5.0 DISCUSSION

5.1 PREVALENCE OF MRSA

A prevalence of 34.8% MRSA was established by this study in Kumasi. Kyei in 2004 reported 12.1% rate in Kumasi. Kesah et al, in 2003 reported rates of 20-30% in Nigeria, Kenya and Cameroon, and in Morocco, Senegal, and Cote' D'Ivoire recorded rates of 10-20 % (Kesah et al, 2003). Rates of below 10% were reported for Algeria, Tunisia and Malta (Kesah et al, 2003).

Rates similar to the one obtained in this study have been reported in other parts of the world. Voss et al, in 1994 reported 34% in France. Madani et al in 2001 and Austin et al, in 2003 published 33% rate in Saudi Arabia.

MRSA is generally reported to be high in North America. Kuehnert et al, in 2005 reported a prevalence of 43.7%. The European Antimicrobial Resistance Surveillance System in 2002 and Voss et al, 1994 published a prevalence of 43.2% in southern European countries. Prevalence of between 50-70% has been reported in Japan in 2000, Malaysia in 1992, Latin America in 2000, Ethiopia in 1991 and Sri Lanka in 1998 (Takeda et al, 2000, Hanifah, et al, 1992, Gales et al, 2000, Geyid et al, 1991, Hart et al, 1998). Stefani et al, in 2003, reported more than 30% in Spain and Italy and below 1% in Scandinavia. The reason for the increased prevalence in this study may be due to the indiscriminate use of antibiotics, lack of awareness and self medication before coming to the hospital might be contributing factors. This study was however not designed to

identify risk factors for MRSA prevalence but in a country where prevalence is low, this has been associated with restriction of antibiotic use, strict infection control measures and high ratio of nurses to patients (Vandenbroucke-Graul, 1998). All these do not exist at KATH and could have contributed to the increase in MRSA infections in the current study.

5.2 MRSA PREVALENCE BY TYPES

Traditionally, MRSA has been considered a major nosocomial pathogen in healthcare facilities, but in the past decade, it has been observed emerging in the community as well (Saravolatz et al, 1982). In this study, 34.8% (87) prevalence rate was established; however the prevalence rate of HA-MRSA was 26.8% (67) while 8% (20) rate was obtained for CA-MRSA. Similar rates of 20.8% HA and 8.1% CA prevalence have been reported in Trinidad (Fitzroy et al, 2006). Huang et al, 2006 reported 55.1% HA prevalence and 44.9% CA prevalence, in California which is higher than what was obtained in this study.

The isolation of MRSA from hospitalized patients was quite high (74.7%) as compared to outpatients, which was 25.3%. Baddour et al, 2006 reported a similar isolation of 77.5% from inpatients as against 22.5% from outpatients. The rate of MRSA isolation from inpatients and outpatients might have accounted for the difference in rate of HA and CA isolation, although the difference was not statistically significant, given a p value equal to 0.9. However, the high prevalence of MRSA in hospitalized patients might be due to long stay in the hospital, surgery or invasive procedures. Again the role of hospital personnel

as carriers also needs a special mention because many outbreaks of MRSA infections in hospitals have been traced to hospital personnel (Lacksley, 1982).

5.3 ANTIBIOTIC RESISTANCE PATTERNS

Resistance to multiple antibiotics among the *S. aureus* isolates in hospitals has been recognized as one of the major challenges in controlling hospital infections. The pattern of bacterial resistance is important for epidemiological and clinical purposes (Braun et al, 2003). In general there was no significant difference in HA and CA isolates in terms of antibiotic resistant patterns with a p value equal to 0.76. However Herold et al, (1998) has reported that CA isolates are more susceptible to antibiotic classes other than β-lactam antibiotics. Most documented MRSA infections were acquired nosocomially with CA-MRSA restricted to patients with frequent contact with health facilities, such as residents of long-term care facilities and intravenous drug users (Levine et al, 1982). In 1993, novel MRSA strains were reported from Western Australia. The strains had been isolated from indigenous Australian patients who had not been previously exposed to the health-care system (Udo et al, 1993). Publication of this information heralded the worldwide recognition of the striking evolution of genuine CA-MRSA, which were transmitted in the community and differed from conventional endemic nosocomially acquired MRSA but according to Zetola et al, (2005), CA-MRSA apparently did not evolve in the community but represents a hybrid between MRSA which escaped from the hospital environment. This might have accounted for CA-MRSA not differing from HA-MRSA in antibiotic resistance which is in contrast with what was reported by Herold et al, 1998.

Drug resistance was highest with penicillin and ampicillin having 100% resistance in both HA and CA isolates. This high level of resistance has been reported in several other places worldwide. These results are in agreement with Udo et al who reported 100% resistance to penicillin and ampicillin (Udo et al, 2001). Kumari et al, (1997) also reported 100% resistance to penicillin and ampicillin. Cotrimoxazole resistance was 80.5% and 70% in HA and CA isolates respectively. Anupurba et al, (2003) have published more than 80% resistance to cotrimoxazole in their study.

Resistance of HA and CA isolates to tetracycline was 74.6% and 80% respectively. A similar resistance of 78.7% has been reported in Trinidad (Fitzroy et al, 2006). However Leski et al in (1998) reported 40.5% resistance. The result obtained in this study probably reflects the heavy use of tetracycline at KATH.

Gentamicin resistance was 70.1% and 25% in HA and CA isolates respectively. According to Turnidge et al, (2000), Gosbell et al, (2001) and Colliginon et al, (1998) in-vitro resistance to gentamicin is a good surrogate marker of nosocomial acquisition of MRSA, and conversely community–acquired strains of MRSA are usually gentamicin susceptible in-vitro. This has been reiterated in this study where 70.1% of HA-isolates were resistant and 25% of CA isolates were resistant to gentamicin. Among aminoglycosides, 90% resistance has been established in Eastern Uttar Pradesh. (Anupurba et al, 2003).

Flucloxacillin is the drug of choice for the treatment of *S. aureus* infections at KATH. Resistant rate of 55.2% and 60% in HA and CA-isolates, respectively gives a cause for

concern. The current resistant rate may be attributed to the over prescription of the drug and the subsequent abuse by patients, especially in the communities.

Cefuroxime resistance was 22% and 30% in HA and CA isolates respectively. The relatively low resistance rate obtained might be due to the fact that the drug is expensive as such clinicians at KATH do not prescribe it as frequently as they do with the cheaper drugs such as penicillin, ampicillin, gentamicin and tetracycline. Again due to the high price of the drug, potential drug abusers may refrain from its purchase.

Erythromycin resistance in HA and CA isolates were 31% and 45% respectively. This result probably reflects the abuse of the drug in the community. Leski et al in (1998) reported 48.7% erythromycin resistance. Erythromycin resistance of 86.7% has also been reported in Trinidad (Fitzroy et al, 2006).

Overall erythromycin and cefuroxime exhibited moderate efficacy while high rate of resistance were obtained from penicillin, ampicillin, cotrimoxazole, tetracycline, gentamicin and flucloxacillin. According to CLSI, MRSA and methicillin-resistant coagulase-negative Staphylococcus should be reported as resistant to all other penicillins, carbapenems, cephems and other β-lactam or β-lactamase inhibitor combinations, regardless of in-vitro test results with these agents. This is because most cases of documented methicillin-resistant infections have responded poorly to β-lactam therapy and convincing clinical data have yet to be presented that document clinical efficacy for β-lactam against MRSA (CLSI, 2006).

The results of the antimicrobial resistance in this study give serious cause for concern because the predominant MRSA isolates were resistant to the commonly available antimicrobial agents.

Bacterial resistance threatens the ability to treat both common and serious infections. Although new antibiotics can effectively treat some resistant pathogens and more research is needed to develop novel antimicrobials, bacteria will eventually develop resistance to any antibiotic with time. The misuse and overuse of antibiotics drive the emergence and spread of resistance. Eliminating inappropriate antibiotic use and promoting more judicious use are essential parts of the solution.

5.4 THE MIC OF FIFTY MRSA ISOLATES TO FOUR ANTIBIOTICS

Dilution methods have the ability to detect certain resistance patterns that may not be detected by disc diffusion or automated systems (Sahm et al, 1989). They are not routinely applied to all microorganisms but rather are used in unusual situations. Results of these tests may aid in determination of optimal antimicrobial therapy, elucidation of resistant mechanism, or epidemiologic analysis of resistant isolates (Charles et al, 1982). They are used both in the clinical setting and in research. In research, they are most often used to predict antimicrobial dose responses (Charles et al, 1982).

Results of oxacillin E-test showed that all 50 isolates tested were resistant with MIC ranging from 4 - ≥256μg/ml. This was in the resistant range of ≥ 4μg/ml according to CLSI standards.

Gentamicin MIC for susceptible isolates ranged from 0.125-4μg/ml which is within the susceptible range of ≤4μg/ml according to CLSI. At MIC 8μg/ml which is the intermediate criteria for CLSI, 3 isolates were obtained. The resistant range obtained in this study was 24 - ≥ 256μg/ml, which was slightly higher than resistant value quoted by CLSI, which is ≥16μg/ml.

Trimethoprim sulfamethoxazole MIC for susceptible isolates ranged from 0.064-2μg/ml. This result was in the susceptible range of ≤ 2μg/ml according to CLSI. Isolates which showed resistance had MIC ranging from 4- ≥32μg/ml, which conformed to CLSI criteria of ≥ 4μg/ml.

Ceftriaxone breakpoint for *S. aureus* has not yet been established by CLSI. Results of MIC obtained in this study ranged from 1.5- ≥32μg/ml. However the control *S. aureus* ATCC 25923 gave an MIC of 2μg/ml.

5.5 DEMOGRAPHICS

The mean age of the study was 11 years. This is lower than the mean age of 35.7years quoted by Bukharie and Abdelhadi (Bukharie et al, 2001). Khairulddin et al, 2001, reported that the rate of methicillin resistance of *S. aureus* from children aged less than 15 years increased from 0.9% in 1990 to 13% in 2000, and was most notable in infants. MRSA outbreaks in neonatal intensive care units are well reported (Anderson et al, 2002). Ross et al and Adcock et al have reported that young children tend to have higher

colonization rates, probably because of their frequent contact with respiratory secretions (Ross et al, 1974, Adcock et al, 1998).

The increase in proportion of MRSA bacteraemia in children is a cause for concern for both patients and clinicians. This is due to the fact that MRSA bacteraemia is associated with a higher mortality, longer hospital stay, and a significant independent risk factor for death (Cosgrove et al, 2003). Although some of these may be confounding factors, undoubtedly MRSA bacteraemia in infants may have serious sequelae (Cosgrove et al, 2003).

MRSA isolation was 50.6% in the age group of less than one year. Age group of 1-9 years had 24.1%. The results obtained, may be due to the following: The microbiology department of KATH receives the bulk of its blood samples from the mother and baby unit (MBU) and paediatric emergency unit (PEU). In this study *S. aureus* was isolated mostly (84%, 210 / 250) from blood samples and subsequently, 86.2% (75 /87) of MRSA isolates was obtained from blood. Out of the 87 MRSA isolates 46.3 % (31) and 40.3% (27) isolates were obtained from the MBU and PEU respectively. The MBU caters mostly for patients who are less than one year. Patients between the ages of 1-9 years are mostly catered for at the PEU.

The 87 MRSA isolates were distributed among the sex groups as follows: 39% were recovered from male patients while 61% was from female patients. These results are in contrast to those reported in other parts of the world. Vanbelkum et al and Madani et al

reported a procurement of 64.4% from males as against 35.6% from females and 65.8% male and 34.2% females respectively from some hospitals in Saudi Arabia. (Vanbelkum et al, 1997, Madani et al, 2001). Tentolouris et al, 2006 also reported 60.7% male patients as against 39.3% females.

The results obtained in this study probably reflect the gender distribution of MRSA infections at KATH with female patient predominance, indicating greater exposure to the infection. However no obvious reason has been reported in literature as to the impact of gender in the prevalence of MRSA in the community and hospital setting (Osmon et al, 2004).

5.6 RELATIONSHIP OF SEX AND AGE ON PREVALENCE

The MRSA status was treated as binary outcome with regards to the individual's sex and age. In the univariate model, females were 1.4 times more likely to have MRSA as males; however this was not statistically significant. Age groups 1-9 were 40% less likely to have MRSA than children less than 1 year old, however this association was not statistically significant. After controlling for sex in the multivariate model only age group 30-39 was associated with MRSA and this was significant.

5.7 RATE OF ISOLATION OF MRSA FROM CLINICAL SPECIMENS

Maximum isolation of MRSA was from blood specimens with 86.2% of the total 87 isolates whiles 13.8% was isolated from miscellaneous samples (Pus, 1.2%, wound swab 9.2% nasal swab, 1.2% and ear swab 2.3%). In a report on the surveillance and

epidemiology of MRSA bacteraemia in the UK, the most striking finding from the surveillance was the dramatic increase in proportion of *S. aureus* isolates from blood culture that were methicillin resistant which occurred during the last 12 years (Woodhead et al, 2004). A surveillance study carried out by the BSAC, revealed that high proportion of MRSA among blood culture isolates of *S aureus* in recent years has been confirmed in their study which involved 25 sentinel laboratories geographically, dispersed throughout the UK and Ireland. They reported that, rates of MRSA among cases of *S aureus* bacteraemia in 2001, 2002 and 2003 were 43%, 41% and 40% respectively (BSAC, 2005, Reynolds et al, 2004).

The high rate of isolation from blood in this study was in accordance with a lot of the study population being diagnosed with blood stream infections. The top clinical diagnosis in the study was sepsis with 51.7%, followed by septicaemia with 5.7% of the total 87 MRSA isolates. The probable reason for low isolation of *S. aureus* and subsequently MRSA from miscellaneous specimens can be attributed to prior taking of antibiotics by patients before reporting to the hospital, which is a popular habit of patients attending this hospital.

5.8 CONCLUSION

There is a progressive increase in MRSA prevalence in Kumasi but the current rate is still low in comparison to reports in some other countries. In view of the high resistance rates of MRSA to penicillin, ampicillin, flucloxacillin, tetracycline, gentamicin and cotrimoxazole, treatment of MRSA infections at KATH with these antibacterial agents would be unreliable in cases where no prior antibiotic testing has been done. However cefuroxime and erythromycin showed moderate efficacy. Vancomycin is still the drug of choice for treating multidrug resistant MRSA infections; even though the cost makes it difficult to be used routinely. The study emphasizes the fact that the problem of MRSA has become important. There is therefore the need for constant monitoring of its prevalence and antimicrobial susceptibility patterns as the data from such studies will help the clinicians in effective treatment of MRSA infections.

5.9 RECOMMENDATIONS

Hand washing has been shown to be the most effective tool in hospital infection control; this must be rigidly enforced in the hospitals. This is because health-workers' hands is widely believed to be the predominant method by which MRSA is transmitted to patients.

Health-care workers should be routinely screened. Those found to be carriers, should be kept from intensive care units and the baby units; since many outbreaks in these units have been traced to them. Failure to identify health-care-workers who are persistently colonized or infected can lead to continuing transmission despite implementation of barrier precautions and hand hygiene.

Surveillance to monitor the prevalence, epidemiology and antimicrobial resistance of MRSA infections, should be implemented in hospitals. This knowledge will allow the establishment of recommendations for antimicrobial prescribing within local communities and for the implementation of rational antibiotic policies. Education on judicious use of antibiotics should be intensified in the communities since antibiotic resistance in CA isolates has been shown to be as intense as the HA isolates in this study.

Not much investigation of outpatients infections are done routinely to detect and monitor MRSA. Efforts should be made to obtain cultures from all patients with infections that may be caused by *S. aureus,* whereas microbiology laboratories should routinely test all *S. aureus* isolates for resistance to methicillin (oxacillin).

Against the background of CLSI's recommendation of not using any beta lactam antibiotic for a proven MRSA case, it is recommended strongly that local clinical studies be conducted to confirm the situation, since the practice is not currently being followed by clinicians in Ghana.

Further studies using molecular studies to monitor the epidemiology of MRSA in hospitals in the country is highly recommended. The diagnosis of MRSA using the detection of the mecA is acclaimed as the method of the future.

REFERENCES

Adcock PM, Pastor P, Medley F, Patterson JE, Murphy TV. (1998). Methicillin-resistant *Staphylococcus aureus* in two child care centers. *J Infect Dis.* 178 :577 –580.

Almeida,R. J., J. H. Jorgenson and J. E. Johnson (1983). Evaluation of the AutoMicrobic System Gram-positive Identification Card for species Identification of coagulase-negative Staphylococci. J. Clin. Microbiol. 18:438-439.

Andersen BM, Lindemann R, Bergh K. (2002). Spread of methicillin-resistant Staphylococcus aureus in a neonatal intensive care unit associated with understaffing, overcrowding and mixing of patients. *J Hosp Infect*; 50:18–24.

Anupurba S, Sen MR, Nath G, Sharma BM, Gulati AK, Mohapatra TM. (2003). Prevalence of methicillin resistant *Staphylococcus aureus* in a tertiary referral hospital in eastern Uttar Pradesh. Indian J Med Microbiol .21:49-51.

Austin TW, Austin MA, McAlear DE. (2003). MRSA prevalence in a teaching hospital in western Saudi Arabia. *Saudi Med J* 24:1313-1316.

Baddour M.M Abuelkheir, M.M, Fatani A.J. (2006). Trends in antibiotic susceptibility patterns and epidemiology of MRSA isolates from several hospitals in Riyadh, Saudi Arabia. Annals of Clinical Microbiology and Antimicrobials; 5:30.

Baker CN, (1991). Comparison of the E-test to agar dilution, broth microdilution and agar diffusion susceptibility testing techniques by using a special challenge set of bacteria. J Clin Microbiol; 29:533-538.

Baldwin JN, Rheins MS, Sylvester RF (1957). Staphylococcal infections in newborn infants. Am. J. Dis. Child. 94: 107 – 116.

Barber M, Rozwadowska-Dowzenko M. (1948). Infection by penicillin-resistant *Staphylococci.* Lancet.; 641–644.

Barry, A. L. (1986). Procedures for testing antimicrobial agents in agar media; theoretical considerations, p.1-26. In V. Lorian (ed), Antibiotics in laboratory medicine, 2nd ed. The Williams and Wilkons Co., Baltimore.

Barry, A. L., and R. N. Jones. (1987). Reliability of high- content discs and modified broth dilution tests for detecting staphylococcal resistance to the penicillinase-resistance penicillins. J. Clin. Microbiol. 25:1897-1901.

Bekkaoul F, McNevin J P, Leung C H, Peterson G J, Patel A, Bhatt R S, Bryan R N. (1999). Rapid detection of the *mecA* gene in methicillin resistant staphylococci using a colorimetric cycling probe technology. Microbiol Infect Dis 34:83–90.

Blot S, Vandewoude K, Hoste E, Colardyn F (2002). Outcome and attributable mortality in critically ill patients with bacteremia involving methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. *Arch Intern Med* 162 (19): 2229–35.

Bohach, G.A., and Foster, T.J. (1999). *Staphylococcus aureus* exotoxins in *Gram positive bacterial pathogens.* American Society for Microbiology, Washington, D.C., USA. 367–378.

Bouchillon SK, Johnson BM, Hoban DJ, Johnson JL, Dowzicky MJ, Wu DH, Visalli MA, Bradford PA. (2004). Determining incidence of extended spectrum β-lactamase producing Enterobacteriaceae, vancomycin-resistant Enterococcus faecium and methicillin-resistant Staphylococcus aureus in 38 centres from 17 countries. Int J Antimicrob Agents. 24:119–124.

Boyce J.M., Landry M., Deetz T.R. and Dupont H.L. (1981). Epidemiological studies of outbreaks of nosocomial methicillin-resistant *Staphylococcus aureus* infections. Infect. Control, 2: 1 10, 1981.

Bratu S, Eramo A, Kopec R. (2005). Community-associated methicillin-resistant *Staphylococcus aureus* in hospital nursery and maternity units. Emerg Infect Dis 11:808-813.

Braun R, Hassler D. (2003). Methicillin-resistant *Staphylococcus aureus* infections spread in USA. Dtsch Med Wochenschr 28:855.

British Society for Antimicrobial Chemotherapy (BSAC) (2005). Resistance Surveillance: Bacteraemia Menu. http://www.bsacsurv.org.

Brown, D. F., and P. E. Reynolds. (1980). Intrinsic resistance to beta-lactam antibiotics in *Staphylococcus aureus*. FEBS Lett. 122:275-278.

Brumfit W, Hamilton-Miller J. (1989). Methicillin-resistant *Staphylococcus aureus*.

Bukharie HA, Abdelhadi MS. (2001). The epidemiology of Methicillin-resistant *Staphylococcus aureus* at a Saudi University Hospital. *Microb Drug Resist.* 2001;7:413–416.

Burnett GW, Henry WS, Schuster SG (1996). *Staphylococcus* and Staphylococcal infections. In Oral microbiology and infectious disease. First edition, the Williams and Wilkins, pp. 405 – 416.

Centers for Disease Control and Prevention. (1997). *Staphylococcus aureus* with reduced susceptibility to vancomycin-United States, MMWR Morb Mortal Wkly Rep;46:765-766.

Centres for Disease Control and Prevention. (2002). National Nosocomial Infections Surveillance System (NNIS) report, Am J Infect Control. 31:481-498.

Centers for Disease Control and Prevention. (2003). Public health dispatch: outbreaks of community-associated methicillin-resistant *Staphylococcus aureus* skin infections- Los Angeles, California,.MMWR Morb Mortal Wkly Rep.52:88.

Centres for Disease Control. (2004). Vancomycin-resistant *Staphylococcus aureus*-New York. MMWR, Morb Mortal Wkly Report, 23; 53 (15): 322-323.

Chambers HF (2001). The changing epidemiology of *Staphylococcus aureus*. Emerg Infect Dis 7 (2): 178-82.

Charles PG, Ward PB, Johnson PD, Howden BP,Grayson. (2004). Clinical features associated with bacteraemia due to heterogenous vancomycin-intermediate *Staphylococcus aureus*.Clin Infect Dis 38:448-451.

Charles W.S.,R.C.Cooksey. (1982). Susceptibility tests: Special tests. Manual of Clinical Microbiology 5th Ed., pp1153-1165.

Cheesbrough M. (2000). Medical Laboratory Manual for Tropical Countries PT 2, 2nd ed., Macmillan Publishing Co., New York. pp, 64-86, 132-141.

Chung M, Dickinson G, de Lencastre H., Tomasz A. (2004). International Clones of Methicillin-Resistant *Staphylococcus aureus* in two Hospitals in Miami, Florida *J. Clin. Microbiol.* 42:542-547.

Clinical and laboratory Standards Institute (2006). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; 7th ed. Vol. 23 No. 2.

Cockerill III FR, Hughes JG, Vetter EA, Mueller RA, Weaver AL, Ilstrup DM. (1997) Analysis of 281,797 consecutive blood cultures performed over an eight-year period: trends in microorganisms isolated and the value of anaerobic culture of blood. Clin Infect Dis 24:403-418.

Cosgrove SE, Qi Y, Kaye KS, Harbarth S, Karchmer AW, Carmeli Y. (2005). The impact of Methicillin Resistance in Staphylococcus aureus Bacteremia on Patient Outcomes: Mortality, Length of Stay, and Hospital Charges. Infection Control and Hospital Epidemiology 26: 166–174.

Cosgrove SE, Sakoulas G, Perencevich EN. (2003). Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia; a meta-analysis. Clin Infect Dis 36:53–59.

Colliginon P, Gosbell I, Vickery A. (1998). Community-acquired methicillin resistant Staphylococcus aureus in Australia.Lancet 352:145-146.

Crouch, S. F., T. A. Pearson, and D. M. Parham. (1987). Comparison of modified Minitek system with Staph-Ident system for species identification of coagulase-negative staphylococci. J. Clin. Microbiol. 25: 1626-1628.

Curran JP, Al-Salihi FL (1980). Neonatal Staphylococcal scalded skin syndrome: massive outbreak due to an unusual phage type. 66 (2): 285-290.

D'Amato, R. F., L. Hochstein, J. R. Vernalio, D. J.Cleni, A .A. Wallman, M.S.Gradus , and C. Thornsberry. (1985). Evaluation of the BIOGRAM antimicrobial susceptibility test system. J. Clin Microbiol. 22:793-798.

de Haas, C.J. (2004). Chemotaxis inhibitory protein of *Staphylococcus aureus*, a bacterial anti-inflammatory agent. *J. Exp. Med.* 199:687-695.

Diekema, D. J., B. Boots Miller, T. Vaughn, R. Woolson, J. Yankey, E. Ernst, S. Flach, M. Ward, C. Franciscus, M. A. Pfaller, and B. N. Doebbeling. (2004). Antimicrobial resistance trends and outbreak frequency in United States hospitals. Clin. Infect. Dis. 38:78-85.

Doig CM (1981). Nasal carriage of *Staphylococcus* in a general surgical extra corporal circulation. Pneumonol. Alergol. Pol. 63: 371 – 377.

Duckworth G. (2003). Controlling methicillin-resistant Staphylococcus aureus. British Med Journal.; 327:1177–1178.

Dufour P, Gillet Y, Bes M. (2002). Community-acquired methicillin-resistant *Staphylococcus aureus* infections in France: emergence of a single clone that produces Panton-Valentine leukocidin. *Clin Infect Dis.*; 35:819 –824.

Ena J, Dick RW, Jones RN. (1993). The epidemiology of intravenous vancomycin usage in a university hospital: a 10-year study. JAMA, 269: 598 – 602.

Enright M C. (2003). The evolution of a resistant pathogen – the case of MRSA. *Curr Opin pharmacol* 3: 474-479.

European Antimicrobial Resistance Surveillance System. (2002). *Annual Report. On-going surveillance of S. pneumoniae, S. aureus, E. coli, E. faecium, E. faecalis.* Bilthoven EARSS; extra corporal circulation. Pneumonol. Alergol. Pol. 63: 371 – 377.

Fedtke, I., Gotz, F., and Peschel, A. (2004). Bacterial evasion of innate host defenses-the *Staphylococcus aureus* lesson. *Int. J. Med. Microbiol.* 294:189-194.

Fitzroy A Orrett, Michael Land. (2006). Methicillin-resistant S*taphylococcus aureus* prevalence: Current susceptibility patterns in Trinidad. BMC Infect Dis.; 6: 83.

Foster, T.J., and Höök, M. (1998). Surface protein adhesions of *Staphylococcus aureus. Trends Microbiol.* 6:484-488.

Fridkin SK, Hill HA, Volkova NV. (2002). Temporal changes in prevalence of antimicrobial resistance in 23 US hospitals. Emerg Infect Dis 2002:8:697-701.

Gales, Ac.;Jones, RN.;Pfaller, MA.;Gordon, KA.; Sader, HS.(2000). Two-year assessment of the pathogen frequency and antimicrobial resistance patterns among organisms isolated from skin and soft tissue infections in Latin American Hospitals. *Int J Infect Dis.*; 4:75–84.

Geyid A, Lemeneh Y. (1991). The incidence of methicillin-resistant *Staphylococcus aureus* strains in clinical specimens in relation to their β-lactamase producing and multiple drug resistance properties in Addis Ababa. *Ethiop Med J.* 29:149–161.

Goodyear, C.S., and Silverman, G.J. (2003). Death by a B cell super antigen: in vivo VH-targeted apoptotic supraclonal B cell deletion by a Staphylococcal Toxin. *J. Exp. Med.* 197:1125-1139.

Gosbell IB, Mercer JL, Neville SA. (2001). Non-multiresistant and multiresistant methicillin-resistant *Staphylococcus aureus* in community-acquired infections.Med J Aust 174:627-630.

Gradelski, E., I. Aleksunes, D. Bonner, and J. Fung-Tomc. (2001). Correlation between genotype and phenotypic categorization of staphylococci based on methicillin susceptible and resistance. J. Clin. Microbiol. 39:2961-2963.

Groom AV, Wolsey DH, Naimi TS, Smith K, Johnson S, Boxru. (2001) Community-acquired methicillin-resistant Staphylococcus aureus in a rural American Indian community. JAMA. 286:1201-1205.

Hamilton-Miller JM. (1995). Errors arising from incorrect orientation of E-test strips. J Clin Microbiol; 33:1966-1967

Hanifah YA, Hiramatsu K, Yokota T. (1992).Characterization of methicillin-resistant *Staphylococcus aureus* associated with nosocomial infection in the University Hospital, Kuala Lumpur. *J Hosp Infect.*; 21:15–28.

Hart CA, Kariuki S. (1998). Antimicrobial resistance in developing countries. *BMJ.* 317:647–650.

Herold BC, Immergluck LC, Maranan MC, Lauderdale DS ,Gaskin RE, Boyle-Vavra S. (1998). Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA.1998; 279:593-598.

Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. (1997). Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. J Antimicrob Chemother 40:135-136.

Huang H, Flynn N.M, H. King J.F. (2006). Comparisons of Community-Associated Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Hospital-Associated MSRA Infections in Sacramento, California. Journal of Clinical Microbiology, (44)7:2423-2427.

Ito, T. (2004). Novel type V staphylococcal cassette chromosome mec driven by a novel cassette chromosome recombinase, ccrC. *Antimicrob. Agents Chemother.* 48:2637-2651.

Jessica M, Buck, Kathryn Como-Sabetti , (2003) Community-associated methicillin resistant *Staphylococcus aureus*.Emerging Infectious Disease vol. 11.

Jevons M. (1961). Celbenin-resistant staphylococci. BMJ. 1:124-125.

Johnson AP, Aucken HM, Cavendish S, Ganner M, Wale MC, Warner M, Livermore DM, Cookson BD. (2001). Dominance of EMRSA-15 and -16 among MRSA causing nosocomial bacteraemia in the UK. J Antimicrob Chemother 48 (1): 143-144.

Jorgensen JH. (1991). Quantitative antimicrobial susceptibility testing on *Haemophilus influenzae* and *Streptococcus pneumoniae* by using E-test. J Clin Microbiol. ; 29 :109-114.

Kaplan SL, Hulten KG,Gonzalez BE. (2005). Three-year surveillance of community-acquired Staphylococcus aureus infections in children Clin Infect Dis 40:1785-1791.

Kesah C, Ben Redjeb S, Odugbemi TO. (2003). Prevalence of methicillin-resistant *Staphylococcus aureus* in eight African hospitals and Malta. Clin Microbiol Infect.; 9(2):153–156.

Kessler CM, Nussbaum E, Tuazon CU. (1991). Disseminated intravascular coagulation associated with Staphylococcus aureus septicemia is mediated by peptidoglycan-induced platelet aggregation. J Infect Dis;164:101-107.

Khairulddin N, Bishop L, Lamagni T.L, Sharland M and Duckworth G. (2001). Emergence of methicillin resistant *Staphylococcus aureus* bacteraemia among children in England and Wales. 89:378-379.

Klodkowaska-Farner E, Zwolska-Kwiet Z, Wojciechowka M, Bestry I, Pacocha W, Podsiadlo B, Otto T (1995). Pneumonia in Patients after extra corporal circulation. Pneumonol. Alergol. Pol. 63: 371 – 377.

Kloos , W E, and J. H. Jorgensen. (1985). Staphylococcus; p. 143-153. In E. H. Lennette, A. Barlows , W. J. Hausler jr. and Shadomy H. J. , Manual of Clinical Microbiology, 4th ed.American Society for Microbiology , Washington D.C.

Kloos , W E, and J. H. Jorgensen, (1986). Staphylococcus; p. 143-153. In E. H. Lennette, A. Barlows , W. J. Hausler ,jr. , and Shadomy H. J. Manual of Clinical Microbiology,4th ed.American Society for Microbiology , Washington D.C.

Kloos WE, Bannerman T (1995). *Staphylococcus* and *Micrococcus*. Manual of Clinical Microbiology. 6th ed. ASM, 282-298.

Kuehnert MJ, Hill HA, Kupronis BA, Tokars JI, Solomon SL, Jernigan DB. (2005). Methicillin-resistant-*Staphylococcus aureus* hospitalizations, United States. *Emerging Infectious Diseases;* 11:868–*872.*

Kumari D.N.P., Keer V., Hawkey P.M. (1997). Comparison and application of ribosome spacer DNA amplicon polyrnorphisms and'pulsed-field gel electrophoresis for differentiation of methicillin-resistant Staphylococcus aureus strains. Clin. Microbiol. 35: 881.

Kyei F. (2004). The prevalence rate of methicillin resistant *Staphylococcus aureus* in Kumasi: A dissertation submitted to the Kwame Nkrumah University of Science and Technology.

Lacksley RM. (1982). Multiple antibiotic resistant Staphylococcus aureus : introduction,transmission and evolution of Nosocomial infections. Ann-Int. Med. 97: 317-324. Lancet Infect Dis 5: 275–286.

Layton MC, Hierholzer WJ, Jr, Patterson JE. (1995). The evolving epidemiology of methicillin-resistant *Staphylococcus aureus* at a university hospital. Infect Control Hosp Epidemiol 16:12-17.

Lee G. and Bishop P. (1997): Nosocomial infections. Microbiology and Infection Control For Health Professionals. Prentice Hall. p 269.

Leski T., Oliveira D., Trzcinski K. (1998). Clonal distribution of methicillin-resistant Staphylococcus aureus in Poland. J. Clin. Microbiol., 36: 3532.

Levine DP, Cushing RD .Jui J. Brown WJ. (1982) Community-acquired methicillin-resistant Staphylococcus aureus endocarditis in the Detroit Medical Center. Ann Intern Med :97:330-338.

Lina G, Piemont Y, Godail-Gamot F. (1999). Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 29:1128-1132.

Lord Soulsby of Swaffham Prior (2005). Resistance to antimicrobials in humans and animals. *Brit J Med* 331: 1219–1220.

Lowy FD. Staphylococcus aureus infections. (1998). N Engl J Med; 339: 520 – 532.

MacKenzie F. M., Greig P., Morrison D., Edwards, G., Gould, I. M. (2002). Identification and characterization of teicoplanin-intermediate Staphylococcus aureus blood culture isolates in NE Scotland. *J Antimicrob Chemother* 50: 689-697.

Madani TA, Al-Abdullah NA, Al-Sanousi AA, Ghabrah TM, Afandi SZ, Bajunid HA. (2001). Methicillin-resistant *Staphylococcus aureus* in two tertiary-care centers in Jeddah, Saudi Arabia. *Infect Control Hosp Epidemiol.* 22:211–216.

Marples RR, Reith S. (1992). Methicillin-resistant Staphylococcus aureus in England and Wales.CDR .2 :25-29.

Matsuhashi M., Song M.D., Ishino F. (1986): Molecular cloning of gene of a penicillin binding protein supposed to cause high resistance to (3-lactam antibiotics in *Staphylococcus aureus*. J. Bact., 167: 975.

Mazmanian, S.K (2003). Passage of haeme-iron across the envelope of *Staphylococcus aureus. Science.* 299**:** 906-909.

Miller L, Perdreau-Remington F, Rieg G, Mehdi S, Perlroth J, Bayer A, Tang A, Phung T, Spellberg B (2005). Necrotizing fasciitis caused by community-associated methicillin-resistant Staphylococcus *aureus* in Los Angeles. *N Engl J Med* 352 (14): 1445-1453.

Naimi TS, LeDell KH ,Boxrud DJ , Groom AV,Steward CD , Johnson SK. (2003). Epidemiology and clonality of community-acquired methicillin –resistant Staphylococcus aureus in Minnesota.Clin Infect Dis .2001;33:990-6

Novick, R.P. (2003). Mobile genetic elements and bacterial toxinoses: the superantigen-encoding pathogenicity islands of *Staphylococcus aureus. Plasmid.* 49:93-105.

Ogston A. (1883). Micrococcus Poisoning. J Anal physiology 17:24-58.

Oliveira DC. Tomasz A, de Lencastre H. (2001). The evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*: identification of two ancestral genetic backgrounds and the associated *mec* elements.*Microb Drug Resist* 7:349-61.

Oliveira, K., G. W. Procop, D. Wilson, J. Coull, and H. Stender. (2002). Rapid identification of *Staphylococcus aureus* directly from blood cultures by fluorescence in situ hybridization with peptide nucleic acid probes. J. Clin. Microbiol. 40:247-251.

Osmon S, Ward S, Fraser VJ, Kollef MH. (2004). Hospital Mortality for Patients with Bacteremia due to *Staphylococcus aureus* or Pseudomonas aeruginosa. *Chest.* 125:607–616.

Pavillard R., Harvey K., Doughlas D. (1982). Epidemic of hospital acquired infection due to methicillin resistant *Staphylococcus aureus* in major victorian hospitals. Med. J. Aust., 1: 451.

Payne MC, Wood HF, Karakawa W, Gluck L. (1966). A prospective study of staphylococcal colonization and infections in newborns and their families. Am J Epidemiol 82:305-316.

Peacock, S.J., de Silva, I., and Lowy, F.D. (2001). . What determines nasal carriage of *Staphylococcus aureus*. Trends Microbiol. 9:605-610.

Reynolds R, Livermore D, BSAC Working Party. (2004). Bacteraemia Resistance Surveillance.Comparative activity of BAL9141, daptomycin and linezolid versus S. aureus from bacteraemias in the UK and Ireland.In Abstracts of the Forty-fourth

Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, Abstract E-2035, p.180.

Robinson DA, Enright MC. (2003). Evolutionary models of the emergence of methicillin-resistant Staphylococcus aureus. *Antimicrob Agents Chemother* 47:3926-34.

Rolinson GN.Forty years of β-lactam research. (1998). *J Antimicrob Chemother* 41:589-603.

Ross S, Rodroguez W, Controni G, Khan W. (1974). Staphylococcal susceptibility to penicillin G: The changing pattern among community isolates. JAMA 229:1075-1077.

Ryan KJ; Ray CG. (2004). *Sherris Medical Microbiology*, 4th ed., McGraw Hill.

Sahm, D. F., J.Kissinger,M. S. Gilmore, P.R.Murray,R.Mulder,J. Solliday,and B. Clarke. (1989). In vitro susceptibility studies of vancomycin-resistant Enterococcus faecalis. Antimicrob. Agents Chemother.33:1588-1591.

Sanford MD, Widmer AF, Bale MJ, Jones RN, Wenzel RP. (1994). Efficient detection and long-term persistence of the carriage of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis; 19:1123-1128.

Saravolatz LD, Pohlold DJ, Arking LM. (1982). Community-acquired methicillin-resistant *Staphylococcus aureus* infections: a new source for nosocomial outbreaks. Ann Intern Med 97:325-329.

Scott RD, Solomon SL, Cordell R, Roberts RR, Howard DH, McGowan .J Jr. (2005). Measuring the attributable costs of resistant infections in hospital settings. New York:Marcel Dekker 141-181.

Scragg JN, Appelbaum PC, Govender DA. (1978). The spectrum of infection and sensitivity of organisms isolated from African and Indian children in a Durban hospital. *Trans Trop Med Hyg* ;72:325–328.

Shahin R, Johnson IL, Jamieson F, McGeer A ,Tolkin ,Ford-Jones EL. (1999). Methicillin – resistant *Staphylococcus aureus* carriage in a child care center following a case of disease ..*Arch Pediatr* Adolesc Med 153:864-868.

Shanson D.C. (1981). Antibiotic resistant *Staphylococcus aureus*. J. Hosp. Infect., 2: 11.

Sheretz RJ, Reagan DR, Hampton KD. (1996). A cloud adult: the *Staphylococcus aureus*-virus interaction revisited. Ann Intern Med 1996; 124:539-547.

Shieradzki k , Tomasz A. (1997). Inhibition of cell wall ternover and autolysis by vancomycin I a highly vancomycin-resistant mutant of Staphylococus aureus. *J* Bacteriol 8:2557-66.

Stefani S, Varaldo PE. (2003). Epidemiology of methicillin-resistant staphylococci in Europe. Clin Microbiol Infect 9: 1179-1186.

Takeda S, Yasunaka K, Kono K, Arakawa K. (2000). Methicillin resistant *Staphylococcus aureus* (MRSA) isolated at Fukuoka University Hospital and hospitals and clinics in the Fukuoka city area. *Int J Antimicrob Agents.* 14:39–43.

Talaro K, Arthur Talaro. (1993). Foundations in Microbiology, pp 478-481. WCB Publishers.

Tarzi S, Kennedy P, Stone S,Evans M. (2001). Methicillin-resistant *Staphylococcus aureus* psychological impact of hospitalization and isolation in an older adult population.J. Hosp Infect.49:250-254.

Tentolouris N, Petrikkos G, Vallianou N, Zachos C, Daikos GL, Tsapogas P, Markou G, Katsilambros N. (2006). Prevalence of methicillin-resistant *Staphylococcus aureus* in infected and uninfected diabetic foot ulcers. *Clin Microbiol Infect.* 12:186–189.

Tiemersma EW, Bronzwaer SL, Lyytikainen O. (2002). Methicillin-resistant *Staphyloccocus aureus* in Europe, 1999-2002. Emerg Infect Dis ;10:1627-34.

Turnidge JD, Bell JM. (2000) Methicillin-resistant *Staphyloccocal aureus* evolution in Australia over 35 years. Microb Drug Resist; 6: 223-229.

Tuo P, Montobbio G, Callarino R, Tumolo M, Calero MG, Massone MA. (1995). Nosocomial infection caused by multi–resistant staphylococci in a neonatal and pediatric intensive care unit. Pediatric-Med. Clin. 17: 117 – 122.

Udo EE, Jacob LE, Mathew B. (2001). The spread of a mupirocin-resistant/methicillin-resistant *Staphylococcus aureus* clone in Kuwait hospitals. *Acta Tropica.* 80:155–161.

Udo EE. Pearman J W, Grubb WB. (1993). Genetic analysis of community isolates of methicillin-resistant Staphylococcus aureus in Western Australia. J Hosp Infect 25:97-108.

Update: (1997). Staphylococcus aureus with reduced susceptibility to vancomycin-United States,.MMWR Morb Mortal Wkly Rep 1997; 46:813-815.

van Belkum A, Vandenbergh M, Kessie G, Qadri H, Lee G, vanDen Braak N, Verbrugh H, Al-Ahdal MN. (1997). Genetic homogeneity among methicillin-resistant *Staphylococcus aureus* strains from Saudi Arabia. *Microbial Drug Resistance.* 3:365–369.

Vandenbroucke-Grauls C. (1998). Management of methicillin-resistant *Staphylococcus aureus* in the Netherlands. *Rev Med Microbiol* 9:109–16.

Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo G, Hefferman H. (2003). Community-acquired methicillin-resistant *Staphylococcus aureus* carrying the Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis. 9(8):978-84.

von Eiff, C., Becker, K., Machka, K., Stammer, H., and Peters, G. (2001). Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. *N. Engl. J. Med.* 344 :11-16.

Voss A, Milatovic D, Wallrauch-Schwarz C, Rosdahl VT, Braveny I. (1994). Methicillin-resistant *Staphylococcus aureus* in Europe. *Eur J Clin Microbiol Infect D is.*;13:50–55.

Wadlvogel FA. (2000). *Staphylococcus aureus* (including staphylococcal toxic shock). In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. 5th ed. Philadelphia: Churchill Livingstone, p.2072-2073.

Washington, J.A. Synder I.J., Kohner P.C., Wiltsie C.G., D.M.Ilstrup D.M, and J.T. McCall. (1978). Effect of cation of agar on the activity of gentamicin,tobramycin,and amikacin against pseudomonas aureginosa. J.Infect.Dis.137:103-111.

Woodhead M, Fleming D, Wise R. (2004). Antibiotics resistance, and clinical outcomes. BMJ; 328:1270-1271.

Wu S, Piscitelli C, de Lencastre H, Tomasz A. (1996). Tracking the evolutionary origin of the methicillin resistance gene: cloning and sequencing of a homologueof mec A from a methicillin susceptible strain of *Staphylococcal sciuri* .*Microb Drud Resist* 2:435-441.

Zetola N, Francis F.S, Nuermberger E.L, W. R. Bishai. (2005) Community-acquired meticillin-resistant *Staphylococcus aureus*: an emerging threat. Lancet Infect Dis 5: 275–286.

APPENDIX 1A. Results obtained in the study.

	PATH NO	AGE	SEX	SPECIMEN	DIAGNOSIS	OXACILLIN	PENICILLIN	SOURCE
	325	9 yrs	F	blood	septicaemia	16	12	PEU
	333	35 yrs	F	wound swab	wound discharge	14	12	OPD
	406	3 yrs	F	blood	septicaemia	22	11	PEU
	474	24 yrs	F	blood	sepsis	16	16	A5
	490	5 yrs	F	blood	sepsis	23	19	PEU
	925	10 yrs	F	blood	sepsis	17	16	PEU
	930	5 yrs	F	blood	sepsis	24	13	PEU
	3182	24 yrs	F	pus	wound infection	22	16	OPD
	3204	15 yrs	F	wound swab	wound infection	18	0	OPD
0	7306	80 yrs	F	blood	sepsis	19	15	OPD
1	7355	30 yrs	F	blood	osteomyelitis	20	18	OPD
2	7739	1 yr	F	blood	sepsis	18	22	PEU
3	7752	1 yr	F	blood	sepsis	19	13	PEU
4	7754	4 mths	F	blood	neonatal jaundice	24	13	PEU
5	7783	5 yrs	F	blood	sepsis	13	15	MBU
6	7784	11 mths	F	blood	bronchopneumonia	17	20	MBU
7	7810	3 mths	F	blood	bronchopneumonia	14	13	MBU
8	7858	9 yrs	F	blood	septicaemia	13	12	PEU
9	7869	day old	F	blood	sepsis	17	16	MBU
0	7934	2 yrs	F	blood	sepsis	13	12	PEU
1	7973	5mths	F	blood	sepsis	11	17	PEU
2	7976	9 yrs	F	blood	bronchopneumonia	16	19	PEU
3	8003	day old	F	blood	sepsis	15	22	MBU
4	8004	2 mths	F	blood	sepsis	16	19	MBU
5	8072	dayold	F	blood	sepsis	14	23	MBU
6	8142	3 wks	F	blood	meningitis	16	18	MBU
7	8453	11 wks	F	blood	septicaemia	24	16	PEU
8	8455	1 wk	F	pus	sepsis	12	18	MBU
9	8630	6 mths	F	blood	sepsis	25	27	PEU
0	8675	31 yrs	F	blood	haemolytic anaemia	15	20	OPD
1	8730	2 yrs	F	blood	sepsis	21	20	PEU
2	8811	3 mths	F	blood	bronchopneumonia	17	15	PEU
3	8878	6 mths	F	blood	septicaemia	18	19	PEU
4	9042	2 wks	F	blood	sepsis	17	18	MBU
5	9069	1 yr	F	blood	sickle cell disease	18	22	PEU
6	9086	50 yrs	F	blood	sepsis	15	21	B2
7	9157	day old	F	blood	sepsis	21	16	MBU
8	9166	2 yrs	F	blood	fever	16	24	PEU
9	579	1 yr	F	peritoneal fluid	appendicitis	23	14	PEU
0	917	10 mths	F	blood	bronchopneumonia	25	39	PEU
1	899	11 mths	F	blood	sepsis	15	17	PEU
2	938	15 yrs	F	blood	septicaemia	20	22	PEU
3	1252	2 mths	F	blood	septicaemia	20	19	PEU
4	1004	2 wks	F	blood	sepsis	20	32	PEU
5	4092	2 wks	F	blood	sepsis	13	14	MBU
6	4073	2 yrs	F	blood	sepsis	18	19	PEU

APPENDIX 1A. Results obtained in the study.

7	3297	2 yrs	F	blood	sepsis	18	12	PEU
8	3224	2 yrs	F	blood	sepsis	23	18	PEU
9	8453	20 yrs	F	blood	sepsis	20	17	A5
0	7353	20 yrs	F	blood	sepsis	13	19	OPD
1	8866	21 yrs	F	blood	septic arthritis	22	17	OPD
2	8152	22 yrs	F	blood	septicaemia	17	11	A4
3	9153	27 yrs	F	blood	bronchopneumonia	14	13	B2
4	1101	28 yrs	F	pleural aspirate	appendicitis	16	12	OPD
5	3490	29 yrs	F	wound swab	osteomyelitis	13	15	OPD
6	3448	29 yrs	F	wound swab	wound infection	14	23	MBU
7	4164	3 days	F	blood	neonatal sepsis	16	19	MBU
8	9112	3 days	F	blood	sepsis	19	19	MBU
9	7741	3 yrs	F	blood	sepsis	23	16	PEU
0	2979	3 yrs	F	knee aspirate	osteomyelitis	12	18	PEU
1	9525	31 yrs	F	blood	bronchopneumonia	22	17	OPD
2	985	32 yrs	F	blood	sepsis	16	12	OPD
3	8850	34 yrs	F	blood	bronchopneumonia	13	17	OPD
4	8009	36 yrs	F	blood	sepsis	14	23	OPD
5	4809	4 yrs	F	blood	sepsis	20	12	PEU
6	7742	4 yrs	F	blood	sepsis	15	19	PEU
7	1086	40 yrs	F	wound swab	wound infection	21	17	PEU
8	169	46 yrs	F	pus	wound discharge	16	21	OPD
9	111	5 days	F	blood	neonatal jaundice	18	17	MBU
0	284	50 yrs	F	pus	pustular discharge	19	17	OPD
1	3295	55 yrs	F	blood	sepsis	15	23	OPD
2	8235	7 mths	F	blood	sepsis	17	13	PEU
3	9184	7 mths	F	blood	septicaemia	19	20	PEU
4	3177	7 yrs	F	blood	septicaemia	22	18	PEU
5	1462	70 yrs	F	peritoneal fluid	appendicitis	15	16	D5
6	8187	8 yrs	F	blood	sepsis	21	17	PEU
7	3229	9 mths	F	blood	bronchopneumonia	18	14	PEU
8	9063	9 yrs	F	blood	septicaemia	16	21	PEU
9	9158	day old	F	blood	sepsis	19	18	MBU
0	478	day old	F	blood	asphyxia	24	19	MBU
1	8880	day old	F	blood	sepsis	17	14	MBU
2	8820	day old	F	blood	asphyxia	15	23	MBU
3	9067	day old	F	blood	sepsis	11	18	MBU
4	8672	day old	F	blood	sepsis	20	14	MBU
5	834	dayold	F	blood	sepsis	16	21	MBU
6	115	50 yrs	M	pus	wound discharge	16	18	D5
7	313	18 yrs	M	blood	sepsis	16	12	C5
8	2951	1 yr	M	ear swab	otitis media	19	14	PEU
9	3117	30 yrs	M	wound swab	wound infection	15	12	OPD
0	3166	12 yrs	M	wound swab	wound infection	16	21	C2
1	3194	20 yrs	M	wound swab	wound infection	22	18	D2
2	3333	12 yrs	M	wound swab	wound infection	19	16	OPD
3	3372	27 yrs	M	nasal swab	nasal discharge	17	14	OPD

APPENDIX 1A. Results obtained in the study.

4	7355	2 yrs	M	blood	sepsis	19	25	PEU
5	7744	2 wks	M	blood	sepsis	18	16	MBU
6	7747	2 yrs	M	blood	sepsis	16	16	PEU
7	7769	71 yrs	M	blood	sepsis	14	18	MEU
8	7774	3 yrs	M	blood	sepsis	15	21	PEU
9	7859	5 days	M	blood	dehydration	22	19	MBU
00	7877	30 yrs	M	blood	sepsis	19	15	D2B
01	8002	2 mths	M	blood	sepsis	16	18	PEU
02	8145	39 yrs	M	blood	fever	17	16	OPD
03	8174	7 yrs	M	blood	severe malaria	17	19	PEU
04	8443	3 yrs	M	blood	sepsis	15	18	PEU
05	8627	8 yrs	M	blood	sepsis	23	21	PEU
06	8826	50 yrs	M	blood	PTB	15	13	OPD
07	8827	50 yrs	M	blood	PTB	15	21	OPD
08	8841	day old	M	blood	sepsis	17	15	MBU
09	9056	4 yrs	M	blood	sepsis	20	16	PEU
10	9064	2 mths	M	blood	septicaemia	20	25	PEU
11	9077	day old	M	blood	sepsis	15	23	MBU
12	9092	21 days	M	blood	sepsis	17	17	MBU
13	9108	1 yr	M	blood	sepsis	14	17	PEU
14	9139	2 yrs	M	blood	septicaemia	16	18	PEU
15	9149	4 yrs	M	blood	sepsis	15	17	PEU
16	9151	day old	M	blood	sepsis	16	19	MBU
17	9165	29 yrs	M	blood	septicaemia	17	14	OPD
18	9187	16 yrs	M	blood	chronic osteomyeliti	16	17	PEU
19	8156	1 yr	M	blood	bronchopneumonia	15	19	PEU
20	7761	1 yr	M	blood	septicaemia	17	15	PEU
21	109	1 yr	M	blood	sepsis	18	23	PEU
22	8180	10 mths	M	blood	bronchopneumonia	17	20	PEU
23	3181	10 yrs	M	wound swab	wound infection	14	15	PEU
24	8162	11 yrs	M	blood	sepsis	15	17	OPD
25	9212	12 yrs	M	blood	sepsis	18	16	B3
26	9161	12 yrs	M	blood	sepsis	19	21	B3
27	916	15 yrs	M	blood	pneumonitis	17	16	OPD
28	890	16 yrs	M	blood	malaria	15	13	OPD
29	4073	18 yrs	M	blood	sepsis	17	19	OPD
30	1088	18 yrs	M	wound swab	cervical abscess	15	12	C5
31	7741	2 mths	M	blood	pneumonitis	16	19	MBU
32	520	2 yrs	M	blood	chronic osteomyeliti	21	18	PEU
33	8859	2 yrs	M	blood	sickle cell disease	16	15	PEU
34	9046	20 yrs	M	pus	purulent blister	19	18	OPD
35	439	20 yrs	M	ear swab	otitis media	17	14	OPD
36	1107	27 yrs	M	ear swab	ear discharge	21	20	OPD
37	938	3 days	M	blood	sepsis	17	19	MBU
38	3178	3 days	M	blood	sepsis	19	18	MBU
39	499	3 mths	M	blood	sepsis	18	19	MBU
40	409	3 mths	M	blood	sepsis	17	15	B3

APPENDIX 1A. Results obtained in the study.

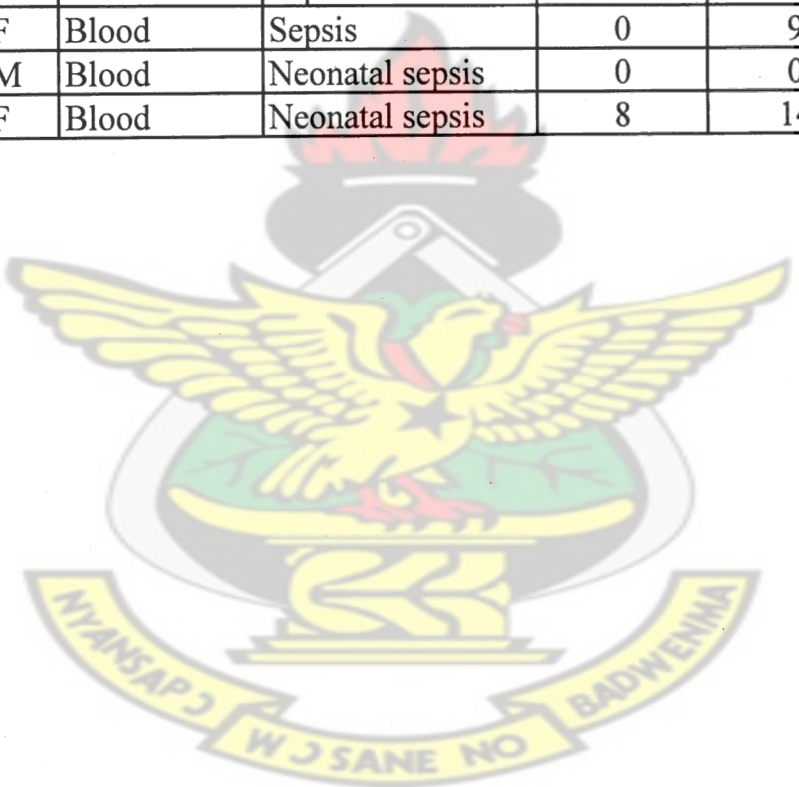
41	2805	3 mths	M	blood	sepsis	21	18	MBU
42	1384	30 yrs	M	pus	wound discharge	18	15	OPD
43	879	30 yrs	M	blood	septicaemia	17	16	OPD
44	3496	30 yrs	M	ear swab	otitis media	15	18	OPD
45	361	31 yrs	M	blood	sepsis	15	13	OPD
46	355	32 yrs	M	blood	pyomyositis	14	17	D3
47	9182	33 yrs	M	blood	sepsis	17	19	D4
48	8159	4 days	M	blood	sepsis	16	17	MBU
49	9155	4 yrs	M	blood	sepsis	17	17	PEU
50	8677	4 yrs	M	blood	severe malaria	21	19	PEU
51	8188	4 yrs	M	blood	sepsis	13	15	PEU
52	4093	4 yrs	M	blood	fever	12	14	PEU
53	8231	6 days	M	blood	sepsis	13	16	MBU
54	4163	6 mths	M	blood	sepsis	14	18	MBU
55	918	6 yrs	M	blood	sepsis	18	15	PEU
56	7773	7 yrs	M	blood	septicaemia	17	21	PEU
57	7432	7 yrs	M	blood	severe malaria	19	21	PEU
58	4072	day old	M	blood	asphyxia	18	15	MBU
59	9066	day old	M	blood	sepsis	17	15	MBU
60	7877	day old	M	blood	asphyxia	16	15	MBU
61	7384	day old	M	blood	sepsis	17	14	MBU
62	8005	day old	M	blood	asphyxia	19	18	MBU
63	7308	5 yrs	M	blood	sepsis	16	17	PEU
64	8591	24yrs	F	Blood	BV+end DP	0	0	D5
65	8120	65yrs	F	Blood	Enteric fever	0	0	OPD
66	8602	10mths	M	Blood	Septic arthritis	0	0	PEU
67	875	1day	F	Blood	Neonatal Sepsis	0	0	MBU
68	2803	1day	F	Blood	Sepsis	10	14	MBU
69	77 92	1day	F	Blood	Asphyxia	0	10	MBU
70	7927	1mth	M	Blood	Sepsis	7	7	MBU
71	9089	1wk	F	Blood	Neonatal sepsis	0	0	MBU
72	3912	1wk	F	Blood	Asphyxia	0	0	MBU
73	8437	1yr	M	Blood	Bronchopneumoni	0	0	PEU
74	7930	1yr	M	Blood	Sepsis	0	8	PEU
75	352	1yr	M	Blood	Sepsis	10	15	PEU
76	882	1yr	M	Blood	Sepsis	0	0	PEU
77	7760	1yr	F	Blood	Sepsis	0	0	PEU
78	315	1yr	M	Blood	Sepsis	0	0	PEU
79	7279	1yr	F	Blood	Septicaemia	10	5	PEU
80	7756	1yr	M	Blood	Sepsis	7	6	PEU
81	8707	1yr	F	Blood	Sepsis	0	0	PEU
82	8141	20yrs	M	Blood	Infected burns	0	0	D4
83	3272	20yrs	M	Wound swab	Pustular discharge	0	7	OPD
84	513	20yrs	F	Wound swab	Pintract infection	7	17	OPD
85	825	21days	F	Blood	Neonatal jaundice	0	0	MBU
86	3357	22yrs	M	Wound swab	Laparatomy	0	0	B2
87	3358	22yrs	M	Nasal swab	Nasal discharge	0	0	OPD

APPENDIX 1A. Results obtained in the study.

88	677	22yrs	M	Ear swab	Ear discharge	0	0	OPD
89	9145	24yrs	F	Blood	Septicaemia	5	12	OPD
90	282	24yrs	F	Wound swab	Pintract infection	0	0	OPD
91	3179	26yrs	F	Wound swab	Septic wound	0	18	OPD
92	9052	2days	F	Blood	Neonatal jaundice	8	14	MBU
93	943	2days	F	Blood	Neonatal jaundice	0	6	MBU
94	7807	2days	F	Blood	Sepsis	0	0	MBU
95	7870	2mths	F	Blood	Bronchopneumoni	0	0	MBU
96	8780	2mths	F	Blood	Pneumonitis	0	0	MBU
97	7353	2yrs	M	Blood	Sepsis	0	0	PEU
98	327	2yrs	F	Blood	Sepsis	0	0	PEU
99	7300	2yrs	M	Blood	Sepsis	0	13	PEU
00	8139	2yrs	M	Blood	Sepsis	0	0	PEU
01	7907	2yrs	M	Blood	Septicaemia	0	9	PEU
02	8738	2yrs	F	Blood	Sepsis	0	0	OPD
03	7878	30yrs	M	Blood	Sepsis	10	16	OPD
04	7761	31yrs	F	Blood	Sepsis	0	0	OPD
05	8680	32yrs	F	Blood	PROM	9	0	A4
06	84OO	3days	F	Blood	Sepsis	0	0	MBU
07	8401	3days	F	Blood	Sepsis	0	0	MBU
08	8501	3mths	F	Blood	Bronchopneumoni	0	0	PEU
09	413	3wks	F	Blood	Bronchopneumoni	9	10	MBU
10	8032	3wks	F	Blood	Osteoarthritis	0	0	MBU
11	8728	3yrs	M	Blood	Septicaemia	10	9	B2
12	9449	42yrs	M	Blood	Sepsis	0	0	OPD
13	8689	46yrs	F	Blood	HIV	0	0	D5
14	873	4mths	F	Blood	Sepsis	0	0	PEU
15	1319	50yrs	M	Wound swab	Chronic ulcer	0	0	OPD
16	9140	57yrs	F	Blood	Sepsis	10	11	MEU
17	7301	59yrs	F	Blood	Sepsis	0	0	OPD
18	7287	5days	F	Blood	Neonatal jaundice	0	0	MBU
19	4184	5mths	F	Blood	Severe	0	0	PEU
20	8478	5wks	F	Blood	Sepsis	0	0	MBU
21	7292	5yr	F	Blood	Sepsis	0	0	PEU
22	9118	5yrs	M	Blood	Sepsis	0	0	OPD
23	8329	6days	F	Blood	Sepsis	0	0	MBU
24	1307	6mths	F	Blood	Pneumonia	0	0	PEU
25	9144	6yrs	F	Blood	Sepsis	7	8	OPD
26	426	7mths	F	Blood	Sickle cell with	8	15	OPD
27	869	7mths	M	Pus	Abscess	0	0	PEU
28	3233	7mths	M	Blood	Septicaemia	0	13	PEU
29	7385	7mths	M	Blood	Sepsis	0	0	PEU
30	1320	7yrs	F	Ear swab	Bloody ear	0	15	OPD
31	7208	80yrs	F	Blood	Sepsis	9	7	OPD
32	650	80yrs	F	Wound swab	Ulcers of right	0	0	OPD
33	545	84yrs	M	Wound swab	Liver abscess	7	37	OPD
34	7757	9mths	M	Blood	Sepsis	10	19	PEU

APPENDIX 1A. Results obtained in the study.

35	7374	9mths	M	Blood	Sepsis	0	15	PEU
36	609	9mths	M	Blood	Sickle cell with	7	10	PEU
37	9004	9mths	M	Blood	Sepsis	0	0	OPD
38	9081	9yr	M	Blood	Sepsis	10	10	PEU
39	8733	Day old	F	Blood	Sepsis	0	0	MBU
40	927	Day old	F	Blood	Sepsis	0	0	MBU
41	9176	Day old	F	Blood	Maternal PROM	8	8	MBU
42	9060	Day old	M	Blood	Neonatal sepsis	0	0	MBU
43	8075	Day old	F	Blood	Sepsis	0	0	MBU
44	8238	Day old	F	Blood	Haemorrhagic	8	13	MBU
45	9061	Day old	M	Blood	Neonatal sepsis	0	0	MBU
46	8264	Day old	F	Blood	Mild asphyxia	0	0	MBU
47	8817	Day old	F	Blood	Sepsis	0	0	MBU
48	1247	Day old	F	Blood	Sepsis	0	9	MBU
49	258	Day old	M	Blood	Neonatal sepsis	0	0	PEU
50	3488	Day old	F	Blood	Neonatal sepsis	8	14	MBU



APPENDIX 1 B. Hospital associated infections (HA- MRSA)

NO	PATH NO.	AGE	SEX	SPECIMEN	PENICILLIN	OXACILLIN	DIAGNOSIS	SOURCE
1	84OO	3days	F	Blood	0	0	Sepsis	MBU
2	1307	6mths	F	Blood	0	0	Pneumonia	PEU
3	8733	Day old	F	Blood	0	0	Sepsis	MBU
4	413	3wks	F	Blood	10	9	Bronchopneumonia	MBU
5	7353	2yrs	M	Blood	0	0	Sepsis	PEU
6	327	2yrs	F	Blood	0	0	Sepsis	PEU
7	9052	2days	F	Blood	14	8	Neonatal jaundice	MBU
8	8141	20yrs	M	Blood	0	0	Infected burns	D4
9	7300	2yrs	M	Blood	13	0	Sepsis	PEU
10	8437	1yr	M	Blood	0	0	Bronchopneumonia	PEU
11	943	2days	F	Blood	6	0	Neonatal jaundice	MBU
12	7936	1yr	M	Blood	8	0	Sepsis	PEU
13	7292	5yr	F	Blood	0	0	Sepsis	PEU
14	8139	2yrs	M	Blood	0	0	Sepsis	PEU
15	9140	57yrs	F	Blood	11	10	Sepsis	MEU
16	8329	6days	F	Blood	0	0	Sepsis	MBU
17	426	7mths	F	Blood	15	8	Sickle cell with fever	OPD
18	927	Day old	F	Blood	0	0	Sepsis	MBU
19	873	4mths	F	Blood	0	0	Sepsis	PEU
20	875	1day	F	Blood	0	0	Neonatal Sepsis	MBU
21	352	1yr	M	Blood	15	10	Sepsis	PEU
22	2803	1day	F	Blood	14	10	Sepsis	MBU
23	882	1yr	M	Blood	0	0	Sepsis	PEU
24	825	21days	F	Blood	0	0	Neonatal jaundice	MBU
25	869	7mths	M	Pus	0	0	Abscess	PEU
26	3233	7mths	M	Blood	13	0	Septicaemia	PEU
27	9176	Day old	F	Blood	8	8	Maternal PROM	MBU
28	9081	9yr	M	Blood	10	10	Sepsis	PEU
29	7807	2days	F	Blood	0	0	Sepsis	MBU
30	7927	1mth	M	Blood	7	7	Sepsis	MBU
31	7760	1yr	F	Blood	0	0	Sepsis	PEU
32	7757	9mths	M	Blood	19	10	Sepsis	PEU
33	8602	10mths	M	Blood	0	0	Septic arthritis	PEU
34	315	1yr	M	Blood	0	0	Sepsis	PEU
35	7870	2mths	F	Blood	0	0	Bronchopneumonia	MBU
36	7279	1yr	F	Blood	5	10	Septicaemia	PEU
37	9060	Day old	M	Blood	0	0	Neonatal sepsis	MBU
38	8075	Day old	F	Blood	0	0	Sepsis	MBU
39	7374	9mths	M	Blood	15	0	Sepsis	PEU
40	7385	7mths	M	Blood	0	0	Sepsis	PEU
41	7792	1day	F	Blood	10	0	Asphyxia	MBU
42	8780	2mths	F	Blood	0	0	Pneumonitis	MBU
43	8501	3mths	F	Blood	0	0	Bronchopneumonia	PEU
44	8238	Day old	F	Blood	13	8	Haemorrhagic capue	MBU
45	9061	Day old	M	Blood	0	0	Neonatal sepsis	MBU
46	9089	1wk	F	Blood	0	0	Neonatal sepsis	MBU
47	8264	Day old	F	Blood	0	0	Mild asphyxia	MBU
48	7907	2yrs	M	Blood	9	0	Septicaemia	PEU
49	7756	1yr	M	Blood	6	7	Sepsis	PEU
50	8032	3wks	F	Blood	0	0	Osteoarthritis	MBU
51	8707	1yr	F	Blood	0	0	Sepsis	PEU
52	3357	22yrs	M	Wound swab	0	0	Laparatomy	B2

Oxacilin Zone of inhibition in mm: ≤ 10 - MRSA; 11-12 - intermidiate; ≥13 - Sensitive

Penicillin Zone of inhibition in mm: ≤ 28 Resistant; ≥ 29 -Sensitive.

APPENDIX 1 B. Hospital associated infections (HA- MRSA)

53	8728	3yrs	M	Blood	9	10	Septicaemia	B2
54	8680	32yrs	F	Blood	0	9	PROM	A4
55	8591	24	F	Blood	0	0	BV+end DP infective	D5
56	8817	Day old	F	Blood	0	0	Sepsis	MBU
57	609	9mths	M	Blood	10	7	Sickle cell with fever	PEU
58	9004	9mths	M	Blood	0	0	Sepsis	OPD
59	8401	3days	F	Blood	0	0	Sepsis	MBU
60	8478	5wks	F	Blood	0	0	Sepsis	MBU
61	7287	5days	F	Blood	0	0	Neonatal jaundice	MBU
62	8689	46yrs	F	Blood	0	0	HIV encephalopathy	D5
63	1247	Day old	F	Blood	9	0	Sepsis	MBU
64	258	Day old	M	Blood	13	0	Neonatal sepsis	PEU
65	3912	1wk	F	Blood	0	0	Asphyxia	MBU
66	4184	5mths	F	Blood	0	0	malnourishment	PEU
67	3488	Day old	F	Blood	14	8	Neonatal Sepsis	MBU

APPENDIX 1C. Community-associated infections (CA-MRSA)

NO	PATH NO	AGE	SEX	SPECIMEN	PENICILLIN	OXACILLIN	DIAGNOSIS	SOURCE
1	9449	42yrs	M	Blood	0	0	Sepsis	OPD
2	1319	50yrs	M	Wound swab	0	0	Chronic ulcer	OPD
3	8120	65	F	Blood	0	0	Enteric fever	OPD
4	3179	26yrs	F	Wound swab	18	0	Septic wound	OPD
5	8738	2yrs	F	Blood	0	0	Sepsis	OPD
6	545	84yrs	M	Wound swab	37	7	Liver abscess	OPD
7	9118	5yrs	M	Blood	0	0	Sepsis	OPD
8	9144	6yrs	F	Blood	8	7	Sepsis	OPD
9	3358	22yrs	M	Nasal swab	0	0	Nasal discharge	OPD
10	7878	30yrs	M	Blood	16	10	Sepsis	OPD
11	7301	59yrs	F	Blood	0	0	Sepsis	OPD
12	9145	24yrs	F	Blood	12	5	Septicaemia	OPD
13	7761	31yrs	F	Blood	0	0	Sepsis	OPD
14	3272	20yrs	M	Wound swab	7	0	Pustular discharge	OPD
15	7208	80yrs	F	Blood	7	9	Sepsis	OPD
16	282	24yrs	F	Wound swab	0	0	Pintract Infection	OPD
17	650	80yrs	F	Wound swab	0	0	Ulcers of right foot	OPD
18	513	20yrs	F	Wound swab	17	7	Pintract infection	OPD
19	677	22yrs	M	Ear swab	0	0	Ear discharge	OPD
20	1320	7yrs	M	Ear wab	15	0	Bloody ear discharge	OPD

APPENDIX 2

DETERMINATION OF MIC$_{(50)}$ AND MIC$_{(90)}$

The formula used to determine the median position for each MIC antibiotic was:

(N+1)/2, where N is the total number of observations.

From the table, N=50

Therefore (50+1)/2=25.5

This implies that the median lies between the 25th and 26th observations.
Therefore the average of the 25th and 26th values were calculated.

For oxacillin, median= (48+48)/2= 48 µg/ml
Similarly, for gentamicin median= (32+32)/2= 32 µg/ml
For SXT, median = (4+4)/2 = 4 µg/ml
For Ceftriaxone, median= (8+8)/2= 8 µg/ml

Calculations for Mean MICs

The formula used to calculate the mean was:

$(\sum fx)/N$,

Where:
f = frequency of a specific MIC value
N = Total number of observations
x = Specific MIC value

The mean for oxacillin was calculated this way:

[(2×4) + (9×8) + (4×12) + (5×16) + (4×32) + (3×48) + (18×192) + (5×256)]/50 = 104.32 µg/ml

Using similar method, means for:

1. gentamicin = 52.4 µg/ml
2. SXT = 6.74 µg/ml
3. Ceftriaxone = 9.97 µg/ml

MIC at 90%

The MIC at which 90% of the isolates were inhibited was calculated using the formula:
(90/100)*(N +1)---------- (A),

Where N is the total number of observations.

This formular was used to locate the MIC at 90.

For oxacillin at 90 the following calculations were done:

Firstly, the position of oxacillin at 90 was determined using the formula in (A)
(90/100)*(50 + 1) = 45.9

This implies that the oxacillin at 90 lies between the 45th and 46th observations. Therefore the average of the 45th and 46th values were calculated.

The oxacillin at 90 is given by 224µg/ml

Similarly MIC at 90 for:

1. gentamicin is given by 256 µg/ml
2. SXT is given by 8 µg/ml
3. Ceftriaxone is given by 32 µg/ml

Table 2 Age distribution of study population

Age (Years)	Number of isolates	(%)
<1	101	40.4
1-9	69	27.6
10-19	15	6.0
20-29	26	10.4
30-39	19	7.6
40-49	4	1.6
50-59	9	3.6
60-69	1	0.4
70-79	2	0.8
80-89	4	1.6
Total	250	100.0

Table 3 Distribution of HA-MRSA from the various departments

Source	HA-MRSA (n= 67)	
	Number of isolates	%
MBU	31	46.3
PEU	27	40.3
OPD	2	3
B2	2	3
D5	2	3
A4	1	1.5
D4	1	1.5
MEU	1	1.5
Total	67	100

Table 4 Distribution of MRSA isolates among the age groups of patients

Age(Years)	CA-MRSA (n =20)		HA-MRSA (n =67)		Total	
	Number of isolates	%	Number of Isolates	%	Number of Isolates	%
<1	0	0	44	65.7	44	50.6
1-9	4	20	17	25.4	21	24.1
10-19	0	0	0	0	0	0
20-29	7	35	3	4.4	10	11.5
30-39	2	10	1	1.5	3	3.4
40-49	1	5	1	1.5	2	2.3
50-59	2	10	1	1.5	3	3.4
60-69	1	5	0	0	1	1.1
70-79	0	0	0	0	0	0
80-89	3	15	0	0	3	3.4
Total	20	100	67	100	87	100

Table 5 Distribution of MRSA in relation to presenting condition

Condition	CA-MRSA (n = 20)		HA-MRSA (n = 67)		Total	
	Number of isolates	%	Number of isolates	%	Number of isolates	%
Sepsis	8	40	35	52.2	43	49.4
Neonatal Sepsis	0	0	6	9	6	6.9
Septicaemia	1	5	4	5.9	5	5.7
Asphyxia	0	0	3	4.5	3	3.4
Neonantal Jaundice	0	0	4	5.9	2	2.3
Pintract Infection	2	10	0	0	2	2.3
Sickle cell with fever	0	0	2	3	2	2.3
Abscess	0	0	1	1.5	1	1.1
BV+end DP infective	0	0	1	1.5	1	1.1
Chronic Ulcer	1	5	0	0	1	1.1
Ear infections	2	10	0	0	2	2.3
Enteric Fever	1	5	0	0	1	1.1
Haemorrhagic capue	0	0	1	1.5	1	1.1
HIV encephalopathy	0	0	1	1.5	1	1.1
Infected burns	0	0	1	1.5	1	1.1
Laparotomy	0	0	1	1.5	1	1.1
Liver Abscess	1	5	0	0	1	1.1
Maternal PROM	0	0	2	3	2	2.3
Nasal discharge	1	5	0	0	1	1.1
Osteomyelitis	0	0	1	1.5	1	1.1
Pneumonia	0	0	6	8.9	6	6.9
Pustular discharge	1	5	0	0	1	1.1
Septic Arthritis	0	0	1	1.5	1	1.1
Septic wound	1	5	0	0	1	1.1

Severe malnourishment	0	0	1	1.5	1	1.1
Ulcers of the feet	1	5	0	0	1	1.1

Table 6 Distribution of MRSA isolates from clinical specimens

Specimen	HA-MRSA (n=67)	%	CA-MRSA (n=20)	%	Total	%
Blood	65	97	10	50	75	86.2
wound swab	1	1.5	7	35	8	9.2
Nasal swab	0	0	1	5	1	1.2
Ear swab	0	0	2	10	2	2.3
Pus	1	1.5	0	0	1	1.2

Table 7 Proportion of males and females in CA and HA-MRSA

Sex	CA-MRSA (n=20)	HA-MRSA (n=67)	p-value
Male (%)	40.0	38.8	0.923
Female (%)	60.0	61.2	0.923

Table 9 Geometric mean of HA and CA isolates

CA-MRSA (n=20)	HA-MRSA (n=67)	
Geometric Mean (SD)	Geometric Mean (SD)	p-value
1.1 (3.7)	1.0 (3.6)	0.791

Table 10 Summary of MIC of MRSA isolates to four antibiotics

Antibiotic Strip	MIC µg/ml		
	Range	MIC(50)	MIC(90)
Oxacillin	4 - ≥256	48	≥256
Gentamicin	0.125 - ≥256	32	≥256
SXT	0.064- ≥32	4	8
Ceftriaxone	1.5-≥32	8	≥32

Table 11 MIC of fifty MRSA isolates to Oxacillin as determined by the E-test.

MIC(µg/ml)	4	8	12	16	32	48	192	≥256
No of isolates	2	9	4	5	4	3	18	5

Table 12 MIC of fifty MRSA isolates to gentamicin as determined by the E-test

MIC(µg/ml)	0.125	0.38	1.5	4	8	12	24	32	≥256
No of isolates	2	1	1	9	3	3	5	19	7

Table 13 MIC of fifty MRSA isolates to trimethoprim-sulfamethoxazole (sxt) as determined by the E-test.

MIC(µg/ml)	0.064	0.094	0.25	0.50	2	4	8	≥32
No. of isolates	4	1	1	1	2	16	22	3

Table 14 MIC of fifty MRSA isolates to ceftriaxone as determined by the E-test

MIC(µg/ml)	1.5	2	4	8	≥ 32
No. of isolates	3	7	14	17	9

Table 15 CLSI interpretive criteria for three antibiotic test strips

Antibiotic	Susceptible	Intermediate	Resistant
Oxacillin	≤ 2	-	≥ 4
Gentamicin	≤ 4	8	≥ 16
SXT	≤ 2	-	≥ 4

Table 16 MIC of control strain

Antibiotic strip	MIC µg/ml
Oxacillin	2
Gentamicin	0.125
SXT	0.064
Ceftriaxone	2

APPENDIX 3. Excel analysis

Anova: Single Factor (Prevalence)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	10	95	9.5	124.7222
Column 2	10	104.7	10.47	461.6046

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.7045	1	4.7045	0.016047	0.900599	4.413873
Within Groups	5276.941	18	293.1634			
Total	5281.646	19				

Anova: Single Factor (Antibiotic resistance)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	545	68.125	718.9821
Column 2	8	510	63.75	848.2143

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	76.5625	1	76.5625	0.097706	0.759207	4.60011
Within Groups	10970.38	14	783.5982			
Total	11046.94	15				

APPENDIX 3B

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF CLINICAL MICROBIOLOGY

MRSA QUESTIONAIRE FORM

Patient s Particulars

Name ……

Age…… Sex……

Locality……

Hospital associated infections (HA-MRSA)

1. Have you undergone surgery? [YES] [NO]

   If yes, when? ……

2. Have you been hospitalized previously? [YES] [NO]

   If yes, when? ……

   For how long……

3. Cause of Infection

   Septicaemia……Osteomyelitis……

   Sepsis……Cellulites……

   Pneumonia…… Others ……

4. Past medical history

   Any History of the following?

   Diabetes [YES] [NO]

   Lung disease [YES] [NO]

   Heart disease [YES] [NO]

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF CLINICAL MICROBIOLOGY

MRSA QUESTIONNAIRE FORM

Patient's particulars

Name……..

Age…… Sex……

Locality……

COMMUNITY ASSOCIATED INFECTIONS

1. Have you undergone surgery before? [YES] [NO]

If yes, when ?.....

2. Have you been hospitalized before? [YES] [NO]

If yes, when?.....

For how long?.....

3. Cause of Infection

Wound infection…….. Carbuncles……

Furuncles (boils)…… Abscesses……

Cellulites …… Others ……

4. Past Medical History

Diabetes [YES] [NO]

Lung disease [YES] [NO]

Heart disease [YES] [NO]

Others……